Developing a Protocol for the Use of Benthic Invertebrates in São Paulo State's Reservoirs Biomonitoring. I. Habitat, Sampling Period, Mesh size and Taxonomic Level.

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ABSTRACT: Developing a Protocol for the Use of Benthic Invertebrates in São Paulo State's Reservoirs Biomonitoring. I. Habitat, Sampling Period, Mesh size and Taxonomic Level. In order to adopt an ecological approach in monitoring programs, the Environmental Agency of São Paulo State (CETESB) has developed studies for the establishment of protocols to standardize the biomonitoring. In this paper, the results of the evaluation of four fundamental items for a protocol of reservoirs' biomonitoring with benthic invertebrates are presented: habitat (sublittoral and profundal), period of sampling (winter, spring, summer and autumn), mesh size (0.25 and 0.5mm) and identification level of the Chironomidae larvae (subfamilies/tribes and genera). Field sampling took place in 6 reservoirs which comprised a quality gradient. The sublittoral environment proved to be the most adequate for systematic sampling, the profundal being an alternative for places where there was the need for a diagnosis of the sediments quality. The period indicated for the sampling was winter, when generally greater richness occurred. The community selected by a 0.5mm sieve presented enough information for an efficient diagnosis, as well as the identification of members of the Chironomidae to the genera level.
Key-words: biomonitoring, reservoirs, zoobenthos, protocol.

RESUMO: Desenvolvimento de Protocolo para a Aplicação da Comunidade Bentônica no Biomonitoramento de Reservatórios do Estado de São Paulo. I. Habitat, Período, Malha e Nível Taxonômico. Diante da necessidade de adoção de uma abordagem ecológica em programas de monitoramento, a CETESB tem desenvolvido estudos para o estabelecimento de protocolos que padronizem esse biomonitoramento. Nesse trabalho são apresentados os resultados da avaliação de quatro ítens fundamentais para um protocolo de biomonitoramento com a comunidade zoobentônica: habitat (sublitoral e profundal), período de amostragem (inverno, primavera, verão e outono), malha de seleção (0,25 e 0,5mm) e nível de identificação das larvas de Chironomidae (sub-famílias/tribos e gêneros). Foram realizadas coletas em 6 reservatórios que compreendiam um gradiente de qualidade. O ambiente sublitoral mostrou-se o mais adequado para amostragens sistemáticas, sendo o profundal alternativa para locais onde houver necessidade de um diagnóstico de qualidade dos sedimentos. O período sugerido para a amostragem foi o inverno, quando em geral ocorreram as maiores riquezas. A comunidade selecionada pela malha de 0,5mm mostrou suficiente informação para um diagnóstico eficaz, assim como a identificação de membros da família Chironomidae em nível genérico.
Palavras-chave: biomonitoramento, reservatórios, zoobentos, protocolo.

Introduction

The State of São Paulo presents a large number of dams and reservoirs, most of them classified in Class I Water Quality (Decree number 10.755, São Paulo, 1977), which includes as one of the legal requirement "the preservation of the natural balance of aquatic communities". Therefore conservation of the biota must be taken into consideration when monitoring the quality of these environments. The classical approach employed in monitoring programs restricted to the measurement of physical, chemical and microbiological variables, is insufficient to assess biological integrity of aquatic environments, and an ecosystemic approach (Prat et al., 1986) of quality is thus necessary.

That approach combines biological indicators with physical and chemical variables, providing greater consistency to management decision making (Lucey, 1987; Ghetti & Ravera, 1994; Prat et al., 1997). The insertion of biological components in a monitoring program is justified by at least four factors: 1) they integrate the action of various pollutants, both as regards industrial as well as domestic discharge, that is the most common situation, which could present synergic or antagonic effects; 2) they respond to the concentration of contaminants not detected by chemical methodology, as well as of new contaminants for which analytical methodology has not yet been developed; 3) they indicate the occurrence of intermittent disposal, such as nocturnal discharge of industrial effluents, thus inserting a temporal factor into monitoring, and 4) they detect the impact of physical alterations such as flow modifications and deforestation (Metcalfe-Smith, 1994).

