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Succession of aquatic microbial communities as a result of the water quality variations in continuous water

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Abstract: The changes of structural and functional parameters of aquatic microbial communities in continuous water on campus of Tsinghua University, China are investigated by polyurethane foam unit(PFU) method. The measured compositions of the communities include alga, protozoa, and some metazoa(such as rotifers). The measured indicators of water quality include water temperature, pH value, dissolved oxygen(DO), potassium permanganate index(COD_{Mn}), total nitrogen(TN), total phosphorus(TP) and chlorophyll-a(Chla). The trophic level, expressed by the trophic level indices(TLIc), is assessed with analytic hierarchy process and principal component analysis (AHP-PCA) method. The changing trends of the structural and functional parameters of aquatic microbial communities, such as Margalef index of diversity(D), Shannon-weaver index of diversity(H), Heterotropy index (H), number of species when the colonization gets equilibrium(S_{eq}), colonizing speed constant(G) and time spent when 90 percent of S_{eq} colonized in PFU($T_{90\%}$), are also analyzed. The experimental results showed the succession of aquatic microbial communities along the water flow is consistent with the water quality changes, so the parameters of microbial community can reflect the changes of water quality from the ecological view.

Keywords: succession; aquatic microbial community; polyurethane foam unit (PFU) method; water quality; biodiversity; biomonitoring

Introduction

A recent worldwide development is the introduction of in-stream biological effects or response monitoring in water resources management. This type of response monitoring, commonly referred to as biomonitoring, is increasingly being recognized as an important component in the overall monitoring and assessment of water resources.

Biomonitoring uses biological variables to survey the environment and is a complement to chemical monitoring. Assessment of ecosystem responses to environmental stresses is a new branch of biomonitoring (Gerhardt, 1999). There are many methods to assess the ecosystem responses to environmental stresses, one of them is the polyurethane foam unit(PFU) method. This method was first brought forward by Cairns, J. Jr. (Cairns, 1969). By comparison with a wide variety of artificial substrates previously used in ecological studies in freshwater ecosystem, it was found that PFUs were suited for collecting complex aquatic microbial communities (Cairns, 1979; Pratt, 1985; Xu, 1998; 1999). PFUs were used to sample the aquatic microbiota communities. The microbiota communities, which are easier to be investigated, are important components of aquatic microbial communities. Microbiota was defined as the microorganisms in the aquatic ecosystems, which can be inspected by optical microscopy, such as alga, protozoa, and some metazoa (Shen, 1990). From an ecological viewpoint,

freshwater microbiota is an interesting group, forming virtually self-contained communities that exhibit many characteristics of structure and function of entire aquatic ecosystems (Cairns, 1980; Shen, 1990). Although often neglected in water resources studies, it has been recognized that changes in these communities may significantly affect other components of the aquatic food web, and thus may influence the distribution and abundance of both lower and higher organisms (Cairns, 1980; Carrick, 1992). Therefore, to a certain extent, the changes of ecological characteristics of microbiota communities can reflect the aquatic ecosystem responses to the changes of water quality.

The objective of this investigation was to investigate the succession of aquatic microbial communities along a continuous water before and after cascade aeration, by using the PFU method, in order to show how the aquatic ecosystems response to the stresses of water quality changes.

1 Materials and methods

1.1 Materials

Artificial substrates (PFUs) were used throughout the study to collect the aquatic microbial colonizers from the water. Although providing "unnatural" refuge from normal predation, PFU offer a very effective method for sampling a new habitat. The PFUs are in size of 75 mm \times 65 mm \times 50 mm, the pore diameter of which is about 100—150 micron.

1.2 Sampling sites and method

The investigation was conducted in a continuous water on Tsinghua Campus. The surface area of the water is about 13700 m². The length of the flow is about 1800 m. PFUs were anchored 30 cm under the water surface at six sampling sites(Fig.1). Sample site No.1 was set near the upstream of a 2 m high water fall, other sample sites were set along the water flow direction. After the water flow reached the sampling site No.6, it was pumped back to the water fall through a connecting pipe. Then the water flow formed a loop. The direction of water flow is shown in Fig. 1.

