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ORIGINAL PAPER

Determination of the optimum period for ciliated protozoa colonizing of an artificial substrate in a tropical aquatic ecosystem

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Abstract The optimum period for ciliated protozoa colonizing of an artificial substrate, the polyurethane foams have been assessed in a tropical aquatic ecosystem, the Ekozoa stream of the Mfoundi River Basin in Yaounde (Cameroon). 5 days were calculated as the highest period for the biological indicators of pollution to optimally colonize the artificial substrate. This time interval is the same for all the sampling stations assessed from upstream to downstream and the various microhabitats along the water course. The statistical method applied is that of the completely randomized blocks. The colonization of the substrate increases from the first day to the fifth day, before decreasing to the tenth day. The statistical analysis of variance between the maximum day and the other sampling period was significant at 5 % while the calculation of the value between different points of the same station was not significant. The average number of ciliated protozoan ranges from 20 to 23, from upstream to downstream.

Keywords Ciliated protozoa · Colonization · Artificial substrate · Tropical aquatic systems

Introduction

The degree of pollution in a stream can be measured with the aid of physico-chemical analysis (Holtrop and Fischer 2002). The results recorded from the abiotic components are instantaneous and do not exactly categorize the pollution of the medium over time, even though they can be precise on the type of pollutant. Biological analyses are used to complete the interpretation of the results (Pucciarelli et al. 2008). Water pollution is transmitted by a simplification of the biocenoses, which is relative to a differentiation of the pollution-sensitive species (Cifluentes et al. 2006; Devaux 1976).

The biological method enables an evaluation of the responses of organisms to changes under its environmental conditions. It is based on indicator organisms, absence of sensitive organisms, or the structure of the organisms present in the sample. There is an increasing recognition of the importance of protozoa in monitoring and evaluating the ecological health of aquatic ecosystems (Jian Guo Jiang and Yun-Fen Shen 2006). Ciliated protozoa are known to be ubiquitous in most water bodies (Pinheiro et al. 2007) and are a primordial component to the secondary production compartments of the trophic status of most limnetic and lotic systems. They are an important link in food webs because they are the major consumers of bacteria, pico and nano photosynthetic plankton, diatoms, dinoflagellates, and amoebas and are eaten in turn by animals such as Crustacea in zooplankton and larval fish (Lance and Hersha 2007). Ciliated protozoa are at the basis of the microbial loop between micro and macro-invertebrates as presented by Tarbe et al. (2011).

They have been considered as the most abundant group of microfauna in wetlands treating primary, secondary, and tertiary effluents (Puigagut et al. 2007). These protists have been known to present a quantitative and qualitative distribution in aquatic ecosystems that have been subjected to a pollution stress (Cairns 1978; Kim et al. 2007).

In a lotic environment, it is difficult to carry out replicable and representative sampling because of the great variation of the natural substrates (stones, plants, sand, and mud). To overcome these difficulties, several micro fauna

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sampling techniques have been developed. Madoni and Rosi (1997) realized a sampling by an aspiration pump for the quantitative study of ciliates for streams in a torrential regime. This method does not, however, allow the sampling of ciliates on aquatic plants where the infusorian population is very important. Other artificial substrates for ciliates sampling include immersion slides (Heaton and Parry 2002). Due to the turbulence of water during periods of droughts, the results obtained are not representative, besides the fact that it does not allow for the reaping of thigmotactic species and swimming forms, where a large majority of them are eliminated during the removal of the slides from the sampling sites (Agamaliev 1974).

The method of Petri dishes was introduced by Spoon and Burbank (1967) for the study of sessile ciliates. It consists of using Petri dishes of 12 × 50 mm. This method gives room only for the sampling of sessile ciliates such as *Stentor* and *Peritrichia*. The method applied in this study is that of the polyurethane foams. It was proposed by Cairns et al. (1969) to study the colonization of protists in relation to the quality of water. Some Peritrich ciliates have been known to colonize living things or to attach to non-living substances in freshwater (Ult 2008).

They enable quantitative and qualitative estimations as well as the comparisons of the communities of different aquatic systems, so as to assess the quality of water (Bamforth 1982). Ciliates are the most successful heterotrophic protozoan group even in extreme situations (Roberts et al. 2004). They are therefore very important as biological indicators of aquatic pollution, which is an acute problem in developing countries (Ajeagah et al. 2007). Despite the applicability of polyurethane foams in ciliate harvesting, the time interval necessary to obtain optimal counts remains an important setback. The objective of our work is to statistically determine the period in which we can obtain the highest population dynamics of ciliates by the application of polyurethane foams. This periodicity is necessary in the replicability of sampling for water quality assessment in aquatic ecosystems.