Amongst aquatic communities, the benthic has been widely used in biomonitoring (Rosenberg & Resh; USEPA, 2002). Several protocols and manuals, standardizing methodology for comparing results, are available (Plafkin et al., 1989; Klemm et al., 1990; Gibbons et al., 1993; Rosenberg et al., 1997; Gerritsen et al., 1998; Gerritsen et al., 2000).

In 1998, CETESB, the agency responsible for controlling the quality of the environment in the State of São Paulo, started projects in order to develop protocols for the insertion of biological communities in its monitoring network. This paper intended to test the following items for the protocol of biomonitoring using zoobenthos in reservoirs: the sampling habitat (sublittoral and profundal zones); the sampling period (winter, spring, summer and autumn), the mesh size (0.25 and 0.50mm), and the taxonomic identification level for Chironomidae larvae (subfamilies/tribes and genera).

Material and methods

Data of the benthic macrofauna in 6 representative reservoirs of an environmental gradient were obtained, in which the degree of industrial and domestic sources of degradation increased from Ponte Nova (PN) ® Cachoeira (CA) ® Atibainha (AT) ® Itupararanga (IT) ® Billings (BL) to Pirapora (PI) Reservoirs. Five sample surveys were undertaken from June 1997 to June 1998 (1 = June/July 1997; 2 = September-October 1997; 3 = December 1997; 4 = March 1998; 5 = June 1998). Additional data on the description of sites and about the physical, chemical and biological diagnosis of the reservoirs can be found in Coelho-Botelho et al. (in press). Three replicate sediment samples for the analysis of sublittoral and profundal communities were obtained, with a modified Peterson grab (600cm^2), in the shallow zone and, with a Ekman-Birge grab as modified by Lenz (200cm^2) in the profundal zone. Both regions were defined following the limnological system (Brinkhurst, 1974). Neutralized formol in a final concentration between 4-10% v/v was used for conservation of samples.

In order to compare the efficiency of different mesh sizes in the capture of indicator organisms useful for environmental diagnosis, sublittoral and profundal samples from the June 1997 and December 1997 were passed through a 0.25mm sieve after that of 0.5mm. In other collecting periods, samples were only washed in a 0.5mm net. After washing, the samples were preserved in 70ºGL alcohol and dyed with rose bengal until sorting, identification and counting under a stereomicroscope.

Samples from the sublittoral zone, rich in gross inorganic particles were submitted to the flotation method in a saturated NaCl solution, for the elimination of gravel and sand particles. For the identification of organisms, the keys published by Pennak (1989), Thorp & Covich (1991), Lopretto & Tell (1995a and b), Epler (1995), Trivinho-Strixino & Strixino (1995), and Merritt & Cummins (1996) were used.

Population densities were transformed into number of individuals/m² and the following indexes were calculated: a) Total density (TD), as being the sum of all benthic macrofauna organisms; b) Richness (S), as the sum of taxonomic categories found in the sample; c) Dominance (DOM), as the highest value of relative abundance in the sample, and d) Diversity, by the Sequencial Comparison Index (SCI) (Cairns & Dickson, 1971), using a software package (Henrique-Marcelino et al., 1992).

The Mann-Whitney U test (α = 0.05) (Siegel, 1975) was applied in the comparison of values of richness between habitats. Differences between richness of sublittoral samples grouped by spring, summer and winter periods was tested by Kruskal-Wallis analysis of variance by ranks (Zar, 1996). In cases where the null hypothesis was rejected, a Q nonparametric test of multiple comparisons was carried out in order to determine significant differences (α = 0.05) between samples (Zar, 1996).

Results

Habitat

Tab. I presents a list of organisms found in the two sampling zones of each of the reservoirs. Sublittoral communities were, in general, richer in number of taxa than those of the profundal, except in the case of the Pirapora reservoir. In this reservoir, the sublittoral presented an azoic condition, whereas in the profundal certain taxa occurred but were not associated to the communities of this region, such as Gymnometriocnemus cf, an terrestrial Orthocladiinae-Chironomidae larvae (Cranston et al., 1983).