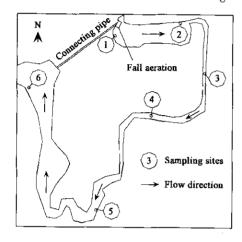


Fig. 1 Map of investigated water (showing sampling sites 1 to 6)

The PFUs were set in the water from 7 to 30 July, 2002 and were sampled on the day 1, 2, 3, 5, 8, 12, 15, 19, and 22 respectively after immersion. When sampling, two replicate PFUs were picked randomly from each sampling Simultaneously, oxygen concentration and water temperature were measured with an O2/temperature probe (YSI MODELS 54 ARC). The water flow velocities were measured with an tachometer. The collected PFUs were carefully placed into clean plastic bags and immediately taken to the laboratory for microscopic inspection. For each site and time, water and microorganisms absorbed in the two PFUs were squeezed into a 200 ml glass beaker, mixed and allowed to settle for about 10 min. Three drops of mixed material taken from the bottom of the settling beaker were examined for the microbiota species via microscopy within three hours. The river water was sampled at the same depth as PFUs with self-made water sampling equipment. The water samples were carried back to laboratory and stored in refrigeratory at 4°C before they were examined for water quality indices.

1.3 Analysis methods

1.3.1 Water quality indices

The investigated water quality indicators included temperature (T), dissolved oxygen (DO), pH, potassium permanganate index (COD_{Mn}), total nitrogen (TN), total phosphorus (TP) and chlorophyll-a (Chla). T and DO were measured using DO online monitor (YSI MODELS 54 ARC), pH values were determined using pH meter (Thermo Orion model 868). COD_{Mn} , TN, TP and Chla were determined

according to Standard Methods (American Public Health Association, 1992).

The degree of eutrophication was assessed by trophic level index (TLI) method using analytic hierarchy process and principal component analysis (AHP-PCA) (Jin, 1995). The principal assessing factor included Chla, TP, TN and COD_{Mn} . The trophic level index (TLI) was calculated applying Eq. (1).

$$TLI_i = 10(a_i + b_i \ln C_{ir}), \tag{1}$$

where TLI_j is the trophic level index of assessing factor j; C_{ji} is the concentration of assessing factor j; a_j , b_j are constants determined by geographic features of the water. The overall trophic level index (TLI_c) was calculated as Eq. (2).

$$TLI_{c} = \sum_{i=1}^{m} W_{i}TLI_{i}, \qquad (2)$$

where TLI_c is the overall trophic level index of the water; W_j is the weight coefficient of assessing factor j; TLI_j is the trophic level index of assessing factor j; m is the number of assessing factors. The values of a_j , b_j and W_j for each assessing factor are listed in Table 1, which suited for waters in Beijing area (Jin, 1995). Table 2 lists the standard for classifying trophic levels according to the TLI_c values (Jin, 1995).

Table 1 Values of a_i , b_i and W_i for each assessing factor

Assessing factor	a_i	b_i	\overline{W}_i
Chla	2.500	1.086	0.54
TΡ	9.436	1.624	0.28
TN	5.453	1.694	0.09
COD_{Mn}	0.109	2.661	0.09

Table 2 Classification of trophication types according to TLIc values

Trophication type	Oligotrophication	Mesotrophication	Eutrophication
TLI_{\circ}	< 30	< 50	> 50

1.3.2 Structural parameters of aquatic microbial communities

The main investigated structural parameters of aquatic microbial communities comprised biodiversity indices, evenness index(EI) and heterotropy index(HI).

The biodiversity of microbial communities at the six sampling sites were compared using the Margalef index of diversity (D) and Shannon-weaver index of diversity (H), which were calculated respectively as Eq. (3) and (4) (Huang, 2001).

$$D = \frac{S - 1}{\ln N},\tag{3}$$

where S is the number of species and N is the total number of individuals.

$$H = \sum_{i=1}^{s} (n_i/N) \log_2(n_i/N), \qquad (4)$$

where S is the number of species; n_i is the total number of specie i and N is the total number of individuals. It was

shown that community stability would essentially always rise with species diversity because of the statistical averaging of the fluctuations in species' abundances (Doak, 1998; Chen, 2001). Biodiversity indices could partially reflect the stability of the investigated community.

