Monitoring of a swamp river contaminated by multiple waste

Monitoramento de um rio de banhado contaminado por despejos múltiplos

Terra, NR.[1], Feiden, IR.[1] and Fachel, JMG.[2]

[1]Fundação Estadual de Proteção Ambiental Henrique Luis Roessler – FEPAM,
Av. Dr. Salvador França, 1707, Bairro Jardim Botânico, CEP 90690-000, Porto Alegre, RS, Brazil
e-mail: nara.terra@ufrgs.br, ilda.feiden@gmail.com

[2]Departamento de Estatística, Instituto de Matemática, Universidade Federal do Rio Grande do Sul – UFRGS,
Av. Bento Gonçalves, 9500, CEP 91509-900, Porto Alegre, RS, Brazil
e-mail: fachel@ufrgs.br

Abstract: Aim: The objective of the present study was to establish a comparison between the studied points and sampling times as to the responses of this water flea. **Methods:** Between July/03 and May/05, 15 sediment samples were collected in order to run a series of chronic semi-static bioassays exposing 450 *Daphnia magna* individuals to the samples. The microcrustaceans were placed in stress situations during 21 days from the beginning of their lives (2-26 hours old). Comparisons were established between collection points as well as between sampling months, taking into consideration biological parameters such as survival and reproduction. **Results:** Reproduction showed to be more sensitive than survival, often indicating presence of chronic toxicity (51%) and absence of acute toxicity (89%). Sampled points showed significant differences between months. Comparison between locations has shown Gr34 as producing fewer individuals when compared to control. It represents only 34% of neonates. **Conclusions:** The results obtained with the exposure of *Daphnia magna* it become evident of the inadequate conditions for the development of cladocerans in the studied stretch.

Keywords: Cladoceran, ecotoxicology, chronic-assay, sediment, Gravataí River.

Resumo: Objetivo: A qualidade da água do rio Gravataí é monitorada sistematicamente pela FEPAM, órgão responsável pela proteção ambiental do Rio Grande do Sul, visando avaliar o impacto da atividade humana sobre os recursos hídricos do Estado. O objetivo do presente estudo foi estabelecer comparações nas respostas de *Daphnia magna* quando considerados os pontos de amostragem e o tempo. **Métodos:** Entre julho/03 e maio/05 foram coletadas 15 amostras para realizar uma série de bioensaios crônicos, semi-estáticos expondo 450 indivíduos de *Daphnia magna* a estas amostras. Os microcrustáceos foram colocados em situações de stress durante 21 dias desde o início de suas vidas (2-26 horas de vida). Ao final do trabalho foram estabelecidas comparações entre os pontos de coleta e entre os meses de amostragem, considerando os parâmetros biológicos como sobrevivência e reprodução. **Resultados:** A reprodução mostrou-se mais sensível que a sobrevivência, indicando em muitas ocasiões presença de toxicidade crônica (51%) e ausência de toxicidade aguda (89%). Os pontos amostrados apresentaram diferenças significativas entre os meses. A comparação entre os locais aponta para Gr34 como aquele que gerou menos indivíduos quando comparado com o grupo controle, representando apenas 34% de neonates. **Conclusões:** Os resultados obtidos após a exposição de *Daphnia magna* mostraram condições inadequadas para o desenvolvimento deste cladócero no trecho estudado.

Palavras-chave: Cladocera, ecotoxicologia, ensaios crônicos, sedimento, Rio Gravataí.

1. Introduction

FEPAM conducts ecotoxicological studies in different areas of the Rio Grande do Sul state, Brazil and emphasizes the study of the water bodies that form the Guaíba Lake hydrographic basin. In this basin is observed anthropic action capable of altering the balance of biocenoses and the consequent loss of environmental homeostasis. The most frequent perturbations in this ecosystem are caused by agriculture processes, urbanization, domestic and industrial waste discharges, riverbed dredging, and water column aeration.

This paper is the result of a study of Gravataí River sediment. The Gravataí River is one of the forming rivers of the above mentioned basin, is used as a source of water supply, and is a primary contact recreation for both the population of Porto Alegre, state capital of Rio Grande do Sul, and its metropolitan region. The River receives pollutants of various types and levels. It springs in a swamp zone; its flow is 5.14 m^3/s (IPH, 2002), and it is slower than the rivers in higher regions (Pineda and Schäfer, 1987), which is a differential characteristic in relation to most rivers in the state. This peculiarity regarding its formation makes the river very sensitive to sources of pollution, due to its reduced aeration and low capacity of diluting pollutants. Its spring region has a semi-lentic structure with a high concentration of nutrients, although it sometimes presents oligotrophic characteristics (Salomoni et al., 2007). The river extends throughout municipalities with some developed urban and industrial areas and runs through highly polluted places, as well as through others that are less subject to anthropic interference (Pineda and Schäfer, 1987).

The hydrographic sub-basin of this river is 2,200 km^2 (i.e., 2.6% of the area of the hydrographic basin of Guaíba Lake), including totally or partially the following municipalities: Santo Antônio da Patrulha, Taquara, Glorinha, Gravataí, Alvorada, Viamão, Cachoeirinha, Canoas, and Porto Alegre (FEPAM, 1996).

