Potential of the tropical cladocerans *Latonopsis australis* Sars, 1888 and *Macrothrix elegans* Sars, 1901 as biomonitors of an acidic lake

Potencial de dois cladóceros tropicais *Latonopsis australis* Sars, 1888 e *Macrothrix elegans* Sars, 1901 como biomonitores de um lago acidificado

Araújo, CVM., Cohin-de-Pinho, SJ., Chastinet, CBA., Delgado Santana, F. and Da Silva, EM.

Instituto de Biologia, Universidade Federal da Bahia – UFBA,
Campus de Ondina, CEP 40170-115, Salvador, BA, Brazil
e-mail: cvmaraujo@yahoo.com.br, salpinho@gmail.com, daphnia@ufba.br,
flaviadelsan@yahoo.com.br, dasilva@ufba.br

Abstract: Two cladoceran species, *Latonopsis australis* and *Macrothrix elegans*, were used to assess and compare their potential as biomonitors of an acidic small lake (Dunas Lake, Camaçari, Bahia, Brazil). Monthly, laboratory bioassays were carried out assessing the toxicity of surface lake water, using cladoceran neonates younger than 24 hours of age. Along with the exposure, during different time intervals, organisms were observed and mortality/immobility recorded to determine the ET_{50} (median effective time) values. Dunas Lake mean ET_{50} and 95% confidence intervals values for *M. elegans* and *L. australis* were 0.97 (0.83-1.11) hours and 1.75 (1.30-2.21) hours, respectively. ET_{50} mean values showed a significant statistical difference ($p < 0.05$), thus demonstrating a higher sensibility of *M. elegans*, which seemed to be the species with more potential to be used as biomonitor in these waters. These results were then compared with bioassays carried out simultaneously using fingerlings of a secondary consumer, *Poecilia reticulata*, which has been used in the Dunas Lake monitoring program since 1994, and *M. elegans* proved again to be more sensitive and suitable as a test organism for neotropical aquatic ecosystems.

Keywords: acidic waters, acute toxicity, *Latonopsis australis*, *Macrothrix elegans*, tropical cladocerans.

Resumo: Duas espécies de cladóceros, *Latonopsis australis* e *Macrothrix elegans*, foram usadas para avaliar e comparar o potencial de ambas como biomonitores de um lago acidificado (Lagoa de Dunas, Camaçari, Bahia, Brasil). Bioensaios mensais, laboratoriais foram realizados para avaliar a toxicidade da água superficial da lagoa, usando neonatos com idade menor que 24 horas. Durante a exposição, os organismos foram observados e registrou-se a mortalidade e imobilidade para posterior cálculo da ET_{50} (tempo mediano efetivo). Os valores médios de ET_{50} e intervalos de confiança a 95% da lagoa de Dunas para *M. elegans* e *L. australis* foram 0,97 (0,83-1,11) horas e 1,75 (1,30-2,21) horas, respectivamente, com diferença significativa entre si ($p < 0,05$), demonstrando que *M. elegans* é mais sensível e parece ser mais adequado para ser usado como biomonitor em águas ácidas. Maior sensibilidade de *M. elegans* foi comprovada quando se compararam estes resultados aos bioensaios realizados simultaneamente com alevinos de *Poecilia reticulata*, o qual tem sido usado no programa de monitoramento da lagoa de Dunas desde 1994. *M. elegans* provou ser uma espécie bastante promissora como organismo-teste para o uso em estudos de ecossistemas aquáticos neotropicais.

Palavras-chave: águas ácidas, toxicidade aguda, *Latonopsis australis*, *Macrothrix elegans*, cladóceros tropicais.

1. Introduction

Ecotoxicity tests or bioassays, employed to detect deleterious effects of substances on organisms, are a powerful tool in identification, understanding, evaluation and prediction of the environmental risks of toxic compounds (Lambolez et al., 1994; Da-Silva et al., 1998). Basically, the major advantage of these tests over traditional chemical analyses is their direct assessment of biological availability and the predictability of effects on the biological communities (Manusadžianas et al., 2003). Indeed, biological and biochemical methods are more ecologically relevant, sensitive, reliable, reproducible, and easy to apply (Kapanen and Itävaara, 2001). The test choice used may be directly related with the information that is required (Rojičkova-Padrtová et al., 1998). That is, environmental risk analysis of sediments should take into account the benthic community (Moreno-Garrido et al., 2003), while, when water is the main route of uptake of the chemical compounds,

the use of an ecotoxicity test with planktonic organisms is recommended (Walker et al., 2001). The use in the neotropical region of standardised tests originally developed for temperate climates, using *Daphnia* species, for instance, (ISO, 1996; OECD, 1996; ABNT, 1993), which are not found in neotropical ecosystems, seems to be inappropriate. Indeed, Lopes et al. (2004) demonstrated the genetic erosion of local cladoceran population exposed to acid mine drainage (AMD), confirming the importance of the use of local organisms in risk assessment analysis. Therefore, the application of traditional cladoceran bioassays using *D. magna* or *D. similis* lacks ecological relevance in tropical waters, inducing errors in risk assessment studies (Oliveira-Neto and Botta-Paschoal, 2000), as the experimental conditions do not reproduce tropical and subtropical ecosystems variables (Lacher-Jr. and Goldstein, 1997), and community response is different. Indeed, results extrapolation and the prediction of the impacts derived from the use of temperate organisms in tropical conditions are inadequate as climatic, edaphic, physical, chemical characteristics and biological diversity are distinct from region to region (Preza and Smith, 2001). In spite of that, tropical ecotoxicology is still largely influenced by methods and techniques developed in temperate ecosystems (Lacher-Jr. and Goldstein, 1997). Nevertheless, it is very important to understand the restrictions and potentials of bioassays (Kapanen and Itävaara, 2001). Different kinds of organisms are not equally susceptible to the same toxic substances (Pardos et al., 1999; Hadjispyrou et al., 2001). Thus, it seems to be inadequate to standardise a bioassay to universal use, being more important to seek a species more sensitive and relevant to studies of a specific site (Gray, 1989).