The Heterotrophy index(HI) was calculated as Eq.(5) (Shen, 1990; Huang, 2001).

$$HI = \frac{B}{Chla} = \frac{\frac{ATP}{2400}}{Chla},\tag{5}$$

where ATP is the concentration of Adenosine Triphosphate (ATP) in the sample($\mu g/L$); B is the biomass expressed by ATP concentration (mg/L); Chla is the concentration of chlorophyll-a (mg/L). HI reflects the ratio of hetertrophic microorganisms in total aquatic microbial community biomass.

1.3.3 Functional parameters of aquatic microbial communities

The colonization of aquatic microorganisms in PFUs can be described by MacArthur-Wilson island biogeographical colonization equilibrium model (MacArthur, 1963), as follows:

$$S_i = S_{eq} (1 - e^{-Gi}),$$
 (6)

where S_t is the number of species at time t; $S_{\rm eq}$ is the number of species when the colonization gets equilibrium; G is the colonization rate constant. $S_{\rm eq}$ and G are used as functional parameters of aquatic microbial community in biomonitoring(GB/T 12990-91). $S_{\rm eq}$ indicates the maximum number of species which can colonize in PFUs, while G represents the colonization rate of microorganisms. Besides, the time spent when 90 percent of $S_{\rm eq}$ colonized in PFU ($T_{90\%}$) is also used as a functional parameter of aquatic microbial community. $S_{\rm eq}$, G and $T_{90\%}$ are all investigated in this research.

2 Results and discussion

2.1 Water flow velocity and water quality

The water flow velocity at each sampling site changed due to the difference of their sectional area. The water flow velocity was the highest at No.5 sampling site and was about 0.06 m/s(Fig.2), but was below 0.01 m/s at No.2 and No.6 sampling site.

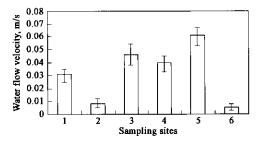


Fig. 2 Water flow velocity of each sampling site

During the period of the experiment, pH value and water temperature of each sampling site varied minutely, as

shown in Fig. 3 and Fig. 4, pH value was about 7.8 and water temperature was about 25 °C. However, the DO concentration at each sampling site (Fig. 5) decreased gradually along the river except No.2 sampling site, the DO concentration at No.1 sampling site was 1—2 mg/L higher than other sites as the result of cascade aeration, and along the river oxygen consumption was higher than oxygen dissolution, so the DO concentration decreased gradually. While at No.2 sampling site probably because the water flow velocity was lower there and the oxygen dissolution effect was smaller than No.3, so it had a lower DO concentration.

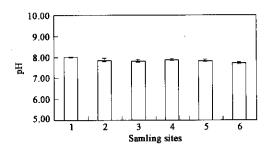


Fig. 3 pH values of each sampling site

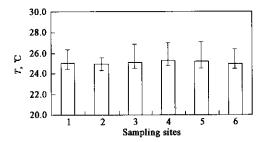


Fig. 4 Temperature of each sampling site

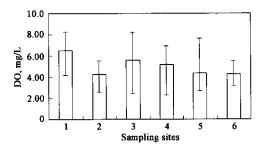


Fig.5 $\,$ DO of each sampling site

The potassium permanganate indices (COD_{Mn}), total nitrogen (TN), total phosphorus (TP) and chlorophyll-a (Chla) of each site are shown in Fig. 6, Fig. 7, Fig. 8 and Fig. 9 respectively. These water quality indicators showed the similar changing trends along the water from sampling site No.1 to No.6. From sampling site No.1 to No.4, all these indicators showed a gradually increase. But at sampling site No.5, there was a drop of all the values, then at sampling site No.6, all the values rebounded to a higher level. These trends reflected the water quality changes along the water. The water quality deteriorated along the flow except that there

was an amelioration at sampling site No.5.

