

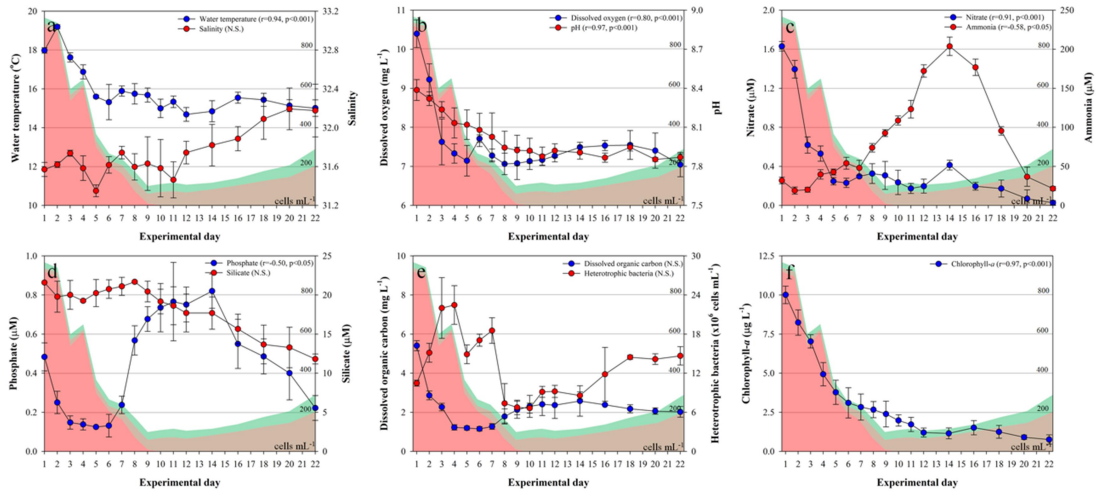
**Supplementary information**

**Dynamic bacterial community response to *Akashiwo sanguinea* (Dinophyceae) bloom in indoor  
marine microcosms**

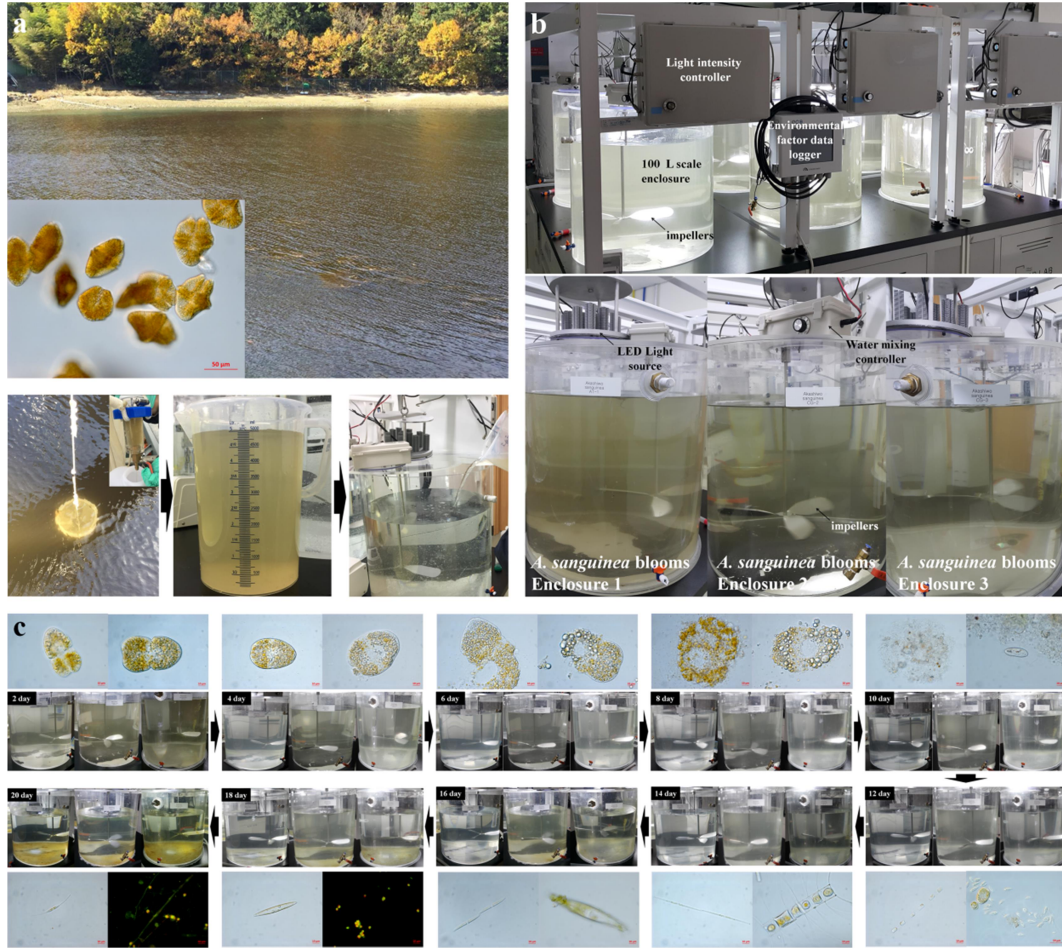
Seung Won Jung<sup>1,\*</sup>, Junsu Kang<sup>1,2</sup>, Joon Sang Park<sup>1</sup>, Hyoung Min Joo<sup>3</sup>, Sung-Suk Suh<sup>4</sup>, Donhyug Kang<sup>5</sup>,  
Taek-Kyun Lee<sup>6</sup>, Hyun-Jung Kim<sup>1</sup>

