Thesis for the Degree of Master of Science

eDNA metabarcoding analysis of fish assemblages and benthic microbiomes in the furrowed seabed area

by

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The Graduate School

Pukyong National University

August 2020

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(eDNA 메타바코딩을 통한 어류 군집과 저서 미생물에 대한 해저 지형변화의 영향 분석)

Advisor: Prof. Hyun-Woo Kim

by

Hyun Sagong

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Metabarcoding analysis of fish assemblage in the aggregate extraction area

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Abstract

Although physical changes in the seabed caused by artificial activity are known to have broad impacts on the marine ecosystem, its scientific understanding for the reliable assessment of their effect still lacks in Korean waters. However, traditional survey methods require a high degree of cost and labors to obtain factual data. Alternatively, we here adopted the environmental DNA metabarcoding (eDNA metabarcoding) technique to analyze the fish and benthic microbial assemblages. Fish and microorganism were analyzed by Illumina MiSeq system using Mifish primer and 16S rDNA primer, respectively, for environmental samples collected in the furrowed area (3H and 3I), nearby area (4D, 4G, and 5H) and distance located area (N, E, E1, W, and S) in September and November 2019. A total of 86 fish species were identified from 20 sites from the MiFish pipeline. Based on the similarity analysis, three fish assemblage clades were identified; those in furrowed, in September, and in November. Heat map analysis revealed that the *Pagrus major* is the species statistically abundant in the furrowed area compared with the other two clades. The difference of fish assemblage in the furrowed area from the other two clades appeared to be to the environmental changes by artificial physical activity, such as changes in seabed topography and hydrodynamic characteristics, providing a favorable environment for them. Besides, the furrowed clade showed significantly low biodiversity compared with those of the others. Different from the fish assemblage, there was no detectable difference in microbiomes of the seabed between furrowed area and unaffected ones suggesting its fast recovery in the surface of the seabed. Instead, higher microbial biomass was identified in site E1, where the remote area of the furrowed one.Further study is needed to determine whether this result is due to the impact of suspended sediment caused by artificial physical activity in the seabed transported by ocean currents or due to regional

characteristics. From this study, we have identified that the changes of fish assemblage induced by the physical change in the seabed by the artificial impacts in Korean waters using eDNA metabarcoding analysis, which suggested that this technique is useful for the estimating the changes in the marine ecosystem with low cost and labors. However, it is too early to conclude by a single short-term study and further study should be conducted to obtain better results.

메타바코딩 분석을 통한 골재채취 해역에서 어류상 분석 연구

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요약

인위적 활동에 의한 해저의 물리적 변형은 해양생태계에 광범위한 영향을 주는 것으로 알려져 있으나 한국 해역에서의 영향에 대한 과학적이고 객관적인 분석 자료가 부족한 실정이다. 본 논문은 남해의 해저에서 이루어 지고 있는 물리적 활동이 해양 생태계에 미치는 영향을 평가하기 위해 전통적인 생태학적 접근법의 대안으로 떠오르고 있는 환경 DNA 메타바코딩 (eDNA metabarcoding) 방법을 이용하여 어류와 저층 미생물의 군집 구조를 비교분석하였다. 2019년 9월과 11월에 해저가 물리적 변형된 해역 (3H, 3I)와 인근 해역 (4D, 4G, 5H), 그리고 약 20km 떨어진 해역 (N, E, E1, W, S) 에서 채집한 환경 시료에 대해 어류는 MiFish primer, 저서 미생물은 16S rDNA primer를 이용하여 Illumina MiSeq system을 통해 분석하였다. MiFish pipeline분석을 통해 20개의 정점에서 전체 어류 86종이 확인되었고 군집다양성의 유사분석도을 통해 해저가 물리적 변형된 해역, 9월 그리고 11월 해역의 3개의 group을 확인하였다. Heatmap 분석을 통해 참돔이 해저가 물리적 변형된 해역과 다른 group을 구분하는 대표종으로 확인되었다. 이러한 어류 군집의 특이성은 인위적인 물리적 활동에 의한 해저의 지형과 수리수문학적 특성의 변화가 이들에게 유리한 환경을 제공하였기 때문이라고 생각된다. 또한 생물다양성 통계분석을 통하여 해저가 물리적으로 변형된 해역에서 다른 해역에 비해 매우 유의하게 낮은 생물다양성이 확인되었다. 따라서 해저에서의 물리적 변형은 서식지의 인위적인 파괴로 인하여 종 다양성에 악영향을 끼치는 것으로 파악된다. 저서 미생물 군집 구조 분석을 통해 보았을 때 해저의 물리적 변화에 의한 유의한 차이가 확인되지 않았으며 계절에 따른 구분이 확인되었다. 이는 해저의 물리적 변형의 영향이 저서 미생물 군집 구조의 변화에는 크게 작용

하지 않은 것으로 파악된다. 다만 해저가 물리적으로 변형된 해역과 먼 곳에 위치한 E1 지역에서 특이적으로 높은 미생물량을 확인하였다. 이와 같은 결과가 해저에서의 물리적 활동으로 발생한 부유사가 해류에 의해 이동한 결과인지 아니면 지역적 특성 요인에 의한 것인지에 대한 추가적이 연구가 필요하다. 본 연구를 통하여 한국 해역의 생태계에 대한 해저의 인공적인 물리적 변형의 영향을 확인하였으며 환경 DNA를 이용한 분석법이 환경 교란에 따른 생태계의 변화를 분석하는데 뛰어나다는 것을 확인하였다. 하지만 본 연구는 단 기간의 연구이며 해양 생태계는 지역적 환경에 크게 좌우된다. 그러므로 지역적 환경을 고려한 장기간의 추가 연구가 필요하다.

INTRODUCTION

Seabed contains various mineral resources (e.g., aggregates such as sand and gravels) and energy sources (e.g., oil and gas), and diverse biotas are distributed depending on the depth of the water. On the seabed, activities to supply these resources have been made (e.g., aggregate extraction, oil drilling, and Bottom trawl), and these activities bring artificial physical changes in the seabed (Todd et al. 2019). Physical events in the seabed for the acquisition of resources often accompanied the creation of various sizes of pits or furrows or the changed sediment composition near the sites (Birklund and Wijsman 2005, Kim and Grigalunas 2009). Additionally, the suspended sediments containing organic matter, nutrients, or other contaminants (e.g., heavy metals) were also released during physical activities, which can spread up to approximately 10 km away (Birklund and Wijsman 2005, Jones et al. 2016, Won et al. 2017).

In order to assess the potential environmental and ecological effects of physical change in the seabed and provide its guidelines, studies have been conducted (Birklund and Wijsman 2005, Mensah 1997, Phua et al. 2002, Byrnes et al. 2004). First, physical activity on the seabed changes the sediment composition changing benthic fauna (Desprez et al. 2010)

and diversity and abundance of crustacean species (Son and Han 2007). Besides benthos, fish diversity in the area is also impacted negatively (Hwang et al. 2014). By contrast, Newell et al. (2004) reported that the method of dredging might have little effect on the assemblage composition of macrofauna as well as an increase in species diversity, density, and biomass around the area where the seabed is physically changed. Those contrasting results have been mainly dependent on the traditional survey methods (e.g., visual surveys and trawling), which requires a high amount of budget as well as time-consuming analysis with well-trained specialists to the reliable result. That weakness of the traditional survey method was a significant challenge to obtain extensive scale data, which would be used to draw statistically reliable results. Therefore, conflicting results often produced from the different research groups with limited information making it difficult to draw reliable conclusions that the majority could agree.

As an alternative way of the traditional survey methods, environmental DNA (eDNA) metabarcoding methods are currently being applied to analyze biodiversity from a marine environment. eDNA refers to all the genetic materials in the environment (e.g., water, soil, or air), which from skin tissue, scales, hair, mucus, and excreta that have been

removed from organisms (Bohmann et al. 2014, Taberlet et al. 2012). eDNA metabarcoding can analyze a biota directly with high sensitivity from the environmental samples (water, soil, or air) without environmental destruction (Stat et al. 2017, Djurhuus et al. 2018, Ficetola et al. 2008). Besides, this method allows rapid and accurate analysis of an enormous amount of marine species composition in the study area and understand the change of biodiversity (Harvey et al. 2017, Stoeckle et al. 2017, Thomsen et al. 2012). Therefore, the eDNA metabarcoding approach is suitable for analyzing species composition of fishery resources in the area where the seabed is physically changed and surrounding areas.

In this study, we conducted the eDNA metabarcoding analysis for assessment of the impact of physical change in the seabed change on the marine ecosystem in the southern sea of Korea water. Assemblage composition and biodiversity were analyzed from environmental samples, and the areas where the seabed is physically changed and surrounding control areas were compared. First, to confirm the impact on the water layer, the fish assemblage was analyzed from the seawater sample using the mitochondrial 12S ribosomal RNA region. Then, to confirm the impact on the seabed sediment, we analyzed the benthic microbial assemblage from sediment samples using the 16S ribosomal RNA region.

MATERIALS AND METHODS

Sample collection and DNA extraction

Seawater and sediment samples were collected from the southern sea of Korea water in September and November 2019 by the National Institute of Fisheries Science (NIFS). Sampling was carried out in the two "Furrowed area" with a physical change in the seabed (3I and 3H), and three "Nearby area" where adjacent from the "Furrowed area" to 2.41 - 7.89 km (4D, 4G, and 5H). The other sampling sites were five "Distance located area" about 20 km from other areas (N, E, E1, W, and S) (Fig. 1). Seawater samples were collected two liters (one liter each at surface layer and 50m depth) using on each site. Sediments were collected using Van Veen grab sampler (0.1 m^3), and collected sediment samples were stored in sterile 50 ml conical tubes. The seawater samples were filtered through 0.45 μm pore-sized GN-6 membrane filter (47mm, Pall Corporation, USA), and the filtered membranes were stored at -70℃ until DNA extraction is conducted. The membrane filters, Lysis buffer (630 μL of ATL buffer), and 70 μL of Protenase K and ceramic sphere were added to

a sterile 2 mL microtube, and the mixture was further homogenized using FastPrep-2 (MP Biomedicals™, USA). Homogenized filters were extracted genomic DNA using DNeasy® Blood & Tissue Kit (Qiagen, Germany) following the manufacturer's protocol. The sediment samples were extracted genomic DNA for 0.3g of sediment using DNeasy® PowerSoil Kit (Qiagen, Germany) following the manufacturer's protocol. Extracted genomic DNA of seawater and sediment samples were quantified using a Nano-drop spectrophotometer ND-1000 (Thermo Scientific, USA) and then stored at -70℃.