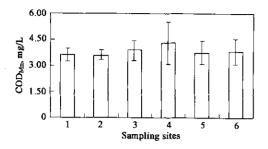


Fig. 6 COD_{Mo} of each sampling site

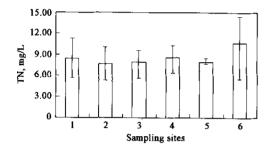


Fig. 7 TN of each sampling site

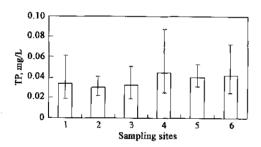


Fig. 8 TP of each sampling site

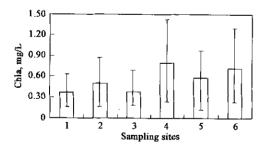


Fig. 9 Chla of each sampling site

The trophic level indices of each sampling sites were calculated and the degrees of eutrophication were assessed according to the standards listed in Table 3. Close to the water fall, No. 1, No. 2 and No. 3 sampling sites had comparatively lower trophic level than other sampling sites that were gradually polluted by non-point sources. The overall trophic level of the investigated water was oligo-meso trophic.

2.2 Structure of the microbial communities

2.2.1 Composition

The taxonomic composition and total number of species of phytoplankton and zooplankton collected from PFUs at the

six sampling sites during the study period are listed in Table 4 and Table 5 respectively.

Table 3 Tropic level indices of each sampling site

Sampling sites	TLI (Chla)	TLI (TP)	TLI (TN)	TLI (COD _{Mn})	TLI _C	Trophic level
1	14.03	39.46	85.83	31.69	29.20	Oligo
2	17.39	37.61	85.50	31.78	30.48	Meso
3	14.35	38.59	85.96	33.98	29.35	Oligo
4	22.47	43.89	88.08	36.67	35.65	Meso
5	19.03	42.19	87.20	34.23	32.45	Meso
6	21.35	42.98	89.36	33.30	34.60	Meso

Table 4 Taxonomic composition and total numbers of species of phytoplankton at the six sampling sites

Names of species	Sampling sites					
rames of species	1	2	3	4	5	6
Chlorophyceae						
Chorococcales						
Pediastrum duplex	+	-	-	-	+	-
Scenedesmus opoliensis	+	+	+	+	+	+
Scenedesmus ucuminatus	+	+	+	+	+	+
Ankistrodesmus falcatus	+	-	-	+	-	-
Golenkinia radiata	+	+	+	+	+	+
Coelastrum reticulatum	+	+	+	+	+	+
Coelastrum microporum	=	+	+	+	-	-
Scenedesmus quadricauda	-	+	-	+	-	-
Scenedesmus ecornis	+	+	+	+	+	-
Oocystis sp.	+	+	+	+	+	+
Dimorphococcus lunatus	+	+	+	+	+	+
Pediastrum Biwae	+	+	-	+	+	+
Pediastrumduplexvar . Clathratum	+	-	-	+	+	+
Micractinum pusillum	-	-	-	-	+	-
Crucigenia irregularis	+	-		-	-	+
Volvocales						
Gonium sociale	-	-	-	-	-	+
Pandorina morum	+	-	-	-	-	+
Tetrasporales						
Sphaerocystis schroeteri	+	+	+	-	+	-
Closteriopsis longissima		+	-	+	-	+
Ulotrichales						
Ulothrix aegoalis	+	+	+	+	+	+
Coniugales						
Spirogyra fluviatilis	-	-	+	+	_	_
Spirogyra varians	+	_	+	+	+	+
St . tetracerum	+	+	+	+	+	+
Cyanophyceae						
Chroococcales						
Hyella caespitosa	_	+	_	-	_	-
D . rupestris	+	+	+	+	+	+
Nostocales						
Oscillatoriatenuis	+	+	+	+	+	+
Dinophyceae						
Peridiniales						
Peridinium inconspicuum	+	+	+	+	+	+
Ceratium hirundinella	-	_	+	+	-	+
Euglenophyceae						
Euglenales						
Euglenaproxyma	+	+	+	_	+	+
Trachelomonashispida	-	+	_	_	+	-