<sup>1</sup>Library of Marine Samples, Korea Institute of Ocean Science & Technology, Geoje, 53201, <sup>2</sup>Department  
of Oceanography, Pukyong National University, Busan 48513, Republic of Korea, <sup>3</sup>Division of Polar  
Ocean Science, Korea Polar Research Institute, Incheon 21990, Republic of Korea, <sup>4</sup>Department of  
Bioscience, Mokpo National University, Muan 58554, Republic of Korea, <sup>5</sup>Maritime Security Research  
Center, Korea Institute of Ocean Science & Technology, Busan 49111, Republic of Korea, <sup>6</sup>Risk  
Assessment Research Center, Korea Institute of Ocean Science & Technology, Geoje 53201, Republic of  
Korea

**\*Correspondence to:** S.-W. Jung, diatoms@kiost.ac.kr, Fax: +82-55-639-8429



**Supplementary Figure S1. Changes in the environmental characteristics of the indoor microcosm bloom dynamics of *Akashiwo sanguinea*.** (a) Water temperature and salinity, (b) dissolved oxygen and pH, (c) nitrate (nitrate + nitrite) and ammonia, (d) phosphate and silicate, (e) dissolved organic carbon and heterotrophic bacteria and (f) chlorophyll *a*. The *r* value in each figure (upper right) is the Pearson correlation coefficient ( $n = 17$ ). Data are presented as the mean (three replicates) and error bars indicate the standard deviation. N.S. indicates no significance.



**Supplementary Figure S2. Experimental design of the 100-L indoor microcosm bloom dynamics of *Akashiwo sanguinea*.** (a) *A. sanguinea* bloom in the Jangmok Bay time-series monitoring site (JB-TMS: 34°59'37'' N and 128°40'27'' E) and microcosm using concentrated *A. sanguinea* cells in natural seawater. (b) Triplicate indoor microcosm experiments in seawater. Each enclosure consisted of a LED light with light intensity controller, impellers with water mixing controller and data logger of environmental factors. (c) Time-series photographs of the three replicate microcosms and microscopic photographs of common phytoplankton.

**Supplementary Table S1.** Abbreviations and node sizes of the significant factors ( $P<0.01$ ;  $Q<0.05$ ) identified in the Network Analysis provided in Figure 4.

