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Colonization of periphytic ciliated protozoa on an artificial substrate in mariculture waters with notes on responses to environmental factors

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Abstract

Colonization of periphytic ciliates and their usefulness for monitoring water quality were evaluated using the glass slide method in two enclosed mariculture ponds used to culture crab larva and in a natural seawater reservoir. The results revealed that (1) the ciliate species composition and colonizing process differed between the culturing ponds and the natural reservoir and (2) the dominant ciliate species showed a greater distribution in terms of both the abundance and the occurrence frequency. This study also demonstrated that structural parameters were strongly related to water conditions. For example, the abundance was positively associated with NO_3^-–N and soluble reactive phosphate ($P < 0.05$), whereas the species number, species diversity and evenness were negatively correlated with nutrients (e.g. NO_3^-–N and NH_3–N) and temperature ($P < 0.05$). These findings support the evidence that periphytic ciliates are useful bioindicators of water quality in enclosed mariculture ecosystems.

Keywords: Periphytic ciliates; Colonization process; Environmental factors

1. Introduction

Ciliated protozoa are important components of the aquatic ecosystem and play a crucial role in the functioning of microbial food webs by mediating the flow of substances and energy [1,2]. Intensive aquaculture ponds possess unique ecological features that are designed to facilitate the growth and survival of the target species. However, it is necessary to gain a better understanding of the microbial communities and their relationships to the changes of environmental factors in aquaculture ecosystems. In previous studies, it has been confirmed that the structural and functional parameters of ciliate communities can be used to assess the water quality of natural and mariculture waters. During colonization of substrates, ciliate communities generally represent a temporal succession. However, little information is available regarding ciliate colonization in intensive aquaculture systems, and no comparative studies have been conducted to compare the colonization process of periphytic ciliates in natural systems to that in intensive mariculture systems.

In the present study, the species composition, community structure, and the significance as indicators of water quality were evaluated in two enclosed crab-larva-culturing mariculture ponds and a natural seawater reservoir using the glass slide method. The main aims were to reveal (1) the colonization process of marine periphytic ciliates; (2) their responses to the changes of environmental factors in intensive mariculture ponds. The information was to determine that the periphytic ciliates can be used as bioindicators of water quality in enclosed mariculture ecosystems.

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2. Materials and methods

2.1. Sampling sites and conditions

This study was carried out during a 20-day period (April 19 to May 9, 2003) in Weihai (Fig. 1; 37°30'N; 122°6'E), north China. Two identical crab larva culture ponds (CP1 and CP2) and a seawater reservoir (RP) were used for this study. The mariculture ponds were cuboid in shape and had a size of $6 \times 4 \times 3$ m, while the seawater reservoir had a cylindrical configuration with a diameter of 10 m and a depth of 5 m.

2.2. Sampling

Glass slides (2.6×7.6 cm) were used as artificial substrates for the collection of periphytic ciliates from each pond [3]. During the study period, samples were collected every 2 days. The identification and enumeration of ciliates were completed within 4 h.

Table 1
Principal component analyses (varimax rotation) showing the relationships among physical–chemical parameters.

	Component	
	1	2
NO_3^-–N	+0.870	+0.066
SRP	+0.848	−0.128
NH_3–N	+0.832	−0.083
NO_2^-–N	+0.795	+0.286
Temperature	+0.881	+0.271
Salinity	+0.012	+0.867
pH	−0.831	+0.264
Bacterial count	+0.001	+0.664
Variance (%)	53.4	18.0
Cumulative (%)	53.4	71.4

2.3. Analyses of environmental factors

Temperature, salinity, and pH were measured *in situ* at a depth of 1 m using appropriate sensors (WTW). In addition, samples were analyzed for NO_3^-–N, NO_2^-–N, NH_3–N, and soluble reactive phosphate (SRP) following the standard methods. Finally, bacterial counts were conducted using 10 ml water samples [4].

2.4. Identification and enumeration of ciliates

Ciliates were identified based on the characteristics of living individuals observed under a microscope [5,6].