N	Sampling sites					
Names of species	1	2	3	4	5	6
Euglena gracilis	+	+	+	-	+	+
Euglena sanguineae	+	+	+	+	+	+
Phacustrypanon	+	+	+	+	+	+
Chlorvgoniule longatum	+	+	+	+	+	+
Synediarumpens	. +	+	+	+	+	+
Diatom						
Achnanthes	+	-	+	+	+	+
Anomoeoneis	+	_	-	+	-	-
Asterionella	+	+	+	+	-	-
Bacillaria		+	_	_	-	-
Coscinodiscus	+	+	+	+	+	+
Cyclotella	+	+	+	+	+	-
Cymatopleura solea	_	-	_	+	-	-
Cymbella	+	+	+	+	+	+
Diatoma	+	-	+	-	+	+
Diploneis	+	-	-	-	+	-
Ditylum	-	+	+	+	+	+
Epithemia	+	+	+	+	+	+
Eucocconeis	-	-	_	-	-	-
Eunotia	+	+	+	+	+	+
Fragilaria	+	+	+	+	+	+
Frustulia	+	+	+	+	+	+
Hantzschia		_	+	+	_	+
Melosira	-	+	+	+	+	+
Meridion	+	+	+	+	+	+
Navicula	+	_	+	+	+	-
Neidium	+	+	+	+	+	+
Nitzschia	+	+	+	+	+	+
Opephora		_	+	+	+	+
Pinnularia	+	+	+	+	+	+
Rhizosolenia	+	+	+	+	+	+
Rhoicosphenia	+	+	+	+	+	+
Rhopalodia	_	_	_	_	_	+
Surirella	+	+	+	+	+	+
Synedra	+	+	+	+	+	+
Tabellaria	_	_	_	_	_	+
Other algal genera	8	4	4	4	5	5
Total number	55	48	50	53	52	52

Notes: Presence is denoted by (+), absence by (-)

The ratio of the number of each main species to the whole species number of phytoplankton and zooplankton are showed in Fig. 10 and Fig. 11. It can be seen that the main phytoplankton species in the waters were *Diatom* and *Chlorophyceae*, at all the six sampling sites, this two types of algae together can take about 70% of the total algae species number and their proportion to the whole phytoplankton species increase from sampling site No. 1 to No. 4 and decrease at sampling site No. 5, this changing trend is similar to the water quality changing trend. *Zoomastigophorea* and *Rotifer* were the main species of zooplankton. They take about 60% of the whole species and the changing trend of their proportion to the whole zooplankton species is also similar to the changing trend of water quality indicators.

2.2.2 Biodiversity

The biodiversity indices of aquatic microbial communities in the PFUs at each site are calculated. Fig. 12

shows the Margalef biodiversity indices (D) of different sampling sites at different times. As a whole, the Margalef biodiversity indices(D) decline along the flow direction from sampling site No. 1 to No. 6. Fig. 13 depicts the Shannon-Weaver biodiversity indices(H) of different sampling sites. By comparing Fig. 12 and Fig. 13, it is evident that the variation trends of H at different sites are similar to those of D. But the fluctuation of H values is smaller than that of D values. Partly because the richness of each species is considered when calculating H, the changes of species number are counteracted by the changes of individual number of each species, whereas the individual number is not considered when calculating D. Both of these figures showed that the biodiversity decrease along the water as the water quality worsen gradually

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Table 5 Taxonomic composition and total numbers of species of zooplankton at the six sampling sites

	Sampling sites					
Names of species	1	2	3	4	5	6
Phytomastigophorea				**		
Euglena uiridis	+	+	+	+	+	+
Zoomastigophorea						
Vorticella convallaria	+	-	+	+	+	+
Litonotus obtusus	+	+	+	+	+	+
Ghecamoeba quadrillineata	-	-	+	-	+	-
Prorodon virides	+	-	+	+	+	-
Litonotus cygnus	-	+	+	-	+	+
Arcella sp.	+	+	+	+	+	+
Steutor roeseli	-	-	-	-	٠+	+
Rotifer						
Brachionus sp.	+	-	-		-	-
Synchaeta	-	-	-	+	-	-
Rotaria sp.	+	+	+	+	+	+
Euchlanis	+	+	+	+	+	+
Monostyla lunaris	+	+	+	+	+	+
Colurella uncinata	+	+	+	+	+	+
Bdelloidea	-	+	+	-	+	-
Conochilus sp.	-	+	-	+	+	-
Lepadella sp.	-	+	-	+ .	-	+
Philodina roseola	-	-	-	-	+	-
Lecne sp.	+	+	+	+	+	-
Trichocerca sp.	+	+	+	+	+	+
Gastrotricha						
Chaetonotus sp.	+	+	+	+	+	+
Nematoda						
Nematoda sp.	+	+	+	+	+	+
Tardigrada						
Macrobiotus	+	-	-	+	+	+
Crustacea						
Daphnia	+	+	-	+	-	-
Alona sp.	+	+	+	+	+	-
Cyclops sp.	+	+	+	-	-	-
Acroperus harpae	-	+	-	-	-	-
Other species	1	1	1	1	1	1
Total number	18	20	19	19	22	16

