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Colonisation and succession of marine biofilm-dwelling ciliates in response to environmental variation

Matthew G. Watson[1,*], Andrew J. Scardino[2], Liliana Zalizniak[1], Jeff Shimeta[1]

[1]School of Applied Sciences, RMIT University, PO Box 71, Bundoora, Victoria 3083, Australia
[2]Maritime Division, DSTO Defence Science and Technology Organisation, Fishermans Bend, Victoria 3207, Australia

ABSTRACT: Protozoan assemblages and successional dynamics are important components of biofouling that require better understanding. We studied marine ciliates in temperate Australia as they colonised artificial substrates for 21 d during 2 different seasons, with 2 different aspects of orientation. Sessile and planktonic taxa established within 7 d, whereas vagile taxa colonised throughout the period. Abundances reached 366 ciliates cm^{-2}. Colonies of the peritrichs *Zoothamnium* and *Vorticella*, and the hypotrichs *Aspidisca* and *Euplotes* were the most abundant. The north aspect received more light than the south aspect during summer, but assemblages did not differ significantly. Assemblage structure was different between seasons, and it developed more quickly and reached greater abundances during summer. A storm late in summer abruptly reduced abundances and affected functional groups differently, but diversity was largely unaffected. Thus, diversity of an established assemblage can be maintained through disturbances, despite abundances being subject to great fluctuation.

KEY WORDS: Ciliates · Functional group · Marine microbial ecology · Marine organisms · Microbial assemblage structure

INTRODUCTION

The settlement and metamorphosis of many marine invertebrates is strongly influenced by physical and chemical cues associated with microbial biofilms (Rittschof et al. 1998, Qian et al. 2007). Chemical cues released by the pioneering colonisers of biofilms in particular are widely considered to have the greatest influence on the settlement of macrofoulers (Rittschof et al. 1998, Wieczorek & Todd 1998, Hadfield and Paul 2001, Qian et al. 2007). In most cases these 'microbial biofilms' are mixed assemblages of bacteria, microalgae, protozoa and fungi bound to a solid surface by their extracellular products (Arndt et al. 2003, Dobretsov 2013).

Protozoa, a ubiquitous component of microbial biofilms, are able to rapidly colonise new substrata, and over a short period of time reach high abundances within marine biofilms (Arndt et al. 2003, Gong et al. 2005, Xu et al. 2009). Heterotrophic protozoa are, in quantitative terms, considered the most important grazers of microbes in aquatic environments (Berninger et al. 1991, Sherr & Sherr 1994, Corno & Jürgens 2006). There is increasing evidence that the presence of protozoa can influence biofilm structure and population dynamics of microbial biofilms (Jackson & Jones 1991, Lawrence & Snyder 1998, Kiørboe et al. 2003, Huws et al. 2005). The impact of biofilm-dwelling protozoan assemblages, however, largely depends on their structure, both in terms of abundance and their taxonomical and functional composition (Arndt et al. 2003).

Selective grazing on particular prey types via both mechanosensory and chemosensory cues has been

*Corresponding author: s3225789@student.rmit.edu.au

observed in protozoa (Fenchel 1980, Ayo et al. 2001). These mechanisms allow protozoa to select the size and composition of the food particles on which they feed (Fenchel 1980, Bernard & Rassoulzadegan 1990, Gonzalez et al. 1990, Ayo et al. 2001), and in turn allow protozoa to exert a strong influence on the composition of microbial assemblages within biofilms. Recent research conducted in simplified bioassays has shown that the grazing activities of protozoa increase the spatial and temporal heterogeneity of bacterial biofilms, altering the taxonomic composition of bacterial assemblages, and in extreme cases creating areas of clearance or sloughing on the substrate (Lawrence & Snyder 1998, Corno & Jürgens 2006).

Given the impacts of protozoan activities within biofilms, their influence could extend to the settlement of invertebrate larvae either indirectly by influencing the structure, abundance or community composition of microbial biofilms, or directly through physical or chemical interactions with larvae. We recently showed that the presence of a mixed assemblage of ciliates both inhibited and, in some cases, induced the settlement of several different invertebrate foulers (Shimeta et al. 2012). However, while these mixed assemblages of ciliates had strong effects on invertebrate settlement, they appear to be highly varied and species-specific (Shimeta et al. 2012). These results point to a need for a better description of species assemblages and successional processes in marine biofilms.

Investigations of succession on marine hard substrata have contributed considerably to understanding of the biofouling process. An array of direct and indirect species interactions, stochastic recruitment events and environmental disturbance ultimately dictates the successional trajectories of microbial biofilms (Henschel & Cook 1990, Stoodley et al. 2002, Jenkins & Martins 2010). Succession driven by biological interactions is considered the primary determinant of assemblage structure (Stoodley et al. 2002, Arndt et al. 2003, Qian et al. 2007). As new substrates are immersed, they are gradually colonised by a number of species. These early species may in turn facilitate or inhibit colonisation by later species (Sousa 1984, Arndt et al. 2003, Jenkins & Martins 2010).

Currently, the literature on biofilm-dwelling protozoa, while considerable in size, deals almost exclusively with freshwater systems. Relatively little is known about assemblage dynamics in the marine environment (Arndt et al. 2003). To understand the complex interactions of ciliates within microbial biofilms, special attention must be paid to the taxonomic structure and successional dynamics of the assemblages present. Although ubiquitous within biofilms, the density and species composition of ciliate assemblages are known to vary seasonally (Gong et al. 2005, Xu et al. 2009). With their rapid growth and delicate external membranes, ciliate assemblages are strongly influenced by environmental variation (Arndt et al. 2003, Gong et al. 2005). Environmental factors including temperature, flow velocities, nutrient loadings and surface composition have all been shown to significantly influence the structure of ciliate assemblages (Franco et al. 1998, Gong et al. 2005, Norf et al. 2009, Risse-Buhl et al. 2009, Xu et al. 2009).

An understanding of the structure and function of ciliates in marine microbial biofilms is central to interpreting and predicting the impacts of these assemblages on the surrounding marine ecosystem. Here, we report on the temporal succession of ciliate assemblages at genus level resolution in Port Phillip Bay, Melbourne, Australia. The aims of this study were as follows: (1) to document the taxonomic structure and succession of ciliate assemblages in temperate Australian waters; (2) to describe the successional patterns during 2 contrasting weather conditions, i.e. by studying time-series in summer and in winter, including identifying the impact of north and south orientations; and (3) to investigate the response of established ciliate assemblages to a natural disturbance event, which occurred during the summer deployment.