Table I: Occurrence of taxa and richness (S) in benthic communities, with generic identification of Chironomidae, of the sublittoral (SL) and profundal (P) zones of the reservoirs (PN = Ponte Nova; CA = Cachoeira; AT = Atibainha; IT = Itupararanga; BL = Billings; PI = Pirapora).

	PN		CA		AT		IT		BL		PI	
	SL	P	SL	P	SL	P	SL	P	SL	P	SL	P
BRYOZOA	x		x		x		x					
TURBELLARIA					x				x			
NEMERTINEA	x											
GASTROPODA	x											x
OLIGOCHAETA	x		x		x	x	x		x	x		x
HIRUDINEA	x				x		x		x			
HYDRACARINA	x		x		x		x		x			
EPHEMEROPTERA					x		x					
ODONATA	x				x		x					
Psychodidae					x							x
Brachycera												x
Ceratopogonidae					x							
Chironomus	x		x		x				x			
Cladopelma	x	x	x		x		x			x		
Cryptochironomus	x		x		x		x		x			

Table I: Cont.

	PN		CA		AT		IT		BL		PI	
	SL	**P**	**SL**	**P**	**SL**	**P**	**SL**	**P**	**SL**	**P**	**SL**	**P**
Demicryptochironomus												
Endotribelos			x						x			
Fissimentum	x		x		x		x		x			
Goeldichiromus	x						x		x			
Harnischia	x	x	x		x		x					
Nilothauma	x		x		x		x		x			
Parachironomus	x						x		x			
Paralauterborniella			x		x							
Polypedilum (Asheum)	x				x				x			
Polypedilum (Tripodura)	x	x	x	x	x		x		x			
Saetheria	x		x				x	x	x			
Stenochironomus					x		x					
Zavreliella					x							
Caladomyia	x	x	x	x	x		x		x			
Stempellina	x		x				x	x	x			
Tanytarsus	x		x		x		x	x	x			
Ablabesmyia	x	x	x		x		x		x			
Clinotanupus					x				x	x		
Coelotanypus	x		x	x	x		x		x	x		
Djalmabatista	x				x		x		x			
Labrundinia	x				x		x		x	x		
Procladius	x	x	x		x		x	x	x			
Tanypus			x							x		
Gymnometriocnemus cf												x
Nanocladius							x					
S	26	6	21	3	30	1	26	3	23	6	0	5

Results of the Mann-Whitney U test confirmed the significant difference for richness (S) between the two habitats in the studied reservoirs (Tab. II).

Fig. 1 shows total densities as well as the occurrence of azoic data for each site. In the sublittoral region, only in Pirapora, benthic macrofauna was absent in all months of sampling.

Table II: Results of the Mann-Whitney U test applied to richness of the profundal and sublittoral communities in the PN(Ponte Nova); CA(Cachoeira); AT(Atibainha); IT(Itupararanga); BL(Billings); PI(Pirapora) Reservoirs.

Reservoirs	**PN**	**CA**	**AT**	**IT**	**BL**	**PI**
n1/n2	15/15	15/15	15/15	15/14	12/12	15/15
Uc	225	225	217	186.5	144	165
Ut	161	161	161	151	107	161

