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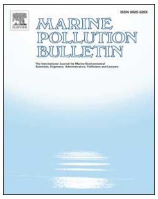

Insight into tidal disturbance on colonization surveys for marine bioassessment using periphytic ciliates based on biological trait analysis

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ABSTRACT

To understand tidal disturbance on the colonization dynamics in community functioning of the ciliates in marine ecosystems, a 1-month survey was conducted using the conventional slide system (CS) and the polyurethane foam enveloped slide system (PFES) in Korean coastal waters. The results showed considerable differences were detected in community functioning parameters between the two systems. The vagile, flattened forms feeding on periphytic microalgae showed a higher community-weighted mean (CWM) value, and the solitary-colonial pattern represented a more stable temporal variability during the colonization in the PFES system than the CS system. The temporal shift in community functioning of the ciliates showed different ways in both systems. The functional diversity measures generally levelled off at more stable values in the communities with ages of 13–15 days in the PFES system than the CS system. These results imply that the PFES system may be a workable method for bioassessment in marine ecosystems.

1. Introduction

Aquatic ecosystems have large amounts of biodiversity, however, they are increasingly impacted by multiple anthropogenic stressors. To conserve these systems, it is urgent for us to get knowledge, not only of which species are present, but of how the systems work and the overall functioning of an ecosystem. As such, biological traits analysis (BTA) is developed to provide a description of community functioning and ecosystem process under the influence of environmental changes at taxon-free levels (Bremner et al., 2006a, 2006b; Wan Hussin et al., 2012; Alves et al., 2014). It does this by utilizing species trait distribution in functional space to summarized community structures and functional diversity (Petchey and Gaston, 2006; Hewitt et al., 2008; Gusmao et al., 2016; Zhong et al., 2017).

Recent investigations have demonstrated that BTA is a robust tool to explore community functioning and ecosystem process in various types of marine environments (Bremner et al., 2003; Villéger et al., 2010; Dimitriadis et al., 2012; Gusmao et al., 2016; Zhong et al., 2017). Compared with traditional community research based on observed species abundance/biomass data, BTA can significantly reduce the strong disturbance from high "signal to noise" ratios due to functional redundancy in monitoring programs (Mason et al., 2005; Petchey and Gaston, 2006; Schleuter et al., 2010; Wan Hussin et al., 2012; Zhong et al., 2017).

In aquatic ecosystem, ciliated protozoa act as a crucial component of microbial food webs (Pomeroy et al., 2007). Due to their cosmopolitan distribution and sensitivity to environmental changes, ciliates have been widely used as useful bioindicators of aquatic ecosystems. However, most previous studies were conducted relying on species-abundance information, and it is commonly subject to high "signal to noise" ratio (Norf et al., 2009; Xu et al., 2014). Thus, the BTA technique is urgently required in order to remove the disturbance from high functional redundancy of the ciliate communities in monitoring surveys (Zhong et al., 2017). However, for bioassessment using periphytic ciliates, sampling methods using a conventional artificial substratum are commonly subject to strong tidal current and circulation, and thus less efficient for exploring their colonization dynamics in marine ecosystems (Xu et al., 2009a, 2009b). For reducing this kind of disturbance, Xu et al. (2009a, 2009b) developed a modified slide method, *i.e.*, polyurethane foam enveloped slide system (PFES). Compared with the convenient slide system, the PFES system is a more advanced sampling system and could avoid the influence of the tidal current and circulation by the polyurethane foam in marine ecosystems (Xu et al., 2002; Gong et al., 2005; Xu et al., 2009a, 2009b). So far, however, although several

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studies based on observed species data have been conducted using the modified sampling method, little information is known about the tidal influence on community functioning of periphytic ciliates in colonization surveys for marine bioassessment using BTA (Xu et al., 2009a, 2009b).

In this study, the temporal shift in community functioning of the ciliates during colonization process was investigated in Korean coastal waters of the Yellow Sea based on a 1-month survey. The main objectives of this survey are: (1) to the explore the influence of tidal events on the functional trait distribution of periphytic ciliates during their colonization process; (2) to compare the colonization parameters of the ciliates between the modified and naked glass slide systems based on BTA technique; and (3) to determine the disturbance of tidal event on colonization surveys for BTA-based bioassessment using periphytic ciliate marine ecosystem.