MATERIALS AND METHODS

Study site and sampling

The study took place at the Defence Science and Technology Organisation (DSTO) Marine Coatings exposure raft on Booth Pier, Williamstown, Victoria, Australia (37° 51′ 41.40″ S, 144° 54′ 38.06″ E). The floating raft lies in Hobsons Bay, the northernmost part of Port Phillip Bay, which is a large inland bay covering 2000 km^2 with a narrow opening into Bass Straight. The site is approximately 6 m in depth with a tidal range of approximately 1 m. The assemblage analysis was conducted during June and July (Australian winter) 2012 and again in February and March (Australian summer) 2013. The influence of aspect was investigated by deploying the artificial substrates in different orientations (north–south alignments), exposing the surfaces to different amounts of light.

Ciliate sampling was conducted using sealable plastic Petri dish microscope slides (Analyslide™,

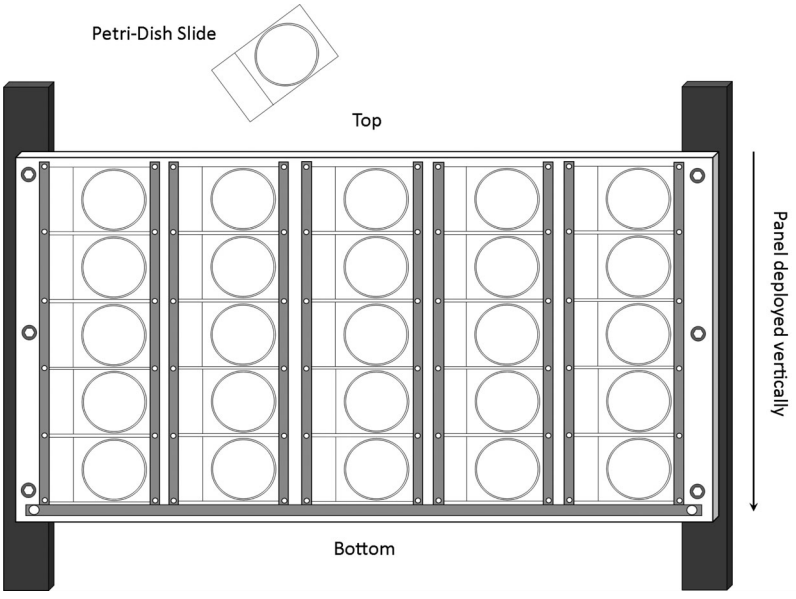

Fig. 1. Panel design illustrating how each individual Petri dish slide was held in place by rails mounted on the PVC panel which was then attached to an aluminium frame and suspended vertically 1 m underneath a raft

Pall Corporation) as artificial substrates for biofilm development. A total of 50 Petri dishes (25 on each aspect) were used to collect ciliates from a depth of 1 m below the water surface. Each Petri dish slide was secured vertically to a polyvinyl chloride (PVC) rack, which was designed to attach to an aluminium frame suspended vertically beneath a raft (Fig. 1). A total of 5 samplings were carried out over a 21 d period in each season. Assuming there would be no significant differences between ciliate assemblages colonising slides within the same frame, 5 randomly selected replicate Petri dish slides from each aspect were collected on each sampling date. The slides were sealed with a cap under the water surface and transferred into a cool box for transportation to the laboratory.

Temperature and luminosity (lux) were measured every 10 min throughout the deployment period with data loggers (HOBO UA-002-64, Onset). Salinity, pH, turbidity and dissolved oxygen were recorded *in situ* on each sampling date with a multi-parameter probe (Hydrolab DS5X, Aqualab Scientific) (see Table 2).

Identification and enumeration

Ciliates were initially observed live at 45× magnification under a stereomicroscope (Leica MZ9.5) to determine the classifications and abundances of sessile ciliate genera. Live observations provided insight into the behaviour, movement and ecological niches occupied by vagile and planktonic ciliates in the samples. After live observation, the samples were fixed in 2% glutaraldehyde solution within 1 to 2 h of sample collection. Identification and enumeration were by quantitative protargol staining (QPS) techniques. The fixed samples were first concentrated onto cellulose filters and embedded in agar, and the filters were then post-fixed in 10% Bouin's solution prior to protargol impregnation. Staining followed the QPS method described by Skibbe (1994).

Protargol impregnations were mounted in Permount and examined under a phase contrast microscope (Leica DM2500) at 100 to 1250× magnification to reveal details of kinetid patterns and other morphological characteristics required for genus identification. Enumeration was conducted at 200× magnification, and 20 fields of view per slide were randomly selected for counting. The ciliate abundances were determined based on the counts of all 5 replicate Petri dish slides collected at each sampling to confirm cell densities (cells cm^{-2}). Specimens were also photographed and catalogued for taxonomic classification using keys published by Carey (1992), Lee et al. (2000), and Lynn (2008).

Data analysis

Species diversity (H') of samples was calculated as follows:

$$H' = -\sum_{i=1}^{S} P_i(\ln P_i) \quad (1)$$

where S is the total number of species and P_i = proportion of the total count arising from the ith species (Pielou 1966).

Abundance data were $\log_{10}(x + 1)$ transformed when required to reduce heterogeneity of variances. A repeated-measures ANOVA (SYSTAT v.13) was used to establish whether there were significant differences in genera abundances or composition between time points, aspect and season. Where one of these factors or an interaction was significant at $\alpha = 0.05$, Tukey's pairwise comparisons were run.

Multivariate analyses were performed with Primer v.6.0 (Plymouth Marine Laboratory) software. To prevent bias caused by highly abundant taxa, ciliate

assemblage data were transformed prior to analysis. Square root transformations down-weighted the importance of the highly abundant taxa, allowing rarer taxa to exert some influence in the diversity and similarity calculations. Similarity percentage (SIMPER) analysis was performed to determine the percentage of dissimilarity between ciliate genera within aspects or season and the percentage contribution of each genus to the overall dissimilarity. Spatial and temporal patterns in community structure were examined via hierarchal cluster analysis and non-metric multidimensional scaling (MDS) ordinations, also generated from this transformed data set.

