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Acute Effects of *Microcystis aeruginosa* from the Patos Lagoon Estuary, Southern Brazil, on the Microcrustacean *Kalliapseudes schubartii* (Crustacea: Tanaidacea)

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Abstract. Toxic blooms of the cyanobacterium *Microcystis aeruginosa*, a microcystin producer, have been observed in the past two decades in the Patos Lagoon estuary (southern Brazil). This cyanobacterium reaches the estuary from northern waters and accumulates as toxic blooms in the shallow margins of the environment. Microcystins are phosphatase (PP1 and PP2A) inhibitors and cause animal death via alteration of the liver cell cytoskeletons and intrahepatic hemorrhage. The massive accumulation of toxic material affects the survival of several benthonic estuarine local organisms. The tanaidacea *Kalliapseudes schubartii* is a benthonic estuarine species which occurs at high densities throughout the year in mixohaline areas of the Patos Lagoon. This microcrustacean is of high ecological relevance and plays an important role in the estuarine food web, as it is consumed on a large scale by estuarine fish. This work verifies the acute toxicity of aqueous extracts of *M. aeruginosa* RST9501 and of sediments spiked with lyophilized material of the same strain on *K. schubartii*; it also evaluates the sublethal effects on tanaidacean oxygen consumption rates and glycogen levels under acute exposure to *M. aeruginosa* aqueous extracts. The strain *M. aeruginosa* RST9501 was cultured in BGN/2 medium. The aqueous extracts were prepared using the lyophilized material from the strain cultures. Acute tests were performed over 96 h at a salinity of 15, at six toxic concentrations, and resulted in an average 96-h LC50 of 1.44 mg ml^{-1}. The spiked sediment tests were performed with a 10-day duration, using the lyophilized material in three proportions of powder/sediment and showed an average LC50 of 1.79 mg ml^{-1}. Oxygen consumption was determined after 24 and 48 h of incubation in adult organisms exposed to sublethal aqueous extract concentrations and showed a significant increase at the highest concentrations. This suggests alterations in the organism's metabolism by exposure to the cyanobacterium extract. The glycogen levels were determined with a commercial kit (Glicox 500; DOLES Ltd.); after 24 and 48 h the dosages were administered in the same organisms utilized in the oxygen consumption test and did not demonstrate significant differences. The results demonstrate the possible risks of intoxication to which the natural populations of *K. schubartii* were exposed in the environment and emphasize the importance of studies involving sublethal concentrations of *M. aeruginosa* to other organisms of the trophic web in this aquatic system.

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Cyanobacterial blooms have several consequences for water quality and their collapse frequently causes high mortality among aquatic animal populations (Vasconcelos *et al.* 2001). Among many species of cyanobacteria that can develop toxic blooms, *Microcystis aeruginosa* is one of the most common and is a matter of great concern (Chorus and Bartram 1999). The cyanobacterium *Microcystis aeruginosa* produces toxins called microcystins, monocyclic heptapeptide molecules, of which over 65 structural variants are known. Most of the variants are potent hepatotoxins and tumor promoters in mammals (Codd *et al.* 1999; Sivonen and Jones 1999). At the molecular level, microcystins are serine/threonine phosphatase PP1 and PP2A inhibitors, resulting in hyperphosphorylation of proteins, affecting intracellular signaling, cell growth, and differentiation processes, and inducing cell disturbance (Toivola and Eriksson 1999).

Previous works have shown that microcystin can be conjugated with the tripeptide glutathione (GSH), through a conjugation reaction catalyzed by glutathione-*S*-transferase (GST) (Pflugmacher *et al.* 1998; Takenaka 2001; Vinagre *et al.* 2003). The microcystin–GSH complex has a less inhibitory effect on phosphatases, clearly showing that this conjugation reaction is involved in detoxification processes (Metcalf *et al.* 2000). The enzyme (GST) is reported to be involved in detoxification reactions of several kinds of pollutants, such as metals, fungicides, and pesticides (Freedman *et al.* 1989; Gallagher *et al.* 1992; Maracine and Segner 1998).