Materials and methods

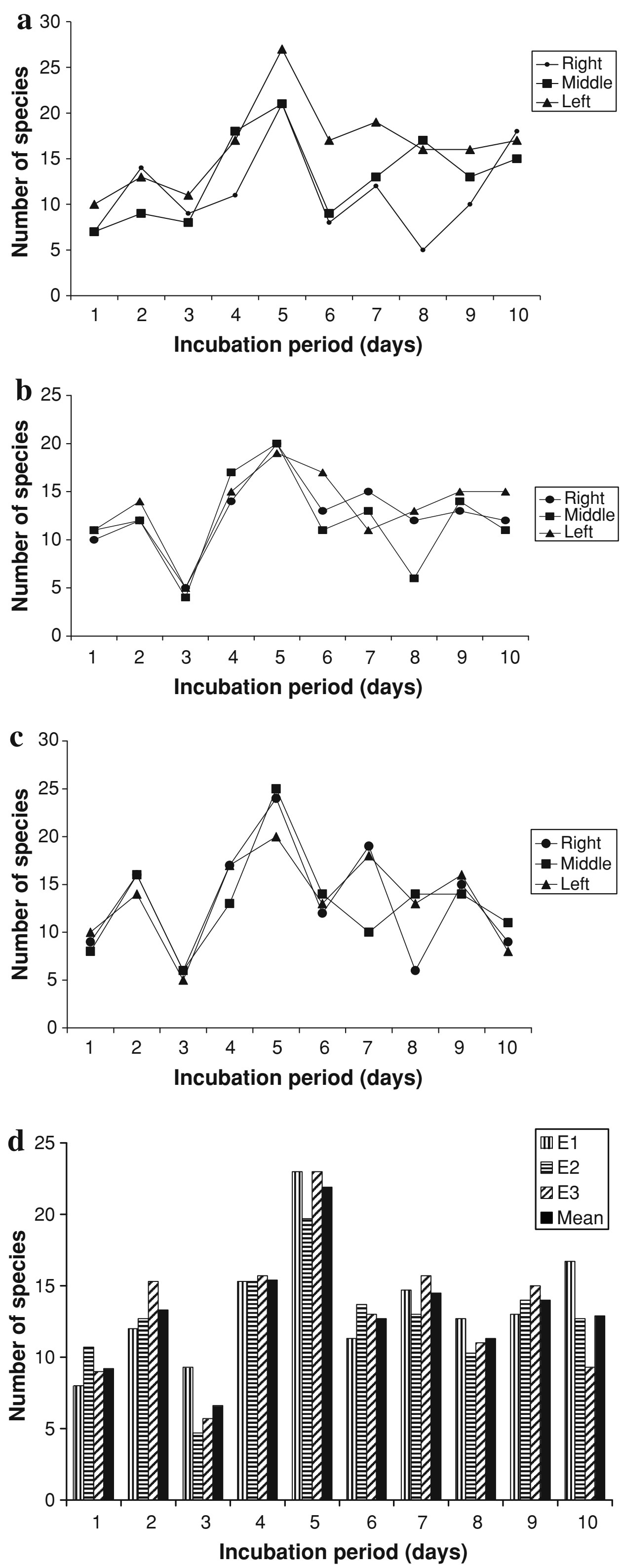
The research was carried out in the Ekozoa stream of the Mfoundi river basin Yaounde and bio-statistically assessed in 2011 as an in depth contribution to the attachment and detachment of micro-zooplankton on particulate matter in hydro-ecosystems. The stream has a length of 2.8 km with a course direction of WNW- ESE. Three sampling stations were chosen and designated as Ekozoa 1 (E1), Ekozoa 2 (E2), and Ekozoa 3 (E3) from upstream to downstream respectively. Ekozoa 1 is situated at 10 m from the source, Ekozoa 2 is located at 1.2 km from the source and Ekozoa 3 is located at 0.5 km from Ekozoa 2. At each sampling station, 30 blocks measuring 5 × 6.5 × 7.5 cm were immerged into the stream. The polyurethane foam is inert, non-selective, and allows the development of numerous organisms due to a complex network of compartments and interstitial spaces. They are easily transportable and lodge in a more complete community of protists. Ten blocks were each placed at the right, middle and left points respectively, to enable a sampling of ciliates in a maximum of microhabitats in the station. The blocks were attached to ropes, which were themselves attached to other ropes fixed along the banks of the stream by sticks. Three blocks were recuperated per station on a daily basis for 10 days. They were transported to the laboratory of General Biology of the University of Yaounde 1 in plastic dishes with lids, where they were squeezed, homogenized and then submitted to microscopic examination at 400 then at 1,000 magnifications. The silver ammonium carbonate staining method of Galiano (1976) was applied in the specific identification. The ciliates were determined by the key proposed by Dragesco and Dragesco-Kerneis (1986).