Table 1. (A) Ciliate genera (by order) recorded in a total of 100 samples collected during winter (Jun and Jul 2012) and summer (Feb and Mar 2013) seasons, including functional group occupied (S: sessile, V: vagile, P: planktonic), peak abundance (±SE) and occurrence. (B) Total abundance of ciliate functional groups, including % contribution of each functional group. –: not present

	Functional group	Summer		Winter	
		Peak abundance (ciliates cm^{-2})	Occurrence (%)	Peak abundance (ciliates cm^{-2})	Occurrence (%)
A) Taxonomic composition					
Sessilida					
Zoothamnium	S	224.62 ± 41.38	100	78.76 ± 13.96	100
Vorticella	S	50.97 ± 10.13	100	4.36 ± 0.82	100
Euplotida					
Aspidisca	V	6.92 ± 1.01	100	12.45 ± 1.50	100
Euplotes	V	10.03 ± 0.65	100	5.11 ± 0.78	100
Uronychia	V	0.36 ± 0.19	60	0.55 ± 0.19	40
Diophrys	V	1.68 ± 0.21	60	–	0
Uronstylida					
Holosticha	V	0.66 ± 0.25	80	0.21 ± 0.13	60
Stichotrichida					
Sticotricha	V	7.18 ± 0.67	100	3.05 ± 0.25	100
Heterotrichida					
Folliculina	S	0.72 ± 0.19	100	–	0
Haptorida					
Lacrymaria	V	4.48 ± 0.55	100	1.02 ± 0.27	80
Pleurostomatida					
Amphileptus	V	17.57 ± 1.06	100	17.25 ± 1.93	80
Litonotus	V	1.68 ± 0.31	60	5.22 ± 0.55	80
Dysteriida					
Dysteria	V	1.42 ± 0.33	20	0.74 ± 0.17	60
Strombidiida					
Strombidium	P	1.02 ± 0.14	80	2.17 ± 0.24	100
Philasterida					
Uronema	P	8.40 ± 0.77	100	3.31 ± 0.36	100
Pleuronematida					
Cyclidium	P	5.80 ± 0.35	100	4.63 ± 0.64	100

B) Ciliate abundance				
Functional group	Total abundance	Contribution (%)	Total abundance	Contribution (%)
Sessile	276.31 ± 52.18	80.44	83.12 ± 14.78	60.12
Vagile	51.98 ± 5.23	15.13	45.03 ± 5.77	32.57
Planktonic	15.22 ± 1.26	4.43	10.11 ± 1.24	7.31

RESULTS

Taxonomic composition

The taxonomic composition of the ciliate assemblages recorded during summer and winter deployments are summarized in Table 1A. A total of 16 genera representing 11 orders were recorded following examinations of 100 samples. Sessile ciliates were represented by species of the orders Sessilida and Heterotrichida. Vagile forms belonged primarily to the orders Euplotida, Pleurostomatida and Stichotrichida. Planktonic taxa were represented by the orders Philasterida, Pleuronematida and Strombidiida (Table 1A). The sessile ciliate *Zoothamnium* was the most abundant ciliate genus. At its peak, ciliate abundance reached >350 cm^{-2}. The combined sessile ciliates dominated the assemblage, accounting for 80.4% of the total ciliate abundance at the point when the assemblage reached its peak abundance (Table 1B). Vagile ciliates had low abundance in comparison; however, they were the primary contributors to the variation in genera diversity. Euplotida and Pleurostomatida were the 2 orders represented by the most genera, accounting for up to 25.0 and 12.5% of the total genera present, respectively.

Variation between summer and winter deployments

The ranges of environmental parameters recorded during summer and winter deployments are summarised in Table 2. Among these variables, the mean values of temperature, salinity and dissolved oxygen were significantly different between summer and winter deployments (Table 2). Correlations between the ciliate assemblages and environmental variables were weak as the environmental parameters varied little during summer and winter samplings, and therefore these data are not shown. However,

Table 2. Chronological variation of environmental parameters recorded at the study site in Port Phillip Bay during summer (Feb and Mar 2013) and winter (Jun and Jul 2012) deployments (p-values from paired sample *t*-tests are comparisons of summer and winter means; significant difference at the 0.05 level)

	Summer					Winter					p-value
	Sampling day					Sampling day					
	3	7	10	14	21	3	7	10	14	21	
Temperature (°C)	21.22	22.03	23.55	22.98	22.54	10.88	11.11	11.18	11.76	12.29	0.00
Salinity (PPT)	35.77	35.36	36.27	35.65	36.19	34.05	33.12	32.90	33.43	33.15	0.00
pH	8.07	7.94	8.06	8.11	8.13	7.90	7.97	8.00	8.03	8.01	0.08
Turbidity (NTU)	10.22	11.00	10.30	15.70	8.80	12.40	12.20	13.70	14.71	11.40	0.16
Dissolved oxygen (%)	89.01	87.40	87.20	80.60	84.80	99.10	101.20	104.70	94.10	92.20	0.00

the ciliate assemblages did vary significantly between summer and winter samplings. Abundances ranged around mean values of 109.9 ± 64.9 (SE) and 39.2 ± 25.7 cm^{-2} in summer and winter samplings, respectively, with corresponding maximum abundances of 366.1 ± 50.1 and 138.7 ± 15.83 cm^{-2} (Fig. 2). The abundances were significantly different between summer and winter samplings on Days 3 ($p < 0.001$), 7 ($p < 0.001$), 10 ($p < 0.001$) and 21 ($p = 0.001$), which had comparably higher abundances during summer (Fig. 2). A total of 16 genera were identified during the summer deployment and 14 genera during the winter deployment (Table 1). The cumulative genera represented during both seasons showed little variation, with the exception of Heterotrichida which was only identified in summer samples.

The SIMPER analysis presented in Table 3 breaks down the contribution of each ciliate genera to the observed dissimilarities between summer and winter samplings. The sessile ciliate *Zoothamnium* was the primary contributor to the dissimilarity in abundance/occurrence, due to the much higher abundances reached during the summer deployment despite the fact they were ubiquitous during both seasons. The 5 most dominant genera accounted for 62.7% of the total dissimilarity (Table 3). The vagile ciliates *Amphileptus*, *Aspidisca* and *Euplotes* also represented high contributions to the dissimilarity due to their high frequency of occurrence/abundance.

Cluster analysis based on Bray-Curtis similarities of square root-transformed abundances resulted in summer genera falling into 3 groups (I to III) at a 60% similarity level, and winter genera into 4 groups (I to IV) at 70% similarity (Fig. 3). Summer deployment groups I and II represented genera with high abundance and/or occurrence, whereas group III represented assemblages of genera with low occurrence (Fig. 3). Similarly, in the winter deployment groupings, highly abundant genera are present in groups II and III, while the remaining groups represented those rarer genera (Fig. 3).