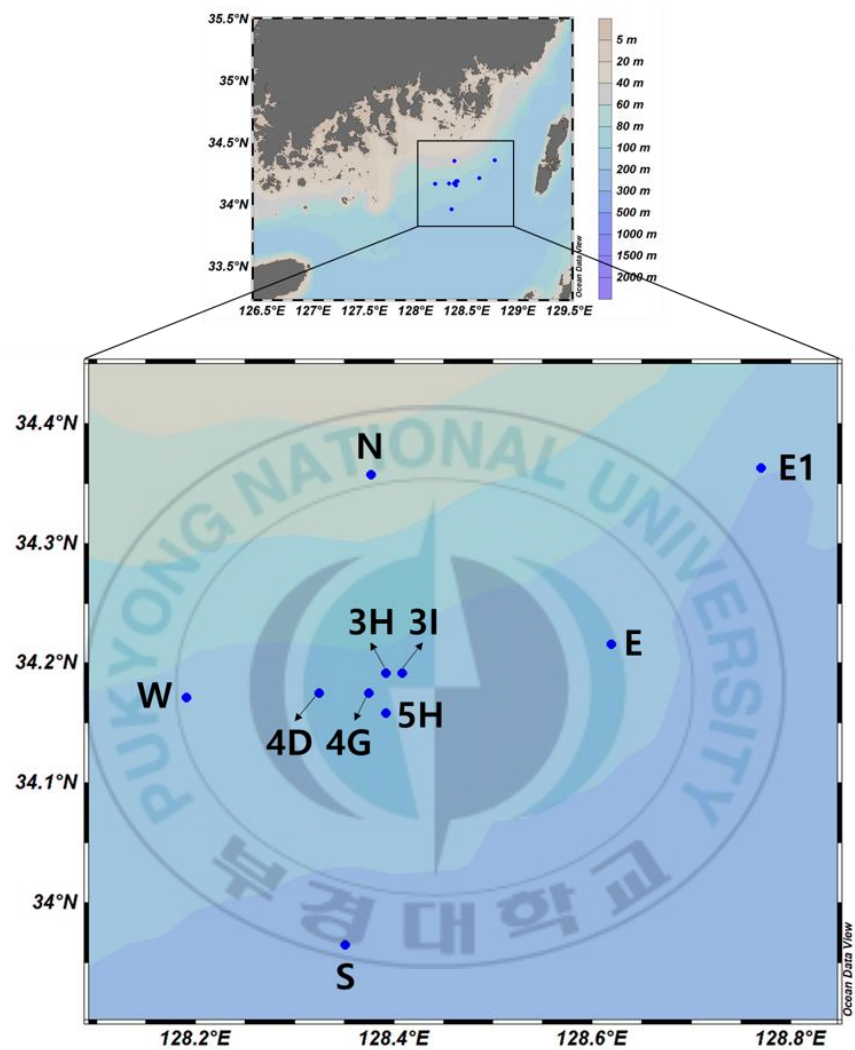

Fig. 1. Sampling sites of furrowed area (site 3H and 3I), nearby area (site 4D, 4G, and 5H) and distance located area (site N, E, E1, W and S) in southern waters of Korea (2019).

Quantitative PCR analysis (sediment sample)

To quantify the total microbes in sediment samples, quantitative PCR (qPCR) was performed using quantitative PCR. For the bacterial PCR amplification, 16S universal primers (Herlemann et al. 2011) were used. qPCR mixture (20 μL) included 4 μL of the genomic DNA, 1.0 μL of each primer (10 pmol), 10 μL of Luna® universial qPCR Master Mix (NEB, USA), and 4 μL of DNase/RNase-free water. qPCR cycling profile after an initial denaturation at 94℃ for 5min was as follows: 35 cycle of denaturation at 94℃ for 30 s, annealing at 55℃ for 30s, and extension at 72℃ for 30s, and a final extension at 72℃ for 5min. The copy number was calculated by substituting Ct values into the standard curve.

Library construction and sequencing

To analyze the effects of physical changes in the seabed on the fish assemblage and microbiome, next generation sequencing (NGS) was used with MiSeq platform (Illumina, USA). Seawater and sediment samples were collected for the analysis of fish and benthic microbes assemblage, respectively. MiFish universal primer set was used for fish assemblage analysis amplifying 12S rRNA region of fish taxa (Miya et al. 2015). The 16S universal primer overhanging adapter sequences were used for the microbiome analysis. Primary PCR mixture (20μL) included template DNA (100ng for MiFish and based on the copy numbers for 16S), 1.0 μL of each MiFish primer (5 pmol), 2.0 μL of dNTPs (each 2.5mM), 2.0 μL of 10× EX Taq buffer, 0.2 μL of EX Taq Hot Start (TaKaRa, Japan) and DNase/RNase-free water. PCR cycle profile of MiFish library was as follows: initial denaturation at 94℃ for 3min; 33 cycle of 94℃ for 5s, 65℃ for 20 sec, and 72℃ for 15s; and a final extension at 72℃ for 5 min. PCR cycle profile of 16 library was as follows: initial denaturation at 94℃ for 5min; 35 cycle of 94℃ for 30 sec, 55℃ for 30 sec, and 72℃ for 30s; and a final extension at 72℃ for 5 min. The primary PCR products were separated by 1.5% agarose gel electrophoresis after stained with Loading

STAR (Dyne Bio, Korea). The expected size (250bp - 350bp for MiFish and 440 - 460bp for 16S) was pooled together the surface and middle layer of the same site and purified using AccuPrep® PCR/Gel DNA Purification Kit (Bioneer, Korea). The second PCR was performed in triplicate using the Nextera XT Index Kit (Illumina, USA). Second PCR mixture (20μm) included purified product (6 μL for MiFish and 4 μL for 16S), 1 μL of each index primer, 0.5 μL of dNTP (each 10mM), 4 μL of 5× Phusion HF Buffer (New England Biolabs, UK), 0.2 μL of Phusion® High-Fidelity DNA Polymerase (New England Biolabs, UK), and DNase/RNase-free water. PCR cycling profile after an initial denaturation at 94℃ for 3 min was as follows: 12 (for MiFish) and 15 (for 16S) cycles of denaturation at 94℃ for 30 s, annealing at 55℃ for 30s, and extension at 72℃ for 30s, and a final extension at 72℃ for 5 min. The agarose gel electrophoresis and gel purification were performed using the same as primary PCR. The concentration of constructed libraries was measured using Quantus™ Fluorometer (Promega, USA) and sequencing was carried out using the MiSeq platform.

Bioinformatic and statistical analyses

Raw reads of MiSeq for assess the fish assemblage structures were paired using Phyton 27 (v. 2.7.1) before uploaded to the MiFish pipeline (http://mitofish.aori.u-tokyo.ac.jp/mifish/). As the first step in MiFish pipeline, the quality of the raw reads were checked using FastQC software (https://www.bioinformatics.babraham.ac.uk/index.html). Low-quality reads (QV ≤ 20) were trimmed using SolexaQA (http://solexaqa.sourceforge.net/). Paired-and reads were assembled by FLASH (https://ccb.jhu.edu/software/FLASH/) and erroneous merged reads that contain N-nucleotides and showing unusual lengths were removed. The merged reads were clustered with a cutoff sequence identity of 99% using Usearch (filtering the size of less than 10), which BLASTN based on GenBank database, after removed chimeric sequence and primer sequence. The obtained haplotypes were assigned to species with cutoff sequence identity of 99% and e-value 10^{-5} using a BLASTn based on NCBI-NT database. Haplotypes with low identity (> 99%) were described as “Unidentified”.

Raw reads of MiSeq for assess the microbial assemblage structures

were paired using Python 27 (v. 2.7.1), and using Mothur software (v. 1.44.0), the reads were merged, filtered based on criteria (400 ~ 500 size length, least 7 bp, zero mismatches) and trimmed the primer sequences (pdiffs = 1). OTUs clustering was carried out with a cutoff sequence identity of 98% and chimeric sequences were removed using USEARCH (v. 8.1.1861). The taxonomic assignment was carried out using BLASTn (v. 2.10.0) based on the NCBI-NT database. The OTUs were assigned to species names with 99% and assigned to the top-hit genus or taxon with between 90% and 98% sequence identity. If the sequence identity was less than 90%, the OTU was described to "Unknown".

Assemblage similarity analysis of fish species and microbial phylum was performed according to Bray-Curtis similarity using PRIMER-E software v.6 (Primer-E Ltd., UK). The non-metric multidensional scaling (nMDS) analysis was performed based on the Bray-Curtis similarity with group average on square-root transformed abundance data to summarize the similarity between fish assemblage in two dimensions using PRIMER-

E software v.6. Nonparametric alpha diversity estimates for fish species were calculated using the DADA2 (1.16.0) in R (4.0.0) (Callahan et al. 2016) and Mothur (v. 1.44.0; http://www.mothur.org; Schloss et al. (2009)). Subsample for normalizing was conducted based on a sample of

the smallest read number (93,472 reads) before alpha diversity calculation. Two-sample t-test was performed to confirm that the difference of alpha diversity between samples was significant using XLSTAT (Version 2020.1.3, Addinsoft, USA). Heat maps of fish species and microbial phylum were generated using XLSTAT. (Version 2020.1.3, Addinsoft, USA). A one-way ANOVA followed by a Tukey's HSD test was performed to confirm that the difference of copy number of microorganisms between sites using the IBM SPSS Statistics Subscription.

RESULTS

Environmental Parameters

In September, water temperatures ranged from 23.09 to 25.07℃ in the surface layer and from 23.50 to 24.91℃ in 50m depth, respectively. Salinities oscillated between 31.57 and 32.84 psu in surface water and 32.51 and 33.53 psu in the water at 50m in depth, respectively. In November, water temperatures were lower than those in September ranging from 20.09 to 22.73℃ in the surface layer and from 16.10 23.07℃, respectively. By contrast, higher salinities were identified in November oscillating between 32.62 and 34.17 psu in the surface layer and between 33.31 and 34.32 psu in the 50m layer in depth, respectively (Table S2).