Its drainage system presents three different sets of behavior: a) spring (+/– 400 m of altitude); b) Banhado Grande, and c) lower course or the Gravataí River itself. According to DNOS/GTZ (1985), Banhado Grande, which serves as flow regulator, had an area of 450 km^2, presently reduced to only 50 km^2. The differences between existing drainage systems influence the way the river responds to environmental aggressions.

The movement of pollutants resulting from the water flow usually prevents their detection in liquid samples due to the time gap between discharge and sampling. On the other hand, persistent substances that are deposited in the riverbed are accumulated in the sediments; and as a result, a positive response is more likely to be obtained, since the time passed between the discharge of the pollutant and sampling becomes less relevant. According to Burton (1992), the holistic nature of the perturbation of toxic substances in aquatic ecosystems and the significant alteration between water and sediment compartments is essential for the impact on nonbenthic communities. Contaminants associated to the sediment arrive to the environment through several routes, such as storms, dredging, currents, or bioturbation-related ones, like resuspension, desorption, incorporation into benthic biota that work as preys of nonbenthic species, and sediment ingestion by species that occasionally act as epibenthics, like bottom fish and cladocerans.

Chronic biological assays simulating natural environment enables detection of actions on the development of living beings. Long chronic assays identify reproductive alterations that are not detected in acute assays (Terra and Schäfer, 2000). Assays with *D. magna* are appropriate for sediment evaluation due to their behavior, since they manifest the action of pollutants in their survival, and especially, in their reproduction, even when these are within the standards permitted by the legislation.

Many studies have considered *D. magna* as an excellent trial species and have recommended its use as a tool in routine tests with sediment (Nebeker et al., 1984). This study evaluated the quality of a stretch of Gravataí River based on reproductive and survival responses of *Daphnia magna* exposed to sediment samples. The option for the use of this tool was based on quality, reproducibility, and response sensitivity of organisms to water contaminants. Another objective of the present study was to establish a comparison between the studied stations and sampling times as to the responses of this water flea.

2. Material and Methods

D. magna was exposed to sediment samples because this compartment is able to retain pollutants and give responses when cladocerans are submitted to sediment.

Between July/03 and May/05, 15 sediment samplings were conducted in three places on Gravataí River: two included in Class 2 (Gr06 and Gr08) and one in Class 1 (Gr34), according to Resolution CONAMA n. 357 (CONAMA, 2005). According to Brazilian law (CONAMA, 2005), Class 1 consists of water for primary contact recreation, protection of aquatic communities, and human consumption after simplified treatment, and Class 2 are rivers that provide water supply for human consumption after conventional treatment, protection of the aquatic community, and primary contact recreation.

To name these sites, the two first letters of the name of the river (Gr) were used, followed by the number of kilometers from the mouth (06, 08, and 34 km). The collected material was stored in glass flasks and transported in ice to the laboratory where it was kept in the dark at 4 °C for up to one month.

2.1. Sampling stations

The description of the sampling points was based on information of the Basic Network of Environmental Monitoring of Superficial Waters of FEPAM (Figure 1).

2.1.1. Gr34 – 29° 57' 55" S and 50° 56' 52" W

In this area, the river is 30 m wide and has a sandy riverbed; it is used for primary contact recreation, aquatic life protection, amateur fishing, rice irrigation, and cattle rising. The region is known as "Passo dos Negros".

2.1.2. Gr08 – 29° 57' 16" and 51° 07' 36" W

Also 30 meters wide and having a sandy riverbed, the river is used for navigation; it receives domestic, pluvial, cloacal and industrial sewage and waste deposit from areas of the municipalities of Alvorada, Cachoeirinha, and Gravataí. When there is the reflux of the river, it suffers the influence of sewages from the north zone of Porto Alegre (FEPAM, 1996). Its waters are also used in industrial supply for cooling off machinery.

2.1.3. Gr06 – 29° 57' 37" and 51° 08' 34"

In this place, the river is 100 meters wide, and its riverbed varies between sandy and muddy. The river is used