The statistical test of significance was calculated by applying the method of completely randomized blocks (CRB) with three repetitions. The treatment is the period of incubation, the repetitions are the sampling points (left, middle, and right) and the sampling stations and the variables are the number of colonizing species of ciliates. These calculations provide an assessment of the variation of the daily abundance of ciliates per sampling period in each station and in each sampling point.

Results and discussion

The results show a representative harvesting of ciliates in all the three stations and in the sampling points per station (right (R) middle (M) and left (L)) from the first day to the tenth day. The lowest values are four species/block in day 1 of Ekozoa1 while the highest value obtained is 27 species per bloc in day 5 of Ekozoa 1. Intermediary values are noted between these extremes along the stream course as represented in Fig. 1a–c. The results recorded show the first day of incubations as having the lowest number of species in all the three stations and this increases to day five where the ciliates species was at its maximum, before decreasing to other lower values (Fig. 1d). The average values obtained for the ten observations and per station are 13.6 in Ekozoa 1, 12.7 in Ekozoa 2 and 13,7 in Ekozoa 3 (Fig. 1d).

The analysis of variance from the optimal number of species during the 10-day period of colonization by the application of the experimental model of completely randomized blocks with three repetitions per station indicated



◀ **Fig. 1** **a** Variation of number of species with time (days) in polyurethane foams at the various points (microhabitats) of Ekozoa 1 (*E1*). **b** Variation of number of species with time (days) in polyurethane foams at the various points (microhabitats) of Ekozoa 2 (*E2*). **c** Variation of number of species with time (days) in polyurethane foams at the various points (microhabitats) of Ekozoa 3 (*E3*). **d** Comparison of variation of number of species with time at various stations (*E1*, *E2*, *E3*) of the Ekozoa stream

a difference of 3.2 species giving a 5 % significance, while a 1 % significance was obtained for a difference of 4.4 species (Table 1).

Table 1 Analysis of variance of colonizing species during 10 days of incubation

Source of variation	Degree of liberty	Sum of squares	Squared average	Fb calculated	F-read 5 %	F-read 1 %
Repetitions	2	4.353	2.18			
Treatments	9	439.60	48.84	13.80**	2.46	3.60
Error	18	63.76				
Total	29					

PP DS (0.05) = 3.2

PP DS (0.001) = 4.4

CV = 14.3 %

** Significant at 1 %

A comparison of the average number of species identified during the optimal incubation period of 5 days and other incubation period showed a high significance, with respect to the maximum incubation period. This comparison with the general values of species obtained in the Ekozoa stream is highly significant with respect to the average number of species obtained in the three stations as expressed in Tables 2, 3. The various microhabitats do not show any significant variation in the number of species. The statistical proofs between the same points per station are not significantly different in the watercourse as represented in Tables 4, 5.

The results obtained by using the experimental model of CRB with three repetitions has been applied in a tropical aquatic ecosystem, in order to study the effect of incubation period on the colonization of polyurethane foams by ciliated protozoan. 5 days of incubation are statistically validated as having the optimum number of species and there is no significant variation in the various microhabitats per station as analyzed from Tables 1, 2, 3, 4, and 5. Ciliates have been observed on submerged macrophytes, filamentous chlorophytes and debris in aquatic ecosystems (Zaleski and Claps 2001). The colonization of artificial substrates by ciliated protozoan have been known to present successional patterns in hydrosystems that have been subjected to various types of organic and inorganic pollution (Bharati et al. 2006). The high qualitative and quantitative identification of the ciliates with the artificial substrate gives a reasonable consideration for the application of the biotic indices in the evaluation of pollution. This value of 5 days falls within the range of 4–14 days that have been calculated in France (Chakli 1987) and in the

Table 2 Comparing the average number of species, during the optimal incubation periods (5 days) and that of other incubation periods

Period of incubation	Average[a] (number of species)			Difference with that of optimal period[b]		
	E1	E2	E3	E1	E2	E3
1	8.0	10.7	9	15**	9**	14**
2	9.3	12.7	15.3	11*	7**	7.5**
3	9.3	4.7	5.7	13.7**	15**	17.3**
4	15.3	15.4	15.7	7.7**	1.3**	7.3**
6	11.3	13.7	13	11.7**	6**	10**
7	14.7	13.0	15.7	8.3**	6.7**	7.3**
8	12.7	10.3	11	10.3**	9.4**	12**
9	13.0	14.0	15	10**	5.7**	8**
10	16.7	12.7	9.3	6.3**	7**	13.7**

[a] Average of three stations

[b] ** 1 % significance

Table 3 General comparison on the Ekozoa stream of the optimal periods with other periods of incubation