n1/n2 = sample sizes from profundal (1) and sublittoral (2) communities

Uc = calculated value of U

Ut = critical value of U

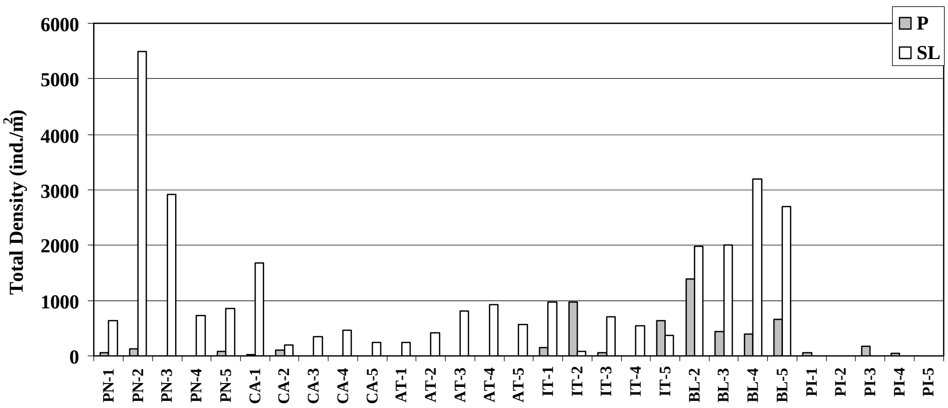

Figure 1: Total densities of the benthic communities of the sublittoral (SL) and profundal (P) zones of the reservoirs. (PN = Ponte Nova; CA = Cachoeira; AT = Atibainha; IT = Itupararanga; BL = Billings; PI = Pirapora; 1= June-Juy 1997; 2 = September-October 1997; 3 = December 1997; 4 = March 1998 and 5 = June 1998).

Period and frequency of sampling

No significant differences were found between richness of sublittoral samples of spring, summer and winter periods, except in the Ponte Nova (PN) reservoir (H = 6.03). The Q nonparametric test of multiple comparisons carried out in the PN reservoir samples detected a significant difference (Q = 3.23) between spring and summer richness. In fact, the most conspicuous differences between benthic richness in sublittoral samples was noted when sample of spring and summer periods were compared (Fig. 2).

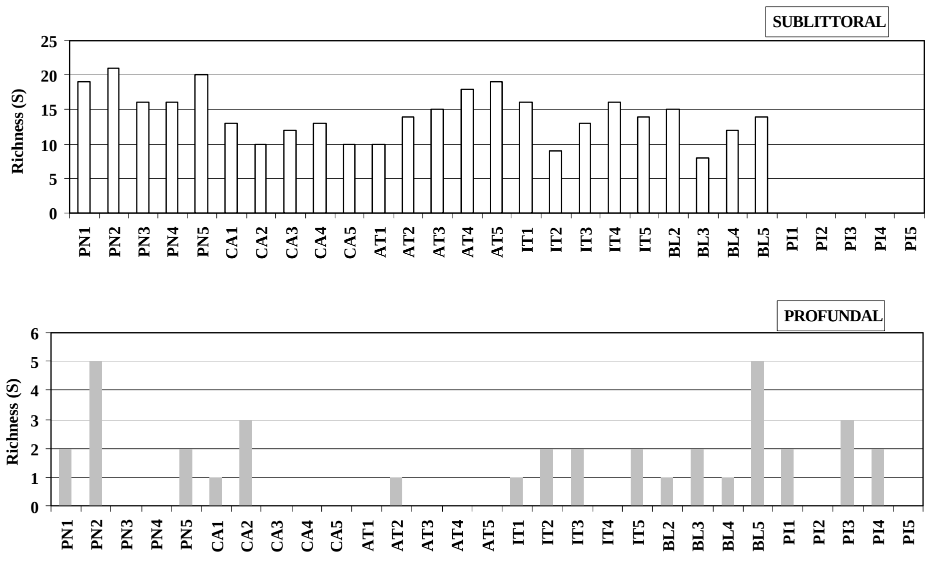

Figure 2: Richness (S) of the benthic communities of the sublittoral and profundal zones of the reservoirs (PN = Ponte Nova; CA = Cachoeira; AT = Atibainha; IT = Itupararanga; BL = Billings; PI = Pirapora; 1 = June-July 1997; 2 = September-October1997; 3 = December 1997; 4 = March 1998; and 5 = June 1998).

Mesh Size

Tab. III presents the results of the comparison between material retained in the sieves with mesh-openings of 0.5mm and 0.25mm.