Tropical aquatic ecotoxicology must create its own methodology because tropical aquatic ecosystems contain a large part of world biodiversity (Lacher-Jr. and Goldstein, 1997), and is the place where biodiversity is at highest risk (Primack and Rodrigues, 2001). Within the neotropical region, in Brazil, the largest country, there is an urgent need to develop ecotoxicological studies using tropical species in order to establish water quality standards (Knie and Lopes, 2004; CONAMA, 2005; Bertoletti and Zagatto, 2006). Lewinsohn and Prado (2002) stated that 14% of the world's species can be found in Brazil. However, most of this extraordinary biodiversity is still poorly known, as the precise number of species in Brazilian inland waters is unknown and difficult to estimate: numerous hydrographic basins have never been sampled; the number of researchers and the infrastructure required for sampling and monitoring are insufficient; aquatic inventories have, until recently, been few; information is scattered and often difficult to access; and a number of groups need major taxonomic revisions (Agostinho et al., 2005).

Cladocerans have been the preferred aquatic organism to be used in aquatic ecotoxicity tests (Gray, 1989; Baird et al., 1991; Soares et al., 1992; Barata et al., 2002), especially because these organisms dominate the water column in temperate lakes and in some cases are responsible for the total clearing of the water due to their overgrazing activity as primary consumers (Moss, 1998). Moreover, cladocerans present a continuous availability of neonates due to their parthenogenetic reproduction, easiness to work with and low costs to keep laboratory cultures, a high sensitivity to an array of toxic substances and high reproducibility of results for similar clones (Soares and Calow, 1993). *Latonopsis australis* (Cladocera, Sididae) and *Macrothrix elegans* (Cladocera, Macrothricidae) are quite representative of the zooplankton in tropical aquatic ecosystems (Korovchinsky, 1992; Güntzel et al., 2003, 2004).

According to Da-Silva et al. (2000), in the early 90's large quantities (ca. 34 t) of both industrial and domestic solid waste including sulphur, iron, titanium dioxide and ilmenite residues were deposited on the dunes adjacent to Dunas Lake (Camaçari, BA, Brazil). After contamination, a rehabilitation program was carried out (1992-1993) to recover ground- and surface water quality and reduce contamination (Gomes, 1994; Da-Silva et al., 1999b). Initially, the residues were partially removed and the contaminated dune was sealed with impermeable layers of clay and topsoil (hydraulically encapsulated). An additional action was to pump the groundwater to reduce the contaminated plume (Gomes, 1994; Da-Silva et al., 1999b, 2000). The results of the established biomonitoring program, including bioassays with guppy fingerlings (*P. reticulata*), were described by Da-Silva et al. (1999a, 2000) and Araújo et al. (2006).

The aims of this study were: i) to assess and compare the sensibility of *L. australis* and *M. elegans* in toxicity acute bioassays; ii) to assess the potential of the selected species as biomonitors in acidic freshwater; and iii) to choose the species to be incorporated into biomonitoring studies of Dunas Lake.

2. Materials and Methods

2.1. Study site and process contamination

The acidic lake (Dunas Lake) is located in Camaçari (BA, Brazil) (Figure 1) between geographic coordinates 12° 48' 09" to 12° 48' 12.3" S and 38° 13' 09" to 38° 13' 14" W, lying within a depression, forming a narrow and shallow body of freshwater between dunes along the Atlantic Ocean (Silva et al., 2000).

2.2. Sampling

Monthly sampling of the Dunas Lake, during August 2003 to June 2004, except in September and October 2003 (n = 9), were carried out. Water surface samples were transported to the laboratory without any further treatment and kept at 4 ± 1 °C until the assay day when they were