2. Materials and methods

2.1. Sampling and data collection

Samples were obtained from western coastal water of Korea in April 2007 (Fig. 1). This area is commonly influenced by strong tidal current and circulation with a tidal interval of 3 m and high turbidity because of the mud-sandy bottom. The water depth is about 8 m.

Two anchoring systems for glass slides, the conventional slide system (CS) and polyurethane foam enveloped slide system (PFES), were designed for gathering periphytic ciliates (for details, see Xu et al., 2009a, 2009b). As the transparency is about 1 m *in situ*, these two systems were placed at a depth of 1 m below the water surface and collected at the same time.

For each sampling system, a total of 40 glass slides were used as artificial substrates for collecting periphytic ciliates. Every 10 glass slides were held vertically in one polyvinyl chloride (PVC) frame as back-to-back pairs (Xu et al., 2009a, 2009b). Thus, a total of four PVC frames were used to hold all 40 glass slides, with two parallel sampling replicates. For each replicate, two slides were randomly collected from each PVC frame at the time interval of 1, 3, 5, ..., 19 days during the exposure period, respectively. A total of 10 samplings were carried out. For each sampling the slide samples were transferred into Petri dishes containing water from the sampling site, and then stored in a cooling box before transporting to the laboratory within 12 h for identification and enumeration (Xu et al., 2009a, 2009b).

Ciliate species were firstly observed at a 45-fold magnification using a stereomicroscope (Olympus SZH10 research stereo) to examine their body form, color, movement and behavior. They were then transferred using a micropipette onto a clean glass slide and placed under a microscope (Leica DM2500) to reveal details of the cell size and other morphological characters at 100–1250-fold magnification (Song et al., 1999). To aid species identification, protargol stain was used, if necessary (Song et al., 1999). Taxonomic classification of ciliates was based on the published references such as Song et al. (1999). The enumeration of ciliates *in vivo* was performed under an inverted microscope after sampling generally within 2–4 h. Using bright field illumination, 20 fields of view per slide were randomly chosen for counting and the abundances were calculated from all two replicates to confirm the average cell densities (cell cm^{-2}).

2.2. Functional traits

A total of 7 biological traits related to ecological functioning were chosen to establish functional trait database based on observations and literatures (*e.g.*, Bremner et al., 2003; Song et al., 2009; Dimitriadis et al., 2012; Zhang et al., 2012; Zhong et al., 2017). Four morphological features (body size, form, flexibility and sociability) and three behavior characteristics (feeding, food resource and movement), which can be sub-divided into 17 categories, were included in the functional trait system (Table S1).

The fuzzy coding trait system was used when a species represents more than one trait within each variable based on literatures such as Bremner et al. (2003) and Zhong et al. (2017). In the fuzzy coding system, the scoring range of 0 to 3 was adopted, with 0 reflecting no affinity for the given trait category, 1 or 2 partial affinity and 3 total exclusive affinity. For example, the ciliate *Euplotes vannus* feeds on both bacteria and microalgae but prefers the former, so it was coded 2 (bactivores), 1 (algivores), 0 (predators) for the trait "feeding type".