Different aquatic organism species from all trophic levels in the web are susceptible to microcystins, these include bacteria, protozoa, macrophytes, green algae and diatoms, and micro- and mesozooplankton, as well as invertebrates and fish (Christoffersen 1996). A number of reports have demonstrated the sensitivity of different species of marine and freshwater crustacea to *Microcystis aeruginosa* cells and microcystins (Delaney and Wilkins 1995; Salomon *et al.* 1996; DeMott

1991; Hietalla *et al.* 1997; Yogui 1999; Vasconcelos *et al.* 2001).

Microcystis aeruginosa blooms have been observed in the past two decades in the Patos Lagoon estuary (RS-Brazil) (Yunes *et al.* 1996, 1998), and their toxicity and microcystin contents have been reported previously (Yunes *et al.* 1992). This cyanobacterium reaches the estuary of the Patos Lagoon from northern waters and accumulates toxic blooms and scums in the shallow margins of the estuary. This massive accumulation of toxic material in the sediment puts the survival of several local benthonic estuarine organisms at risk.

The Patos Lagoon estuary has a diversified fauna and is an important nursery for marine and limnic species. *Kalliapseudes schubartii* Mañe-Garzon 1949 (Crustacea–Tanaidacea) is among the most interactive benthonic organisms (Fig. 1). This tanaidacean is a benthonic tube-forming filter-feeding species, which occurs at high densities year-round in mixohaline regions of the Patos Lagoon (Zamboni 2000). The organism carapace encloses the branchial chamber along the thorax. Respiration through the gills and filter feeding on detritus and plankton (McLaughlin 1980) are the possible routes for the uptake of cyanotoxins. The species plays an important role in the estuarine food web, as it is consumed on a large scale by estuarine fish (*Micropogonias furnieri*, *Odontesthes bonariense*s, etc.) and estuarine crustacea (*Farfantepenaeus paulensis*, *Callinectes sapidus*). All of them are very important fishing resources in the Patos Lagoon estuary. The easy adaptability of the species *Kalliapseudes schubartii* to laboratory maintenance has led other authors (Costa 1998; Zamboni 2000) to use this estuarine tanaidacean as a test organism for heavy metal toxicity tests and water quality bioassays.

The objective of this work was to determine the acute toxicity of an aqueous extract of *Microcystis aeruginosa* RST9501 (isolated from the Patos Lagoon estuary in 1995 and kept as an axenic strain at the Unit of Research on Cyanobacteria of FURG, Rio Grande, Brazil) and the toxicity of sediments spiked with lyophilized material obtained from the same strain cultures, by measurements in *Kalliapseudes schubartii.* It also aimed to evaluate the sublethal effects of acute exposure to *Microcystis aeruginosa* aqueous extracts on the tanaidacean oxygen consumption rates and glycogen levels.

Materials and Methods

Sampling and Adaptation of Organisms

Using a shovel, test organisms were collected on the sandy margins of the Patos Lagoon estuary (southern Brazil), and organisms and sediments were separated with a 500-μm mesh net. The organisms were immediately transferred to the laboratory and separated into three groups: adults, juveniles, and females with eggs. Only adult organisms measuring between 0.7 and 1 cm long were used. Depending on the salinity of the site from which the sample was taken, the adaptation to salinity 15 (‰) was made by gradually adding brine, obtained from melting filtered seawater, or with filtered freshwater (1‰ every 2 h). Thus, these adults were adapted to salinity 15 and maintained in plastic containers approximately 10 L in volume, containing a 2-cm-deep layer of sediment sifted through a 500-μm mesh net, with a maximum of 100 organisms in each recipient. They were maintained under a controlled temperature (22°C), a light/dark cycle of 12:12 h, and constant aeration for a minimum of 5 days and a maximum of 7 days prior to the toxicity tests. Animals were fed with a solution of two commercial rations for trout and fingerling (Alcon). For each lot of 100 organisms, 2 ml of this solution was supplied every 48 h.