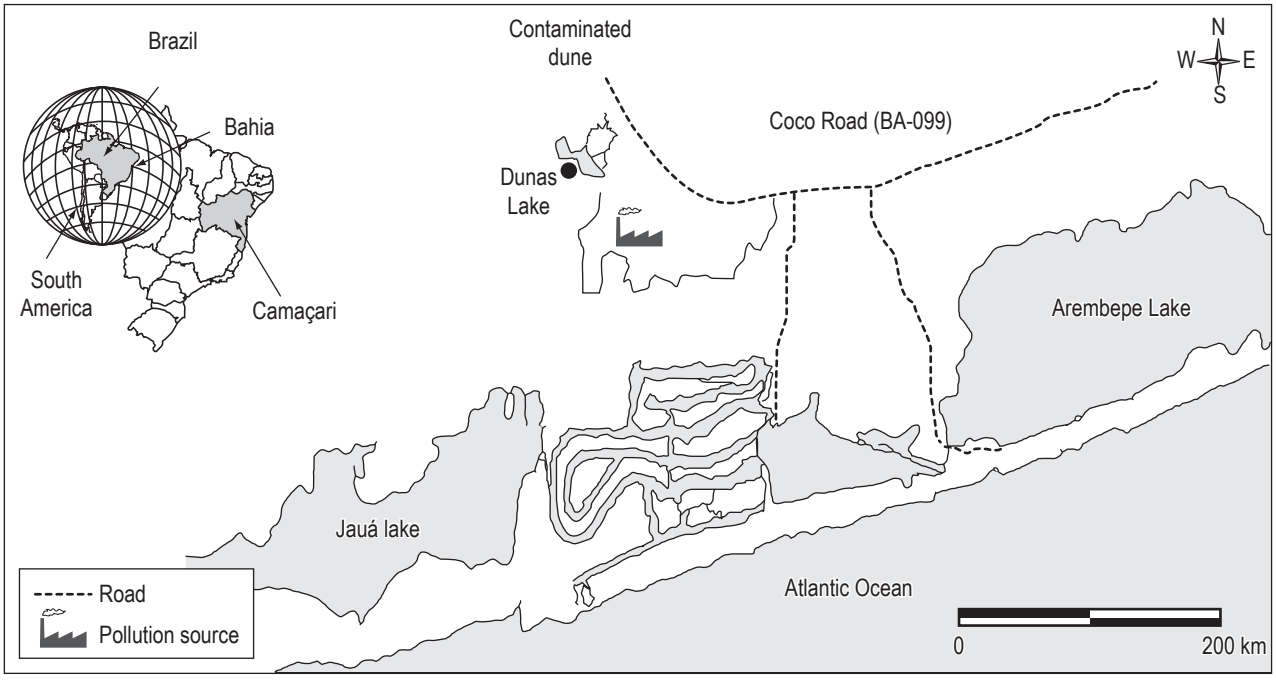

Figure 1. Location of the study site (lake) between dunes and the adjacent wetlands.

removed from the refrigerator and left outside to reach laboratory temperature (25 °C).

2.3. Culture and acclimation

L. australis and *M. elegans* were collected from Capivari Creek, at Cruz das Almas (BA, Brazil), and isolated from submersed fronds of the floating aquatic fern, *Salvinia oblongifolia* (Andrade, 2003). Organisms have been maintained in the laboratory since 2001, in 500 mL glass flasks, with Capivari Creek glass fibre filtered water at a temperature of 25 ± 1 °C and photoperiod of 12:12 hours (light:dark), containing 150 to 200 individuals. Organisms were fed ad libitum with an algal suspension of *Pseudokirchineriella subcapitata* (final density of 1×10^6 cells.L^{-1}), in alternated days.

2.4. Bioassays with Cladocera

The methodology adopted followed standard procedure, with minor changes, to the *Daphnia similis* bioassays (ABNT, 1993) and Andrade's work (2003). Bioassays were performed using only neonates (<24 hours old) in 50 mL glass vessels with 40 mL of Dunas Lake sample, which was not treated or diluted. To each sample, three replicates were established, with four to five organisms in each, randomly distributed. A total of 18 bioassays were carried out under the same culture condition. Time was the independent variable, and during bioassays organisms were checked for mortality/immobility each 10 minutes. Dead organisms were counted and removed immediately to avoid adverse effect due to decomposition. Organisms were considered dead when they remained immobile during 15 seconds of gentle prodding with plastic Pasteur pipette. Bioassays were carried out until the last organism died or the 48 hours period was completed. Capivari Creek water, in which the organisms have been cultured since 2001, was used as control due to the better development of theses organisms in this medium in relation to other synthetic media (Andrade, 2003).

2.5. Physical and chemical analysis

Dissolved oxygen content (WTW, Inolab Oxi Level 2), water hardness (APHA, 1998), pH (Digi-Sense, Cole Parmer) and conductivity (Hanna HI 9033) of the Capivari Creek water and Dunas Lake samples at the beginning and the end of the experiments were measured. The results were validated when there was no pH variation higher than 10% of initial pH value, according to Ribeiro et al. (2000). Physical-chemical characterisation of the Capivari Creek and Dunas Lake water was carried out (Table 1). Metal analyses were carried out using inductively coupled plasma atomic emission spectrometry (ICP-AES) technique. For Cr(VI), nitrate, nitrite, phosphate and sulphate concentrations were determined using ion chromatography (IC) (APHA, 1998).

2.6. Culture, acclimation and bioassay with P. reticulata

Results with cladocerans were compared to those obtained using fingerlings of the guppy, *Poecilia reticulata* fish, species used in the biomonitoring of the Dunas Lake since 1994 (Silva et al., 1999a; Araújo et al., 2006). Guppy fin-

Table 1. Physical and chemical characterisation of the Capivari Creek and Dunas Lake water ($mg.L^{-1}$).

Parameter	Capivari Creek	Dunas Lake	Parameter	Capivari Creek	Dunas Lake
SO_4	3.7	113.7	Total-Fe	0.51	0.65
Cr(VI)	<0.01	<0.01	Dissolved-Fe	0.49	0.64
NO_2	<0.01	<0.01	Total-K	3.5	0.78
NO_3	0.34	<0.01	Total-Mg	4.2	2.6
Phosphate	<1.0	<1.0	Total-Mn	<0.005	0.5
Total-Al	0.18	1.5	Total-Na	28.0	6.7
Total-Ca	2.1	19.0	Total-Ni	n.a.	<0.016
Total-Cd	<0.005	<0.005	Total-P	<0.33	<0.33
Total-Co	<0.01	<0.01	Total-Pb	n.a.	<0.14
Total-Cr	<0.01	<0.01	Total-Ti	n.a.	0.05
Total-Cu	<0.005	0.014	Total-Zn	<0.01	<0.01

n.a.: not analysed.