MiSeq sequencing and taxonomic assignment

As a result of eDNA metabarcoding analyses from 20 water samples (10 sites x 2 months), a total of 7,152,010 raw reads were obtained (Table 1). After trimmed, 5,906,682 merged reads were generated by the MiFish pipeline among which 5,479,604 reads (92.77 %) encoded fish taxa (3,443,340 in September and 2,036,264 in November, respectively). Among 28,377 haplotypes generated by MiFish pipeline, 23,573 exhibited a high degree of identity to the database (higher than 99 % sequence identity), while 4,804 (7.46 %) with low identity (lower than 90 % identity) were classified as "Unidentified" (Table 1 and supplement 3). Finally, a total of 86 fish species (69 in September and 63 in November) were assigned from 23,573 haplotypes (Table 1 and Table S2). 86 fish species included 79 genera, 52 families, 19 orders.

Differences in fish assemblage by eDNA metabarcoding analysis from 20 sample sites in two months (September and November) were compared. The average species numbers obtained from 2 liters of each sample site were 27.15, ranging from 10 to 38. When E1 site in September with weedy species numbers (10 species) was eliminated from the

calculation, its average species numbers increased up to 28.05 in the area. Slightly higher average species numbers were identified in September (27.7) than those in November (26.6). The highest species numbers were detected at 4G (38 species) in September, followed by at 3H (36 species) in November (Table 1).

Table 1. Summary of taxonomic assignment of MiSeq reads number by site for September and November.

Target (sample type)	Area	Station	September							November						
			Raw reads		Merged reads	Total reads	Yield rate (%)	Haplotypes	Species	Raw reads		Merged reads	Total reads	Yield rate (%)	Haplotypes	Species
			R1	R2						R1	R2					
Fish (Water)	Aggregate extraction	3H	335,768	333,598	306,493	293,765	95.85	1,105	28	444,835	451,064	322,845	282,263	87.43	1,496	36
		3I	419,813	416,636	389,075	378,717	97.34	1,062	30	362,475	366,899	263,458	238,993	90.71	669	23
		4D	374,502	372,879	341,923	323,996	94.76	1,424	33	354,540	363,763	249,676	213,391	85.47	1,449	25
		4G	443,617	441,999	405,670	392,418	96.73	1,299	38	205,860	216,223	135,403	116,536	86.07	700	24
		5H	413,194	411,206	380,934	365,239	95.88	1,033	27	276,026	282,622	192,276	169,713	88.27	840	24
	Control	N	397,696	393,408	361,144	347,203	96.14	1,385	33	262,348	264,548	196,993	172,203	87.42	1,008	20
		E	436,925	434,797	399,061	383,380	96.07	1,618	25	257,733	261,719	189,455	164,921	87.05	910	27
		E1	247,932	244,499	215,443	209,733	97.35	785	10	396,426	402,018	287,167	251,237	87.49	1,379	30
		W	425,468	423,555	390,649	374,569	95.88	1,585	26	399,816	406,967	293,495	257,618	87.78	1,316	27
		S	431,155	425,154	389,140	374,320	96.19	1,537	27	265,881	268,234	196,382	169,389	86.25	973	30

Target (sample type)	Area	Station	Raw reads		Merged reads	Total reads	Yield rate (%)	OTUs	Phylum	Raw reads		Merged reads	Total reads	Yield rate (%)	OTUs	Phylum
			R1	R2						R1	R2					
Microorganism (Sediment)	Aggregate extraction	3H	363,492	369,231	354,248	57,805	16.32	1,466	23	109,838	109,084	108,278	34,308	31.69	6,342	36
		3I	490,995	490,573	474,452	84,220	17.75	1,396	25	170,943	169,300	168,216	59,972	35.65	8,409	34
		4D	371,615	372,181	359,676	66,705	18.55	1,421	22	121,783	120,344	119,618	41,527	34.72	6,961	38
		4G	586,571	581,248	565,611	96,449	17.05	1,639	23	166,900	165,719	164,491	59,947	36.44	8,587	35
		5H	596,866	581,826	568,731	112,936	19.86	1,414	22	168,674	167,539	166,419	57,430	34.51	8,046	39
	Control	N	490,192	490,809	475,145	88,519	18.63	1,360	22	87,099	86,630	85,950	24,146	28.09	5,272	32
		E	262,949	264,277	255,907	45,542	17.80	1,333	23	183,832	182,555	181,285	65,648	36.21	9,342	36
		E1	422,428	421,552	408,094	73,956	18.12	1,339	23	142,964	142,487	141,052	42,723	30.29	5,757	34
		W	412,283	413,398	399,658	71,634	17.92	1,377	25	196,417	165,077	193,776	69,508	35.87	9,518	35
		S	351,582	340,653	332,441	53,917	16.22	1,232	23	155,989	155,758	154,316	43,406	28.13	7,202	33

Comparative analysis of fish assemblage structure and biodiversity

The hierarchical cluster analysis of 20 fish assemblages was conducted using Bray-Curtis similarity method (Fig. 2). As a result, the fish assemblages were further divided into three clades. Clade I included three sites of furrowed area (3H, 3I, and September and 3I in November) and its nearby site (5H). Clade II consisted of the other six sites in September (4D, N, E, E1, W, and S), while clade III included the other eight sites (3H, 4D, 5H, N, E, E1, W, and S) in November. A similar result was also identified in the results of the non-metric multidimensional scaling (nMDS) plot (Fig. 2, 3). Those results indicated that fish assemblages in the area showed similar patterns in each sampling time except for the furrowed and its nearby sites, where a statistically unique fish assemblage from its surroundings.

Total 51, 58, and 66 fish species were identified in the clades I, II, and III, respectively (Fig. 4). Among them, 32 species were commonly identified in all three clades. Eight, eleven, and ten species were identified exclusively from clades I, II, and III, respectively (Fig. 4). *Zoarces gillii*,

Scorpaenopsis neglecta, and *Echelus uropterus* were solely identified in the clade I, while *Tanakius kitaharae*, *Diodon holocanthus*, and *Gadus macrocephalus* were only in the clade II. *Chaeturichthys stigmatias, Maurolicus japonicas,* and *Etrumeus teres* were among the solely detected in the clade III). However, those proportions were negligible in each clade ranging from 0.01 % to 0.41 %. The most abundant 15 species in each group were compared (Table 4 and Fig. 4). In clade I, *Pagrus major* was identified the most abundant fish species, followed by *Trachurus japonicus* and *Psenopsis anomala*. In clade II, *Scomber japonicus* was the most abundant fish species, followed by *P. anomala* and *T. japonicus*. *T. japonicus* was most abundantly detected in clade III, followed by *P. anomala* and *S. japonicus*. The fish species statistically different in each clade were obtained by a heat-map analysis (Fig. 5). *P. major* was identified as a critical species in group I sites, which was significantly different from other groups. *S. japonicus* and *Nuchequula nuchalis* differentiated clade II from the other clades, while *T. japonicus* in group III were significantly different from other groups.

Alpha diversity of fish assemblages from three clades were analyzed using three biodiversity indices (Chao1, Pielou's evenness, Shannon diversity) (Fig. 6). Although no detectable difference among three clades

was found, the statistically low alpha diversity indices in clade I were identified compared with those in the other two clades (Fig. 6).

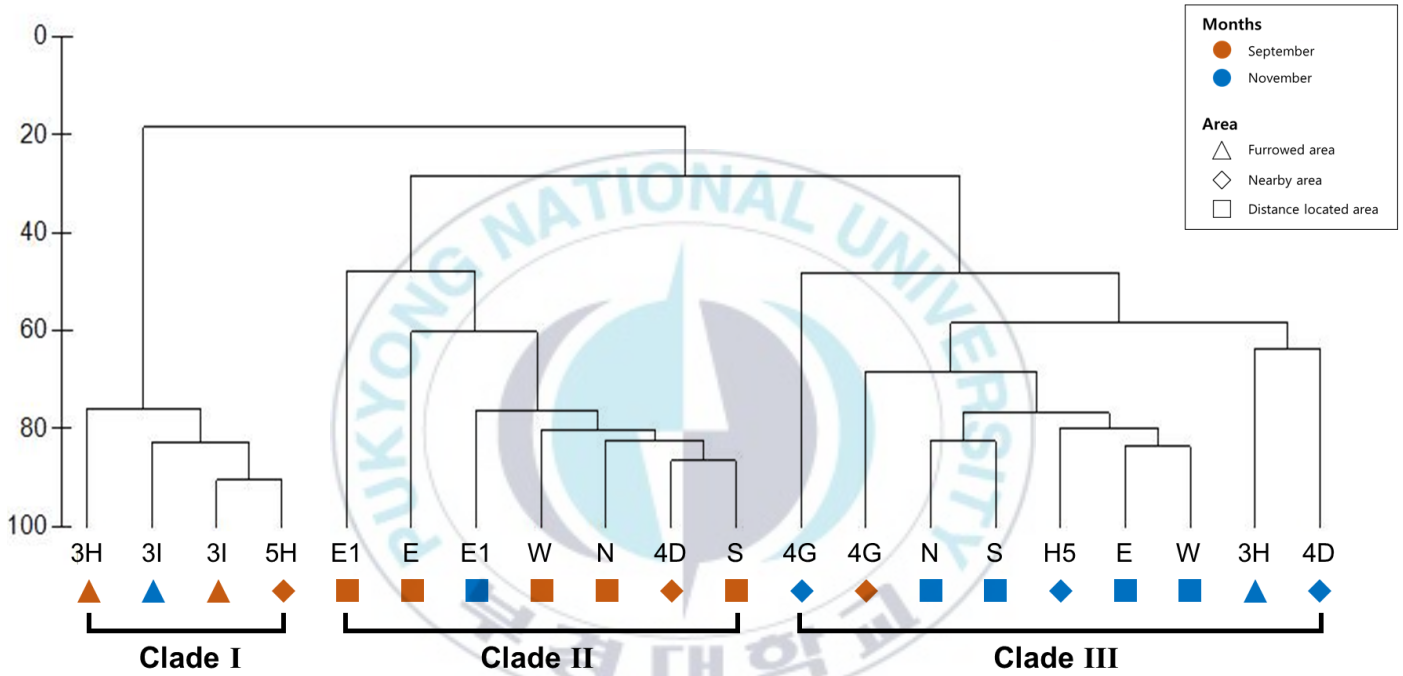

Fig. 2. Similarity analysis for the fish assemblage by sites for September and November together.