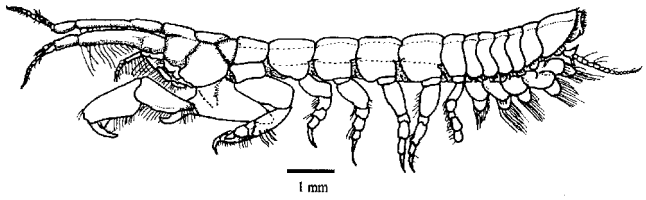

Fig. 1. Adult form of *Kalliapseudes schubartii*

Cyanobacterial Toxic Extract Preparation

The strain *M. aeruginosa* RST9501 (UPC Culture Collection, Brazil) was grown in batch cultures in BGN/2 (BG11 with half the total N) medium (Rippka *et al.* 1979) under constant conditions (salinity 0; temperature, 25°C; radiation, 63 $\mu E\ m^{-2}\ s^{-1}$; and pH 8.0) in 5-L flasks. These unialgal and axenic cultures had a growth rate (μ) of $+0.2\ day^{-1}$ and were lyophilized on reaching 1×10^7 cells ml^{-1}. The aqueous extracts were prepared using lyophilized material from the strain cultures and sterilized water at a salinity of 15 ppm. Samples were frozen and thawed three times and sonicated (50 mHz; Soniprep, USA) at −10°C for 10 min. This suspension was centrifuged at 14,000 rpm on a bench centrifuge (Eppendorf, Germany) at room temperature for 10 min and the supernatant was separated for the tests and stored at −20°C. The microcystin content of the extracts was determined using a commercial enzyme-linked immunoassay (ELISA) with polyclonal antibodies (EnviroLogix Inc., Portland, ME, USA), which has a detection limit of 0.01 $\mu g\ L^{-1}$.

Acute Tests with the Reference Substance

For the toxicity tests presented in this work, many samples of organisms in the environment were necessary. To evaluate whether the sensitivity of the animals collected was the same, they were tested according to Zamboni (2000) using the standard reference toxic substance ($CdCl_2$). Sampling and adaptation of organisms were done, and the water used in the tests was prepared as described above. All tests, following the same procedure described for the toxic extracts, were static, acute over 96 h, at salinity 15, temperature of 22°C, and a light/dark cycle of 12:12 h, and without feeding and aeration. The organisms were tested at five toxic concentrations (0.5, 0.7, 0.98, 1.37, and 1.92 mg L^{-1} and controls), including four replicates at each.

Acute Tests Applying the Microcystis aeruginosa *Aqueous Extracts*

Three acute tests were performed over 96 h, at a salinity of 15 ppm, without feeding, at six toxic concentrations (0.65, 0.84, 1.09, 1.42, 1.85, and 2.4 mg lyophilized material/ml water, plus controls), with four replicates of each. The microcystin concentration in the aqueous extract was 1094.07 $\mu g\ g^{-1}$. The concentrations used were established in previous tests, which determined a range defined by the highest concentration at which no mortality was observed and the lowest concentration at which a mortality of 100% was observed. The water

used in the tests was sterilized in an autoclave for 40 min at 120°C. Polyethylene flasks were used containing 100 ml of test solution, and 10 organisms were put in each flask. No effects due to the volume of the culture and the number of organisms were observed. Tests were maintained with constant aeration, at a controlled temperature (22 ± 2°C), and under light/dark cycles of 12:12 h. The pH was checked at the beginning and end of each experiment and each toxic concentration. The 96-h LC50 was calculated by the trimmed Spearman–Karber method (Hamilton *et al.* 1977).

Acute Tests Using an Aqueous Extract of Aphanotece microscópica

To confirm whether the mortality observed was provoked by the presence of microcystins, acute tests were prepared as were the previous tests, using an aqueous extract of the cyanobacterium strain *Aphanotece microscópica* RSMan 9401, previously characterized as a nontoxic strain using mouse tests and microcystin analysis (Yunes *et al.* 1996).