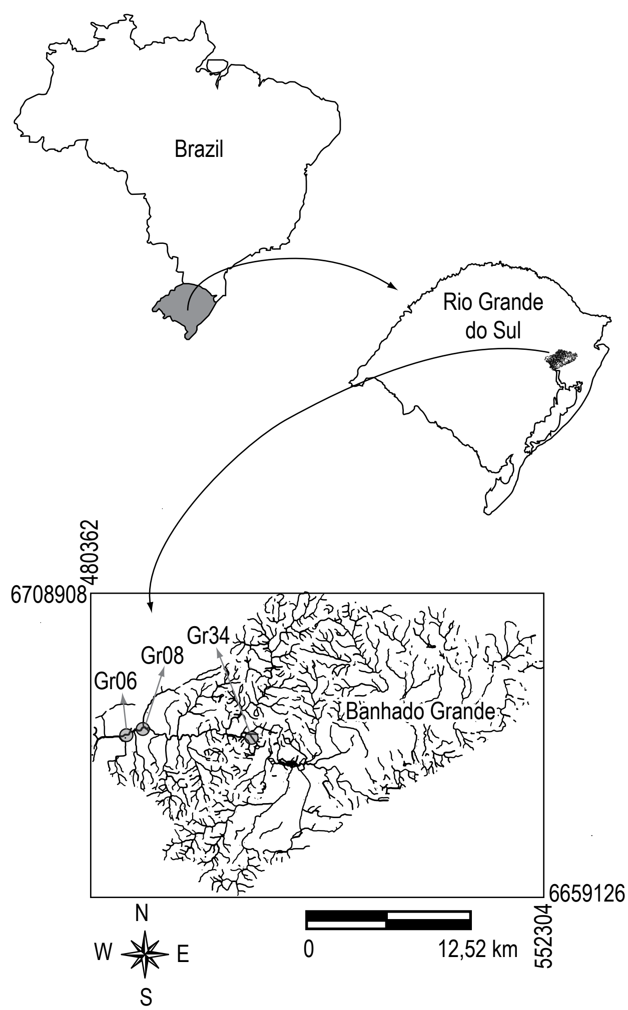

Figure 1. Location of the study sites in Brazil, Rio Grande do Sul state, Gravataí River basin, Sites Gr06, Gr08 and Gr34. (Adapted map of Salomoni, SE., Rocha, O. and Leite, EH, (2007).

for navigation, discharge of pluvial, cloacal and industrial sewage from Cachoeirinha and North Porto Alegre. In this area, river waters are captured, treated, and then used in public supply. It is located in the mouth of Areia Brook.

The microcrustaceans were obtained through cultivation in laboratory with M4 medium (pH 7.8, total hardness 230 mg $CaCO_3$/L) (Elendt and Bias, 1990) in a density of 25 adults in 1000 ml, in germinator programmed for 20 ± 2 °C and 16:00 hours light / 8:00 hours dark photoperiod. These conditions guarantee parthenogenetic reproduction with absence of ephippia. Cultivations and tests were maintained in independent germinators in order to avoid contamination. The lots used in the assays presented LC50–24 hours of 0.91 ± 0.16 mg $K_2Cr_2O_7$. For the calculation of LC50–24 hours, the Trimmed Spearman-Karber Method was applied (Toxstat, version 1.5).

Assays were performed in ten replicas, in beaker of nominal capacity of 50 mL, containing one individual in each. Beakers were covered with laboratory sealing film to avoid evaporation and contamination. The experiment was run for 21 days with daphnids between 2 and 26 hours of life at the beginning of exposure. The proportion of sediment and M4 medium was 1:4 (v:v) as successfully used in other studies (Burton Jr., 1992; Suedel et al., 1996, Terra et al., 2006). This proportion was chosen so that organisms were not debilitated due to nutrient deficiency during the long period of exposure.

Daphnids were monitored every other day, to observe mortality (total lack of movement) and reproduction, and to observe the reproductive stage. For the counting of young and the observation of adults, they were removed along with the old medium, and fresh M4 medium was slowly placed in each beaker through the glass wall using a washer flask. Young were counted and discarded and the adult microcrustacean returned to the original flask. The procedure of substitution of cultivation medium with elimination of the young avoids environment quality alteration and interference in results; moreover, it prevents dispute for space and food after the beginning of reproduction. Test-organisms were fed with green alga *Scenedesmus subspicatus*, "ad libitum" (1 mL; concentration of 10^7cells.cm^{-3}).

Smolders et al. (2005) state that high energy status organisms are more successful in dealing with stress when compared to low energy status organisms.

ANOVA test was used with two factors to detect differences between means of reproduction levels between the sites on Gravataí River and between the months of observation. In this analysis, factors "month" and "site" were fixed, and beakers were considered repetitions (n = 10). The option SLICED of software SAS was used to present the ANOVA solution for the interaction of factors per month and site. Due to high variability, the supposition of homocedasticity for ANOVA test was not attained. Variable transformations were done to bypass non-homocedasticity, and the best response was obtained using the original variable weighed by the inverse of the variance of each group; groups were formed by the combination of each point (3 points) with each month (15 months), resulting 45 groups. For multiple comparisons, the LSD (least significant difference) test was used, with a 5% significance level.

As a supplementary analysis of the data and to define the level of alteration of the ecosystem, the following indicators were calculated: reproductive mean per brood (chronic effect), in which the minimum number of 20 individuals was expected, and survival rate (acute effect), in which the value expected should be equal or above 80%.

Sediment samplings were submitted to the analyses of physical-chemical parameters, including metals in water, which contrary to sediment are not cumulative. Thus, since metals are cumulative, it is possible that in the studied compartment the content of metals and other persistent chemicals results in higher concentrations when compared to those detected in the water samples. Physical-chemical parameters and metals analyzed were defined according to the sources of anthropic contamination located in the study area.

The samples for heavy metal dosage were kept in flasks with a nominal capacity for 1,000 mL, containing 5 mL of nitric acid, while the samples for microbiological analyses were collected in a borosilicate flask with a wide mouth and a 100 mL capacity.

3. Results

The physical-chemical analyses in water samples identified some parameters outside the standards permitted by Brazilian legislation (CONAMA, 2005). In site Gr06, results were above the permitted standard in 62% of the BOD5 analyses; for Total P, this percentage was 85%; Phenols index, 33%; Ammoniacal N, 60%; OD, 50% and Turbidity, 20%. In Gr08, 31% of the BOD5 analyses were above the permitted standard; Total P, 100%; Phenols index, 7%; Ammoniacal N, 47%; OD, 85% and Turbidity, 20%. On the other hand, in Gr34 only two parameters exceeded the permitted values, namely Phenols index (27%) and Turbidity (93%). Table 1 shows the months in which the Phenols index and Turbidity were above the standard permitted by Brazilian legislation (CONAMA, 2005) in Gr34.