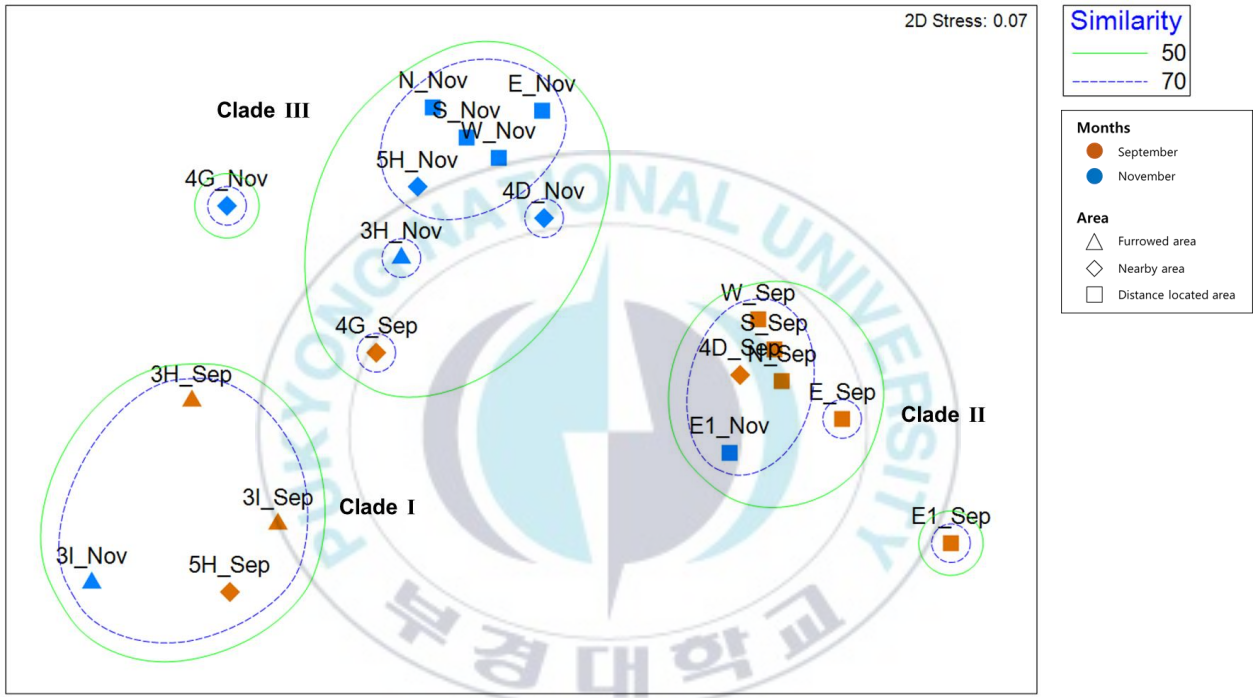

Fig. 3. A non-metric Multi-Dimensional Scaling (nMDS) plot of fish assemblage by sites for September and November together. The solid and broken line represents 50% and 70% similarities, respectively.

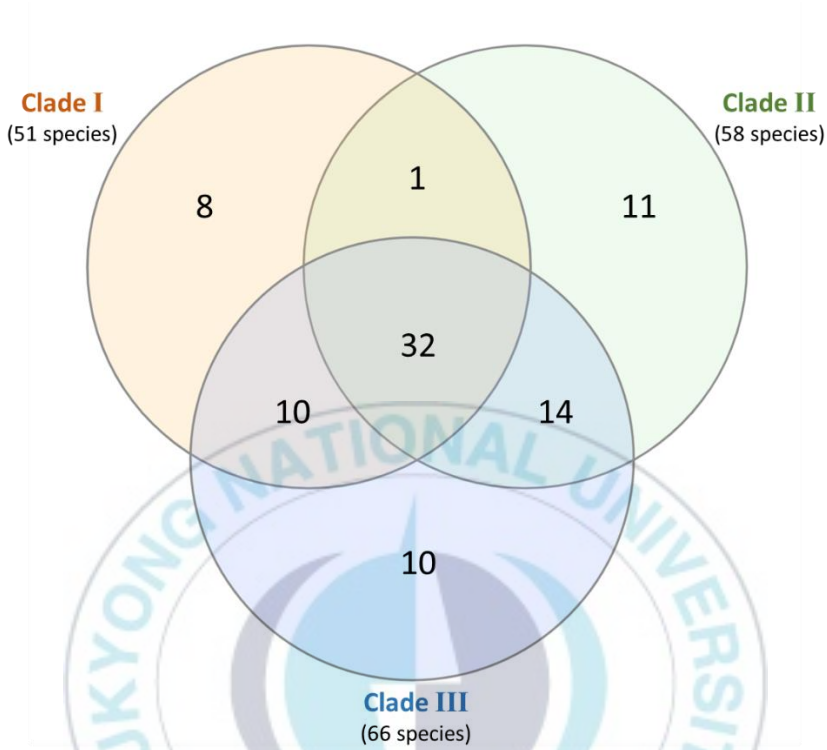

Fig. 4. The Venn diagram of the number of emergence species of three clades in Fig. 2.

Table 4. Top 15 fish species of three clades in Fig. 2.

No.	Clade I	Clade II	Clade III
1	*Pagrus major*	*Scomber japonicus*	*Trachurus japonicus*
2	*Trachurus japonicus*	*Psenopsis anomala*	*Psenopsis anomala*
3	*Psenopsis anomala*	*Trachurus japonicus*	*Scomber japonicus*
4	*Scomber japonicus*	*Nuchequula nuchalis*	*Pagrus major*
5	*Kaiwarinus equula*	*Lophius litulon*	*Benthosema pterotum*
6	*Engraulis japonicus*	*Engraulis japonicus*	*Engraulis japonicus*
7	*Lophius litulon*	*Gnathophis nystromi*	*Pennahia argentata*
8	*Dentex tumifrons*	*Benthosema pterotum*	*Acropoma japonicum*
9	*Zeus faber*	*Pampus* sp.	*Scomberomorus niphonius*
10	*Thamnaconus modestus*	*Zeus faber*	*Saurida wanieso*
11	*Benthosema pterotum*	*Hoplobrotula armata*	*Lophius litulon*
12	*Chelidonichthys spinosus*	*Larimichthys polyactis*	*Chelidonichthys spinosus*
13	*Paralichthys olivaceus*	*Sphyraena pinguis*	*Nuchequula nuchalis*
14	*Nuchequula nuchalis*	*Scomber australasicus*	*Thamnaconus modestus*
15	*Saurida wanieso*	*Dentex tumifrons*	*Dentex tumifrons*

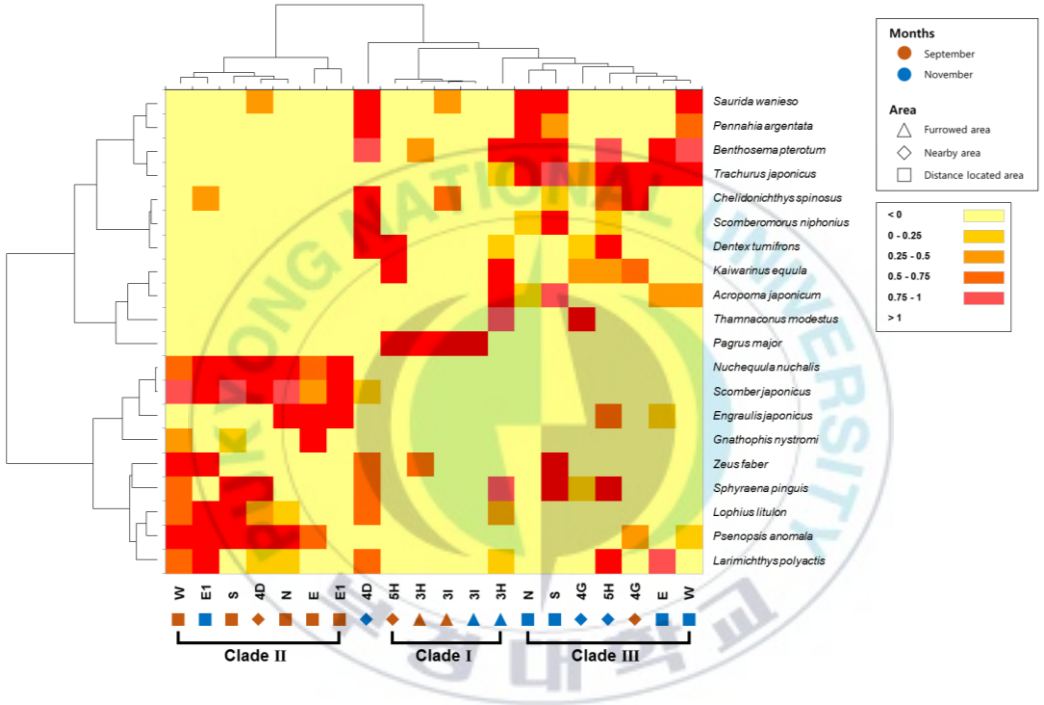

Fig. 5. Heat map of fish assemblage structure by site for September and November together. The plot depicts the relative abundance of species within fish assemblage.

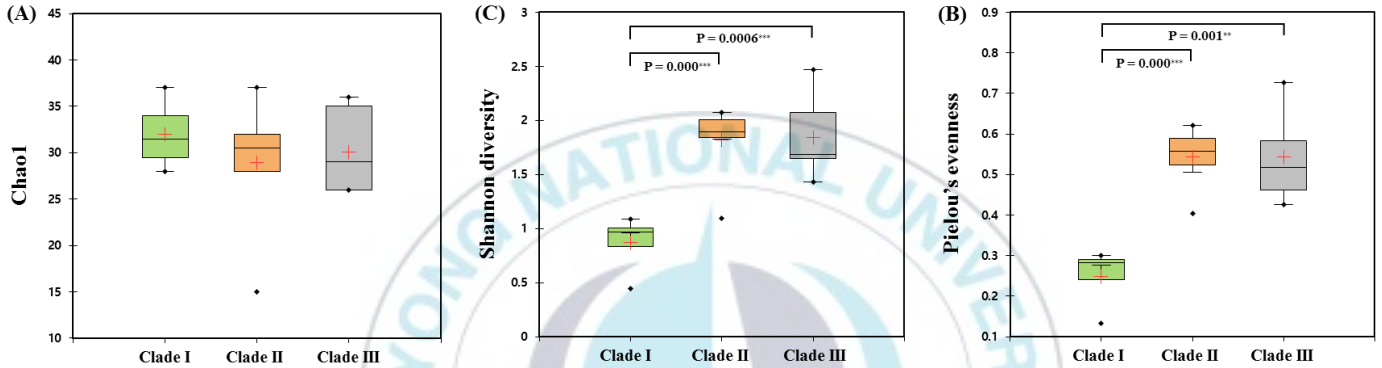

Fig. 6. Box-plot showing alpha diversity for the three clades in Fig. 2. Four different indices including Chao1 (A), Pielou's evenness (B), and Shannon diversity (C). Each clade, Two-sample t-test was conducted (: $p < 0.01$, ***: $p < 0.001$).**

Quantitative analysis of benthic microorganisms from sediment samples.