There was a lower percentage of individual-loss using the 0.5mm mesh size in the profundal compared to the sublittoral zone but, since profundal samples generally presented both low density and richness. The profundal losses contributed greatly in relation to the total. In a few cases (12%), observation of the azoic condition would be altered by the use of a finer mesh.

In the sublittoral, the loss percentage was higher, since organisms of first instars and taxa of a smaller size are more frequent and abundant in this region. As a consequence of greater community densities and richness, losses were proportionally less in relation to the total when compared to the profundal.

Table III: Evaluation of efficiency of the 0,5mm and 0,25mm mesh sizes for benthic macrofauna sorting losses as against the total samples for each habitat.

Zones / Statisitical	Profundal	Sublittoral
Sample size (n)	25	9
Samples with equal number of individuals to both mesh sizes.	16 (64%)	2 (22%)
0.5mm azoic samples but with individuals captured with 0.25mm	3 (12%)	0 (0%)
% loss (density)	33 to 100%	0 to 50%
% loss (richness)	50 to 100%	0 to 33%

Taxonomy

Fig. 3 presents a comparison of data from the benthic communities of the profundal and sublittoral zones, in which larvae of Diptera-Chironomidae were identified, at the level of genera or tribes and subfamilies. On the whole, no information was gained from the profundal region with a finer identification, whereas in the sublittoral, identification of genera generated considerably higher values of S and SCI, and lower DOM.

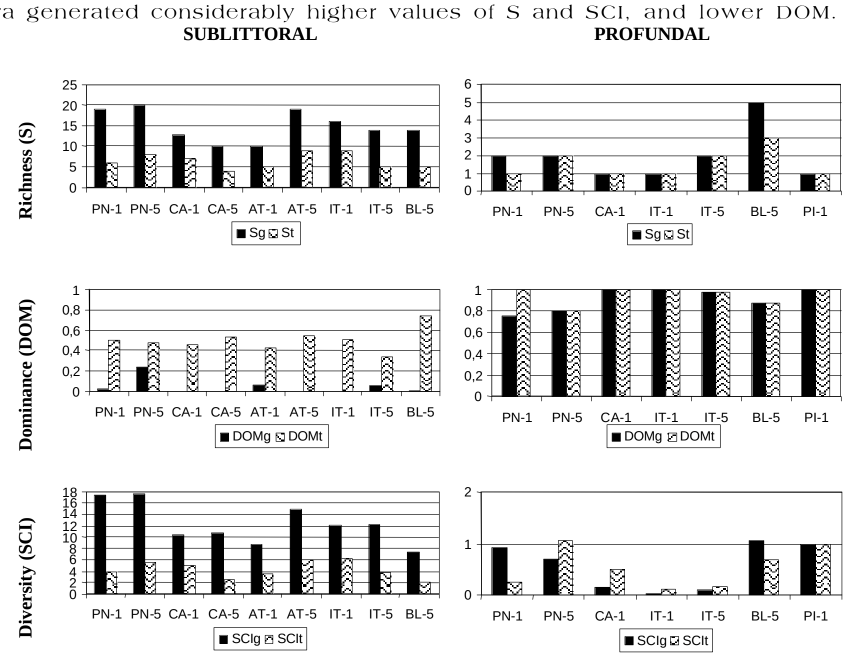

Figure 3: Richness, dominance and diversity indexes according to different taxonomic levels for Chironomidae larvae of the sublittoral and profundal zones of the reservoirs (PN = Ponte Nova; CA = Cachoeira; AT = Atibainha; IT = Itupararanga; BL = Billings; PI = Pirapora. 1 = June-July1997 and 5 = June 1998; INDEXg = Chironomidae identified to genus; INDEXt = Chironomidae identified to tribe and subfamily).