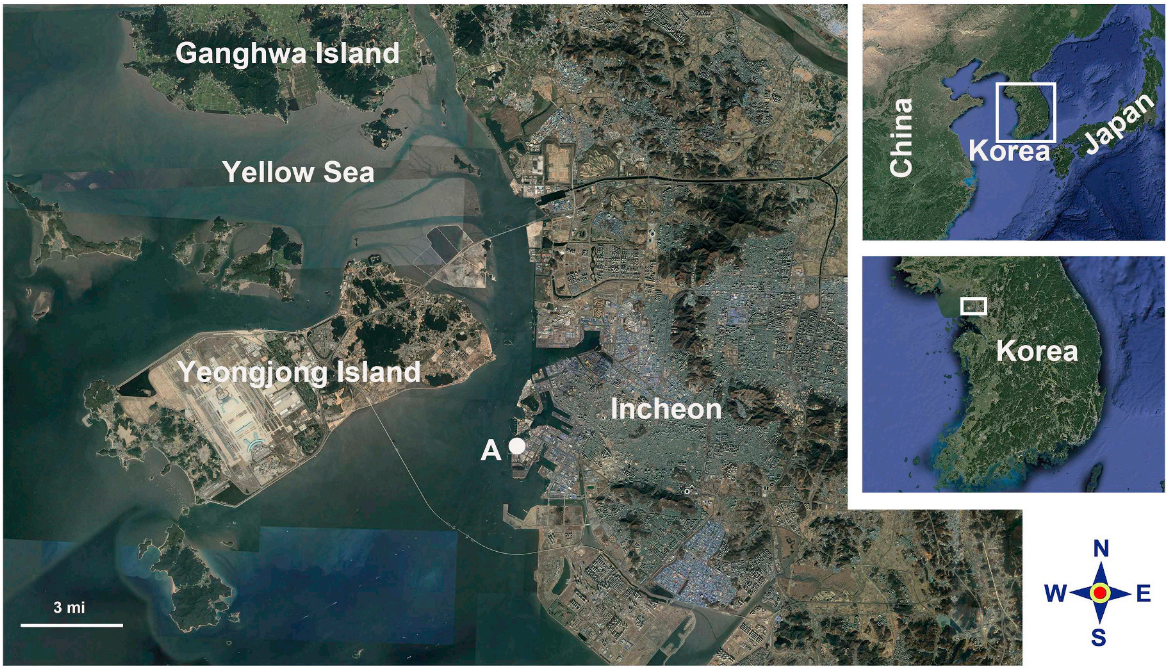

Fig. 1. Sampling site in Korean coastal waters, near Incheon Harbor, Korea. A, sampling site.

2.3. Data analyses

The functional diversity (FD) R package was used to obtain community-weighted means (CWM) of traits and functional diversity indices (Laliberté et al., 2014). Based on both trait data ('weight') and species relative abundance data (%*N*), the CWM values were computed by %*N* × weight (Lavorel et al., 2008).

Multidimensional FD indices, including functional richness (FRic), functional evenness (FEve), functional divergence (FDiv), functional dispersion (FDis) and Rao's quadratic entropy index (RaoQ), were computed by the function FD of R software. It should be noted that because of complex equation expresses for calculations of all five indices, for details please see the literatures Botta-Dukát (2005), Mason et al. (2005), Villéger et al. (2008), Schleuter et al. (2010) and Laliberté and Legendre (2010).

The functional trait data were further analyzed using the software of PRIMER (v7.0.13) (Clarke and Gorley, 2015). Two tests, the dbRDA (distance-based redundancy analysis) and clustering test, were chosen to identify colonization dynamics of ciliate communities. Before analysis, Euclidean distance matrices were computed from log-transformation/normalized CWM data (Clarke and Gorley, 2015). The difference in functional structure of communities between the PFES and CS sampling systems was calculated by the bootstrapped average analysis (Anderson et al., 2008; Xu et al., 2014). SIMPROF test was used to signify differences in community functioning of the ciliates between two sampling systems based on clustering analysis (Anderson et al., 2008).

3. Results

3.1. Functional trait distribution during the colonization process

A total of 28 species were identified in Korean coastal waters of the Yellow Sea during the study period. The biological traits information is summarized in Table 1.

The seven functional traits distributions in the protozoan communities during the colonization process in Korean coastal waters of the Yellow Sea using the CS and PFES sampling systems are shown in Fig. 2. Totally, the species functional traits (especially feeding type, body form, movement, sociability and food resource) showed different temporal dynamics during the colonization process using these two sampling systems.