gerlings, 13 to 21 days old (average length of 1.0 ± 0.2 cm) were obtained from a local aquarist who kept the fish under standardised conditions. In spite of the recommendation by OECD (1992) and ABNT (2002) to use adult *P. reticulata* in acute and static bioassays, fingerlings in early life stages were employed since the early life stages of fish are in many cases the most sensitive to adverse conditions (Farag et al., 1993; Petersen and Kristensen, 1998), especially under pH changes (Vuorinen et al., 2003). The organisms were transported to the laboratory in plastic bags with sufficient air, and acclimated in 20 L glass aquaria of dechlorinated tap water for at least 24 hous prior to the experiments. The fish were not fed during this period or during the experiment. Acclimation and bioassays were performed at 25 ± 1 °C, in constant temperature rooms with photoperiod of 12:12 hours (light:dark). Static acute toxicity assays were carried out according to OECD (1992) and ABNT (2002). Dechlorinated tap water, in which the fish had been reared, was used as control. In all experiments, adequate measures were taken to minimise pain or discomfort and animals were handled according to the recommendation of Baumans (2005).

Glass aquaria of 1.2 L capacity, containing 900-1000 mL of samples were used as test vessels. Five replicates of eight to ten fish each were tested, totalling 40-50 organisms exposed to each sample. The organisms were randomly distributed in the test vessels and fish mortality was checked at reduced time intervals, 10 minutes in the first three hours of the tests, 30 minutes of the third until the tenth hour, and at longer time intervals in the following hours. Time was the independent variable, and samples were not diluted. Dead fish were counted and removed immediately to avoid adverse effects due to the decomposition. Organisms were only considered dead when operculum and gill movements had ceased and there was no swimming response after stimulation with a plastic Pasteur pipette. For all bioassays types a lower mortality rate than 10% in the control was taken into account.

2.7. Data analysis

Median effective time (ET_{50}) was determined by Probit Analysis (Finney, 1971). To compare the mean values of the series, an analysis of variance (One-way ANOVA), followed by Tukey multiple comparison test (Zar, 1996), was carried out. Differences were considered significant at $p < 0.05$ (Zar, 1996), and all values are given as means and 95% confidence interval (95%-CI).

3. Results

Dissolved oxygen values of the Dunas Lake samples, in all bioassays, were always higher than 6.5 $mg.L^{-1}$. In general, dissolved oxygen values registered in the culture water (Capivari Creek) was about 6.0 $mg.L^{-1}$ (equivalent to 71.4% oxygen saturation), demonstrating that the oxygen levels of the samples were acceptable. Samples mean values of pH, conductivity and total hardness, and their respective 95%-CI are summarised in the Table 2.

There was no mortality in the control during the bioassays with cladoceran, and survival in all bioassays control with *P. reticulata* was greater than 90%, thus accomplishing the recommendations from OECD (1992) and ABNT (1993, 2002) to cladoceran, *D. magna* and *D. similis*. Dunas Lake mean ET_{50} and 95%-CI to *L. australis* was 1.75 (1.30-2.21) hours, however, to *M. elegans* these ET_{50} were 0.97 (0.83-1.11) hours. Results demonstrated a significant difference ($p < 0.05$) between the sensitivity of these two species (Figure 2). The cladoceran bioassay results were compared with those obtained with *P. reticulata*, which has been used in the site as biomonitor for over a decade (Silva et al., 1999a, 1999b; Araújo et al., 2006). Mean value and 95%-CI of Dunas Lake ET_{50} to *P. reticulata* was 1.63 (1.21-2.04) hours. In fact, *P. reticulata* presents equivalent sensitivity ($p > 0.05$) to *L. australis*, while, on

Table 2. Mean values (n = 9), and respective 95%-CI, of pH, conductivity and total hardness of the control and Dunas Lake samples of the bioassays with *L. australis*, *M. elegans* and *P. reticulata*.

Parameters	Organisms	Samples	
		Control	Dunas Lake
pH	*L. australis*	6.83 (6.63-7.14)	3.10 (3.00-3.19)
	M. elegans	6.77 (6.57-7.14)	3.10 (3.02-3.18)
	P. reticulata	7.24 (6.80-7.69)	3.07 (3.01-3.13)
Conductivity ($\mu S.cm^{-1}$)	*L. australis*	255.40 (241.60-269.20)	335.80 (315.91-355.69)
	M. elegans	249.40 (231.70-267.10)	332.80 (314.37-351.23)
	P. reticulata	356.11 (319.86-392.36)	353.44 (336.09-370.80)
Total hardness (mg $CaCO_3.L^{-1}$)	*L. australis*	24.86 (24.37-25.34)	83.91 (77.69-90.14)
	M. elegans	24.85 (24.33-25.36)	84.20 (76.60-91.80)
	P. reticulata	115.44 (103.38-127.51)	82.78 (76.42-89.14)

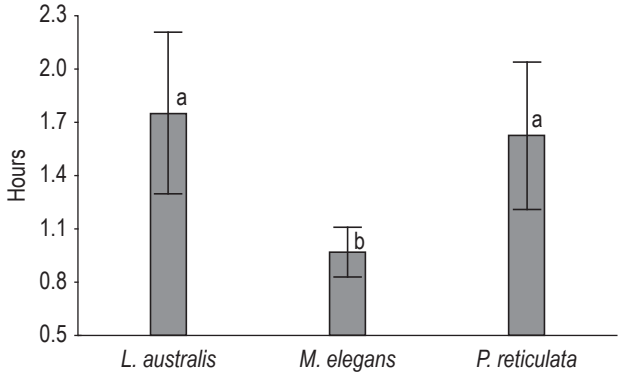

Figure 2. Mean values (n = 9) and respective 95%-CI of ET_{50} of the bioassays with *L. australis*, *M. elegans* and *P. reticulata*, exposed to Dunas Lake samples. Means followed by the same letter do not differ significantly ($p < 0.05$).

the other hand, *M. elegans* bioassays were more sensitive in relation to *P. reticulata* ($p < 0.05$) (Figure 2). *M. elegans* ecotoxicity results were more sensitive and also presented lower variability, therefore being more precise. Variation coefficient of the bioassays with *M. elegans* was 18.55%, while with *L. australis* and *P. reticulata* were 33.71 and 32.51%, respectively.