2.5. Data analyses of samples

The species diversity (H'), evenness (J), and species richness (d) of samples were calculated using the following equations:

$$H' = -\sum_{i=1}^{s} Pi(Ln\ Pi);\ d = (S-1)/\ln N;\ J = H'/\ln S$$

where H' is the observed diversity index; Pi is the proportion of the total count arising from the ith species; S is the total number of species; N is the total number of individuals [7].

SPSS (version 11.5) was used for all statistical analyses. In addition, all data were transformed into logarithmic

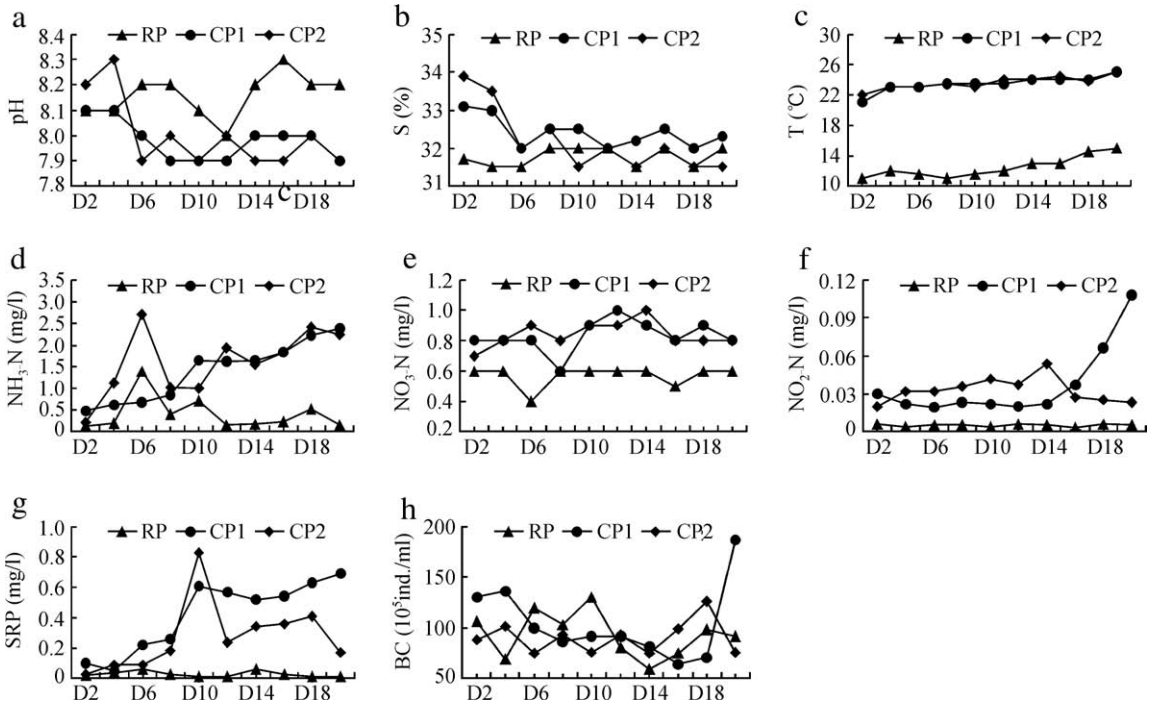

Fig. 1. Temporal variations in environmental factors of water samples collected from three ponds during the colonization process. D2–D20: days of sampling. (a) pH, (b) temperature, (c) salinity, (d) bacteria count, (e) NH_3–N, (f) NO_2–N, (g) NO_3–N, and (h) SRP.

values prior to analysis to meet the assumptions of normality. Principal component analyses (PCA) were performed on environmental variables in all three ponds. For the cluster analyses of the ciliate community structures, the Czekanowski similarity coefficient matrix was calculated from root-transformed species-abundance data. The correlations between structural parameters and environmental variables in each pond were determined by linear regression.