All detected values were considered for heavy metals, even when they were within the limit permitted by the Brazilian legislation or in a month not sampled for ecotoxicological assays; this was due to the cumulative aspect those metals present and their capacity to sediment. According to LeBlanc (1985), it is ecologically important to consider that, even in concentrations that were previously considered safe, prolonged exposure to metals may significantly change delicately balanced ecosystems. However, precipitated metals are not necessarily permanently fixed in sediment. They may be re-suspended by natural phenomena, urban activity, or redox conditions, and may increase bioavailability, as well as toxicity of trace metals for bottom fauna (Sundelin and Eriksson, 2001). The following results were observed in Gr06: Pb (18% of samplings), Cu (54%, i.e., 8% above the permitted value), Total Cr (9%), Ni (12%), and Zn (70%, i.e., 7% above the permitted value). In Gr08, the following results were observed: Pb (6% of samplings), Cu (59%), Total Cr (4%), Ni (12%), and Zn (82%). Finally, in Gr34: Cd (8% of samplings), Pb (13%, i.e., 50% above the permitted value), Cu (15%), Total Cr (15%), and Zn 52%. Of the 83 presences of heavy metals in samples, 40% were detected in Gr08, followed by Gr06 (37%); in Gr06, values of Zn and Cu exceeded the limit permitted by the Brazilian legislation in March/04 and December/04, respectively (CONAMA, 2005). In Gr34, the presence of heavy metals was observed only in 23% of the samples. Hg was not detected. On the other hand, Cd was absent in Gr06 and Gr08, and Ni was not detected in Gr34.

Table 1. Months with index of phenols and turbidity in Gr34 above the value permitted by the Brazilian legislation.

Parameter	Year		
	2003	2004	2005
Phenols index	Sept.	Feb. Dec.	May
Turbidity	July Sept. Nov.	Feb. Mar. Apr. Aug. Sept. Oct. Nov. Dec.	Jan. Feb.

Comparing the three sampled sites, Gr06 showed 67% (10 samplings) of inadequate responses for the maintenance of life due to low rate of survival, followed by Gr34 with 60% (9 samplings), and Gr08 with 13% (Figure 2). Of the 9 samplings with reduced survival in Gr34, five (November 2003, February/04, April/04, July/04, and May/05) presented 100% of mortality in the first five days of November 2003 and at 48:00 hours of exposure in the remaining. Total mortality was also found in Gr08 (August/04) and Gr06 (March/04 and August/04). Moreover, in October in Gr06, mortality rate was 80%, starting on the second day of exposure. The total number of survivors at the end of 15 assays was 74 in Gr34, 126 in Gr08, and 78 in Gr06; the expected was at least 120 individuals.

The sum of neonates in the end of the observations indicated that the organisms exposed to the samples of Gr34 presented reduced reproductive capacity when compared to the other sites (Figure 3). In comparison to the control group, Gr34 generated 31% of the individuals, Gr06 48%, and Gr08 56%.

Reproduction showed great variability between sites mainly in the first months. Located close to the mouth, four samples (27%) in Gr06 and only one sample in Gr08 (7%) presented responses within the expected standard for reproduction.

In Gr08 in February/03, one female specimen died on the 12th test day and after releasing 15 stillborns. In October/04, also in this station, one individual started reproduction on the 12th day, when it generated only one daphnid, resumed reproduction only on the 21st day and again generated one individual.

In February/04, Gr06 maintained only two females alive until their reproductive age; these females died on the 12th day; one generated six stillborns; the other, ten. A similar situation happened in March of the same year, when two females reproduced on the 8th day; one died soon after that (it generated 3 individuals), and the other died on the 10th day (it generated 10 individuals). Besides these, the two remaining daphnids became pale on the 11th day and died