Acute Tests with the Sediment Spiked with M. aeruginosa

Two spiked sediment tests were performed using the lyophilized cyanobacteria at three proportions of powder/sediment. Thus, 110 g of dry sediment was used with 354, 510, or 735 mg of *M. aeruginosa* RST9501 dry lyophilized powder at each test concentration, respectively. These concentrations were based on the CL50 value obtained with the aqueous extract. The microcystin concentration in the lyophilized material was 1086.41 μg g^{-1}. Sediment was collected at the same site as the organisms, sifted in a 500-μm mesh, and dried at 40°C in the laboratory. The methodology used for sediment tests applied to *Kalliapseudes schubartii* followed Zamboni (2000) and consists of acute tests, without water or sediment renewal, and with constant aeration. Adult organisms were exposed for 10 days to the sediment previously spiked with the *M. aeruginosa* powder, with each concentration having four replicates. Tests were done in 400-ml beakers containing 110 g of spiked sediment at the respective concentrations, 300 ml of sterilized water (salinity, 15 ppm), and 10 organisms. Thus, considering the volume of tests, the tested concentrations were 2.45, 1.70, and 1.18 mg ml^{-1}, plus the control. The sediment and flasks used in the tests were previously sterilized at 121°C during 40 min for elimination of microorganism contamination. During the experiment, the medium was maintained with constant aeration, light/dark cycles of 12:12 h, a temperature of 22°C, and feeding every 48 h using the same maintenance diet. The organic fraction was determined by the Strickland and Parsons (1972) method. The LC50 value was calculated by the trimmed Spearman–Karber method.

Oxygen Consumption of Kalliapseudes schubartii

Oxygen consumption was determined after 24 and 48 h of incubation of four average adult organisms exposed to sublethal extract concentrations (0.36, 0.8, and 1.2 mg ml^{-1} and controls), with four replicates each. The microcystin concentration in the lyophilized material was 304.84 μg g^{-1}. The water used in the tests was previously autoclaved for 40 min at 120°C. Organisms were exposed in polyethylene flasks containing 100 ml of test solution and 10 organisms. Tests were performed over 48 h, at a salinity of 15 ppm, with constant aeration, a controlled temperature (22 ± 2°C), and a light/dark cycle of 12:12 h, and without feeding. Adult organisms of *Kalliapseudes schubartii* having an average weight of 7.3 ± 1.4 mg were used. Four Clark-type estatic-system digital respirometers (Rank Brothers, England) (Fig. 2) were used to perform simultaneously four tests at the same concentration and, consequently, four replicates. Following this procedure, the four digital respirometers used were tested for all concentrations, thus avoiding equipment differences. Each system contained a microcathode oxygen electrode within a closed glass jacket connected to an oxygen meter display. At each exposure period of 24 and 48 h, one organism from each of four replicate flasks was transferred to each of four respirometer chambers containing 1.5 ml of aerated test solution. The animal was placed and kept above the magnet stirrer by means of a plastic net adapted to the internal dimensions of the chamber. Each toxic concentration was measured for 30 min, the first 5 min being for adaptation. Thereafter, all organisms were frozen at −20°C for posterior total glycogen analysis. After each experiment, the chamber was soaked with alcohol, then rinsed thoroughly with distilled water to reduce microbial growth and accumulation inside the chamber. The estimated effect of organism weight on oxygen consumption was verified. No correlation between organism weight and oxygen consumption (for body mass) ($p = 0.607$ and $R^2 = 0.0021$) was observed.

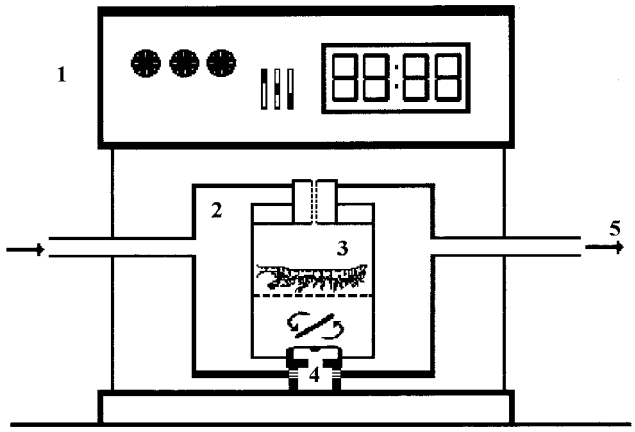

Fig. 2. Respirometer schematic design. (1) Oxygen meter; (2) bath chamber; (3) respirometer chamber and stirrer; (4) electrode; (5) bath water flow

Total Glycogen Analysis

Since phosphatase inhibition maintains the glycogen phosphorylase enzyme activity, causing a collapse in the organism energy supply (MacKintosh and MacKintosh 1994), glycogen concentration measurements in *Kalliapseudes schubartii* were made. All organisms, including the control, used for total glycogen analysis were previously submitted to the oxygen consumption tests described above. The glycogen analyses, made using a commercial kit (Glicox 500; DOLES Ltd, Brazil), were based on glucose equivalents and followed the oxidase glucose method. The methodology for microcrustacea was described by Nery and Santos (1993).