3. Results

3.1. Environmental factors

With the exception of temperature, the environmental factors varied greatly during the sampling period (Fig. 1). In addition, the chemical factors, NO_2^-–N, NO_3^-–N, NH_3–N, and SRP tended to increase with time in all three

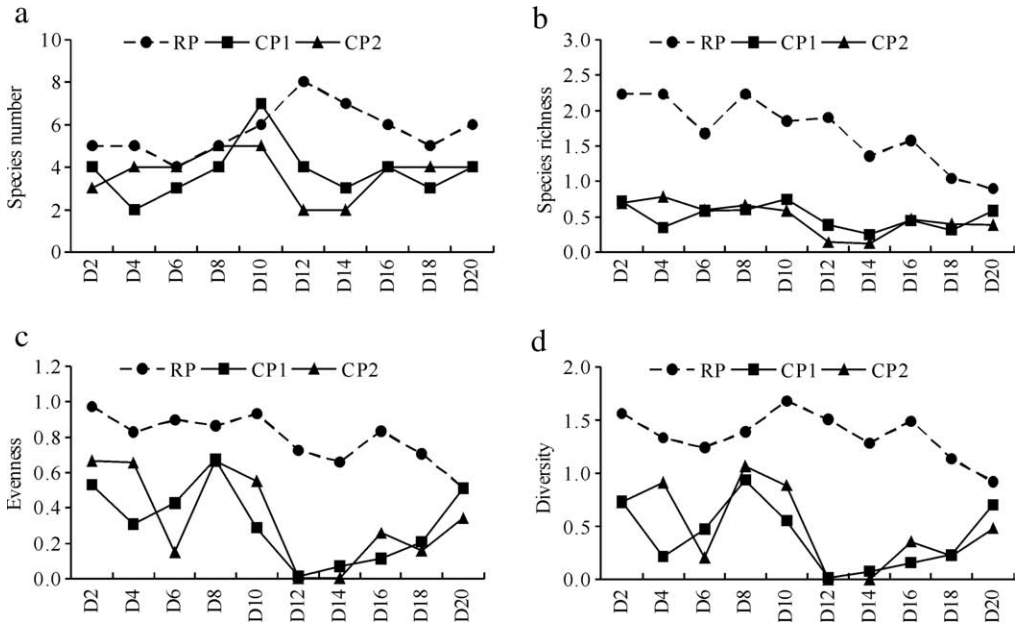

Fig. 2. Temporal variations in species number (a), species richness (b), evenness (c), and species diversity (d) in three ponds throughout the experimental period. D2–D20: days of the sampling time.