4. Discussion

According to the values presented in the Table 2, and due to the bioassay had been carried out at the same time, it can be certain that the organisms were exposed to same conditions, being possible to compare the results. The pH values of the Dunas Lake samples during the study were extremely low. According to Araújo et al. (submitted), *P. reticulata* bioassays were able to demonstrate that the acidity (low pH) is the main factor of toxicity in Dunas Lake. On the other hand, conductivity and hardness values may also have influenced the final toxicity for cladocerans, seeing as they were relatively high in relation to control samples (Andrade, 2003; Araújo, 2005; Cohin-de-Pinho, 2006).

M. elegans bioassays seemed to be more advantageous to be applied in the biomonitoring program at acidified ecosystems, especially in the Dunas Lake program. Ecotoxicity results with this species were more sensitive and also presented lower variability. Variation coefficient values presented by *M. elegans* bioassays reveal quite acceptable repeatability (Dave, 1993; Araújo and Nascimento, 1999; Preza and Smith, 2001; Abessa and Sousa, 2003), thus confirming results of Cohin-de-Pinho (2006), that also found similar variation coefficient in bioassays with *M. elegans*. Although the choice of the test organism is unquestionably a crucial point in ecotoxicology, the sensitivity varies according to geographical region; therefore the standardisation of a universal bioassay method is often inappropriate (Gray, 1989). In many studies, the bioassays have been carried out with organisms that can be easily collected, cultured and tested; the ecological significance being a secondary factor (Chapman, 2002). The use of high relevance species to tropical ecosystems allows higher autonomy to tropical ecotoxicology, not being only an extension of methods development in temperate countries (Lacher-Jr. and Goldstein, 1997), as the differences in sensitivity and ecology between tropical and temperate species favour the search to found more appropriate ecotoxicological methodologies. Although the toxic response of the organisms that belong to the same biological groups have similar sensitivity (Aragão and Araújo, 2006), intraspecific differences can be significant due to toxicokinectic factors (Walker et al., 2001). Recently, there has been increased interest in the use of ecologically relevant bioassays with autochthonous species, mainly in Brazil (Araújo and Nascimento, 1998; Preza and Smith, 2001; Abessa and Sousa, 2003; Cohin-de-Pinho, 2006; Maia et al., 2006; Niemeyer et al., 2006), as these results are more relevant in relation to local impact potential and offer the possibility to establish regional standards necessary for each area (Preza and Smith, 2001).

L. australis lives in the tropics and subtropics of Africa, America, Asia and Australia, mainly in the littoral zone of lakes and reservoirs, in ponds, temporary water bodies, but can also occur in some temperate regions (e.g.: Northern Italy, Yugoslavia, Bulgaria, North of USA – Wisconsin State) (Korovchinsky, 1992; Elmoor-Loureiro, 1997). According to Korovchinsky (1992), *L. occidentalis* Birge, 1892 and *L. breviremis* Daday, 1905 are synonymous of *L. australis*. In relation to the life cycle in our laboratory, the maximum longevity obtained to 25 ± 1 °C, with photoperiod of 12:12 hours (light:dark), was approximately of 30 days (Araújo, 2005). On the other hand, *M. elegans* lives associated to aquatic macrophytes, has scraper feeding habits, and can occur sporadically as a planktonic organism (Elmoor-Loureiro, 1997). According to Güntzel et al. (2003, 2004), this species lives in tropical water bodies, and has a life cycle of 27 days at a temperature of 23 °C. However, Andrade (2003) and Cohin-de-Pinho (2006) registered a maximum longevity of 40 days, cultivating this species under the same condition. According to Güntzel et al. (2004), *M. flabelligera* Smirnov, 1992 and *M. triserialis* Brady, 1886 may be synonymous of *M. elegans*.

Within the neotropical landscape lakes are not the dominant aquatic feature and inundated floodplains are more common (Serafim Jr. et al., 2003). In these plains, macrophyte plants are a much more important source of organic matter than phytoplankton and thus water column zooplankter are not so important. Indeed, organisms associated with aquatic macrophytes root systems, such as *M. elegans* and other cladocerans, play a more decisive role on the organic matter recycling (Moss, 1998; Nandini et al., 2004; 2005). Due to its short life cycle, high frequency of reproduction, easy maintenance in laboratory cultures and suitability as a test-organism (this study and also, Cohin-de-Pinho, 2006), the *M. elegans* bioassay may be suggested as a potential test to be standardised for neotropical aquatic ecosystems.

5. Conclusion

L. australis and *M. elegans* acute bioassays seemed to be useful in ecotoxicological assessment. Due to the continuous availability of their neonates, high sensibility, short time bioassays, and ecological relevance, these species have demonstrated a strong potential to be used as surrogates for the ecotoxicological investigation of neotropical systems.

M. elegans may be preferentially adopted as test organism in the Dunas Lake biomonitoring plan, as well as other acidified ecosystems, as bioassays with this species were more sensitive and precise. On the other hand, both species may be further used in other studies and under a variety of contamination conditions to better assess their potential as biomonitors. These results represent a first step to possible standardisation of the *M. elegans* acute bioassay to provide information on the response of a regional species of neotropical freshwater ecosystems.