Statistical Analysis

Data were submitted to analysis of variance (ANOVA) and previous requirements for analysis (normality and homoscedasticity) were also verified. Treatments that demonstrated significance differences ($p < 0.05$) were submitted to the LSD test. All statistical analyses and correlations were made using the program Statistics for Windows v.5.0. LC50 values for the toxicity tests with *Kalliapseudes schubartii*

were calculated using the trimmed Spearman–Karber program (Hamilton *et al.* 1977).

Results

Establishing Microcystin Concentrations

In the acute toxicity tests done with the aqueous extract and sediment spiked with lyophilized *M. aeruginosa*, the microcystin concentration was estimated as 1094.07 and 1087.43 μg g^{-1} dw, respectively. However, in the sublethal tests of oxygen consumption, the toxic extracts contained 304.84 μg g^{-1} dw of microcystins.

Reference Substance

The organism sensitivity tests for a reference substance ($CdCl_2$) resulted in an average 96-h LC50 of 0.89 μg ml^{-1}. The data confirm the equal sensitivity of different lots of organisms collected in the wild (Table 1).

Acute Tests with Microcystis aeruginosa

Kalliapseudes schubartii exposed to toxicity experiments with the *Microcystis aeruginosa* RST9501 aqueous extract resulted in a 96-h LC50 varying from 1.41 to 1.50 mg ml^{-1} with an average of 1.44 mg ml^{-1}. The pH values were between 7.5 and 8 throughout the test. The 96-h LC50 values of all three tests are listed in Table 2.

Acute Tests with Aphanothece microscópica

Tests with the cyanobacterium *Aphanotece microscopica* RSMan9401 showed a low mortality for *Kalliapseudes schubartii.* The mortality percentages for every 24 h are listed in Table 3.

Acute Tests with Lyophilized M. aeruginosa-*Spiked Sediment*

After 10 days of organism exposition to the sediment spiked with *M. aeruginosa* RST9501, the average LC50 was 1.79 mg ml^{-1} (approximately 537 mg of lyophilized *M. aeruginosa* in 110 g of sediment). The pH values were 6.5–7.5 and 6.7–8 at the beginning and end of the tests, respectively. The organic fraction in the sediment was 1.53%. The results obtained in the two tests are listed in Table 4.

Oxygen Consumption

Acute exposure of *Kalliapseudes schubartii* to *Microcystis aeruginosa* RST9501 aqueous extract at sublethal concentrations caused a significant increase ($p = 0.001023$, $F = 7.52443$) in oxygen consumption at the two highest concentrations (0.8 and 1.2 mg ml^{-1}) in both the 24-h and the 48-h periods (Fig. 3).

Table 1. The 96-h LC50 values (μg ml^{-1}) and respective 95% confidence intervals of *Kalliapseudes schubartii* collected in the wild and exposed to the reference substance ($CdCl_2$)

Test	96-h LC50
I	0.70 (0.85–0.58)[a]
II	0.70 (0.95–0.53)
III	0.94 (1.34–0.66)
Average	0.89

[a] Confidence interval in parentheses.

Table 2. The 96-h LC50 values (mg ml^{-1}) and respective 95% confidence intervals of *Kalliapseudes schubartii* exposed to *Microcystis aeruginosa* RST9501 aqueous extract (microcystin concentration in extract, 1094.07 μg g^{-1})

Test	96-h LC50
I	1.42 (1.34–1.53)[a]
II	1.50 (1.40–1.60)
III	1.41 (1.24–1.61)
Average	1.44

[a] Confidence interval in parentheses.