Notes: Presence is denoted by (+), absence by (-)

2.2.3 Heterotrophy indices

The heterotrophy indices (HI) of the total aquatic

microbial communities at six sampling sites are calculated and illustrated in Fig. 14. As mentioned above, HI reflects the ratio of hetertrophic microorganisms in total aquatic microbial community biomass. The higher the trophic level is, the more algae will emerge in the water, so the autotrophic species will dominate in the aquatic microbial communities, the HI values will be lower. Therefore, in some sense, HI values will increase when the trophic level decrease, as shown in Fig. 14, from sampling site No. 4 to No. 5, the HI values increase while the TLIc values decrease. Although the HI changing trend is in reverse to the trend of TLIc, it reflects the responses of aquatic microbial communities to the changes of water quality.

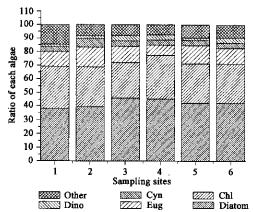


Fig. 10 Ratio of each main phytoplankton species at each sampling site

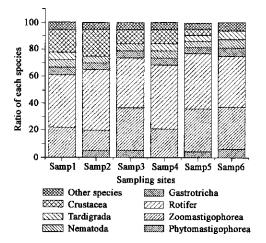


Fig.11 Ratio of each main phytoplankton species at each sampling site

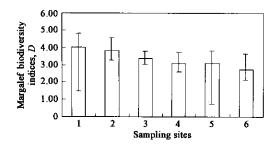


Fig. 12 Margalef biodiversity indices of each sampling site

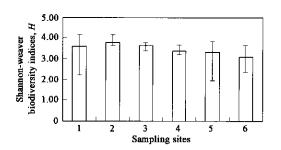


Fig. 13 Shannon-Weaver biodiversity indices of each sampling site

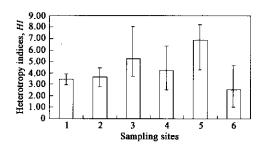


Fig. 14 Heterotropy indices of each sampling site

2.3 Function of the microbial communities

The process of colonization in PFU was simulated with Equation (6). The constants in the Equation (6) were calculated by direct nonlinear regression analysis MATLAB 6.1. The results are given in Table 6. It was clear that from sampling site No. 1 to No. 6, S_{eq} and $T_{90\%}$ decreased gradually on the whole, this reflected that the species number possibly existed in the water decreased from sampling site No. 1 to No. 6, the time needed to get the biogeographical equilibrium also decreased. This could be partially explained by the principle that it took much longer time for more species to colonize in the PFUs and get the biogeographical equilibrium. However, the G values showed an increasing trend on the whole. This indicated that the colonization or immigration rates of the aquatic microbial communities were different at the six sampling sites. As the compositions and structures of the aquatic microbial communities changed, their functions would change accordingly.

Table 6 Functional parameters of aquatic microbial communities

Sampling site	$S_{\rm eq}$	G	T _{90%}
1	36	0.181	12.72
2	36	0.306	7.53
3	35	0.456	5.05
4	32	0.499	4.62
5	30	0.379	6.08
6	27	0.449	5.13

3 Conclusions

In the investigated water, the water quality deteriorate gradually from sampling site No. 1 to No. 6, except at sampling site No. 5 there was a little amelioration. So the trophic level, reflected by *TLIc* values, varies accordingly.

Through analyzing the compositions and proportions of the aquatic microbial communities, it is evident that the structural parameters of the aquatic microbial communities, such as D, H and HI, change correspondingly as the water quality change. The functional parameters of the aquatic microbial communities, such as $S_{\rm eq}$, G and $T_{90\%}$, also exhibit their changes in response to the changes of water quality. The remarkable consistency between the succession of aquatic microbial communities and the water quality changes indicated that the changes of water quality would lead to the structural and functional changes of aquatic microbial communities, which reflect the ecological responses of aquatic ecosystems to the environmental changes, such as, the water quality changes.

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