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References

ABESSA, DMS. and SOUSA, ECPM. Sensitivity of the amphipod *Tiburonella viscana* (Platyischnopidae) to $K_2Cr_2O_7$. *Braz. Arch. Biol. Technol.*, 2003, vol. 46, no. 1, p. 53-55.

ABNT – Associação Brasileira de Normas Técnicas. *NBR 12713.* Água - Ensaio de toxicidade aguda com Daphnia similis Claus, 1876 (Crustacea, Cladocera). Rio de Janeiro: ABNT, 1993. 16 p.

ABNT – Associação Brasileira de Normas Técnicas. *NBR 12714.* Água - Ensaio de toxicidade aguda com peixes – Parte I – Sistema estático. Rio de Janeiro: ABNT, 2002. 15 p.

AGOSTINHO, AA., THOMAZ, SM. and GOMES, LC. Conservation of the Biodiversity of Brazil's inland waters. *Conservation Biology*, 2005, vol. 19, no. 3, p. 646-652.

ANDRADE, AMS. *Estudo sobre a biologia de Macrothrix elegans Sars (1901) (Crustacea: Anomopoda) e uma avaliação sobre sua sensibilidade ao dicromato de potássio. ($K_2Cr_2O_7$).* Salvador: Universidade Federal da Bahia – UFBA, 2003. 49 p. [Monograph].

APHA. *Standard methods for examination of water and wastewater.* 20 ed. Baltimore: American Public Health Association; Port City Press, 1998. 1190 p.

ARAGÃO, MA. and ARAÚJO, RPA. Métodos de ensaios de toxicidade com organismos aquáticos. In: Zagatto, PA. et al. *Ecotoxicologia aquática – princípios e aplicações.* São Carlos: RiMa, 2006. p. 117-152.

ARAÚJO, MMS. and NASCIMENTO, IA. Testes ecotoxicológicos: análise de sensibilidade. *Ecotoxicol. Environ. Restor.*, 1999, vol. 2, no. 1, p. 41-47.

ARAÚJO, CVM. Av*aliação ecotoxicológica da Lagoa de Dunas (Camaçari, Bahia, Brasil)*. Salvador: Universidade Federal da Bahia – UFBA, 2005. 65 p. [Master Thesis].

ARAÚJO, CVM., COHIN-DE-PINHO, SJ., DELGADO, F., SANTANA, LCS., CHASTINET, CBA. and SILVA, EM. In situ and laboratory bioassays using *Poecilia reticulata* Peters, 1859 in the biomonitoring of an acidic lake at Camaçari, BA, Brazil. *Chemosphere*, 2006, vol. 65, no. 4, p. 599-603.

ARAÚJO, CVM., COHIN-DE-PINHO, SJ., CHASTINET, CBA., SANTOS, JS. and SILVA, EM. Discriminating the

pH toxicity to Poecilia reticulata Peters, 1859 in the Dunas Lake (Camaçari, BA, Brazil). *Chemosphere,* in press.

BAIRD, DJ., BARBER, MB., SOARES, AMVM. and CALOW, P. A comparative study of genotype sensitivity to acute toxic stress using clones of *Daphnia magna* Straus. *Ecotox. Environ. Saf.,* 1991, vol. 21, no. 3, p. 257-265.

BARATA, C., BAIRD, DJ., MITCHELL, SE. and SOARES, AMVM. Among and within population variability in tolerance to cadmium stress in natural populations of *Daphnia magna*: implications of ecological risk assessment. *Environ. Toxicol. Chem*., 2002, vol. 21, no. 5, p. 1058-1064.

BAUMANS, V. Science-based assessment of animal welfare: laboratory animals. *Rev. Sci. Tech. Off. Int. Epiz.*, 2005, vol. 24, no. 2, p. 503-514.

BERTOLETTI, E. and ZAGATTO, PA. Aplicação dos ensaios ecotoxicológicos e legislação pertinente. In: Zagatto, PA. et al. *Ecotoxicologia aquática – princípios e aplicações.* São Carlos: RiMa, 2006. p. 347-382.

CHAPMAN, PM. Integrating toxicology and ecology: putting the "eco" into ecotoxicology. *Mar. Pollut. Bull.*, 2002, vol. 44, no. 1, p. 7-15.

COHIN-DE-PINHO, SJ. *Estudo da viabilidade do cladócero tropical Macrothrix elegans Sars, 1901 (Cladocera, Macrothricidae) como organismo-teste em ensaios ecotoxicológicos*. Salvador: Universidade Federal da Bahia – UFBA, 2006. 40 p. [Master Thesis].

CONAMA – Conselho Nacional do Meio Ambiente. *Resolução CONAMA nº 357*, 17 de Março de 2005. Brasília: Ministério do Meio Ambiente, 2005. 23 p.

DA-SILVA, EM., SOARES, AMVM., SOBRAL, OMF., LOPES, IMCA., CORREIA, JFJS., MARCHANTE, EMDC., CHASTINET, CBA. and MORENO, AJM. Ecotoxicological responses of isolated mitochondrial systems to complex effluents. Are they worthwhile? *Chemosphere*, 1998, vol. 37, no. 13, p. 2695-2701.

DA-SILVA, EM., NAVARRO, MFT., BARROS, AF., MOTA, MFV. and CHASTINET, CBA. The utilisation of *Poecilia reticulata* as a biomonitor in the environmental recovery of an aquatic ecosystem. *Ecotoxicol. Environ. Restor.*, 1999a, vol. 2, no. 2, p. 51-55.