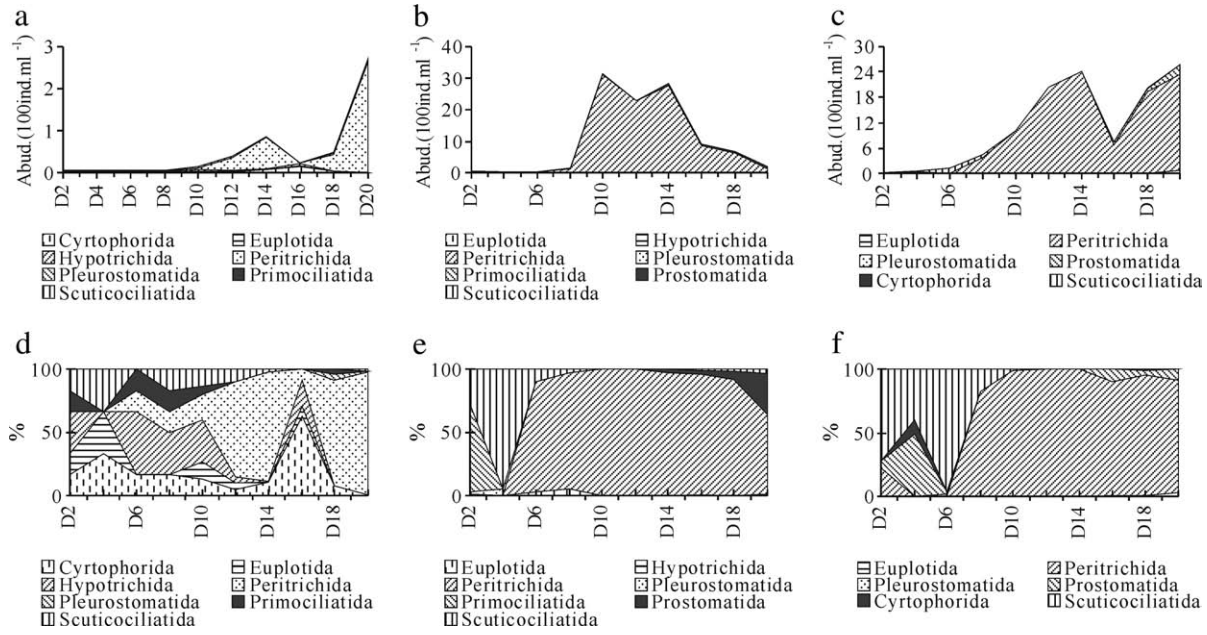

Fig. 3. Temporal variations in the abundance (a)–(c) and contribution (d)–(f) of periphytic ciliates in each order recorded throughout the experimental period for three ponds. D2–D20: days of the sampling time.

ponds. Furthermore, the temperature and nutrient concentrations were higher in the culture ponds than in the natural pond, whereas the pH was higher in the natural pond.

The results of PCA are shown in Table 1. Two factors explaining 71.4% of the environmental variability were identified. Chemical factors (NH_3–N, NO_3^-–N, NO_2^-–N, and SRP) and temperature were grouped in the first factor, which accounted for 53.4% of the total variance. In addition, temperature was positively associated with each of the chemical factors and both factors were negatively correlated with pH. The second factor, which explained an additional 18.0% of the variability, was weight and the positive relationship between salinity and bacterial concentrations.

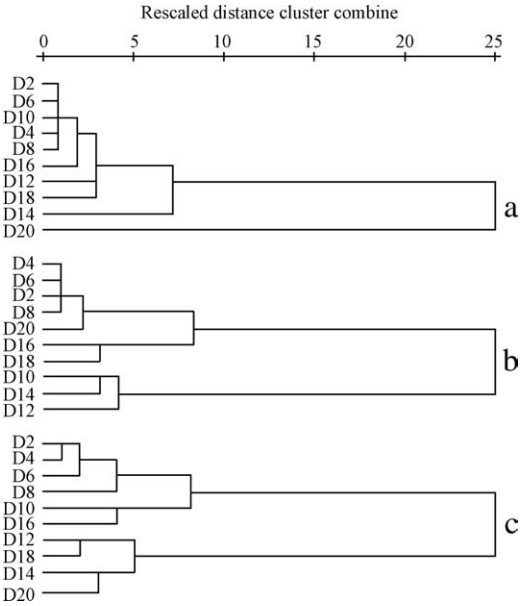

Fig. 4. Dendrogram of sequential versions of the structure of the colonizing community. (a) Pond RP, (b) pond CP1, and (c) pond CP2; D2–D20: days of sampling.

3.2. Structural parameters and similarity of the ciliate communities

Throughout the study period, the curves of community structural parameters (species number, evenness, richness, and diversity) presented wave-shaped oscillations around a mean value. In addition, the curves describing the community structures generally increased with time in ponds CP1 and CP2, while they decreased in pond RP (Fig. 2(a)–(d)). Similarly, the community structural parameters were maintained at a high level, with a lower amplitude and longer period of oscillations in pond RP than in ponds CP1 and CP2.

The ciliate abundances presented two peaks in all three ponds (Fig. 3(a)–(c)). In pond RP and CP2, two peaks appeared on days 14 and 20, respectively, whereas peaks appeared on days 10 and 14 in pond CP1. Peritrichs were the primary contributors to the peaks in all ponds (Fig. 3(d)–(f)). However, the distribution of the major taxonomic groups was more equal in pond RP than that in ponds CP1 or CP2 (Fig. 3(d)–(f)).