Discussion

Habitat

Benthic communities of lentic environments are influenced by parameters which are essential to these aquatic organisms, such as oxygen level, grain size, temperature, light penetration, the presence of macrophytes and hydrodynamics that can change in function of depth. In the littoral and sublittoral zones, oxygen level, thermal variability and diversity of habitats are higher than in profundal. Consequently, communities are more diversified than those of the profundal region (Brinkhurst, 1974; Shimizu, 1981), thus supplying a larger number of elements for diagnosis of the environment quality. Although greater diversity of microhabitats provides greater richness to benthic communities, it also results in higher abiotic as well hydrodynamics variabilities, that can make difficult the visualization of anthropic effects. Since littoral and sublittoral zones are nearer to the margin, they better reflect the quality of the water mass and can be used for identification of the localized effects of discharging pollutants (Mastrantuono, 1986). The first typology studies of reservoirs made use of the profundal fauna in the classification of lakes according to trophic characteristics (Wiederholm, 1980). Profundal benthic communities have served both to the water quality (Mastrantuono, 1986) as well as to the sediment contamination (Burton, 1992) evaluations, once these communities inhabit the fine sediments deposits of profundal zones, where the retention of contaminants is expected. However, in the natural environments with hypoxia and anoxia in the profundal, as a result of the stratification/circulation regime of the water column, the benthic community is very limited. Most protocols dealing with biomonitoring using benthic organisms, have pointed out communities of the sublittoral zone for routine evaluation (Rosenberg et al., 1997; Gerritsen et al., 1998). In environments without natural hypoxia or anoxia conditions and in which the sediment quality evaluation is an important issue, the profundal community should also be considered (Wiederholm, 1980; Gerritsen et al., 1998).

The azoic condition at Pirapora reservoir obviously is a response to its low water and sediment qualities. Except in Billings, all the reservoirs presented azoic data at profundal zone, in several months, due to the water quality (Pirapora/September-October 1997 and PiraporaJune 1998) and presence of oxiclines (Atibaia/December 1997, Atibaia/March 1998 and Atibaia/June 1998; Cachoeira/March 1998 and Cachoeira/June 1998; Itupararanga/March 1998; Ponte Nova/December 1997 and Ponte Nova/March 1998) (Coelho-Botelho et al., in press). Thus, the occurrence of azoic conditions not associated to anthropogenic degradation in the profundal benthos makes these communities inadequate as biomonitoring tool to the studied reservoirs, except for Billings.

The occurrence of Diptera-Psychodidae and Brachycera, both presenting atmospheric oxygen respiration, and Gymnometriocnemus cf, an inhabitant of terrestrial systems (Cranston et al., 1983) were considered accidental in the Pirapora reservoir and should be related to the short retention time of water (4 days according to Coelho-Botelho et al., in press).

Moreover, the profundal fauna was poorer than sublittoral, as has been usually observed (Brinkhurst, 1973; Shimizu, 1981; Gerritsen et al., 1998). Most of the taxa are associated to profundal habitat or soft bottom (Fittkau & Roback, 1983; Pinder & Reiss, 1983; Merritt & Cummins, 1996) and just Tanypus has been considered a member of littoral region (Fittkau & Roback, 1983) and Gymnometriocnemus cf to terrestrial system (Cranston et al., 1983).

The sublittoral fauna exhibited significantly more components than profundal (thirty seven taxa). According to Pinder & Reiss (1983), Stenochironomus is an obligate miner in living or dead vegetation, others taxa can be associated to macrophytes, as Goeldichironomus, Harnischia, Parachironomus, Paralauterborniella, Polypedilum, Tanytarsus and Zavreliella, but can be also found inhabiting fine sediments (Pinder & Reiss, 1983; Merritt & Cummins, 1996). On the other hand, Ablabesmyia, Labrundinia, Polypedilum, Stempellina and Tanytarsus are considered eurytopics living in any kind of habitat (Fittkau & Roback, 1983; Pinder & Reiss, 1983).

Because of the higher richness, the sublittoral zone of reservoirs are preferentially recommended in biomonitoring programs. In reservoirs exhibiting a history of industrial and domestic waste inputs, the profundal community should also be evaluated, since it lives in direct contact with deposits of contaminants.