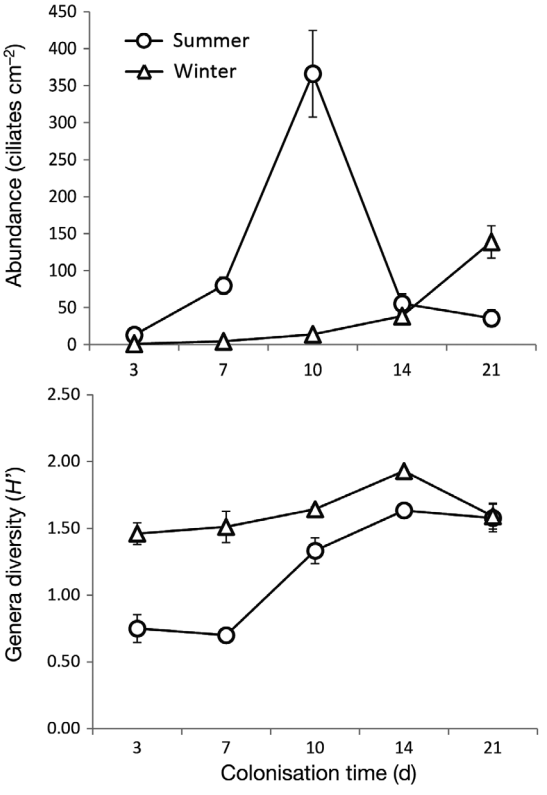

Fig. 2. Summer and winter assemblage comparison of total abundance and genera diversity (*H'*) (Mean ± SE)

Table 3. SIMPER analysis displaying the contribution of the top 9 genera to the average Bray-Curtis dissimilarity in ciliate assemblages between seasons

Genus	Contribution (%)	Cumulative %
Zoothamnium	24.44	24.44
Amphileptus	14.18	38.63
Aspidisca	10.29	48.92
Euplotes	6.91	55.84
Litonotus	6.91	62.74
Vorticella	6.46	69.21
Cyclidium	5.99	75.20
Uronema	5.39	80.59
Stichotricha	5.16	85.75

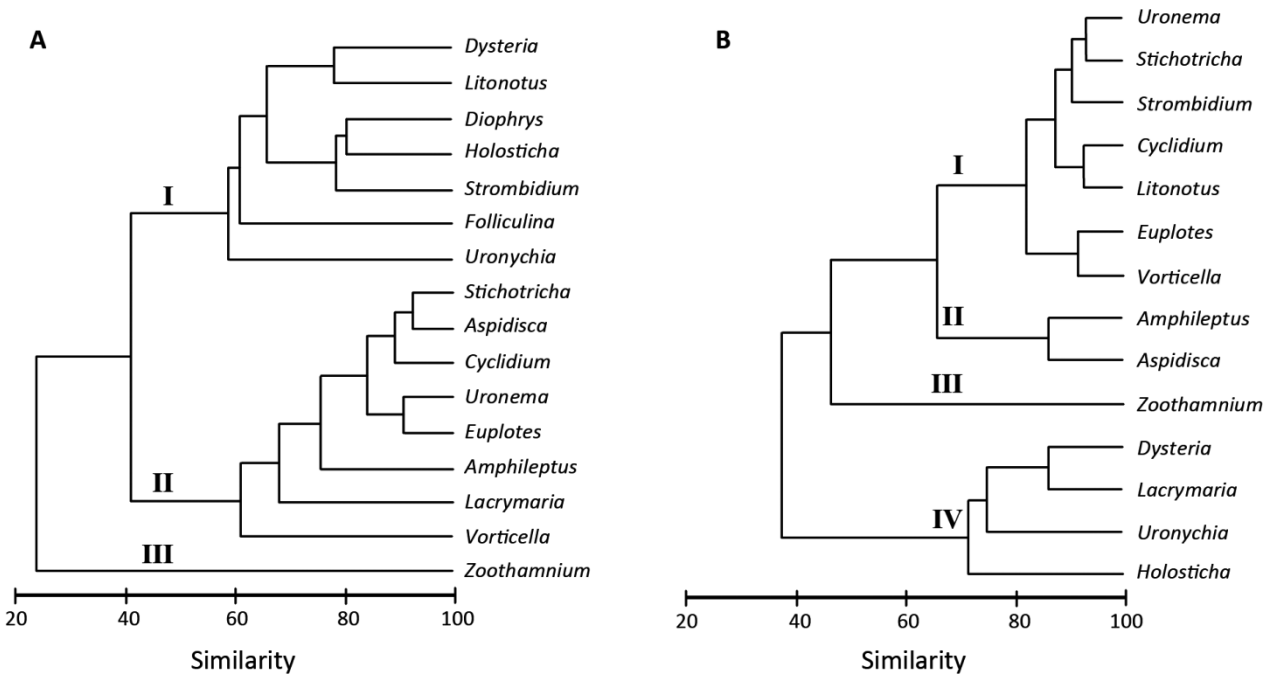

Fig. 3. Dendrograms of ciliate assemblages using group averages based on Bray-Curtis similarity matrix of square root-transformed genera abundances from (A) summer and (B) winter samplings. Roman numerals indicate groups I to IV

Chronological assemblage succession

A clear pattern of chronological succession within ciliate assemblage structures in terms of both genera number and abundance was observed during both summer and winter samplings (Figs. 4 & 5). Sessile ciliates dominated the ciliate assemblages in terms of abundance, particularly during summer samplings, where sessile ciliates accounted for up to 93.0% of the relative abundance (total assemblage) early in the deployment (Fig. 4A). Sessile and planktonic forms colonised the substrates early with few additional recruits observed beyond 7 d (Fig. 4B,D), whereas vagile taxa colonised the substrate throughout the deployment period. Ciliate genera from the orders Euplotida and Pleurostomatida in particular exhibited high variability in occurrence and abundance (Fig. 5).

MDS ordinations of the 5 samples taken during each season were plotted from Bray-Curtis similarities on square root-transformed genera abundances (Fig. 6). The analyses indicated that throughout the winter deployment, ciliate assemblages showed an uninterrupted trend of growth, with each day showing increased dissimilarity to the next as the assemblage developed. The ciliate assemblages during the summer deployment also initially followed this trend on Days 3, 7 and 10. Colonisation, however, occurred at a faster rate during summer with assemblages developing 7 to 10 d earlier than the equivalent winter assemblage, with Day 3 assemblages sharing high similarity to the winter assemblages after 10 d of development (Fig. 6).

Aspect

During the summer deployment, the north aspect (187.3 ± 7.2 [SE] klx d^{-1}) was exposed to significantly more light per day than the south facing aspect (96.2 ± 4.1 klx d^{-1}) ($p < 0.001$). The different aspects were also exposed to significantly more average light per day during summer (141.7 ± 15.5 klx d^{-1}) than winter deployments (61.6 ± 4.6 klx d^{-1}) ($p < 0.001$). The ciliate assemblages between north and south aspects, however, were at no point significantly different during either summer or winter deployments ($p > 0.05$). While no significant difference was found in the assemblages between the north and south aspects, new ciliate recruits tended to colonise the more exposed north aspect first.