DA-SILVA, EM., BARROS, AF., NAVARRO, MFT., MOTA, MFV., COTSIFIS, P. and CHASTINET, CBA. Rehabilitation following industrial contamination: Jauá Lake, a coastal wetland in Camaçari, Bahia, Brazil. In: Streever, W. *An international perspective on wetland rehabilitation.* Netherlands: Kluver Academic Press, 1999b. p.197-203.

DA-SILVA, EM., NAVARRO, MFT., BARROS, AF., MOTA, MFV. and CHASTINET, CBA. Metals in the sediments of Jauá Lake (Camaçari, Bahia, Brazil) following an episode of industrial contamination. *Aquat. Ecosys. Health Manag.*, 2000, vol. 3, p. 509-514.

DAVE, G. Replicability, repeatability, and reproducibility of embryo-larval toxicity tests with fish. In: Soares, AMVM. and Calow, P. *Progress in standardization of aquatic toxicity tests*. Boca Raton: Lewis Pub., 1993. p. 129-157.

ELMOOR-LOUREIRO, LMA. *Manual de identificação de cladóceros límnicos do Brasil*. Brasília: Universa, 1997. 156 p.

FARAG, AM., WOODWARD, DF. and LITTLE, EE. The effects of low pH and elevated aluminum on yellowstone cutthroat trout (*Oncorhynchus clarki bouvieri*). *Environ. Toxicol. Chem*., 1993, vol. 12, no. 4, p. 719-731.

FINNEY, DJ. *Statistical Method in Biological Assay*. London: Charles Griffin and Co., 1971. 668 p.

GOMES, DC. *Poluição de aquífero costeiro de Arembepe-BA por ácido sulfúrico e compostos inorgânicos oriundos da produção de dióxido de titânio*. São Paulo: Universidade de São Paulo – USP, 1994. 116 p. [Master Thesis].

GRAY, JS. Do bioassays adequately predict ecological effects of pollutants? *Hydrobiologia*, 1989, vol. 188/189, no. 1, p. 397-402.

GÜNTZEL, AM., MATSUMURA-TUNDISI, T. and ROCHA, O. Life cycle of *Macrothrix flabeligera* Smirnov, 1992 (Cladocera, Macrothricidae), recently reported in the Neotropical region. *Hydrobiologia*, 2003, vol. 490, 1-3, p. 87-92.

GÜNTZEL, AM., MATSUMURA-TUNDISI, T. and ROCHA, O. 2004. *Macrothrix flabeligera* a newly-recorded Cladocera Macrothricidae in Brazilian freshwaters. *Braz. J. Biol = Rev. Bras. Biol.*, 2004, vol. 64, no. 2, p. 1-6.

HADJISPYROU, S., KUNGOLOS, A. and ANAGNOSTOPOULOS, A. 2001. Toxicity, bioaccumulation, and interactive effects of organotin, cadmium, and chromium on *Artemia franciscana*. *Ecotox. Environ. Saf.*, 2001, vol. 49, no. 2, p. 179-186.

International Organization for Standardization. ISO/6341: 1996. *Water Quality - Determination of the inhibition of mobility of Daphnia magna Straus (Cladocera – Crustacea).* Geneva, 1996. 12 p.

KAPANEN, A. and ITÄVAARA, M. Ecotoxicity tests for compost applications. *Ecotox. Environ. Saf.*, 2001, vol. 49, no. 1, p. 1-16.

KNIE, JLW. and LOPES, EWB. Test*es Ecotoxicológicos*: métodos, técnicas e aplicações. Florianópolis: FATMA/GTZ, 2004. 288 p.

KOROVCHINSKY, NM. *Sididae and Holopediidae. Guides to the identification of microinvertebrates of the continental waters of the world. n. 3.* The Hague, Netherlands: SPB Academic Publishing, 1992. 82 p.

LACHER-Jr., TE. and GOLDSTEIN, MI. Tropical ecotoxicology: status and needs. *Environ. Toxicol. Chem*., 1997, vol. 16, no. 1, p. 100-111.

LAMBOLEZ, L., VASSEUR, P., FERARD, JF. and GISBERT, T. The environmental risks of industrial waste disposal: an experimental approach including acute and chronic toxicity studies. *Ecotoxicol. Environ. Saf.*, 1994, vol. 28, no. 3, p. 317-328.

LEWINSOHN, TM. and PRADO, PI. Biodiversity of Brazil: a synthesis of the current state of knowledge. In: Lewinsohn, TM. and Prado, PI. *Biodiversidade brasileira: síntese do estado*

do conhecimento atual. São Paulo: Contexto Acadêmica, 2002. p. 139-144.

LOPES, I., BAIRD, DJ. and RIBEIRO, R. Genetic determination of tolerance to lethal and sublethal copper concentrations in field populations of *Daphnia longispina*. *Arch. Environ. Contam. Toxicol.*, 2004, vol. 46, no. 1, p. 43-51.

MAIA, CB., ALMEIDA, ACM. and MOREIRA, FR. Avaliação do teor de chumbo em mexilhões da espécie *Perna perna* na região metropolitana da cidade do Rio de Janeiro. *J. Braz. Soc. Ecotoxicol.*, 2006, vol. 1, no. 2, p. 195-198.

MANUSADŽIANAS, L., BALKELYTE, L., SADAUSKAS, K., BLINOVA, I., PÕLLUMAA, L. & KAHRU, A. Ecotoxicological study of Lithuanian and Estonian wastewaters: selection of the biotests, and correspondence between toxicity and chemical-based indices. *Aquat. Toxicol.*, 2003, vol. 63, no. 1, p. 27-41.