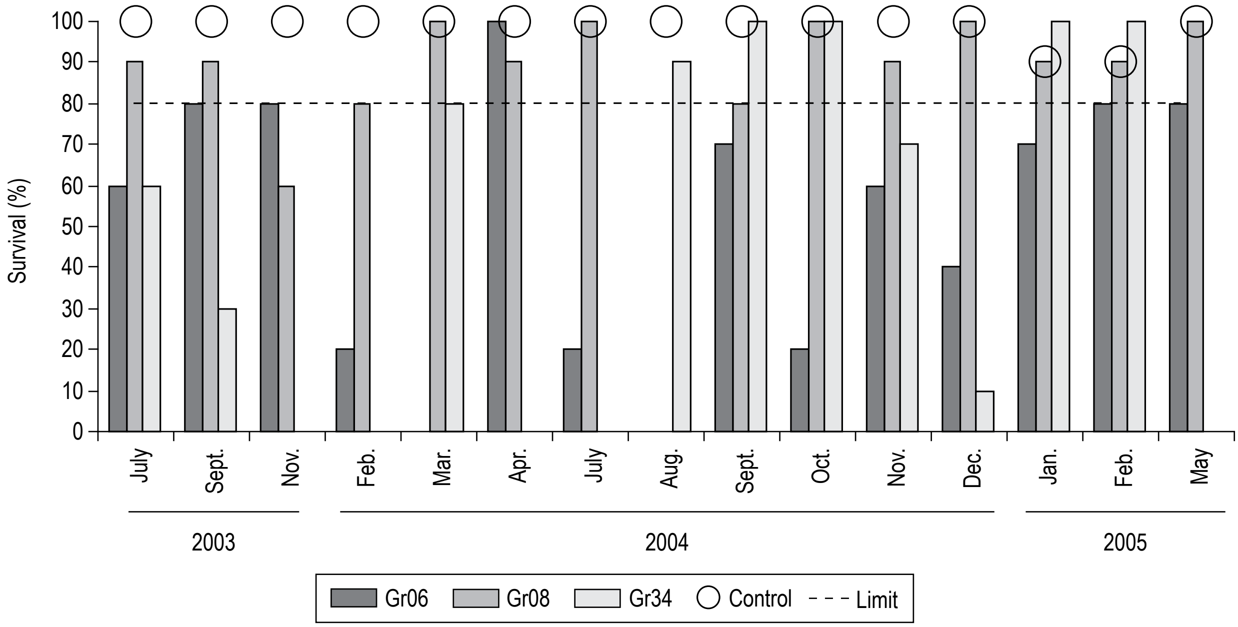

Figure 2. Survival percentage of *Daphnia magna* exposed to Gravatai River sediment samples, control group and expected survival from July/03 to May/05.

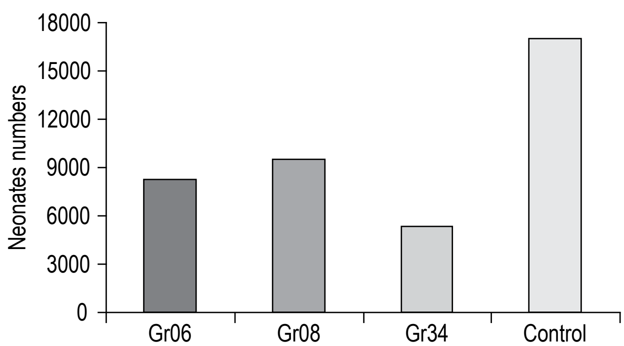

Figure 3. Number of *Daphnia magna* neonates in assays with Gravataí River sediment samples, and control group from July/03 to May/05.

two days later. In July, one individual reproduced on the 6^{th} day and died on the 12^{th} day, and it did not reproduce again. In that same month, five daphnids died on the 9^{th} day after releasing stillborns.

The months of September/04 and October/04 showed a small recovery, which was not maintained in the following months, when results were below expectations in all sites (Figure 4).

Statistical tests evaluated the data in a more detailed way. ANOVA showed that the main factors (month and site) and the interaction between them were significant ($p < 0.001$). In other words, the differences between the means for births observed in each site depended on the moment they were observed.

Table 2 (fixing month and varying site) and Table 3 (fixing site and varying month) provide multiple comparisons by the LSD (Least Significant Difference) test; considering a significance level of 5%, means followed by the same letter were not significantly different. Equation result could not be estimated when values were zero.

4. Discussion

Data analysis showed toxicity levels variability in Gravataí River due to different sources of pollution, and the effect varied from acute to chronic. Toxicity variation was due to different levels of stress to which organisms were submitted according to local characteristics. Alteration in environment quality and health of the trophic chain organisms happens due to the process of ingestion of xenobiotics through the process of filtration or predation followed by metabolization.

Salomoni et al. (2007) described Gravataí River in two different portions: the first formed by the more preserved upper and medium stretches and the second marked by the entrance of organic dumping of anthropogenic source. According to the same authors, a continuum was observed in this river from its spring to its medium stretch and was characterized by a gradual decrease in OD concentrations and increase in Turbidity, Conductivity, Chloride, and BOD.

In 2003 and 2005, the presence of BOD5, Total Phosphorus, Phenols, and Ammoniacal Nitrogen observed in disagreement with the present legislation were less frequent in all the sampled sites. However, especially in 2004,

Table 2. Comparison of means between sites per month. Means followed by the same letter did not differ significantly by LSD test ($\alpha = 0.05$). Months that did not show significant difference were omitted from the table.