Table 3. Average percentage mortality of *Kalliapseudes schubartii* exposed to *Aphanothece microscópica* RSMan 9401 aqueous extract at different concentrations (mg ml^{-1}), $n = 40$

Time (h)	Control	0.65	0.84	1.09	1.42	1.85	2.45
24	0	0	0	5	0	0	0
48	7.5	0	2.5	10	2.5	2.5	2.5
72	7.5	2.5	2.5	15	10	7.5	5
96	10	7.5	5	15	15	15	10

Table 4. LC50 values (mg ml^{-1}) and respective 95% confidence intervals of *Kalliapseudes schubartii* exposed for 10 days in sediment spiked with lyophilized *Microcystis aeruginosa* RST9501 (microcystin concentration in lyophilized material, 1086.41 μg g^{-1})

Test	10-day LC50
I	1.71 (1.50–1.96)[a]
II	1.86 (1.68–2.07)
Average	1.79

[a] Confidence interval in parentheses.

Total Glycogen

Total glycogen results of exposed *Kalliapseudes schubartii* after 48 h at different sublethal concentrations of *Microcystis aeruginosa* aqueous extracts are presented in Figure 4. ANOVA did not indicate significant differences ($p < 0.05$) in the total glycogen levels determined during the exposure time of the four tested concentrations. The values are expressed as milligrams per gram.

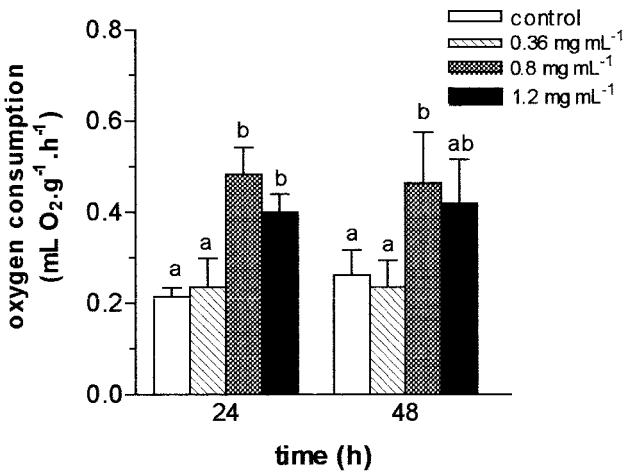

Fig. 3. Oxygen consumption of *K. schubartii* exposed to sublethal concentrations of *M. aeruginosa* extract during 24- and 48-h tests

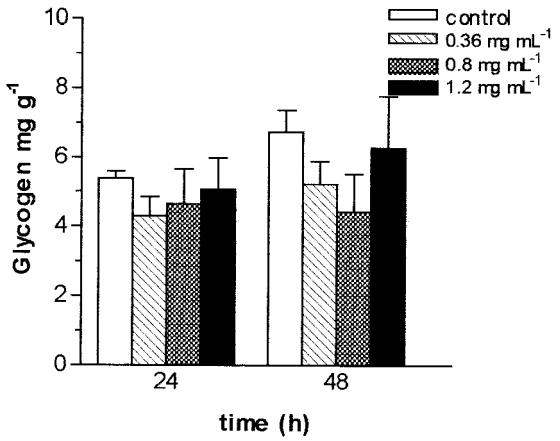

Fig. 4. Glycogen levels of *K. schubartii* exposed to sublethal concentrations of *M. aeruginosa* extracts for 24 and 48 h

Discussion

The results obtained for the toxicity tests with aqueous extracts of *Microcystis aeruginosa* RST9501 showed that *Kalliapseudes schubartii* is equally sensitive (96-h LC50 = 1.44 mg ml^{-1}) compared with other crustacean species tested with the same strain. The decapod crustacean *Farfantepenaeus paulensis* has also been acutely tested with *M. aeruginosa* RST9501 extracts and showed a 24-h LC50 of 2.96 mg ml^{-1}, while the brine shrimp *Artemia salina* presented an 18-h LC50 of 4.73 mg ml^{-1} (Yogui 1999).

The low mortality observed in the tests with the nontoxic extracts of the cyanobacterium *Aphanotece microscópica* RS-Man9401 confirms the toxic action of the *Microcystis aeruginosa* toxins in the organism and also indicates the low sensitivity of *Kalliapseudes schubartii* to other cell components such as pigments and membrane lipopolysaccharides present in the extract.