In terms of the community weighted means for the categories of each trait, a typical functional pattern was identified with high homogeneity in cylindrical, sessile, nonflexible and colonial bacterivores feeding on plankton food during colonization time of 9–19 days using both of the CS and PFES sampling systems. In comparing with CS system, the vagile, flattened forms feeding on periphytic microalgae showed a higher community-weighted mean value, and the solitary-colonial pattern represented a more stable temporal variability during the colonization process in the PFES system. Otherwise, the distinct functional trait patterns were detected at the early stage of colonization (1–7 days) using these two different sampling systems, except that common solitary trait pattern was observed during colonization time of 1–3 days.

3.2. Influence on colonization dynamics in functional pattern

The dbRDA ordinations on the CWMs of protozoan during the colonization process using the CS and PFES sampling methods are shown in Fig. 3. The results showed that for these two sampling methods, dbRDA1 could explain ≥71% of total variation in functional pattern during the colonization process. For the CS sampling method, samples with age of 1 day were separated from those with age of 3 day, and those with ages of 1–3 days were distant from those with ages of > 5 days. As regard to the PFES sampling method, samples with ages of 9–19 days were separated from the other samples, and those with ages of 1–7 days were further divided into four distinct groups (Fig. 3). Moreover, four categories (*e.g.*, cylindrical, sessile, colonial and medium) generally pointed towards samples with ages of 9–19 days at

Table 1

Biological traits of the ciliated protozoa in Korean coastal waters of the Yellow Sea during the study period. A, algivores; B, bacterivores; L, large; M, medium; Sm, small; F, flattened; C, cylindrical; V, vagile; Pl, planktonic; S, sensile; Fl, flexible; N, non-flexible; So, sociality; Co, colonial; I, periphyton food supply; O, planktonic food supply.

Species	Feeding type	Body size	Form	Movement	Flexibility	Sociability	Food resource
Amphileptus gui	A	L	F	V	Fl	So	I
Amphileptus litonotiformis	A	M	F	V	Fl	So	I
Aspidisca leptaspis	B	Sm	F	V	N	So	I
Aspidisca steini	B	Sm	F	V	N	So	I
Condylostoma magnum	A	L	C	V	N	So	I
Condylostoma spatiosum	A	L	C	V	N	So	I
Diophrys appendiculata	A	Sm	F	V	N	So	I
Diophrys scutum	A	M	F	V	N	So	I
Dysteria derouxi	A	M	F	V	N	So	I
Euplotes charon	B	Sm	F	V	N	So	I
Euplotes minuta	B	Sm	F	V	N	So	I
Euplotes vannus	B	L	F	V	N	So	I
Holosticha bradburyae	A	L	F	V	Fl	So	I
Holosticha diademata	A	Sm	F	V	Fl	So	I
Holosticha heterofoissneri	A	L	F	V	Fl	So	I
Litonotus paracygnus	A	L	F	V	Fl	So	I
Loxophyllum rostratum	A	L	F	V	Fl	So	I
Metanophrys similis	B	Sm	C	Pl	N	So	O
Paranophrys magna	B	Sm	C	Pl	N	So	O
Peritromus faurei	A	Sm	F	V	N	So	O
Pleuronema wilberti	B	M	C	Pl	Fl	Co	I
Pseudovorticella paracratera	B	Sm	C	S	N	So	O
Stichotricha marina	A	M	C	S	N	So	O
Strombidium apolatum	A	Sm	C	Pl	N	So	O
Strombidium sulcatum	B	Sm	C	Pl	N	So	O
Thuricola valvata	B	L	C	S	N	So	I
Uronema marinum	B	Sm	C	Pl	N	So	O
Zoothamnium alternans	B	Sm	C	S	N	Co	O