Period of incubation	Average[a] (number of species)	Difference with that of optimal period[b] (21.9 – xi)[c]
1	9.2	12.7**
2	13.3	8.6**
3	6.6	15.3**
4	15.4	6.5**
6	12.7	9.2**
7	14.5	7**
8	11.3	10.6**
9	14	7.9**
10	12.9	9**

[a] Average of three stations

[b] ** 1 % significance

[c] xi average of the ith period of incubation

Table 4 Average number of species per station and per microhabitat

Treatments	EKOZOA I	EKOZOA II	EKOZOA III	Average
Right point (R)	21	20	24	21.7
Middle point (M)	21	20	25	22
Left point (L)	27	19	20	22
Average per station	23	19.7	23	

Chaohu Lake in China (Xu et al. 2005), even though there is no precision on the maximum colonizing period in the above studies. However, the disparity in the precise time interval in the temperate systems and the value determined in this tropical hydrosystem studied could be due to the fact that the ciliates rapidly reach the apex on the fifth day of colonization and then decrease to the tenth day as presented in Fig. 1a–d, due probably to the increase of organic particles in the spaces inside the blocks, predation and other environmental constraints (Kim et al. 2003). This obstructs the attachment of fixing ciliates such as *Vorticella* sp. and the penetration of swimming ciliates like *Paramecium* sp. (Hazen 2002; Pastova et al. 2008). The most predominant ciliates harvested by the polyurethane foams are the sessile and motile forms (Coppellotti and Matarrazzo 2000).Those identified in our studies are mainly *Euplotes amieti*, *Histriculus histriculus*, *Lembadion magnum*, *Oxytricha chlorelligera*, *Paramecium africanum* upstream, *O. chlorelligera*, *Strombidium meganucleatum*, *Urocentrum turbo* midstream, and *Litonotus cygnus*, *O. chlorelligera*, *S. meganucleatum*, *U. turbo*, *Uronema acutum*, *Lembadion leucens* and *Vorticella*. This is in line with the data for ciliate dynamics that is recorded in the different ecological niches of the Antarctic lakes by Kepner et al. (1999).

Table 5 Analysis of variance on the various points (microhabitat) (left, middle, right)

Source of variation	Degree of liberty	Sum of squares	Squared average	Fb calculated	F-read 5 %	F-read 1 %
Repetition	2	22.2	11.1			
Treatment	2	0.3	0.1	0.0104[ns]	19.25	99.30
Error	4	38.5	9.6			
Total	8					

CV = 14.1 %

ns non-significant

The resuspended organic matter from the sediments seems to play a crucial role in ciliate succession and abundance dynamics in the ecosystem as it defines the nutritive status of saprophytic ciliates (Takehito et al. 2001; Verhoeven 2002). A micro-colonization sequence showed an initial accumulation of amorphous particles composed of bacteria, inorganic grains of minerals and unicellular organisms present in the medium (Aguilera et al. 2007; Chen et al. 2008). According to Habdija et al. (2005); Diaz et al. (2011) seasonal changes in ciliates biomass, community structure and trophic composition could be associated to physico-chemical characteristics of the aquatic environment and the periphyton biomass (Liu et al. 2008). The cystic or free mobile active forms can be identified in substrates in both the plankton and Benthos compartments of ecosystems (Doherty et al. 2010). Biotic interactions such as prey–predator relationships (bactivory, omnivory, and carnivory) between the ciliates themselves and other organisms in the environment could influence their occupation of the pores inside the polyurethane foams and also the possibility of fixation on the artificial and natural substrate (Irie et al. 2010; Savadet et al. 2010). The mineral content of the streams also influence the multiplication of the ciliates, as well as their dispersion in the medium (Henglong et al. 2004). The colonization model of a new habitat is rapid at the patch scale (within hours to days) as illustrated by Anne (2000). This could explain the optimal period of 5 days of colonization recorded in this lotic aquatic ecosystem. A difference could be observed in the various depths of a lentic medium (Priit and Ingmar 2000; Bradley et al. 2010). In the Ekozoa stream that has been studied, the colonization of the artificial substrate is independent of the saprobity characteristics, as the upstream is *B*-mesosaprobic (moderate level of pollution) and the downstream is polysaprobic (very high level of pollution) (Ajeagah et al. 2006).

Conclusion

The optimal immersion period statistically obtained from the colonization of the artificial substrate (the polyurethane foams) by ciliates is 5 days. This period is the same for all the sampling stations and also the various microhabitats per sampling station. The periodicity can give a high turnover for sampling ciliated protozoa which are biological indicators of water quality assessment. The maximum colonization period was found to be same in moderate, polluted and highly polluted stations. The distinctive difference does not appear in terms of colonization period among sampling sites. The use of the polyurethane foams for sampling biological indicators of pollution furnishes a fast, cost-effective, and reliable mechanism for data collection that may be useful for measuring responses of organisms to pollution stress and the characterization of aquatic ecosystems.

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