Response to disturbance

A clear disturbance to succession occurred during the summer deployment in the samplings following the 10th day (Fig. 2). Between sampling Days 10 and 14, 38.4 mm of rain fell on site (Bureau of Meteorol-

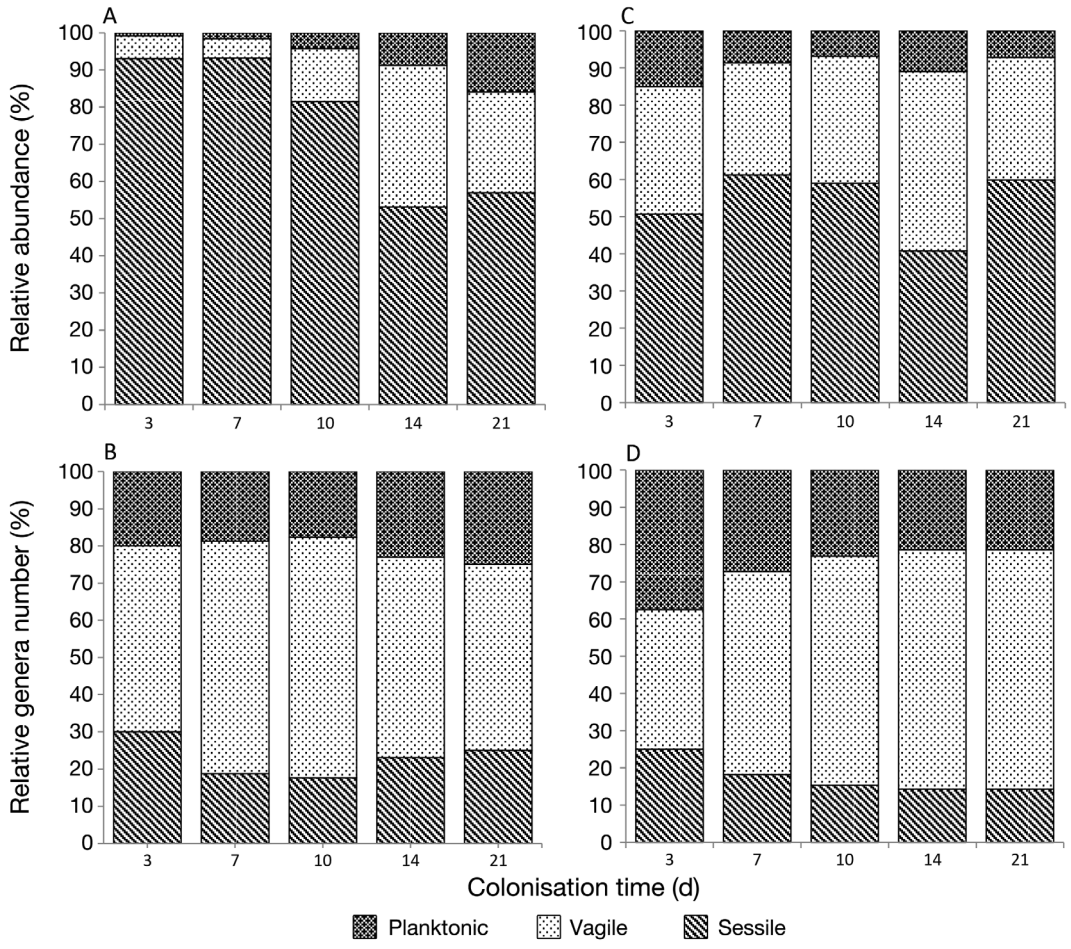

Fig. 4. Chronological variation of (A,C) relative abundance and (B,D) genera number of ciliate functional groups recorded during summer (left column) and winter (right column) deployments

ogy 2013). This event had a considerable impact on the abundance and/or occurrence of many established genera. Subsequently, variability of the ciliate assemblages was distinctly higher during summer samplings (Fig. 2).

Between Days 10 and 14, total abundance dropped from 366.1 ± 50.1 to 55.2 ± 12.2 ciliates cm^{-2} (Fig. 2). Fig. 5A highlights the changes in the relative abundances of the previously established ciliate orders following the disturbance. The relative abundance of Sessilida in particular was heavily reduced in response to the disturbance. In contrast, vagile and planktonic ciliates from the orders Euplotida, Pleurostomatida and Philasterida were largely unaffected, and subsequently increased in relative abundance (Fig. 5A). Despite the considerable reduction in abundance, the diversity of the assemblage increased following the disturbance (Fig. 2).

MDS ordinations of the assemblages plotted from Bray-Curtis similarities on square root-transformed genera abundances show that post disturbance, the ciliate assemblages reverted back to a composition similar to that recorded on Day 7 (Fig. 7B). Ordinations comparing aspects also highlight the impact of the disturbance. The analysis shows that the similarities of ciliate assemblages between aspects remained high throughout the winter and summer deployments, with the exception of the south aspect following the disturbance during the summer deployment (Fig. 7B; D14/South).

DISCUSSION

The colonisation sequences observed here can be understood in terms of ecological niches of the ciliates. The peritrichs *Zoothamnium* and *Vorticella* were among the first ciliates to colonise the substrates, and rapidly increased in abundance over the deployment period. The hypotrichs *Aspidisca* and *Euplotes*, specifically adapted for life on substrates, were also ubiquitous in all samples. Small planktonic ciliates