We also measured total microorganisms from each sediment sample by qPCR technique (Fig. 7). Copy numbers of total microorganisms were higher at all the sediment in November (from 2501.04 ± 323.26 to 11233.31 ± 505.54 copies /10^4) than those in September (form 2552.12 ± 551.10 to 14755.17 ± 785.77 copies/10^4) except for site 5H and S. Average copy number was also higher in November (4357.76 ± 357.89 copies/10^4 in September and 5343.87 ± 591.81 copies/10^4 in November). Interestingly, more than 3-folds higher microbial copy numbers (11,233.31 ± 505.54 copies/10^4 in September and 14,755.17 ± 785.77 copies/10^4 in November, respectively) were identified at E1 sediment compared with those of other sites. By contrast, the lowest copy numbers were shown at 3I in September (2,501.04 ± 323.26 copy numbers/10^4).

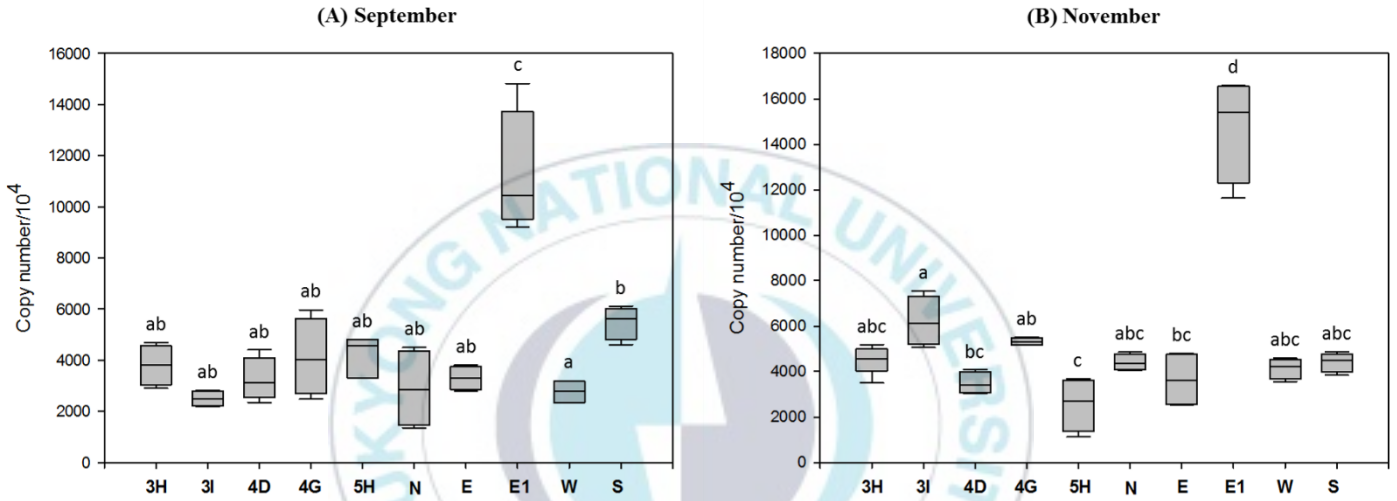

Fig. 7. Copy number of microorganisms by site for September (A) and November (B) (ANOVA $p < 0.05$). Different letters indicate significant differences between the sites (Tukey's HSD $p < 0.05$).

Comparative analysis of benthic microbial assemblage structure

As a result of MiSeq sequencing analysis using 20 sediment samples, a total of 5,853,412 raw reads were obtained (4,348,973 and 1,504,439 reads in September and in November, respectively) (Table 1). After trimming the raw reads, 1,250,298 reads (751,683 in September and 498,615 in November) were finally obtained. As a result of clustering at 98% sequence identity, 89,413 microbial OTUs were generated, which were further classified into 42 phyla (Table 1; Table S3). The higher average phylum numbers were identified in September (31 phyla) compared with those in November (42 phyla). The phylum Proteobacteria accounted for nearly half of the microbiota in the sediment regardless of collection time (43.39% - 67.94%) (Fig. 8). Besides Proteobacteria, Actinobacteria and Nitrospirae in September, and Planctomyces and Acidobacteria in November were among the abundant microbial phyla (Fig. 8).

In order to compare the microbiomes in each sediment sample, a hierarchical cluster analysis was conducted (Fig. 9). Benthic microbial community structures were divided into two clades by sample collection time, September and November. We failed to identify any detectable

regional difference in microbial community structures, which showed a high degree of similarity (> 90 %) among the sediment. A heat map analysis showed a result similar to the hierarchical cluster analysis clustering two clades by the collection time (Fig. 10). Nitrospirae, Proteobacteria, and Spirochaetes were significantly abundant in September, while Acidobacteria, Planctomycetes, Firmicutes, and Lentisphaerae statistically significant in November (Fig. 10).

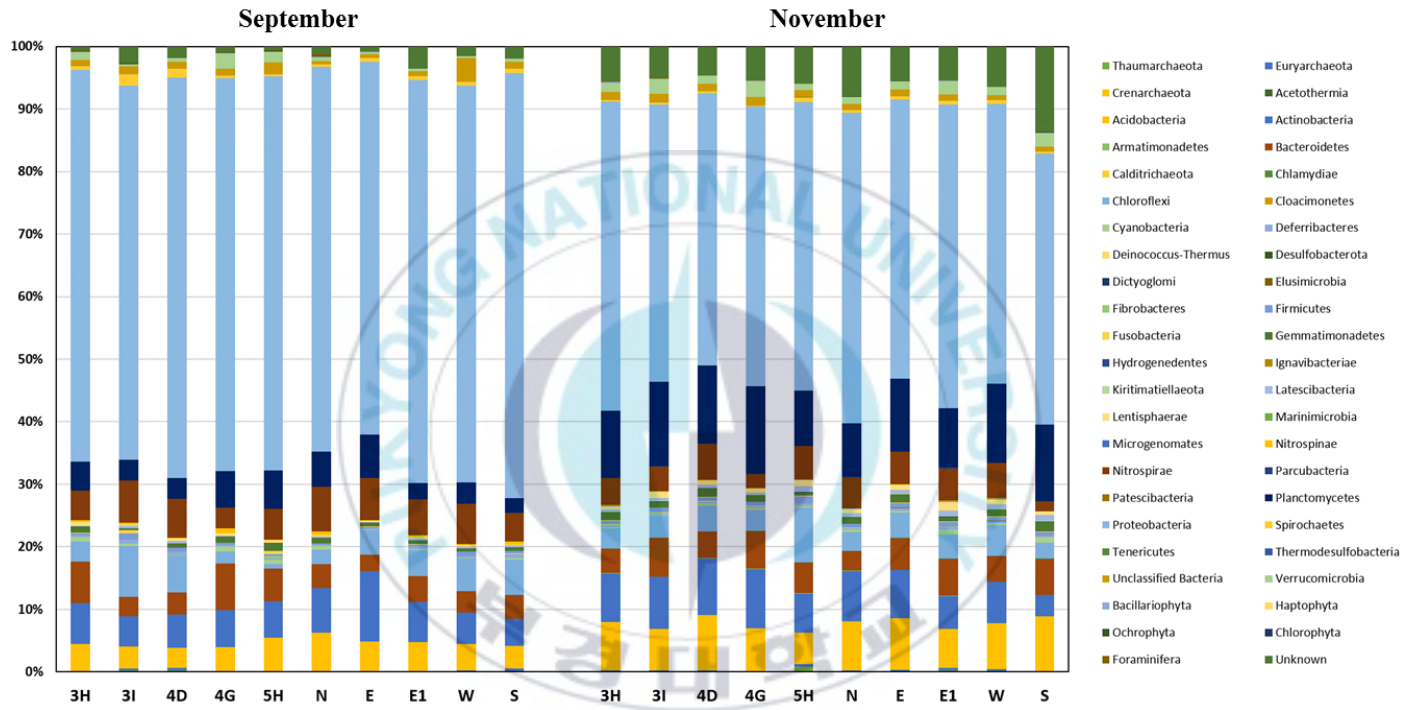

Fig. 8. Microorganisms phylum structures by site for September and November.

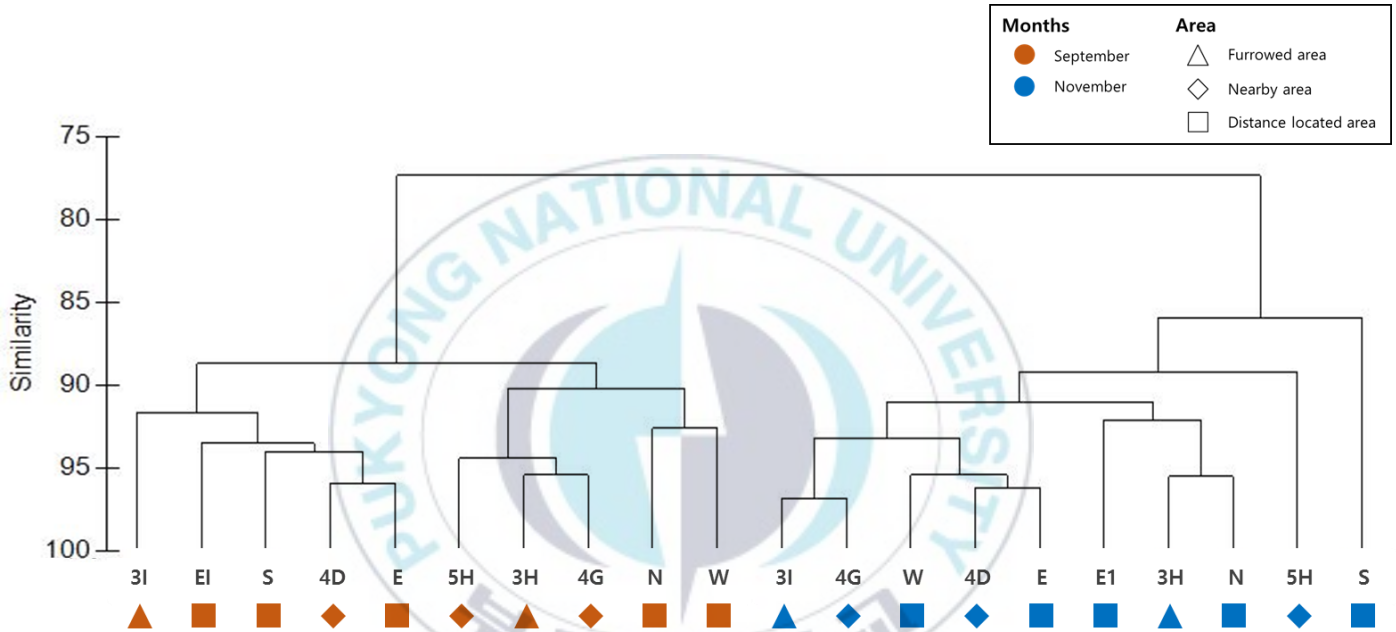

Fig. 9. Similarity analysis for the microbial assemblage by sites for September and November together.