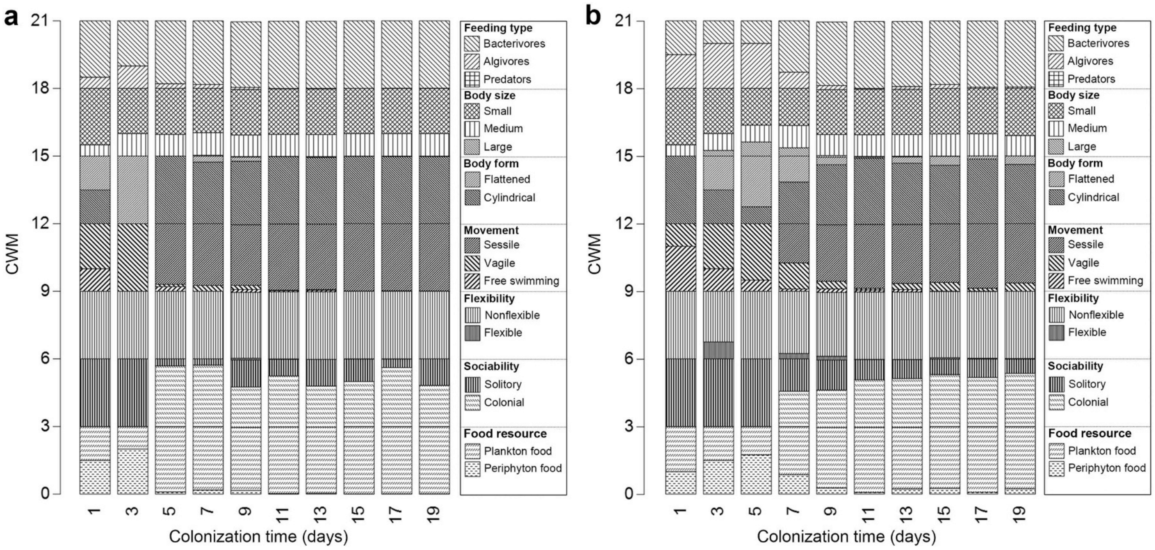

Fig. 2. Temporal variations in community weighted means of multiple functional traits of periphytic ciliate communities during the colonization process using the CS (a) and PFES (b) sampling systems.