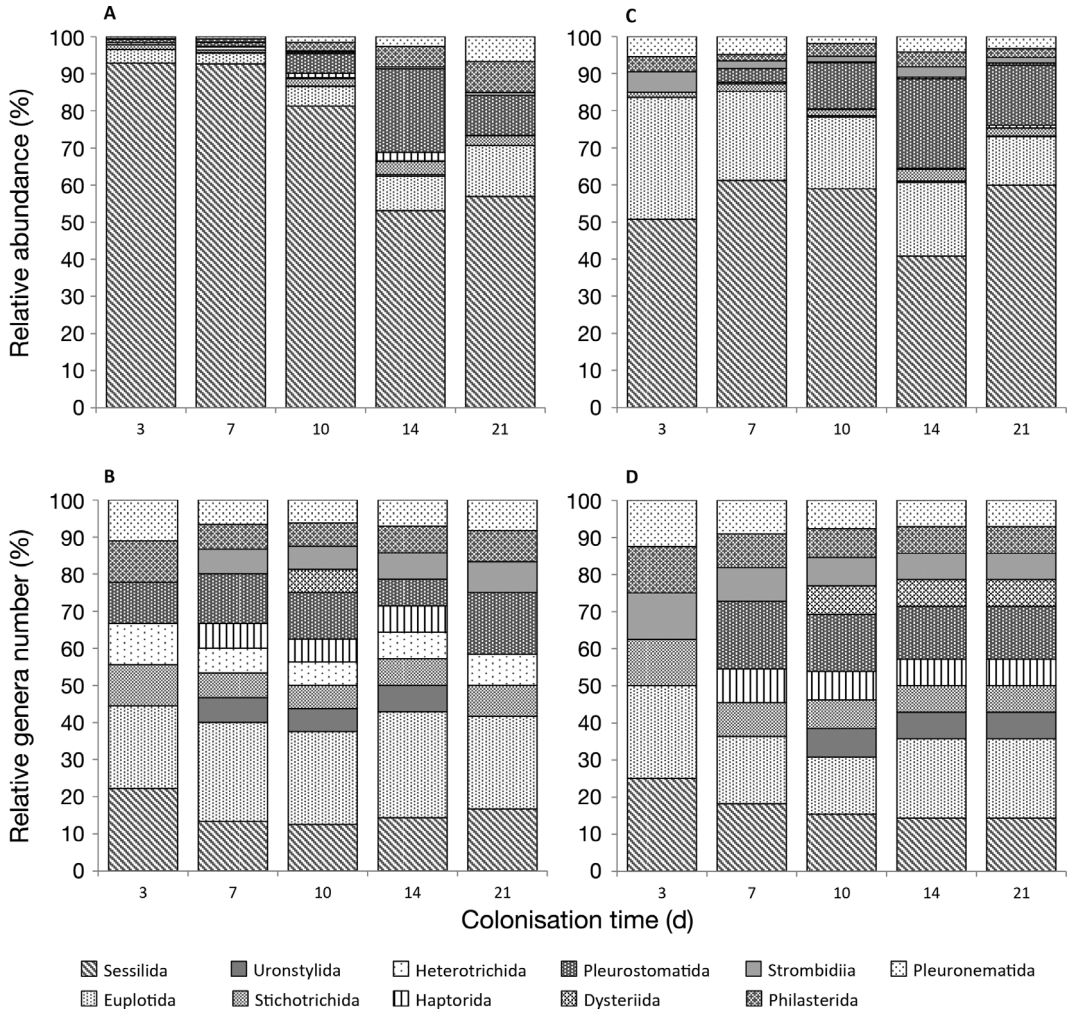

Fig. 5. Chronological variation of (A,C) relative abundance and (B,D) genera number of ciliate assemblages at the level of order recorded during summer (left column) and winter (right column) deployments

Uronema and *Strombidium*, likely swimming in the surroundings of the established biofilm exploiting transitory patches of bacteria (Fenchel 1980), were more unpredictable in their colonisation dynamics but were generally present throughout the deployments. The remaining colonising genera were established in the assemblages for short periods and thus may be considered transient or opportunistic in nature.

When comparing the taxonomic composition observed here with previous works on ciliates colonising artificial substrates in marine environments, our results do not reveal large differences in abundance, diversity or structure of the ciliate assemblages. Despite these works being based on examinations over extended periods of time with substrates located at various depths and sites, many genera were common to all observed assemblages (e.g. *Zoothamnium*, *Euplotes*, *Aspidisca*, *Amphileptus*, *Holosticha*, and *Litonotus*) (Coppellotti & Matarazzo 2000, Gong et al. 2005, Xu et al. 2009). This suggests that habitats providing similar niches will hold comparable ciliate assemblages across a wide range of climates. This

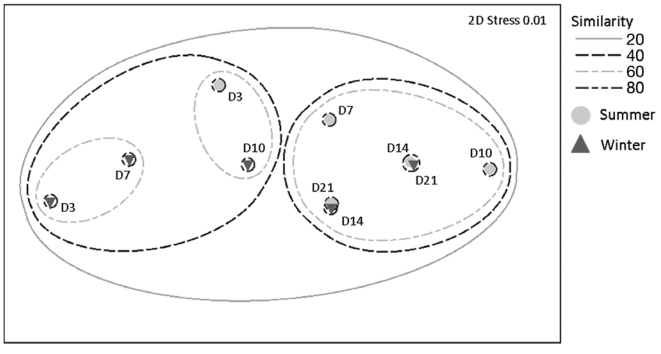

Fig. 6. Multidimensional scaling (MDS) ordination of the 5 samples taken during summer and winter deployments based on Bray-Curtis similarity matrix of square root-transformed genera abundances

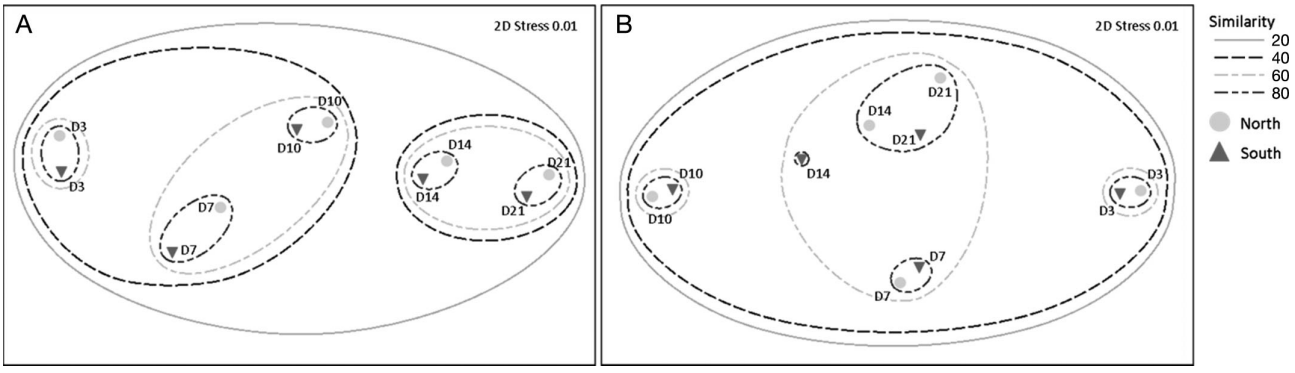

Fig. 7. Multidimensional scaling (MDS) ordination of north and south aspects based on Bray-Curtis similarity matrix, computed from square root-transformed ciliate genera abundances recorded during (A) winter and (B) summer deployments

seems particularly true for the dominance of sessile peritrich and vagile hypotrich ciliates within marine biofilm habitats.

Previous research has shown that ciliate assemblages will eventually reach a state of equilibrium, where few new species will colonise the substrate and abundances of established ciliate groups remain more or less static (Coppellotti & Matarazzo 2000, Gong et al. 2005). The time required for ciliate colonization to reach equilibrium is heavily dependent on environmental factors, but typically takes between 1 and 4 wk (Gong et al. 2005, Xu et al. 2009). In the present study, although the Petri dish slides were exposed for 21 d, the ciliate assemblages did not reach this state of equilibrium. This may have been due to low water temperatures during winter and a significant environmental disturbance during summer likely delaying the onset of equilibrium.