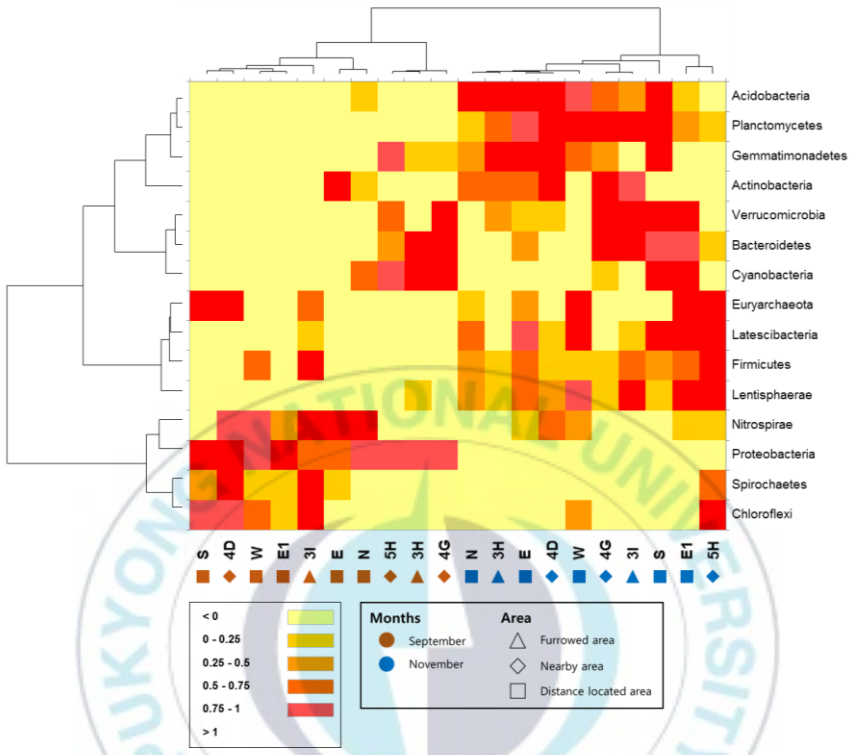

Fig. 10. Heat map of microbial assemblage structure by sites for September and November together. The plot depicts the relative abundance of phyla within microbial assemblage.

DISCUSSION

Analysis of marine ecosystems using the eDNA metabarcoding approach is now widely being adopted as one of the novel alternative methods that can overcome and complement the traditional methods. In this study, 86 fish species were identified through eDNA metabarcoding analysis. The conventional surveys detected total 44 species by five times of bottom trawls and 25 species by two-times of gill net in the Southern sea of Korea (Jeong et al. 2005, Oh et al. 2014). Besides, in the study conducted 11-time of direct observation by scuba diving, 45 species were identified (Lee et al. 2018). We here detected more than 2-folds of species compared with the previous conventional studies indicating the high sensitivity of eDNA metabarcoding. Similar results were also identified in the previous studies supporting the current result (Djurhuus et al. 2018, Yamamoto et al. 2017). eDNA metabarcoding is also useful to detect marine organisms that hard to identify by morphology (e.g. rare species or early life stags such as larvae and egg).

As a result of fish assemblage structures analysis, it was confirmed that it is clearly distinguished by area and month (Fig. 2). Besides, it was possible to distinguish fish assemblage structures between adjacent sites

(3H, 3I, 4D, 4G, and 5H) up to a minimum of 1.5 km. A previous study in Maizuru Bay, Sea of Japan, suggested that the distance between sites that can distinguish fish assemblage was approximately 800 m (Yamamoto et al. 2017). This shows the high resolution of eDNA metabarcoding along with current study results and indicates that narrow and constant interval sampling is possible, unlike the sporadic sampling of the traditional method. Along with eDNA metabarcoding, if the sample size is increased through routine and automatic sampling, statistically reliable accurate results can be presented.

Similarity analysis of fish assemblages showed *Pagrus major* was a critical species on the furrowed site (Fig. 2, 5). A significantly high abundance of *P. major* in the area can reflect the topography of the seabed. Various artificial activities forms pits, and furrows of different scale on the seabed over the world (Kim and Grigalunas 2009, Kubicki et al. 2007, Desprez 2000). Those topographical changes in the seabed often change the hydrodynamic characteristics of the water, including flow velocity, tides, and waves, which further affect the assemblage structure of marine organisms (Kim et al. 2005, Diaz et al. 2004). For instance, some physical changes on the seabed may provide a favorable environment for certain fish species. Besides, the complex seabed structure in the furrow can provide the demersal fish such as *P. major* with the hiding place

(Takahashi and Masuda 2019, Jin et al. 2020). However, it is not clear that increased *P. major* in the furrow is a favorable sign for the marine ecosystem, and further study should be conducted.

Scomber japonicus and *Nuchequula nuchalis* in clade II, and *Trachurus japonicus* in clade III were identified as fish species that distinguish each clade from others (Fig. 5). Average proportions of *S. japonicus* and *N. nuchalis* in clade II were 5.20 and 21.46-folds higher than those in clade III (November), respectively. By contrast, average proportion of *T. japonicus* was 4.46-folds higher in clade III compared with in clade II. Besides those in the furrowed areas (clade I), fish assemblages in clade II and III reflected the sample collection time. These fish species were highly mobile fish, such as oceanodromous (*S. japonicus*, *T. japonicus*) and pelagic-neritic fish (*N. nuchalis*), and have a strong disposition to form a group (Lee and Kim 2011). This study area, southern sea of Korea water is used as migration path, spawning and wintering ground for the fish species such as *S. japonicus* and *T. japonicus* due to characteristics that profoundly affected by Tsushima Current that branched from the Kuroshio Current and passes through the Korea Strait and flowing to the East Sea (Jeong et al. 2005, Moon et al. 2015). Therefore, it is assumed that these results are due to the habitat migration

according to monthly change.

Species richness and species evenness are the main components that determine species diversity, and they can be estimated as various indices (Wilsey and Potvin 2000). In this study, three indices for diversity analysis were used, and significantly low evenness values in the furrowed area (clade I) were identified in both Pielou's evenness index (Pielou 1966) and in Shannon index (Shannon and Weaver 1949). That result suggested that topographic changes of seabed may not affect the species richness, but the evenness by attracting or distracting of a specific fish species changing their local distribution. However, there are some contradictory results in which the species richness decreases by the artificial changes on the seabeds (Son and Han 2007, Hwang et al. 2014). Since eDNA metabarcoding analysis is more sensitive than the traditional methods in detecting fish species, comparison of species richness may not be useful in this study, However, Shannon index contains both richness and evenness values and eDNA metabarcoding result clearly showed its low value in the furrowed area indicating artificially made furrowed on the seabed may lower the evenness values by distracting or attracting a specific fish species.

During the physical activity on the seabed, the resuspended sediment

of the bottom layer and spilled sediments of surface layer form plume. The formed plume moves and settles along with the flow of seawater (Yang et al. 2008). The transporting distance of the plume is affected by the current velocity, turbulence, and types of suspended sediment (e.g. size and adhesion). In the study of Hitchcock and Drucker (1996), the distance of the transported suspended sediments varies from less than 50 m to over 11 km depending on the particle size or current velocity. A survey of Gyeonggi Bay of the Yellow Sea reported that the suspended sediment was transported 20 km from the dredged area by tidal current (Kim and Lim 2009). Those results can explain the significantly high microbial biomass of site E1 (3 - 4 times of other sites) in our study (Fig. 7). It is assumed that the suspended sediments formed by the artificial physical activity in the seabed were transferred by the southern sea currents flowing from west to east and sink to the bottom of the site E1, affecting the microbial biomass. Some previous studies have confirmed that the suspended sediments contain organic matter and nutrients and that the microbial biomass is directly increased by nutrient enrichment (Phua et al. 2002, Nogales et al. 2011). But the site E1 is about 40 km away from the area of physical change in the seabed, a distance that is farther than the transfer distance of suspended sediments reported by previous studies. Further research is needed to determine whether the results of microbial biomass

analysis are due to the transfer of suspended sediments or regional character factors.

In the NGS analysis for benthic microorganism, Proteobacteria was identified as the most dominant phylum comprising about 50% of microbial assemblage structure (Fig. 8), which is consistent with the previous studies in the southern sea of Korea water (Won et al. 2017, Suh et al. 2015), in the East China Sea, and in the Antarctic continental shelf (Feng et al. 2009, Bowman and McCuaig 2003). The relative dominance of phylum Planctomycetes (3% - 13%), Acidobacteria (3% - 9%), Actinobacteria (3% - 9%), Bacteroidetes (3% - 7%) and Chloroflexi (1% - 9%) is also consistent with previous studies (Suh et al. 2015, Feng et al. 2009). This microbial assemblage structure was quite similar regardless of season and area, and the assemblage similarity analysis confirmed that there was a correlation of more than 90% at most sites (Fig. 9). This finding could suggest that the benthic microbial assemblage from the seabed are more stable against changes over space and time (Walsh et al. 2016), or are more resilient to environmental disturbances such as physical activity in the seabed (Won et al. 2017).