Sampling Period and Frequency

For most of reservoirs sampled, the statistical analysis indicated no significant changes in the benthic community richness due to seasonality of the lentic environments. The sampling period with more stable values and some times highest values of richness was the winter.

The period indicated in the literature as being the ideal for collecting benthic organisms within biomonitoring programs is variable. The period of greater stress (Gerritsen et al., 1998; Gibbons et al., 1993); of greater diversity (Gibbons et al., 1993), after recruiting (Gerritsen et al., 1998) or when there are less immature individuals which are difficult to identify (Gibbons et al., 1993; Lenat & Barbour, 1994), can be selected for biomonitoring programs.

The period of greater diversity can be usefull because provides the largest number of elements to the community, each one presenting different tolerance/ resistance level and generating higher sensibility in the identification of subtle alterations promoted by the action of any stress agent. When using profundal communities a biomonitoring program must avoid the period of water mass stratification, when hypoxia or even anoxia simplify or even extinguish the community. In most tropical reservoirs, surface water cooling occurring in the winter promotes circulation of the water column then reoxygenation of the profundal occurs, and, consequently, better conditions for the establishment and development of benthic populations (Paine, 1986; Esteves, 1998).

Significant changes in spring and summer richness of Ponte Nova samples were a statistical evidence to the recommendation of sampling in winter, since periods of rapid changes in the community structure should be avoided (Lenat & Barbour, 1994).

A constant period for sampling throughout a biomonitoring program avoids the seasonal interference on populations in the diagnosis and reduces the biomonitoring cost (Gerritsen et al., 1998).

Mesh Size

Even though certain manuals and protocols adopt finer meshes (0.18 – 0.25mm) (Gibbons et al., 1993) for the selection of the benthic invertebrates sorting. Most of them opt for meshes between 0.5 and 0.6mm, restricting the use of a finer mesh to specific aims. Even though the fine mesh provides a more detailed picture of the populations, much of the retained material is composed of very young individuals, of a reduced size and difficult to identify (Klemm et al., 1990; Lenat & Barbour, 1994; Gerritsen et al., 1998).

In this work, the gain in information through the use of a finer mesh (0.25mm) would not compensate the longer time spent in the sample sorting, identification and counting.

Taxonomy

The identification level to be used in studies of biomonitoring is a controversial subject in the literature (Bailey et al., 2001). The fact is that the lower the taxonomic level, the more accurate the results, and thus, the greater will be the capacity of a sample of the benthic community to detect subtle alterations in quality (Lenat & Barbour, 1994). But sensitivity and the time spent in identification should be considered.

If the lowest taxonomic level is not possible for all groups, it will be necessary to standardize the identification level of the different taxa so that samples could be spatially and temporally comparable (Gibbons et al., 1993). Obviously, the effort to achieve identification to the lowest taxonomic level possible should be centralized on the representative key-groups of the community as a whole and, have genera and/ or species sensitive to different types and intensities of stress (Bailey et al., 2001). In lakes and reservoirs, Oligochaeta and Chironomidae are the two taxonomic groups that fit in with these characteristics (Wiederholm, 1980; 1984; Lenat & Barbour, 1994), and have thus been historically used in the classification of lakes (Wiederholm, 1980; 1984).

The present paper indicates that in reservoirs the generic level for Chironomidae is necessary, mainly in dealing with sublittoral fauna, since higher values for S and SCI indexes are more sensitive to changes in community structure. Identification for the other taxa could be restricted to family, although it must be noted that for Oligochaeta as well, identification to genus/species would be desirable.

It is also recommended that organisms of the micro and meiofauna and/or those not truly benthic, such as Diptera-Chaoboridae, with migrating habit, and Diptera-Psychodidae, Ceratopogonidae, Syrphidae and others, with aerial breathing, as well as organisms of little importance to the community (low relative frequency and abundance) should be removed from the quantitative analysis, since their presence is probably accidental in the samples, and not necessarily related to the degree of degradation of the system.

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