July/03		
site	Mean	
Gr06	56.9	a
Gr08	45	b
Gr34	20	c

September/03		
site	Mean	
Gr06	95.1	a
Gr08	42	b
Gr34	6.6	c

November/03		
site	Mean	
Gr34	127.9	a
Gr06	62.7	b
Gr08	0	c

February/04		
site	Mean	
Gr08	27.4	a
Gr06	16.7	a
Gr34	Non-est	

March/04		
site	Mean	
Gr08	70.8	a
Gr34	60.8	b
Gr06	1.3	c

April/04		
site	Mean	
Gr06	59.8	a
Gr08	53.7	a
Gr34	Non-est	

July/04		
site	Mean	
Gr34	60.8	a
Gr08	16.2	b
Gr06	0	c

August/04		
site	Mean	
Gr08	71.7	a
Gr06	68.8	b
Gr34	43.5	c

September/04		
site	Mean	
Gr06	104.1	a
Gr34	89.3	a
Gr08	88.1	a

October/04		
site	Mean	
Gr08	96.3	a
Gr34	93.2	b
Gr06	23.3	c

November/04		
site	Mean	
Gr08	85.8	a
Gr34	58.7	b
Gr06	46.2	c

December/04		
site	Mean	
Gr08	70.2	a
Gr06	31.8	b
Gr34	3.5	c

January/05		
site	Mean	
Gr34	78.7	a
Gr08	62.9	a
Gr06	57.8	a

February/05		
site	Mean	
Gr34	79.8	a
Gr06	65.2	b
Gr08	49.1	c

May/05		
site	Mean	
Gr08	68	a
Gr06	56.1	a
Gr34	Non-est	

Non-est = Non estimated.

Table 3. Comparison of means between months for each site. Means followed by the same letter did not differ significantly by the LSD test ($\alpha = 0.05$).

Gr06			Gr08			Gr34		
Month	Mean	Comparisons	Month	Mean	Comparisons	Month	Mean	Comparisons
Nov./03	127.90	a	Oct./04	96.30	a	Oct./04	93.20	a
Sept./04	104.10	b	Sept./04	88.10	b	Sept./04	89.30	b
Sept./03	95.10	c	Nov./04	85.80	c	Feb./05	79.80	c
Aug./04	68.80	d	Aug./04	71.70	d	Jan./05	78.70	d
Feb./05	65.20	e	Mar./04	70.80	e	Mar./04	60.80	e
Apr./04	59.80	f	Dec./04	70.20	e	Nov./04	58.70	f
Jan./05	57.80	g	May/05	68.00	f	Aug./04	43.50	g
July/03	56.90	h	Jan./05	62.90	g	July./03	20.00	h
May/05	56.10	h	Nov./03	62.70	g	Sept./03	6.60	i
Nov./04	46.20	i	July/04	60.80	h	Dec./04	3.50	j
Dec./04	31.80	j	Apr./04	53.70	i	Nov./03	0	k
Oct./04	23.30	k	Feb./05	49.10	j	Feb./04	0	k
Feb./04	16.70	l	July/03	45.00	k	Apr./04	0	k
July/04	16.20	l	Sept./03	42.00	l	July/04	0	k
Mar./04	1.30	m	Feb./04	27.40	m	May/05	0	k

it was observed that parameters BOD5, Total Phosphorus, and Ammoniacal Nitrogen, which are specific of anthropic contribution, exceeded the allowed value in Gr06 and Gr08, i.e., places used for discharge of cloacal sewage. Savage et al. (2004) concluded that wastewater is an important source of N and may contribute to the eutrophication process. This was also verified by Sundelin and Eriksson (2004) in discharges of municipal sewage that led to the increase in eutrophication level and the scarcity of oxygen in bottom sediment. According to Routh et al. (2004), organic matter is an important sediment component, due to its association with biota, nutrient cycles, and geochemical processes.

D. magna are nonselective filter feeders, which actively graze the superficial layer of the sediment with ≥48:00 hours of life (Suedel et al., 1996). Although it is a planktonic species, it spends an extensive amount of time feeding on sediment surfaces (Nebeker et al., 1984), when it functions as an epibenthic species (Burton, 1992). In these cases, they release substances that have already been sedimented, and therefore, are an important tool in the evaluation of