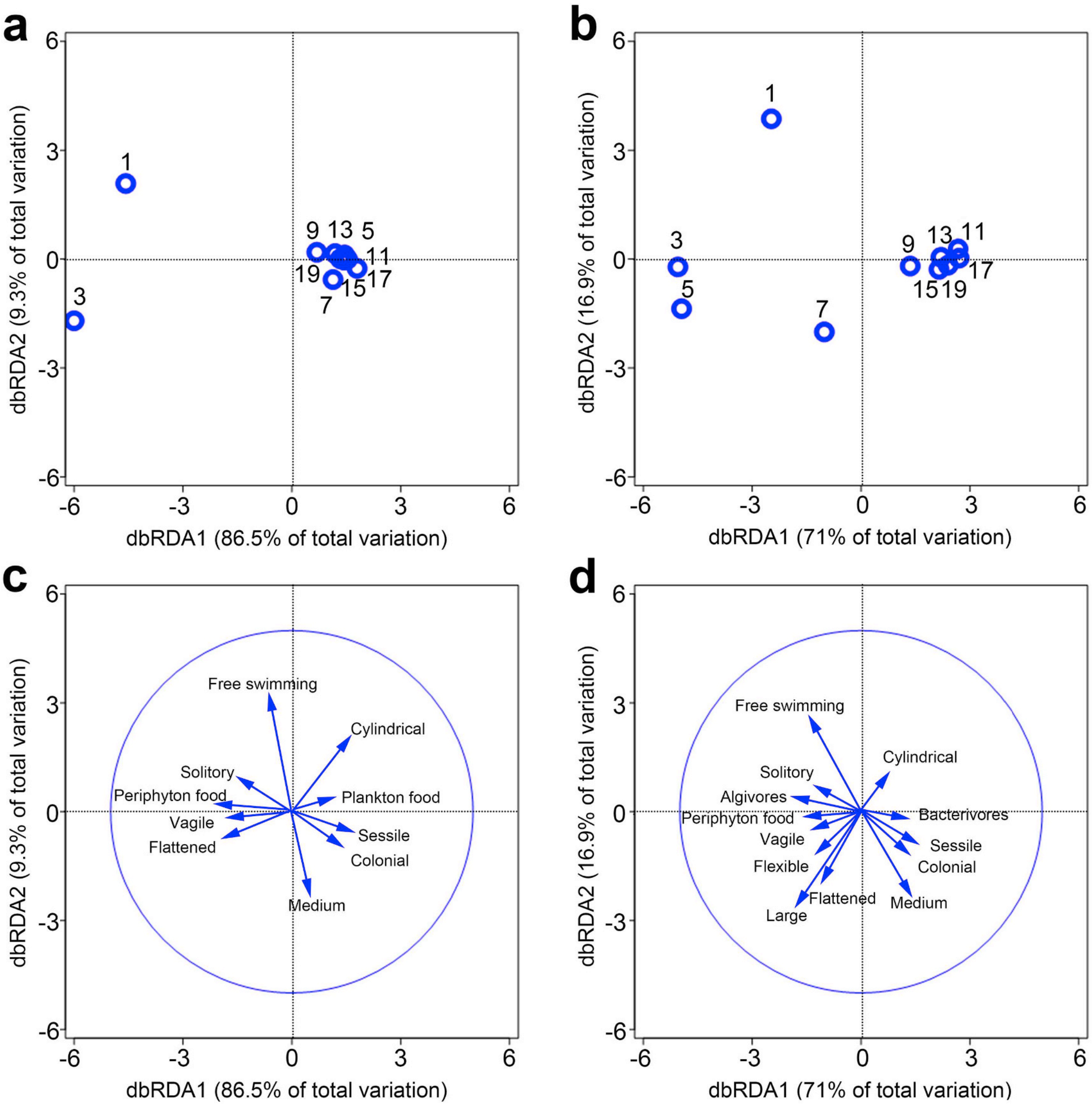

Fig. 3. dbRDA ordinations (a, b) for colonization dynamics of community functioning of periphytic ciliates during the colonization process using the CS (a, c) and PFES (b, d) sampling methods.

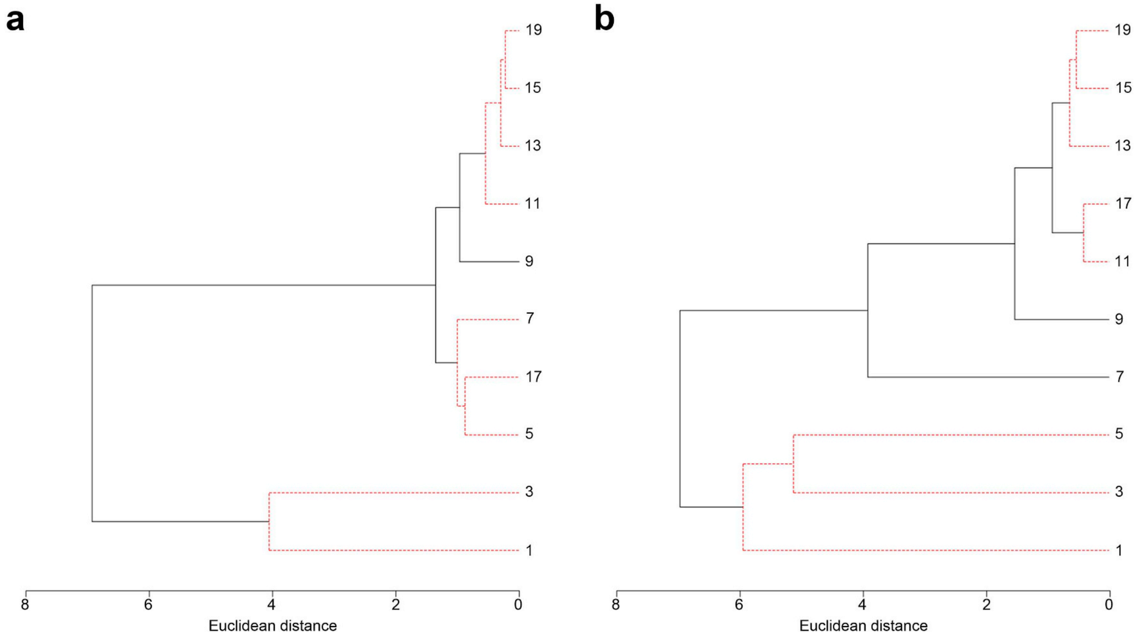

Fig. 4. Clustering analyses with SIMPROF test for colonization dynamics of community functioning of periphytic ciliates during the colonization process using the CS (a) and PFES (b) sampling systems.

level of 0.3 correlation with dbRDA axes (Fig. 3c and d).