Cluster analyses resulted in the ciliate communities falling into two groups for each pond (Fig. 4(a)–(c)). In pond CP1, three samples (day 10, 12 and 14) converged into a single group, while all remaining communities comprised another group (Fig. 4(b)). In pond CP2, one group was comprised of three samples (day 14, 18 and 22), while all remaining communities comprised another group (Fig. 4(c)). However, only communities detected on day 20 appeared in a separate group in pond RP, which indicates that the ciliate communities were more homogeneous in this pond during the study period (Fig. 4(a)).

3.3. Abundance of the dominant species

Dominant species were defined as species with an abundance that exceeded 10% of the total population at some point during the sampling period. There were 7, 7, and 9 dominant species in ponds RP, CP1, and CP2, respectively

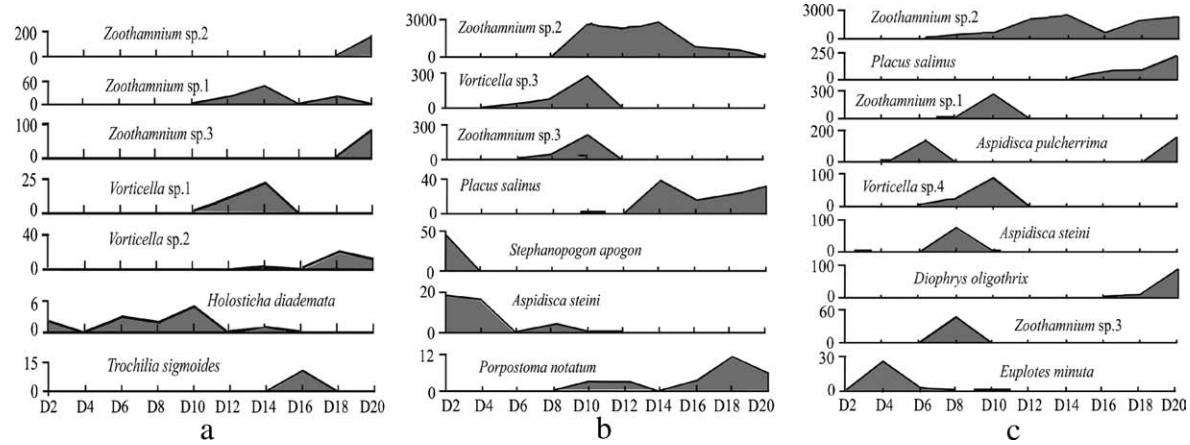

Fig. 5. Temporal variations in the abundance (ind. cm^{-2}) of the dominant periphytic ciliate species in three ponds. See Fig. 4 for abbreviations.

Table 2
Results of the multiple linear regression analyses (step-wise). Correlations between the structural parameters of ciliate communities and environmental factors.

Structural parameters	Environmental factors	R^2	t	P
Species diversity	Temperature	−0.500	−3.020	0.005
	NO_3^-–N	−0.418	−2.523	0.018
Evenness	NO_3^-–N	−0.663	−5.626	0.000
	NH_3–N	−0.295	−2.499	0.019
Abundance	NO_3^-–N	0.513	3.368	0.002
	SRP	0.370	2.429	0.022
Species number	Temperature	−0.610	−4.073	0.000
Species richness	Temperature	−0.933	−13.734	0.000

R^2: standardized regression coefficients.

(Fig. 5(a)–(c)). The maximum abundances observed in pond RP were higher than those observed in ponds CP1 and CP2. Although some ciliate species, such as *Zoothamnium* spp. and *Vorticella* spp., occurred in all three ponds, they occurred earlier in the culture ponds than in pond RP. The species that were found at the beginning of the colonizing process were *Holosticha diademata* in pond RP, *Stephanopogon apogon* and *Aspidisca steini* in pond CP1, and *Euplotes minuta* in pond CP2.