When the microcystin concentrations of the cyanobacterial extracts were considered, a 96-h LC50 of 1.58 μg ml^{-1} was calculated for *Kalliapseudes schubartii.* This value is close to the 48-h LC50 of 2.25 μg ml^{-1} in the pink shrimp *Farfantepenaeus paulensis* postlarvae reported by Salomon *et al.* (1996). In addition, a value of 3.75 μg ml^{-1} for 24-h LC50 was found for *Artemia salina* when pure microcystin-LR (L-Leu and L-Arg in positions 2 and 4) was used (Delaney and Wilkins 1995). Close values were observed in toxicity tests with microcystin-LR on the cladocera *Daphnia pulicaria, D. hyalina,* and *D. pulex* (48-h LC50 of 21.4, 11.6, and 9.6 μg ml^{-1}, respectively) by DeMott *et al.* (1991). The same authors obtained a microcystin-LR 48-h LC50 between 0.45 and 1 μg ml^{-1} in tests with the copepod *Diaptomus birgei.*

Although all crustacea have a distinct liver, no research has yet been done on the effects of hepatoxins and their functions. To evaluate the effects of microcystins on organisms subjected to either acute or chronic tests, the uptake mode of such toxins must be considered. Microcystins are relatively polar molecules with a low cellular membrane permeability and, therefore, require a transport system via bile acids to reach the hepatocytes and liver protein phosphatases (Runnegar *et al.* 1995).

Protein phosphatases serve an important role in maintaining homeostasis in cells (Cohen 1989). In liver cells, intermediate filaments of the cytoskeleton can become hyperphosphorylated, leading to cellular disruption. The progressive loss of the cell architecture disrupts cytoskeleton hepatocytes, leading to the detachment of adjacent cells and sinusoidal capillaries. Thus, blood accumulation in the liver causes a hemorrhagic shock (Falconer and Yeung 1992; Carmichael 1994; Torvola and Eriksson 1999).

Moreover, phosphatase inhibition maintains the glycogen phosphorylase enzyme activity, causing a collapse in the organism's energy supply (MacKintosh and MacKintosh 1994).

The total glycogen content in organisms exposed to sublethal concentrations of *Microcystis aeruginosa* did not vary significantly. These values in *Kalliapseudes schubartii* (average glycogen concentration, 5.41 ± 0.94 mg g^{-1}) are very close to those found in the postlarvae of *Fanfantepenaeus paulensis* (average glycogen concentration, 3.62 ± 0.47 mg g^{-1}) (Pinho 2000) using the methodology described by Nery and Santos (1993).

However, alterations were expected in the glycogen levels of organisms exposed to *Microcystis* extracts, due to a possible inhibition of protein phosphatases and, consequently, an increase in glycogen breakdown. However, considering the low toxin concentrations in the oxygen consumption tests, phosphatase inhibition by microcystins may have been insufficient to unbalance the phosphorylation and desphosphorylation ratios. It is not known how many protein phosphatase molecules have to be inactivated by microcystins in a given incubation period to cause alterations in cellular functions. It is also not known whether there is further control of the synthesis and degradation rates of phosphatase molecules. It may be, however, that the microcystin–phosphatase complex accumulates in the tissues or is degraded (MacKintosh and MacKintosh 1994).

Studies on respiratory rates can reveal important aspects of the metabolic energy states and internal equilibrium in crustacea. In fact, oxygen consumption measurements can be very useful indicators of the sublethal effects of compounds such as cyanotoxins for purposes of validation and toxicology, espe-

cially because compounds like microcystins have never had their effects tested on crustacea respiratory rates.

The presence of *Microcystis aeruginosa* extracts causes an increase in the *Kalliapseudes schubartii* oxygen consumption rate. The significant increase in oxygen consumption rates observed in organisms exposed for 24 and 48 h to sublethal concentrations of *M. aeruginosa* suggests an increase in the total organismic energy expenses, probably due to the expenditure of homeostatic and enantiostatic mechanisms.

When an animal is exposed to a toxic agent, physiological processes go into action to compensate for the toxic stress (the detoxification process). As a consequence, an alteration in the organism's normal health may occur (Depledge 1989). In relation to microcystins, detoxification would be related to the tripeptide glutathione (GSH), through a conjugation reaction catalyzed by GST (Pflugmacher *et al.* 1998). This conjugate with microcystin covalently bound to glutathione has less inhibitory action on protein phosphatases and may be a characteristic of the detoxification process (Metcalf *et al.* 2000). In the same manner, the oxygen consumption rate increase after 48 h of exposure could be a result of activation caused by higher concentrations of *Microcystis aeruginosa* extract. Possibly, the synthesis of new proteins during the detoxification process, as well as the repair of toxin-damaged tissues, increases the total organism energy expenditure, thus reflecting the oxygen consumption.