In conclusion, we explored the effects of physical change in the seabed on fish and benthic microbial assemblage using the eDNA

metabarcoding analysis, and the differences and characteristics of the fish assemblage structure by the impact of physical change in the seabed were confirmed. The eDNA metabarcoding is useful for analyzing the effect of artificial environmental disturbances on the marine ecosystem and is expected to provide more reliable results through an increased sample size. However, the marine ecosystem highly depends on regional environments such as seabed topography and sea currents, and this study is a short-term study during two seasons. It cannot be sure of long-term changes such as habitat recovery. Therefore, further long-term research is needed through qPCR quantitative analysis of dominant fish species, along with analysis considering local environments.

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Supplementary 1. Coordinate information of sampling sites

Station	latitude	longitude
3H	34.19°	128.39°
3I	34.19°	128.41°
4D	34.18°	128.33°
4G	34.18°	128.38°
5H	34.16°	128.39°
N	34.36°	128.38°
E	34.22°	128.62°
E1	34.36°	128.77°
W	34.17°	128.19°
S	33.97°	128.30°

Table S2. Summary of water temperature and salinity per sites

Water temperature (℃)					Salinity (PSU)				
	September		November			September		November	
Site	Suface	Middle	Suface	Middle	Site	Suface	Middle	Suface	Middle
3H	23.28	24.49	22.73	22.68	3H	31.60	33.48	33.78	34.15
3I	23.48	23.59	22.60	22.66	3I	31.57	33.35	34.13	34.16
4D	23.44	23.86	20.45	17.44	4D	32.22	32.51	32.72	33.31
4G	24.05	24.80	22.69	22.68	4G	32.02	33.53	34.17	34.17
5H	23.88	24.13	22.63	22.70	5H	32.44	32.68	33.82	34.20
N	23.09	24.12	20.63	16.10	N	31.72	32.51	32.82	34.32
E	24.29	24.73	20.57	21.12	E	32.65	33.12	32.62	33.66
E1	25.07	24.91	22.47	23.07	E1	32.84	32.90	33.80	34.15
W	23.70	23.50	20.08	17.80	W	32.28	32.56	32.83	33.53
S	24.15	24.69	21.83	22.33	S	32.64	32.98	33.21	33.94
Average	23.84	24.28	21.67	20.86		32.20	32.96	33.35	33.96

Table S3. Read proportion of fish species by site

Species Name	Acc. No.	Read proportion of species for site (%)																			
		September										November									
		Sand mining					Control					Sand mining					Control				
		3H	3I	4D	4G	5H	N	E	E1	W	S	3H	3I	4D	4G	5H	N	E	E1	W	S
Acropoma japonicum	AB974524				0.05	0.04	0.02			0.02	0.05	3.74	0.15	0.20	0.25	0.39	0.46	0.64	0.05	0.78	1.19
Amblychaeturichthys sciistius	LC458190	0.01		0.04	0.04		0.04				0.25										
Apogon semilineatus	LC021144	0.03						0.01													
Auxis rochei	MK548578	0.03	0.42	0.10	0.06	0.02		0.02							0.05			2.15	0.10		0.26
Benthosema pterotum	NC_047480	7.50	0.13	0.13	0.01	0.00	1.10	0.18	0.15	1.10	0.89	11.50	0.76	9.57	3.87	8.82	9.68	13.03	1.52	9.34	13.95
Branchiostegus argentatus	EU861054					0.01															
Branchiostegus japonicus	AP006804				0.02	4.60				0.01				0.03		0.06					0.04
Carangoides equula	LC036988	0.34	0.30		0.71	1.30		0.01				2.57	0.09		0.65	0.55			0.16		
Chaeturichthys stigmatias	LC506694																				0.27
Champsodon snyderi	LC421719			0.01												0.02					
Cheilopogon doederleinii	KU360728				0.05					0.10											
Chelidonichthys spinosus	MN122888	0.16	0.71		1.21	0.01	0.07					0.27	0.03	2.29	0.02	0.72	0.02	0.06	0.57	0.21	0.42
Chelidoperca hirundinacea	LC493912											0.24	0.12								
Coelorinchus multispinulosus	LC340064			0.02		0.00	0.22	0.03		0.12	0.05	0.21		0.20		0.03	0.01	0.06		0.01	0.08
Collichthys niveatus	LC340172																	0.03	0.15		
Conger myriaster	LC278161			0.00				0.03			0.02										
Decapterus maruadsi	LC458355						0.06									0.14					
Dentex tumifrons	LC519481	0.10	0.33	0.18	0.27	1.20		0.38				0.54	0.09	2.91	0.51	1.22					
Diodon holocanthus	LC278283										0.18										
Ditrema viride	LC506672														0.02						
Doederleinia berycoides	LC278041			0.01			0.01														
Echelus uropterus	MF539646					0.02															

Table S3. Continued

Species Name	Acc. No.	Read proportion of species for site (%)																			
		September										November									
		Sand mining					Control					Sand mining					Control				
		3H	3I	4D	4G	5H	N	E	E1	W	S	3H	3I	4D	4G	5H	N	E	E1	W	S
Engraulis japonicus	LC519336	0.05	0.47	1.91	0.45	0.37	15.92	14.82	33.84	0.94	3.86	0.49	0.33	0.80	0.08	10.82	0.08	4.69	0.72	0.77	1.13
Eopsetta grigorjewi	AB972102	0.05	0.05	0.04	0.34		0.09	0.01		0.19	0.13	1.58	0.31								0.11
Erisphex pottii	LC049850																0.02		0.03		
Etrumeus teres	LC385202											0.05									
Foetorepus altivelis	LC193151											0.01									
Gadus macrocephalus	MN122852			0.04															0.09		
Glossanodon semifasciatus	LC020812	0.01		0.27	0.01		0.00			0.03											
Gnathophis nystromi	MF539656			1.28	0.03	0.01	1.30	28.19		3.37	2.37	0.41		0.22					0.01		
Harpadon nehereus	AB970004		0.04			0.01	0.01				0.06							0.14	0.01		0.12
Hemitripterus villosus	NC_046471	0.01																			
Hoplobrotula armata	LC193248	0.03	0.27	0.08	0.25	0.02	0.48	0.36	0.02	0.22	0.55	0.58	0.01	0.04			0.05	0.05	0.06	0.15	0.04
Jaydia lineata	NC_041647										0.05	0.04		0.10	0.23		0.01	0.06	0.04	0.02	0.22
Johnius grypotus	LC036872										0.06							0.01	0.13		
Kareius bicoloratus	LC069803																		0.09		
Larimichthys polyactis	LC036875		0.09	0.30	0.04		0.28	0.02		0.44	0.25	0.25		0.46		0.97	0.03	0.48	1.16	0.08	0.12
Lepidotrigla guentheri	LC458236		0.01	0.04	0.01									0.01							
Lepidotrigla hime	AB974518	0.03	0.17	0.06	0.06		0.08	0.06	0.01	0.09	0.04	0.02								0.18	0.10
Lepidotrigla microptera	LC506627	0.02	0.07		0.11								1.27								
Lophius litulon	AB974498	0.08	1.56	2.44	0.50	0.84	2.17	0.52	0.43	3.13	8.90	2.23	0.06	3.03	0.58	0.08	0.03	0.29	6.91		0.12
Malakichthys elegans	LC021234						0.06														
Maurolicus japonicus	LC021031														0.02			0.03		0.03	
Miichthys miiuy	LC036876																	0.02	0.07	0.01	0.01

Table S3. Continued

Species Name	Acc. No.	Read proportion of species for site (%)																			
		September										November									
		Sand mining					Control					Sand mining					Control				
		3H	3I	4D	4G	5H	N	E	E1	W	S	3H	3I	4D	4G	5H	N	E	E1	W	S
Muraenesox cinereus	LC020903	0.00		0.02	0.08		0.05	0.02		0.10	0.15								0.04		
Myrophis microchir	LC036896				0.02																
Nuchequula nuchalis	LC458248	0.09	0.18	7.07	0.13	0.18	9.25	4.68	8.15	4.83	7.41	0.18		0.11	0.13	0.15	0.06	0.04	7.90	0.44	1.73
Ophichthus asakusae	HQ185624																			0.01	
Pagrus major	LC421694	61.65	73.92	0.15	15.43	72.50	0.00	0.01		0.04	0.01	12.63	81.97	4.40	9.11	7.00	5.72	0.21	0.64	3.19	5.63
Pampus sp.	KJ162571	0.06	0.01	1.14	0.03	0.01	0.01	0.01		11.58	2.10	1.04		0.08			0.05		0.02	0.05	0.06
Paralichthys olivaceus	AB972103	0.22	1.02		1.85													0.07	0.37		
Pennahia argentata	LC519411											0.19	0.01	7.39	0.08	1.32	16.76	1.62		4.25	3.38
Petroscirtes breviceps	AB969915						0.02														
Pholis nebulosa	AB972144			0.07																	
Pisodonophis sangjuensis	MK189459						0.02														
Pomacentrus coelestis	LC069662	0.01																			
Psenopsis anomala	LC340283	0.60	0.84	28.21	16.39	0.86	23.28	18.50	0.89	23.17	25.31	5.95	0.22	10.18	1.32	3.94	8.61	6.88	31.57	13.77	7.01
Pseudorhombus pentophthalmus	LC458339						0.03														0.03
Sarda orientalis	AP012949		0.08									0.18									
Saurida wanieso	LC468900	0.15	0.43	0.48	0.18							0.14	0.01	0.72	0.27	0.09	0.97	0.10		0.78	1.35
Scomber australasicus	LC193283			0.04	0.00		0.03	0.02	0.05	0.03	0.03	0.01		0.02			0.01	0.02	0.10	0.01	
Scomber japonicus	LC385179	0.17	1.04	33.10	2.16	0.71	28.73	21.26	49.85	25.75	28.50	8.49	0.18	14.05	0.61	3.75	5.01	8.47	34.83	7.50	4.89
Scomberomorus niphonius	LC387640						0.08					0.51		9.26	0.07	1.02	1.02			0.70	3.44
Scorpaena neglecta	LC020788	0.01			0.03	0.02						0.16	0.15								
Scorpaenopsis neglecta	LC026722		0.06																		
Sebastiscus marmoratus	LC492345									0.02											