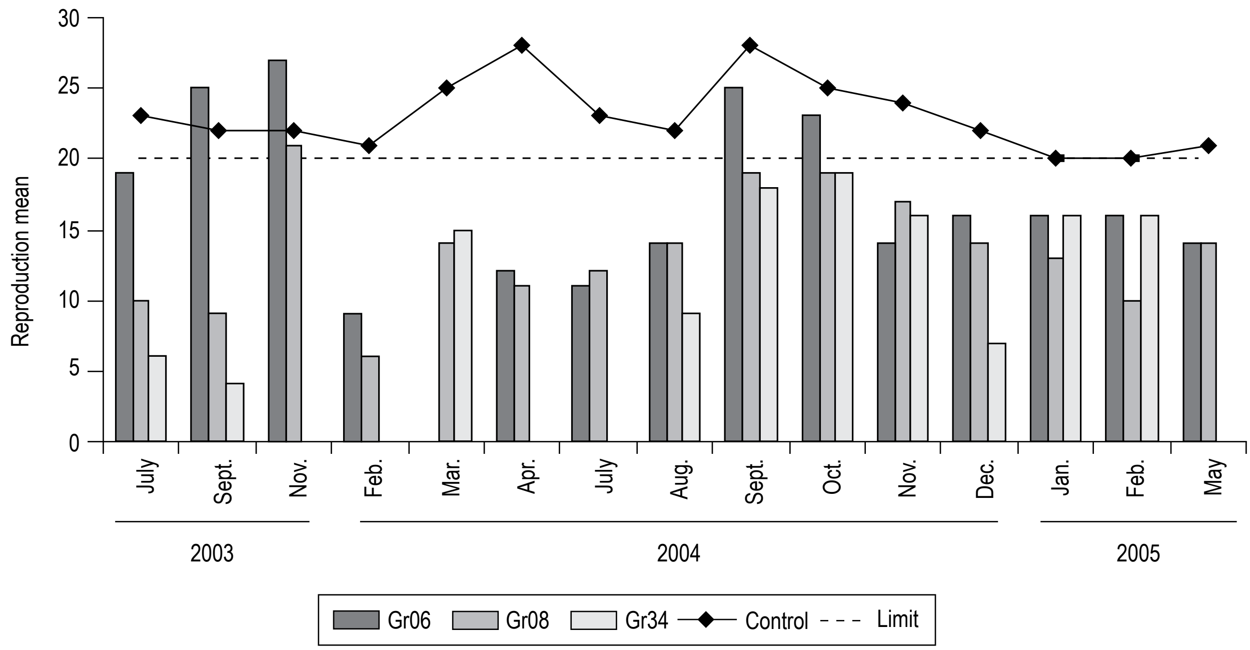

Figure 4. Reproduction mean of *Daphnia magna* exposed to Gravataí River sediment samples, control group and expected mean, from July/03 to May/05.

environmental quality. Ingersoll et al. (2000) observed that amphipods in direct contact with contaminated sediment were more severely affected than those that were in the water column; for this reason, it is important to conduct bioassays with organisms that, despite living in the water column, actively graze on sediment sample surfaces (Suedel, 1996).

In bioassays using whole-sediment, Gillis et al. (2005) found sediment particles in the gut of this water flea indicating ingestion of this material. The same authors also affirmed that, although the species feeds mainly on plankton, it ingests sediment and associated contaminants, including metals, due to the filtration of suspended material or by actively browsing on the sediment-water interface.

This behavior makes *D. magna* an important means for the identification of sediment action when the objective is to know its influence on living beings, be they planktonic, nektonic, or benthonic, since cladocerans are part of the trophic network. Factors, such as body size, growth rate, age differences and spawning, have a strong effect on the excretion and uptake of chemicals. Moreover, in general, higher metabolic activity tends to favor the absorption of substances (Zitko, 1981).

The mortality observed in sites Gr06 and Gr08 in August/04 (rains) probably occurred due to the increase in volume of the liquid mass that have risen the current revolving the riverbed, thus making available substances deposited in superficial layers of the sediment. According to Terra et al. (2004), increase in rainfall with release of substances by the current and hauling of the soil that borders Gravataí River may contribute for contamination of water.

Since sediment works as a depositary system for cumulative pollutants and a potential source of release of these contaminants or of their byproducts into the environment, adsorption of chemical agents to the organic particles may result in their deposit in the bottom of rivers. Depending on the composition of sediments, the action of organisms may present different degrees of responses for the same total quantity of chemical (DiToro et al., 1990). Moreover, high concentration of toxic compounds is not sufficient to interfere in the development of living beings, since their bioavailability is essential. This availability may be facilitated by the dragging of bottom sediment due to current force, and toxic substances may be taken to great distances or return to the liquid mass and may act on benthonic, nektonic or planktonic organisms. This is in agreement with Sundelin and Eriksson (2001), that mentioned that contaminants associated to sediment may slowly return to the water column. Comparing the results presented by this river in a previous study (Terra et al., 2004) it was observed that 2002 was the most critical period for the site Gr34 and that the results have improved in comparison to 2003; on the other hand, in Gr06, quality decreased until 2004 and recovered only in 2005.

Accumulation of Cu, Cd, Ni, Pb, and Zn is an indication of urban growth, industrial discharge, and traffic (Routh et al., 2004). It should be pointed out that these metals may be ingested and assimilated by zooplankton and, as a result, they start to have a major effect on physiology entering into the food chain or being biologically recycled (Twining and Fisher, 2004). The choice of the trophic network by the organism may interfere in the exposure and accumulation of metals (Croteau et al., 2005).

Zinc was the most frequent heavy metal found, though in only one occasion (Gr06) it was detected in a higher level than the value permitted by legislation. This metal is reported to be an inhibitor of the reproduction of water invertebrates (Winner, 1981; Terra and Schäfer, 2000). Since zinc presents a cumulative aspect, its presence may be one of the causes of the reproductive inhibition observed at different moments in Gravataí River. Zn concentration may be elevated in many rivers due to domestic and industrial discharge. Although this metal may be toxic in high concentrations, it is also an essential element to many biological functions (Muyssen and Jansen, 2001). In chronic bioassay with *Pomacea canaliculata* (Terra and Schäfer, 2000) found that reproductive activity was accelerated in water contaminated with 12.5 mg $Zn.m^{-3}$ and that there was a decrease in reproduction with 75 mg $Zn.m^{-3}$.

Another metal detected was copper; its value has also exceeded the levels permitted by legislation in the same site (Gr06). According to Cairns et al. (1984), toxic effect apparently takes place when Cu is mobilized from sediment to water. In a study conducted with *Ceriodaphnia dubia*, it was detected that reproduction was more sensitive than survival only after 14 days of exposure (Suedel et al., 1996), which indicates that assays with at least 14 days of duration are necessary for reproduction to be affected by copper.