By using the clustering analysis with SIMPROF test, differences were detected in functional structure of protozoan communities between the CS and PFES sampling systems during the colonization period (Fig. 4). For example, the results showed that for CS sampling system, there were significant differences between samples with ages of 3–5 days, however, no significant differences were detected between those samples using the PFES sampling system. Also, there were no significant differences between samples with ages of 11–13 days in the CS system, however, significant differences were observed between them using the PFES sampling system. Furthermore, the bootstrapped average analysis revealed that there was a small overlap of colonization pattern between using the CS and PFES sampling systems (Fig. 5).

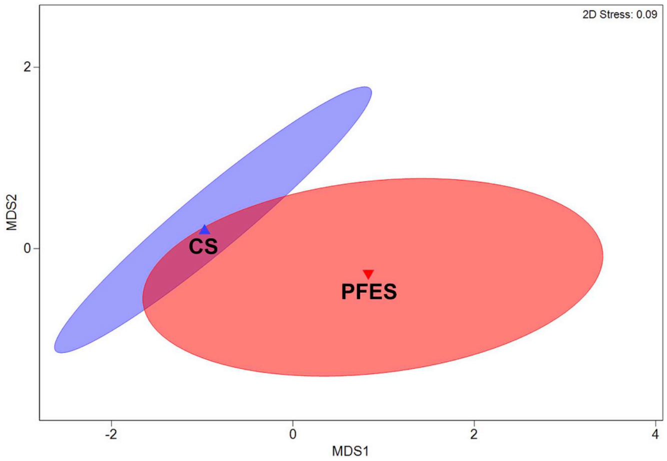

Fig. 5. Bootstrapped average analysis: showing difference in colonization dynamics of community functioning of periphytic ciliates during the colonization process using the CS (a) and PFES (b) sampling systems.

3.3. Influence on temporal pattern of functional diversity indices

The changes of five functional diversity indices during the colonization time are shown in Fig. 6. The functional diversity indices showed different temporal variations during colonization process between those two sampling systems, except for the measure *FDiv* (Fig. 6). In general,

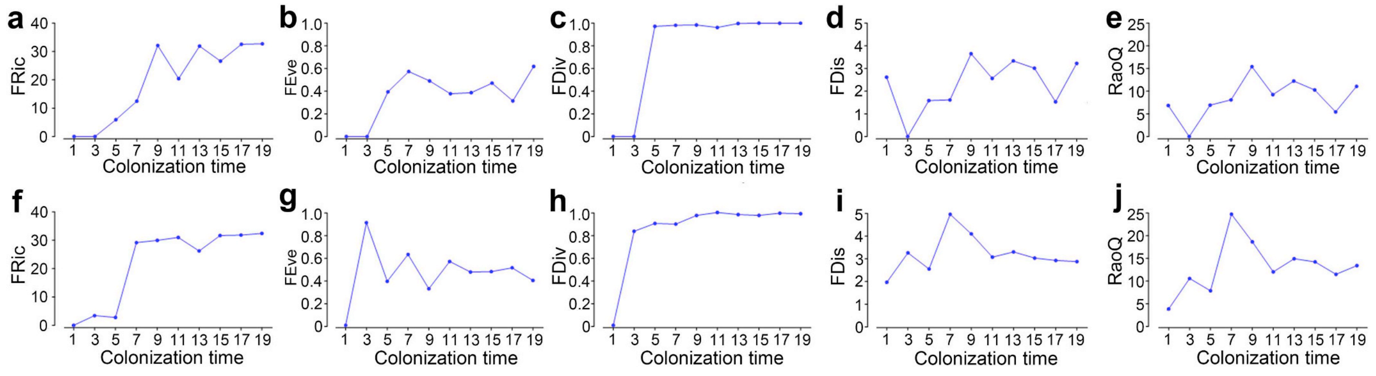

Fig. 6. Temporal variations in five functional diversity indices *FRic* (a, f), *FEve* (b, g), *FDiv* (c, h), *FDis* (d, i) and *RaoQ* (e, j) during colonization process using the CS (a–e) and PFES (f–j) sampling systems.