| Factor                                | Abbreviation  | Node size | Classification       |
|---------------------------------------|---------------|-----------|----------------------|
| Water temperature                     | WT            | 1.99      | Environmental factor |
| Salinity                              | Sal.          | 2.37      |                      |
| pH                                    | pH            | 1.68      |                      |
| Dissolved oxygen                      | DO            | 1.66      |                      |
| Nitrate (including Nitrite)           | NO3           | 0.71      |                      |
| Ammonia                               | NH4           | 2.93      |                      |
| Phosphate                             | PO4           | 0.80      |                      |
| Silicate                              | SiO2          | 2.03      |                      |
| Dissolved organic carbon              | DOC           | 1.19      |                      |
| Chlorophyll- <i>a</i>                 | DO            | 1.19      |                      |
| <i>Akashiwo sanguinea</i>             | <i>A.san.</i> | 1.56      | Autotroph            |
| Diatoms                               | Diatoms       | 2.88      |                      |
| Other phytoplankton                   | Oth.Phyto.    | 2.53      |                      |
| Total bacteria                        | Bacteria      | 1.87      | Bacteria             |
| <i>Marinovum algicola</i>             | OTU #00       | 2.34      |                      |
| <i>Magnetospira thiophila</i>         | OTU #01       | 1.47      |                      |
| <i>Cellulophaga tyrosinoxydans</i>    | OTU #02       | 1.15      |                      |
| <i>Polaribacter marinivivus</i>       | OTU #03       | 0.79      |                      |
| <i>Marivita roseacus</i>              | OTU #04       | 1.06      |                      |
| <i>Methylothermobacter versatilis</i> | OTU #05       | 1.01      |                      |
| <i>Tenacibaculum aestuariivivum</i>   | OTU #06       | 1.42      |                      |
| <i>Halobacteriovorax marinus</i>      | OTU #07.      | 0.77      |                      |
| <i>Fluviicola taffensis</i>           | OTU #08       | 0.60      |                      |
| <i>Polaribacter atrinae</i>           | OTU #09       | 1.10      |                      |
| <i>Polaribacter huanghezhanensis</i>  | OTU #10       | 0.78      |                      |
| <i>Owenweeksia hongkongensis</i>      | OTU #11       | 0.68      |                      |
| <i>Tenacibaculum aiptasiae</i>        | OTU #12       | 1.20      |                      |
| <i>Paracoccus mangrovi</i>            | OTU #13       | 0.94      |                      |
| <i>Pseudohongiella spirulinae</i>     | OTU #14       | 0.82      |                      |
| <i>Polaribacter pacificus</i>         | OTU #15       | 0.84      |                      |
| <i>Nereida ignava</i>                 | OTU #18       | 0.38      |                      |
| <i>Thalassobacter stenotrophicus</i>  | OTU #20       | 0.51      |                      |

37 **Supplementary Table S2.** Local similarity correlations of sub-networks I-III in the Network Analysis in  
38 Figure 4.

| Sub-network | X                             | Y             | LS     | Xs | Ys | Length | Delay | SCC    | P      | Q      |
|-------------|-------------------------------|---------------|--------|----|----|--------|-------|--------|--------|--------|
| I           | <i>A.san.</i>                 | OTU #01       | -0.592 | 1  | 1  | 11     | 0     | -0.964 | <0.001 | <0.001 |
|             | <i>A.san</i>                  | OTU #04       | -0.963 | 1  | 1  | 11     | 0     | -0.853 | 0.009  | 0.010  |
|             | <i>A.san</i>                  | OTU #12       | -0.963 | 1  | 4  | 8      | -3    | 0.189  | 0.009  | 0.010  |
|             | <i>A.san</i>                  | Diatoms       | -0.963 | 1  | 1  | 11     | 0     | -0.853 | 0.005  | 0.008  |
|             | pH                            | <i>A.san.</i> | 1.240  | 1  | 1  | 11     | 0     | 0.823  | 0.005  | 0.008  |
|             | Chl- <i>a</i>                 | <i>A.san.</i> | 0.998  | 1  | 1  | 11     | 0     | 0.863  | 0.002  | 0.005  |
| II          | PO <sub>4</sub> <sup>3-</sup> | Bacteria      | -0.539 | 1  | 1  | 11     | 0     | -0.900 | 0.007  | 0.010  |
|             | PO <sub>4</sub> <sup>3-</sup> | OTU #05       | 0.579  | 1  | 1  | 11     | 0     | 0.909  | <0.001 | <0.001 |
|             | PO <sub>4</sub> <sup>3-</sup> | OTU #11       | 0.562  | 1  | 1  | 11     | 0     | 0.909  | 0.001  | 0.003  |
| II          | NH <sub>4</sub> <sup>+</sup>  | OTU #07       | 0.549  | 1  | 2  | 9      | -1    | 0.484  | 0.006  | 0.009  |
|             | NH <sub>4</sub> <sup>+</sup>  | OTU #10       | -0.568 | 1  | 2  | 10     | -1    | -0.770 | 0.001  | 0.003  |
|             | NH <sub>4</sub> <sup>+</sup>  | OTU #11       | 0.567  | 1  | 1  | 11     | 0     | 0.882  | <0.001 | <0.001 |
| III         | Diatoms                       | Oth.Phyto.    | 0.547  | 1  | 1  | 11     | 0     | 0.808  | 0.001  | 0.003  |
|             | Diatoms                       | OTU #04       | 0.623  | 1  | 1  | 11     | 0     | 0.982  | <0.001 | <0.001 |
|             | Diatoms                       | OTU #07       | 0.535  | 1  | 1  | 11     | 0     | 0.813  | 0.009  | 0.011  |
|             | Diatoms                       | OTU #08       | -0.571 | 1  | 1  | 11     | 0     | -0.936 | <0.001 | <0.001 |
|             | Diatoms                       | OTU #13       | 0.607  | 1  | 1  | 11     | 0     | 0.973  | <0.001 | <0.001 |
|             | Diatoms                       | OTU #14       | 0.523  | 2  | 2  | 10     | 0     | 0.800  | 0.001  | 0.003  |
|             | Sal                           | Diatoms       | 0.551  | 1  | 1  | 11     | 0     | 0.843  | 0.010  | 0.011  |
|             | pH                            | Diatoms       | -0.542 | 1  | 1  | 11     | 0     | -0.815 | 0.004  | 0.008  |
|             | SiO <sub>2</sub> <sup>-</sup> | Diatoms       | -0.548 | 1  | 1  | 11     | 0     | -0.900 | 0.001  | 0.003  |
|             | NO <sub>3</sub> <sup>-</sup>  | Diatoms       | -0.560 | 1  | 1  | 11     | 0     | -0.855 | 0.001  | 0.003  |
|             | Chl- <i>a</i>                 | Diatoms       | -0.578 | 1  | 1  | 11     | 0     | -0.891 | <0.001 | <0.001 |
|             | <i>A.san</i>                  | Diatoms       | -0.963 | 1  | 1  | 11     | 0     | -0.853 | 0.005  | 0.008  |