Table S3. Continued

Species Name	Acc. No.	Read proportion of species for site (%)																			
		September										November									
		Sand mining					Control					Sand mining					Control				
		3H	3I	4D	4G	5H	N	E	E1	W	S	3H	3I	4D	4G	5H	N	E	E1	W	S
Sebastiscus tertius	AB969892											0.09	0.02								
Seriola quinqueradiata	LC036999						0.02													0.02	
Sillago japonica	MF572021												0.02							0.08	0.69
Sillago sinica	KR363151																	0.03			
Sphyraena pinguis	LC506671			0.71	0.01	0.06				0.43	1.04	0.55		0.47	0.33	0.79				0.15	0.62
Spratelloides gracilis	LC506661		0.01																		
Synagrops philippinensis	LC021233		0.01																		
Takifugu vermicularis	LC037110				0.03																
Tanakius kitaharae	AB972095			0.12			0.07	0.01		0.21											
Thamnaconus modestus	LC519448	1.99	0.32		1.57							12.03	0.33		41.96	0.01					
Thamnaconus tessellatus	LC519448				0.00							0.01			0.04						
Thunnus alalunga	KP259549														0.03				0.22		0.10
Trachurus japonicus	LC519400	21.13	13.20	14.01	51.51	8.48	10.50	5.31	1.54	15.03	12.64	23.27	2.93	22.67	32.59	52.13	40.77	51.31	4.18	49.00	39.49
Trichiurus japonicus	MK292708			0.03		0.02	0.01	0.08		0.05	0.07							0.02		0.02	
Upeneus japonicus	LC458158											0.09	0.01								
Uranoscopus japonicus	AP006822					0.02						0.21									
Zebrias zebrinus	LC385317				0.02														0.05		
Zenopsis nebulosa	LC104480		0.08	0.08	0.04	0.11										0.09					
Zeus faber	LC026579	0.71	0.25	0.17	0.25	0.20	0.22	0.01		1.69	0.31	0.20	0.08	0.74	0.06	0.23		0.02	1.24	0.10	1.63
Zoarces gillii	LC093396		0.09																		
Unknown		4.77	3.84	7.66	6.02	8.38	5.78	5.47	5.08	7.31	4.75	9.32	10.83	10.06	7.12	5.67	10.62	9.47	6.98	8.36	11.77
Total		100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100

Table S4. Read proportion of microbial phylum by site

Kingdom	Phylum	Read proportion of species for site (%)																			
		September										November									
		Sand mining					Control					Sand mining					Control				
		3H	3I	4D	4G	5H	N	E	E1	W	S	3H	3I	4D	4G	5H	N	E	E1	W	S
Archaea	Euryarchaeota	0.022	0.266	0.382				0.014	0.017	0.110	0.339	0.137	0.093	0.128	0.063	0.400	0.182	0.210	0.400	0.332	0.131
Archaea	Thaumarchaeota	0.149	0.277	0.246	0.043	0.033	0.061	0.114	0.048	0.125	0.172	0.105	0.092	0.099	0.050	0.792	0.075	0.102	0.213	0.138	0.041
Archaea	Crenarchaeota		0.034		0.019		0.016	0.040								0.010			0.005		
Bacteria	Acidobacteria	4.330	3.524	3.262	3.877	5.442	6.233	4.721	4.718	4.181	3.625	7.704	6.638	8.847	6.901	5.036	7.815	8.255	6.252	7.278	8.692
Bacteria	Actinobacteria	6.537	4.740	5.251	5.964	5.856	7.136	11.252	6.454	5.107	4.346	7.753	8.344	9.088	9.377	6.265	8.051	7.714	5.220	6.617	3.430
Bacteria	Armatimonadetes											0.082	0.088	0.060	0.108	0.064	0.066	0.075	0.143	0.053	0.014
Bacteria	Bacteroidetes	6.574	3.144	3.548	7.462	5.154	3.798	2.567	4.034	3.419	3.832	3.865	6.168	4.214	5.979	4.862	3.119	4.980	5.767	4.073	5.766
Bacteria	Calditrichaeota						0.015							0.007				0.005			
Bacteria	Chlamydiae		0.039					0.014				0.090	0.058	0.051	0.053	0.047	0.046	0.081	0.084	0.062	0.058
Bacteria	Chloroflexi	3.275	8.080	5.958	1.835	0.790	2.309	3.889	4.173	5.228	5.597	3.241	3.442	4.089	3.336	8.776	3.011	4.064	3.869	4.935	2.555
Bacteria	Cyanobacteria	0.687	0.391	0.115	0.837	0.611	0.545	0.113	0.213	0.079	0.306	0.230	0.245	0.359	0.400	0.212	0.340	0.294	0.782	0.259	0.822
Bacteria	Deferribacteres	0.306	0.655	0.483	0.285	0.239	0.254	0.246	0.382	0.272	0.636	0.213	0.192	0.202	0.192	0.298	0.228	0.344	0.386	0.314	0.194
Bacteria	Deinococcus-Thermus	0.019			0.017				0.022		0.035	0.102	0.128	0.132	0.072	0.009	0.075	0.067	0.061	0.035	0.016
Bacteria	Desulfobacterota											0.006		0.007		0.026	0.017	0.008		0.006	
Bacteria	Dictyoglomi											0.009						0.003			
Bacteria	Elusimicrobia											0.061	0.007	0.031	0.007	0.003		0.053	0.014	0.030	0.021
Bacteria	Fibrobacteres		0.018	0.031		0.438					0.050	0.015	0.050	0.022	0.012	0.077	0.008	0.059	0.091	0.042	0.044
Bacteria	Firmicutes	0.280	0.950	0.528	0.263	0.228	0.190	0.161	0.250	0.672	0.389	0.583	0.714	0.588	0.601	1.182	0.625	0.699	0.698	0.552	0.608
Bacteria	Fusobacteria	0.109	0.501	0.057	0.082	0.574	0.000	0.101	0.093	0.066		0.029	0.050	0.060	0.050	0.096	0.017	0.102	0.061	0.089	0.037
Bacteria	Gemmatimonadetes	0.927	0.296	0.696	0.986	1.242	0.911	0.602	0.660	0.463	0.577	1.303	0.874	1.380	1.061	0.564	1.073	1.264	0.730	1.116	1.620
Bacteria	Hydrogenedentes		0.026	0.016						0.026		0.041	0.050	0.026	0.048	0.071	0.008	0.032	0.051	0.068	0.053
Bacteria	Ignavibacteriae							0.021		0.015		0.015	0.017	0.012	0.015	0.007	0.017		0.014	0.019	0.005

Table S4. Continued

Kingdom	Phylum	Read proportion of species for site (%)																			
		September										November									
		Sand mining					Control					Sand mining					Control				
		3H	3I	4D	4G	5H	N	E	E1	W	S	3H	3I	4D	4G	5H	N	E	E1	W	S
Bacteria	Kiritimatiellaeota											0.041	0.097	0.026	0.027	0.101	0.033	0.072	0.096	0.040	0.014
Bacteria	Latescibacteria	0.149	0.451	0.331	0.057	0.063	0.154	0.130	0.241	0.277	0.369	0.300	0.410	0.429	0.297	0.766	0.580	0.609	0.838	0.729	0.901
Bacteria	Lentisphaerae	0.626	0.180	0.394	0.411	0.380	0.221	0.131	0.200	0.226	0.099	0.580	1.004	0.722	0.632	1.024	0.654	0.783	1.393	0.813	0.606
Bacteria	Marinimicrobia															0.003					
Bacteria	Microgenomates											0.044	0.072	0.041	0.025	0.078	0.108	0.052	0.108	0.106	0.009
Bacteria	Nitrospinae	0.272	0.325	0.150	0.830	0.134	0.603	0.140	0.373	0.132	0.471	0.082	0.088	0.082	0.055	0.021	0.066	0.093	0.066	0.076	0.058
Bacteria	Nitrospirae	4.750	6.697	6.247	3.334	4.877	7.163	6.738	5.703	6.442	4.562	4.352	3.915	5.760	2.239	5.318	4.916	5.164	5.208	5.619	1.546
Bacteria	Parcubacteria										0.016	0.035	0.080	0.036	0.100	0.021	0.029	0.058	0.171	0.046	0.018
Bacteria	Patescibacteria			0.027						0.018						0.003					
Bacteria	Planctomycetes	4.560	3.327	3.264	5.806	6.102	5.641	6.896	2.589	3.485	2.380	10.718	13.423	12.520	13.942	8.840	8.552	11.661	9.398	12.653	12.259
Bacteria	Proteobacteria	62.692	59.854	64.100	62.852	63.034	61.484	59.623	64.432	63.388	67.940	49.502	44.357	43.483	44.738	46.096	49.669	44.662	48.604	44.763	43.390
Bacteria	Spirochaetes	0.555	1.757	1.336	0.347	0.328	0.371	0.603	0.649	0.624	0.663	0.146	0.325	0.301	0.133	0.803	0.460	0.442	0.550	0.516	0.311
Bacteria	Tenericutes							0.014	0.022			0.017	0.010	0.022	0.003	0.016		0.009	0.016	0.006	0.005
Bacteria	Thermodesulfobacteria															0.012					
Bacteria	Verrucomicrobia	1.201	0.287	0.523	2.434	1.673	0.584	0.346	0.412	0.329	0.503	1.460	2.383	1.341	2.599	1.003	1.139	1.281	2.104	1.237	2.214
Bacteria	Unclassified Bacteria	1.078	1.351	1.166	1.167	1.951	0.592	0.602	0.742	3.775	1.126	1.303	1.424	1.166	1.363	1.120	0.965	1.101	1.074	0.860	0.783
Chromista	Bacillariophyta	0.062			0.049	0.051	0.032	0.053				0.201	0.023	0.017	0.018	0.012	0.029	0.008	0.075	0.029	0.012
Chromista	Haptophyta													0.005							
Chromista	Ochrophyta	0.109	0.169		0.156	0.142	0.157	0.017	0.000	0.015	0.015	0.017	0.003	0.010	0.008	0.007	0.062	0.087	0.021	0.013	
Plantae	Chlorophyta													0.005	0.048	0.012					0.051
Protozoa	Foraminifera	0.130	0.015		0.122	0.115	0.345				0.054	0.012	0.115	0.005	0.063	0.010		0.087	0.005	0.058	0.005
Unknown	Unknown	0.600	2.644	1.887	0.765	0.544	1.187	0.853	3.570	1.526	1.898	5.608	5.021	4.628	5.383	5.934	7.968	5.421	5.433	6.417	13.712
	Total	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100