MORENO-GARRIDO, I., HAMPEL, M., LUBIÁN, LM. and BLASCO, J. Sediment toxicity tests using benthic marine microalgae *Cylindrotheca closterium* (Ehremberg) Lewin and Reimann (Bacillariophyceae). *Ecotoxicol. Environ. Saf.*, 2003, vol. 54, no. 3, p. 290-295.

MOSS, B. Shallow lakes, biomanipulation and eutrophication. *Scope Newsletter*, 1998, vol. 29, p. 1-27.

NANDINI, S., AGUILERA-LARA, D., SARMA, SSS. and RAMÍREZ-GARCÍA, P. The ability of selected cladoceran species to utilize domestic wastewaters in Mexico City. *J. Environ. Manage.*, 2004, vol. 71, no. 1, p. 59-65.

NANDINI, S., HERNÁNDEZ VALDEZ, M. and SARMA, SSS. Life History Characteristics of Cladocerans (Cladocera) Fed on Wastewaters. *Acta Hydrochim. Hydrobiol.*, 2005, vol. 33, no. 2, p. 133-141.

NIEMEYER JC., DOS-SANTOS, VC., RODRIGUES, JML. and DA-SILVA, EM. Comportamento de *Cubaris murina* Brandt (Crustacea: Isopoda) em solo com glifosato: testes de fuga em laboratório. *J. Braz. Soc. Ecotoxicol.*, 2006, vol. 1, no. 1, p. 13-16.

OECD - Organisation Economic Co-operation and Development. Guideline for testing of chemicals. *Fish Acute Toxicity Test.* Paris, 1992. 9 p.

OECD - Organisation Economic Co-operation and Development. *Daphnia sp. acute immobilization test and reproduction test, Method 202.* Paris, 1996. 16 p.

OLIVEIRA-NETO, AL. and BOTTA-PASCHOAL, CMR. Sensibilidade do cladocera lacustre planctônico Ceriodaphnia silvestrii (Família Daphnidae) aos metais cádmio, cromo e chumbo. In: Espíndola, ELG., Botta-Paschoal, CMR., Rocha, O., Bohrer, MBC. and Oliveira-Neto, AL. (Eds). *Ecotoxicologia*: Perspectivas para o século XXI. São Carlos: RiMa, 2000. p. 537-543.

PARDOS, M., BENNINGHOFF, C., GUÉGUEN, C., THOMAS, R., DOBROWOLSKI, J. and DOMINIK, J. Acute toxicity assessment of Polish (waste) water with a microplate-based *Hydra attenuata* assay: a comparison with the Microtox® test. *Sci. Total Environ.*, 1999, vol. 243/244, p. 141-148.

PETERSEN, G. and KRISTENSEN, P. Bioaccumulation of lipophilic substances in fish early life stage. *Environ. Toxicol. Chem.,* 1998, vol. 17, no. 7, p. 1385-1395.

PREZA, DLC. and SMITH, DH. Use of newborn *Giardia tigrina* (Girard, 1850) in acute toxicity tests. *Ecotoxicol. Environ. Saf.*, 2001, vol. 50, no. 1, p. 1-3.

PRIMACK, RB. and RODRIGUES, E. *Biologia da Conservação.* Londrina, Brasil: Livraria Conceito, 2001. 328 p.

RIBEIRO, R., LOPES, I., PEREIRA, AMM., GONÇALVES, F. and SOARES, AMVM. Survival time of *Ceriodaphia dubia* in acid waters with metal contamination. Bull. *Environ. Contam. Toxicol.*, 2000, vol. 64, no. 1, p. 130-136.

ROJIČKOVA-PADRTOVÁ, R., MARŠÁLEK, B. and HOLOUBEK, I. Evaluation of alternative and standard toxicity assays for screening of environmental samples: selection of an optimal test battery. *Chemosphere*, 1998, vol. 37, no. 3, p. 495-507.

SERAFIM-Jr., M., LANSAC-TÔHA, FA., PAGGI, JC., VELHO, LFM. and ROBERTSON, B. Cladocera fauna composition in a river-lagoon system of the upper Paraná river floodplain, with a new record for Brazil. *Braz. J. Biol = Rev. Bras. Biol.,* 2003, vol. 63, no. 2, p. 349-356.

SOARES, AMVM, and CALOW, P. *Progress in Standardization of Aquatic Toxicity Tests.* Michigan: Lewis Publishers, 1993. 288 p.

SOARES, AMVM., BAIRD, DJ. and CALOW, P. Interclonal variation in the perfomance of *Daphnia magna* Straus in chronic bioassays. *Environ. Toxicol. Chem.*, 1992, vol. 11, no. 10, p. 1477-1483.

VUORINEN, PJ., KEINÄNEN, M., PEURANEN, S. and TIGERSTEDT, C. Reproduction, blood and plasma parameters and gill histology of vendace (*Coregonus albula* L.) in long-therm exposure to acidity and aluminum. *Ecotoxicol. Environ. Saf.,* 2003, vol. 54, no. 3, p. 255-276.

WALKER, CH., HOPKIN, SP., SIBLY, RM. and PEAKALL, DB. *Principles of Ecotoxicology*. 2 ed. London: Taylor and Francis, 2001. 321 p.

ZAR, JH. 1996. *Biostatistical Analysis*. 3 ed. Upper Saddle River, NJ: Prentice-Hall Int, 1996. 662 p.

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