the *FDiv* values showed an increasing trend at the early stage of colonization and maintained a stable level during the colonization periods of 5–19 days using both the CS and PFES sampling methods (Fig. 6c and h).

4. Discussion

The colonization dynamics of ciliates are commonly influenced by environmental changes and thus have been used to indicate water quality in marine ecosystems (Zhang et al., 2012; Xu et al., 2014; Zhong et al., 2014). In this study, colonization dynamics of ciliates were observed with high heterogeneity at the early stage (1–7 days) and high homogeneity at a stable stage (9–19 days) based on the BTA approach. Previous study observed the similar colonization dynamics of periphytic ciliates (Zhong et al., 2017). However, the vagile, flattened forms feeding on periphytic microalgae showed a higher community-weighted mean value, and the solitary-colonial pattern represented a more stable temporal variability during the colonization process in the PFES system in comparison with that in the conventional system. Furthermore, the multivariate analyses demonstrated that there were differences in functional structure of protozoan communities during the colonization periods using the CS and PFES sampling methods. This implies that the ciliate colonization dynamics in functional space followed a different model in the modified glass slide system compared to the CS sampling system. For example, the CWM values of vagile and swimming forms were higher in PFES system than in CS system. This might be due to influence of strong tidal events on these vagile and swimming ciliates is high in CS system compared to that of PFES system.

We suggest that the community functioning of the ciliate assemblages in the CS system was different from that in the PFES system. Considering the PFES system is equipped with caved polyurethane foam (PFU) and the strong tidal current and circulation are observed in the sampling spot, it is possible that some food particles may easily get into the CS system but be rejected by the modified PFES system. However, based on our results, the CWM values of algivores and plankton-food supply were not lower in PFES system than those in CS system. It implies that food supplies were not the primary factor to influence the colonization dynamics in community functioning of the ciliates within the two sampling systems.

Functional diversity indices are reported to be used for comprehensively summarizing the range of species trait distribution in ecosystem process (Tilman, 1996; Petchey, 2003; Mason et al., 2005; Mouchet et al., 2010; Zhong et al., 2017). Our study showed four indices (FRic, FEve, FDis and RaoQ) represented different models in temporal pattern during the colonization process in the CS and PFES systems. For example, all five functional diversity indices generally levelled off at stable values in the communities with ages after 15 days in the PFES system compared to those in the CS system. This finding suggested that the functional diversity measures detected in the CS system were sensitive to the strong disturbance of tidal current and circulation in Korean coastal waters of the Yellow Sea.

It should be noted that the present study was conducted in two artificial substratum systems for finding an effective tools to reduce the influence of tidal events on ciliate colonization for bioassessment of water quality in marine ecosystems. Although the CS system could reflect the *in situ* patterns of the ciliate communities in nature biotopes of marine waters, we still suggest that the PFES system was better in monitoring programs than the naked slide method.

In summary, considerable differences were detected in community functioning and functional diversity measures during ciliate colonization between the PFES system and the conventional slide system. The vagile, flattened forms feeding on periphytic microalgae showed a higher community-weighted mean value, and the solitary-colonial pattern represented a more stable temporal variability during the colonization process in the PFES system in comparison with that on the conventional system. Multivariate analyses demonstrated that the temporal shift in community functioning of the ciliates showed different ways in both systems. The functional diversity measures generally levelled off at more stable values in the communities with ages of 13–15 days in the PFES system than those in the CS system. These results suggest that the PFES system may be used as a feasible method for reducing the disturbance of tidal current and circulation in colonization survey of periphytic ciliates for bioassessment in marine ecosystems.

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