While equilibrium was not reached, the ciliate assemblages showed clear temporal succession throughout colonisation during summer and winter deployments. Colonisation rates were affected by differences between seasons, with the development of ciliate assemblages being much quicker during the summer sampling. While the cumulative genera represented during both seasons showed little variation, the abundances of individual genera varied significantly. This was particularly evident with sessile ciliates *Zoothamnium* and *Vorticella* during the summer sampling, which dominated the assemblage in terms of abundance, accounting for up to 93% of the relative abundance early in substrate colonisation (Fig. 4A). During the winter sampling, when the lowest ciliate concentrations were recorded, the contribution of the different ciliate genera was more evenly distributed. Consequently, the diversity of ciliate genera was higher during winter despite there being fewer genera present overall (Fig. 2). It must be noted that while our brief deployments revealed successional patterns with a fine scale of temporal resolution, caution must be taken at the broader scale in attributing differences between the summer and winter deployment solely to seasonality, as other vagaries of environmental factors and successional stochasticity could have influenced the results.

There is considerable evidence that environmental disturbance is a major source of temporal and spatial heterogeneity in the structure and dynamics of natural biofilm assemblages (Underwood 1998, Sanz-Lázaro et al. 2011, Jones et al. 2013). Typically irregular events, the impacts of disturbances can vary from negligible to extreme depending on the intensity of the disturbance and the vulnerability of the different taxa within the assemblage (Sousa 1984, Turner et al. 1998, Jenkins & Martins 2010). The environmental disturbance experienced during the summer deployment in this study distinctly influenced the colonisation process and the structural and functional parameters of the previously established ciliate assemblages.

Heavy rainfall and the subsequent increase of turbidity, the result of runoff into the nearby Yarra River, had a substantial impact on the abundance of many established ciliate genera. Multivariate analysis highlighted the variations in assemblage structure over time, and revealed how the environmental disturbance impacted the assemblage during summer. While both summer and winter assemblages showed a clear trend of growth over time, following the disturbance during summer (Days 14 to 21) the ciliate assemblages reverted back to a composition similar to that recorded on Day 7 (Fig. 7B).

The abundance of the sessile peritrichs in particular was heavily reduced in the samplings following this event, whereas many of the vagile and planktonic species were more tolerant, and after an initial

decline in abundance showed sustained growth despite the influx of debris. This is likely due to the sessile taxa being physically beaten or smothered by the sudden influx of debris, whereas the motile vagile and planktonic taxa had the advantage of being able to avoid the debris and adjust to the new substrate condition. Ciliate species from the orders Euplotida and Pleurostomatida in particular seemed to benefit, taking advantage of the new substrate condition and exerting a greater presence within the assemblage following the disturbance (Fig. 5C).

Interestingly, while the abundances of many established ciliate genera were substantially reduced, the diversity of the assemblage increased following the disturbance. Similar patterns have been observed in freshwater ciliate assemblage studies (e.g. Cairns et al. 1971, Eddison & Ollason 1978). In stable systems free from natural disturbances, the relative abundance of a small number of genera rise to dominate the ciliate assemblages. Conversely, in systems subject to frequent disturbances the relative abundances of the genera present are comparatively lower than those in stable systems, and the domination of assemblages by a small number of ciliate genera does not occur to the same degree, resulting in higher assemblage diversity (Cairns et al. 1971, Eddison & Ollason 1978, Taylor 1983). The changes in ciliate diversity observed here could be compared with the intermediate disturbance hypothesis, as proposed by Connell (1978), which submits that species diversity is maximised by moderately frequent environmental disturbances which keep the assemblage in a non-equilibrium, non-climactic state.

Therefore, while certainly dependent on the severity of the disturbance, it seems that the diversity of an established assemblage can be maintained or even increase post-disturbance, despite the abundances being subject to great fluctuation. This result also highlights that disturbances do not necessarily have a uniform effect on all ciliate taxa. In this case, the disturbance selectively reduced the abundance of the dominant sessile species, which were out-competing the vagile and planktonic species through the course of succession. Various forms of natural disturbance may result in a proportionally greater loss of certain taxa (Turner et al. 1998, Sousa 2001). Here, we observed that an increase in debris selectively impacted the sessile species. However, had the substrate been exposed to a hydrodynamic disturbance, such as wave action or current flow, the vagile and planktonic species not attached to the surface may have been at greater risk of removal from the assemblage.

Had the deployment period of this study extended further it is likely that new and/or the remaining peritrichs would simply colonise on top of this new layer of debris. Indeed, the subsequent assemblages of peritrichs might even benefit from the increase in ambient debris due to the increase in suspended bacteria present in the water column on which they feed (Arndt et al. 2003). It has been suggested that sessile filter feeders contribute to a tight coupling between the water column and biofilm by channelling the organic carbon from the water column (Augspurger et al. 2008). Hence, the recovery of the peritrichs may be important for the eventual recuperation of the microbial community as a whole.

The recruitment and distribution of sessile marine invertebrates cannot be understood without attempting to understand the ecology of the microbes which condition the immersed substrates for subsequent recruitment. Studies that attempt to understand the influence of biofilms on the recruitment and distribution of sessile marine invertebrates should take into account that ciliate assemblages can vary in complex ways on relatively short time scales. How individual ciliate species influence succession in the surrounding microbial community, and the degree to which these influences interact, could all contribute to the eventual recruitment of sessile invertebrates. Further study of protozoan assemblages across multiple temporal and spatial scales will build a better understanding of these mechanisms within marine fouling assemblages.

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LITERATURE CITED

Arndt H, Schmidt-Denter K, Auer B, Weitere M (2003) Protozoans and biofilms. In: Krumbein WE, Patterson DM, Zavarzin GA (eds) Fossil and recent biofilms: a natural history of life on earth. Kluwer Academic Press, Norwell, MA, p 161–180

Augspurger C, Gleixner G, Kramer C, Kusel K (2008) Tracking carbon flow in a 2-week-old and 6-week-old stream biofilm food web. Limnol Oceanogr 53:642–650

Ayo B, Santamaría E, Latatu A, Artolozaga I, Azúa I, Iriberri J (2001) Grazing rates of diverse morphotypes of bacterivorous ciliates feeding on four allochthonous bacteria. Lett Appl Microbiol 33:455–460

Bernard C, Rassoulzadegan F (1990) Bacteria or microflagellates as a major food source for marine ciliates: possible implications for the microzooplankton. Mar Ecol Prog Ser 64:147–155

Berninger UG, Finlay BJ, Kuuppo-Leinikki P (1991) Protozoan control of bacterial abundances in freshwater. Limnol Oceanogr 36:139–147