For the correct evaluation of the data, it is important to consider the phenomenon of current inversion resulting from the action of the waters of the delta of Jacuí River (FEPAM, 1996) that penetrate into Gravataí River, diluting its waters or preventing sedimentation of substances. This inversion may increase water mass, diluting and/or dislocating pollutants with the consequent decrease in mortality observed in some samples. Nevertheless, there has been an impact on survival in Gr06, since in this stretch it suffers the influence of discharge of the entire region upstream besides punctual contaminants. Located upstream of this site, Gr08 presented the best responses for survival of the species.

The water influx contributes to the mixture of the layers of water mass, constituting an important source of dissolved and particulate material; moreover, the extension of the mixture or the dissipation of influx is controlled mainly by the magnitude and density of influx (Chen et al, 2006).

In the first year of the study, Gr34 was severely affected by local contaminants. In this site, the following were found: Cd, Pb, Cu, Cr, and Zn. It is important to point out that the Cd present in samples of Gr34 is a common metal found in contaminated waters and may substitute Zn in certain enzymes (Twining and Fisher, 2004). The same authors state that water animals may accumulate metals directly from the environment or through the ingestion of preys, and that the physiological effects vary according to the absorption route and may still enter into the trophic network or be biologically recycled. In an experiment conducted with *D. magna*, Smolders et al. (2005) found that absorption of Cd by this daphnid is proportional to the quantity and time of metal exposure, independent of the concentration of food available. Moreover, they found that this metal causes mortality in these cladocerans and decreases growth and production of neonates. Croteau et al. (2005) stated that the increase in Cd within the trophic network may be more common than expected, increasing the vulnerability of consumers at the highest trophic levels. According the Muyssen and Jansen (2001), *Ceriodaphnia dubia* has an ideal range of Cd for its metabolic functioning, and when Cd is above or below these values there may be flaws in homeostatic regulation resulting in organic toxicity or deficiency.

In the first year of the study (July/03 to July/04) only the results of one of the seven samples was within standards in Gr34. However, it was impossible to identify a direct cause besides the seasonality described for this river. Due to this feature, it is expected that Gravataí River presents more critical conditions in more extreme situations (flood or drought), as seen in February (drought) and in July and August (flood), i.e., months reported by Terra et al. (2004) as having high rainfall. The north area of Porto Alegre city presents discharges of domestic sewage and industrial effluents (Salomoni et al., 2007) and this reflects in increases in labile organic matter, characteristic of residual waters discharged in natura. These authors also report increases in BOD values as a consequence.

The low current of this river and its winding structure with many meanderings (FEPAM, 1996) may contribute to the bad quality found especially in GR034, where sedimentation of pollutants is closer to discharge. Rivers with the formation of swamps, such as this one, present elevated values of phenols and turbidity due to decomposition of the present organic matter. This can still be aggravated by anthropic contribution. The presence of complex agents as well as humic acid also affects the uptake of heavy metals (Zitko, 1981). In many samples, values for both phenols and turbidity exceed the limit allowed by Brazilian legislation (CONAMA, 2005).

Comparing the three sites under study, the differences between results show the variability of active environmental and anthropic factors. It was observed that there was sample pressure on the cladocerans mainly in Gr34 and Gr06, while in Gr08 the sum of survivals at the end of the experiment was higher than the minimum expected. Despite of being only 2 km upstream Gr06, Gr08 was less affected. Its responses showed less variation, although at many occasions levels did not reach the desired quality.

Considering time and sites, reproduction means presented a different behavior. Between september 2004 and october 2004, Gr08 showed a trend of increase in brood, decreasing in the following month, perhaps because of the concentration of pollutants in sediment, which is a natural process in the dry season.

Physical-chemical parameters (OD, BOD, Turbidity, Total Solids, Nitrogen, Phosphate) evaluated between 1992 and 1994 indicated a trend of decrease in river quality from upstream to downstream (Leite et al., 1996). Ecotoxicological bioassays showed a gradient inverse to the physical-chemical evaluations probably because these were conducted in water samples and chronic assays with *D. magna* were developed using sediment samples. Although some physical-chemical parameters are outside the standards permitted by Brazilian legislation (CONAMA, 2005), a direct relation with the biological results evaluated was not observed.

The comparison between the present limnological characteristics of Gravataí River and the existing information on previous years (Leite et al., 1996) reveals the occurrence of major changes in the past decade. These changes characterize an accelerated process of eutrophication (Salomoni et al., 2007). In the most upstream region, low reproductive frequency, mainly in the first year, probably occurred due to rice cultivated in the region and the subsequent input of pesticides applied in the cropland. In August/04, for example, the sites presented a small recovery, with Gr06 reaching the level of births expected at healthy environments.

Reproduction is more sensitive to environmental aggressions than the survival/mortality relation, thus making important its evaluation (Terra et al., 2006, 2007; Suedel et al., 1996), mainly when there is reproductive deficiency without mortality in exposed individuals. In order to complement this information, the reproductive data were explored statistically in a more detailed way and a significant difference was found between the reproductive data and the interaction between time and place.

5. Conclusion

Transferring the results obtained with the exposure of *Daphnia magna* to the biota of the studied river, it becomes evident the existence of inadequate conditions for the development of cladocerans in the studied stretch. Consequently, we emphasize the importance of environmental characterization through chronic evaluation for early identification of altered places, even if this alteration is mild. Chronic evaluations help the development of guidelines with more effective measures for the recovery of environments that are subject to human interference.

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