3.4. Analyses of the correlation of biotic data with environmental factors

The results obtained by linear regression indicated that, with the exception of abundance, the structural parameters of ciliate communities were negatively correlated with some environmental factors (Table 2). Specifically, abundance was positively correlated with NO_3^-–N ($P < 0.05$), and SRP ($P < 0.05$). However, species diversity was negatively correlated with temperature and NO_3^-–N ($P < 0.05$). In addition, evenness was negatively associated with both NO_3^-–N and NH_3–N. Finally, species number and richness were negatively correlated with temperature ($P < 0.05$).

4. Discussion and conclusions

Of all artificial substrates, polyurethane foam units (PFU) are the most commonly used for bioassessments; however, the use of PFU is not as efficient in marine waters as in freshwater due to tidal currents and circulation [8]. Recently, the bottled PFU (BPFU) system has been used in marine systems because it effectively protects substrates from tidal conditions and is suitable for studies evaluating the dynamics of community structures in different sampling sites [3,8].

Glass slides have proven to be a robust, inexpensive, and reliable method of collecting periphytic ciliates. In addition, biomonitoring using periphytic ciliates is widely accepted and has many advantages, such as their availability, simple identification, and enumeration, as well as their suitability for use in studies designed to evaluate the dynamics of community structures over long time periods or with large numbers of sampling sites [3]. Previous investigations indicated that a seven-day period would be sufficient to evaluate colonizing marine periphytic ciliates [9]. Therefore, the information collected in this 20-day study should be sufficient to reflect the colonization process in our study ponds. Furthermore, this study documented different characteristics associated with periphytic ciliate colonization among three ponds. This finding was agreed with Burkovskii and Mazei, i.e. ciliate colonization has unique features in different communities [10,11].

To date, it has been demonstrated that populations of ciliates in the aquatic systems with lower eutrophic levels have higher species numbers, diversity, and evenness. In addition, ciliate populations usually have greater diversity and richness in ecosystems with high water quality. Conversely, systems with high-stress environmental conditions typically have reduced species richness and an increased abundance of more tolerant forms of ciliates [12,13].

In the present study, cluster analyses based on the Czekanowski similarity from root-transformed species-abundance data revealed that ciliate communities had a high spatiotemporal heterogeneity. Furthermore, the results suggested that the ciliates were distributed more homogeneously in the culture ponds and that their temporal succession in the culture ponds differed from their succession in the natural pond. Similarly, the distribution of the dominant ciliates differed considerably in both abundance and occurrence between the culture ponds and the natural waters. Moreover, the spatiotemporal pattern of the ciliate community also reflected changes in the structural parameters [10]. For example, the species diversity and evenness indices were lower in the culture ponds than in the natural pond, while the abundance was higher in the culture ponds. These findings indicate that the succession of ciliate community structures responds to the changes in environmental parameters, which indicates that the structural parameters of the colonization process of ciliates could be used to monitor environmental parameters.

The results of the present study also indicated that environmental factors in the culturing ponds were considerably different from those in the natural pond. Specifically, the results of this study demonstrated that the enclosed mariculture waters had higher eutrophic levels than the natural pond. Among the environmental factors, temperature and nutrients were the first principal component, and most of these factors were significantly related to the structural parameters of the ciliate communities. Therefore, these findings suggest that both the succession and community structure of ciliates can be used to monitor the water quality of enclosed mariculture waters [14].

In conclusion, periphytic ciliates showed a clear temporal succession of communities during colonization in both the culturing ponds and the natural marine reservoir; however, different community structures and colonization processes occurred in these systems. Specifically, the species number, diversity, richness, and evenness were generally

low under eutrophic or hypertrophic conditions, while the abundance was generally high under these conditions. These findings confirm that the ciliate colonization process may represent distinctive structural parameters that respond to environmental factors and that ciliated protozoa are useful bioindicators of water quality in intensive mariculture systems.

Acknowledgements

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