Bureau of Meteorology (2013) February 2013 rainfall. (Online) Available at http://www.bom.gov.au/jsp/ncc/cdio/weatherData/av?p_display_type=dataDGraph&p_stn_num=086232&p_nccObsCode=136&p_month=02&p_startYear=2013. Accessed 18 April 2014

Carey PG (1992) Marine interstitial ciliates: an illustrated key. Chapman & Hall, London

Cairns J Jr, Dickson KL, Yonge WH Jr (1971) The consequences of nonselective periodic removal of portions of freshwater protozoa communities. Trans Am Microsc Soc 90:71–80

Coppellotti O, Matarazzo P (2000) Ciliate colonisation of artificial substrates in the Lagoon of Venice. J Mar Biol Assoc UK 80:419–427

Connell JH (1978) Diversity in tropical rain forests and coral reefs. Science 199:1302–1310

Corno G, Jürgens K (2006) Direct and indirect effects of protist predation on population size structure of a bacterial strain with high phenotypic plasticity. Appl Environ Microbiol 72:78–86

Dobretsov S (2013) Mini-review: inhibition of biofouling by marine microorganisms. Biofouling 29:423–441

Eddison JC, Ollason JG (1978) Diversity in constant and fluctuating environments. Nature 275:309–310

Fenchel T (1980) Suspension feeding in ciliated protozoa: functional response and particle size selection. Microb Ecol 6:1–11

Franco C, Esteban GF, Tellez C (1998) Colonization and succession of ciliated protozoa associated with submerged leaves in a river. Limnologica 28:275–283

Gong J, Song W, Warren A (2005) Periphytic ciliates colonization: annual cycle and responses to environmental conditions. Aquat Microb Ecol 39:159–170

Gonzalez JM, Sherr EB, Sherr BF (1990) Size-selective grazing on bacteria by natural assemblages of estuarine flagellates and ciliates. Appl Environ Microbiol 56:583–589

Hadfield MG, Paul VJ (2001) Natural chemical cues for settlement and metamorphosis of marine-invertebrate larvae. In: McClintock JB, Baker BJ (eds) Marine chemical ecology. CRC Press, Boca Raton, FL, p 431–461

Henschel JR, Cook PA (1990) The development of a marine fouling community in relation to the primary film of microorganisms. Biofouling 2:1–11

Huws SA, McBain AJ, Gilbert P (2005) Protozoan grazing and its impact upon population dynamics in biofilm communities. J Appl Microbiol 98:238–244

Jackson SM, Jones EBG (1991) Interactions within biofilms: the disruption of biofilm structure by protozoa. Kiel Meeresforsch, Sonderh 8:264–268

Jenkins SR, Martins GM (2010) Succession on hard substrata. In: Dürr S, Thomason JC (eds) Biofouling. Wiley-Blackwell, Oxford, p 60–69

Jones AC, Liao TSV, Najar FZ, Roe BA, Hambright KD, Caron DA (2013) Seasonality and disturbance: annual pattern and response of the bacterial and microbial eukaryotic assemblages in a freshwater ecosystem. Environ Microbiol 15:2557–2572

Kiørboe T, Tang K, Grossart HP, Ploug H (2003) Dynamics of microbial communities on marine snow aggregates: colonization, growth, detachment and grazing mortality of attached bacteria. Appl Environ Microbiol 69:3036–3047

Lawrence JR, Snyder RA (1998) Feeding behaviour and grazing impacts of a *Euplotes* sp. on attached bacteria. Can J Microbiol 44:623–629

Lee JJ, Leedale GF, Bradbury P (2000) An illustrated guide to the Protozoa, 2nd edn. Society of Protozoologists, Allen Press, Lawrence, KS

Lynn DH (2008) The ciliated protozoa: characterization, classification and guide to the literature, 3rd edn. Springer, Berlin

Norf H, Arndt H, Weitere M (2009) Responses of biofilm dwelling ciliate communities to planktonic and benthic resource enrichment. Microb Ecol 57:687–700

Pielou EC (1966) The measurement of diversity in different types of biological collections. J Theor Biol 13:131–144

Qian PY, Lau SCK, Dahms HU, Dobrestov S, Harder T (2007) Marine biofilms as mediators of colonization by marine macroorganisms: implications for antifouling and aquaculture. Mar Biotechnol 9:399–410

Risse-Buhl U, Scherwass A, Schlüssel A, Arndt H, Kröwer S, Küsel K (2009) Detachment and motility of surface associated ciliates at increased flow velocities. Aquat Microb Ecol 55:209–218

Rittschof D, Forward RB, Cannon G, Weich JM and others (1998) Cues and context: larval responses to physical and chemical cues. Biofouling 12:31–44

Sanz-Lázaro C, Navarrete-Mier F, Arnaldo M (2011) Biofilm responses to marine fish farm wastes. Environ Pollut 159: 825–832

Sherr EB, Sherr BF (1994) Bacterivory and herbivory: key roles of phagotrophic protists in pelagic food webs. Microb Ecol 28:223–235

Shimeta J, Cutajar J, Watson MG, Vlamis T (2012) Influences of biofilm-associated ciliates on the settlement of marine invertebrate larvae. Mar Ecol Prog Ser 449:1–12

Skibbe O (1994) An improved quantitative protargol stain for ciliates and other planktonic protists. Arch Hydrobiol 130:339–347

Sousa WP (1984) The role of disturbance in natural communities. Annu Rev Ecol Syst 15:353–391

Sousa WP (2001) Natural disturbance and the dynamics of marine benthic communities. In: Bertness M, Gaines S, Hay M (eds) Marine community ecology. Sinauer Associates, New York, NY, p 85–130

Stoodley P, Sauer K, Davies DG, Costerton JW (2002) Biofilms as complex differentiated communities. Annu Rev Microbiol 56:187–209

Taylor WD (1983) A comparative study of the sessile, filter-feeding ciliates of several small streams. Hydrobiologia 98:125–133

Turner MG, Baker WL, Peterson CJ, Peet RK (1998) Factors influencing succession: lessons from large, infrequent natural disturbances. Ecosystems 1:511–523

Underwood AJ (1998) Grazing and disturbance: an experimental analysis of patchiness in recovery from a severe storm by the intertidal alga *Hormosira banksii* on rocky shores in New South Wales. J Exp Mar Biol Ecol 231: 291–306

Wieczorek SK, Todd CD (1998) Biofilm cues and larval settlement. Biofouling 12:81–118

Xu H, Min GS, Choi JK, Kim SJ, Jung JH, Lim BJ (2009) Periphytic ciliate colonization of an artificial substrate in Korean coastal waters. Protistology 6:55–65

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