39 Abbreviations: LS, local similarity; Xs and Ys, the initial day of the LS correlation; Length, the duration of  
40 the LS correlation in days; Delay, time-lag in days; SCC, Spearman's Correlation Coefficient; P and Q,  
41 P-value and Q-value, respectively.

42 **Supplementary Table S3.** Primers used in the PCR amplification of V3–V4 regions in the 16S rDNA analysis.

43

| Target region           | Step       | Primer                                                                                                                                                                                                                                                    |                                                                                  | PCR reaction mixtures (25-μL)                                                                                                                                                                                                                           | PCR condition                                                                                                                                                                                                        | References |
|-------------------------|------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|----------------------------------------------------------------------------------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|------------|
|                         |            | Forward                                                                                                                                                                                                                                                   | Reverse                                                                          |                                                                                                                                                                                                                                                         |                                                                                                                                                                                                                      |            |
| 16S rDNA (V3-V4 region) | First PCR  | Illumina preadapter +Sequencing primer sequence+341F (5'-CCTACGGGN GGCWGCAG-3')                                                                                                                                                                           | Illumina preadapter +Sequencing primer sequence+800R (5'-TACCAGGGT ATCTAATCC-3') | 200 μmol L <sup>-1</sup> each dNTP, 1.5 mmol L <sup>-1</sup> MgCl <sub>2</sub> , 0.3 μmol L <sup>-1</sup> each primer, 2.5 U Taq DNA polymerase (TaKaRa, EX Taq, Kyoto, Japan), and DNA template (20 ng μL <sup>-1</sup> )                              | initial denaturation step at 95 °C for 3 min, followed by 30 cycles of denaturation at 95 °C for 10 s, annealing at 52 °C for 45 s, and extension at 72 °C for 1 min, and a final extension step of 72 °C for 5 min. | 1, 2       |
|                         | Second PCR | Illumina preadapter +Sequencing primer sequence+341F (5'-CCTACGGGN GGCWGCAG-3')                                                                                                                                                                           | Illumina preadapter +Sequencing primer sequence+800R (5'-TACCAGGGT ATCTAATCC-3') | 200 μmol L <sup>-1</sup> each dNTP, 1.5 mmol L <sup>-1</sup> MgCl <sub>2</sub> , 0.3 μmol L <sup>-1</sup> each primer, 2.5 U Taq DNA polymerase (TaKaRa, EX Taq, Kyoto, Japan), and DNA template (800 ng), and Nextera XT index Kit (Illumina, CA, USA) | initial denaturation step at 95 °C for 3 min, followed by 12 cycles of denaturation at 95 °C for 10 s, annealing at 52 °C for 45 s, and extension at 72 °C for 1 min, and a final extension step of 72 °C for 5 min. |            |
|                         | Pooling    | Triplicate reaction products (with a consistent volume) were pooled and purified using an Qiaquick PCR purification Kit (No. 28104, Qiagen Inc.). Their DNA concentration was measured in a Bio-analyzer 2100 (Agilent Technologies, Palo Alto, CA, USA). |                                                                                  |                                                                                                                                                                                                                                                         |                                                                                                                                                                                                                      |            |

## References

1. Herlemann, D. P. R. *et al.* Transitions in bacterial communities along the salinity gradient of the Baltic Sea. *The ISME Journal* **5**, 1571-1579 (2011).
2. Ogihara, H. *et al.* Microfloral and chemical changes of salted pickles (Suguki) during its manufacturing process. *Japanese Journal of Food Microbiology* **26**, 98-106 (2009).