The lyophilized *Microcystis aeruginosa* RST9501-spiked sediment was highly toxic to the tanaidacean *Kalliapseudes schubartii* (10-day LC50 of 1.79 mg ml^{-1}). In the environment, sediment contamination by cyanotoxins may occur at bloom termination, when cyanobacterial cells can be deposited on the bottom and margin sediment, and microcystins of intact cells may persist for several months. This process of toxic debris accumulation and persistence in sediment may be hazardous to the benthonic biota. Once deposited on the sediment, cells are subjected to bacterial action, lysis, and toxin transfer (Chorus and Bartran 1999).

A sediment analysis in the environment of material collected 7 cm deep in the sediment surface at the Baia do Casamento (northeast of Patos Lagoon, Brazil), for example, revealed the presence of a cyanobacterial layer deposited several seasons before. The sediment contained up to 0.12 $\mu g\ g^{-1}$ of microcystin contamination (Yunes 2000), a level not far from the LC50 value obtained for the acute tests in the present work. Considering that *M. aeruginosa* toxic blooms and scums accumulate in the narrow mouth of the Patos Lagoon estuary, it is probable that benthic organisms in the environment are subjected to toxic cell deposition and bottom sediment toxicity.

Although this process has received only slight attention from the scientific community, studies on microcystin interaction with sediments and their respective biota and toxin presence are fundamental to benthic community maintenance as well as to environmental health control in regions like the Patos Lagoon estuary.

Despite the limitations involving extrapolation of laboratory toxicity to the environment, the effects verified in this work are from brief acute-mode tests compared to the organism life cycle, however, they can be expected to happen *in situ* in the environment. Lethal and sublethal effects resulting from chronic exposure to the microcystin toxin might be expressed for lower concentrations than those applied in the acute tests. Matthiensen (1996) reported extracellular microcystin concentrations in Patos Lagoon estuary waters of up to 0.245 $\mu g\ ml^{-1}$ during *Microcystis aeruginosa* blooms. These data show that extracellular toxin concentrations in the environment can reach levels within the same range as those used for laboratory tests during oxygen consumption determinations (0.366, 0.244, and 0.110 $\mu g\ ml^{-1}$). These concentrations caused sublethal effects in the organisms and were not so far from the 96-h LC50 value of the 1.58 $\mu g\ ml^{-1}$ obtained in the acute tests.

The results demonstrate the potential risks of intoxication to natural populations of *Kalliapseudes schubartii* in the environment, when heavy blooms are present in the estuary. This tanaidacean is a very important organism in the Patos Lagoon estuary trophic web, and if these and other similar species are compromised by cyanobacterial toxins in the sediments, other important fish and crustacea of economic importance to local fisheries may also be affected.

References

Carmichael WW (1994) The toxins of cyanobacteria. Sci Am 270(1): 64–70

Chorus I, Bartram J (1999) Cyanobacterial toxins. In: Chorus I, Bartram J (eds) Toxic Cyanobacteria in water. A guide to their public health consequences, monitoring and management. E & FN Spon, New York, London, pp 41–111

Christoffersen K (1996) Ecological implications of cyanobacterial toxins in aquatic food webs. Phycology 35(6):42–50

Codd GA, Bell SG, Kaya K, Ward CJ, Beattie KA, Metcalf JS (1999) Cyanobacterial toxins, expose rates and human health. Eur J Phycol 34:405–415

Cohen P (1989) The structure and regulation of protein phosphatases. Annu Rev Biochem 58:453–508

Costa JB (1997) Avaliação da adequabilidade de Kalliapseudes schubartii Mañé-Garzon, 1949 (Crustacea: Tanaidacea) como organismo-teste para utilização em ensaios de toxicidade de sedimentos estuarinos. Trabalho de Graduação, Fundação Universidade Federal de Rio Grande, Rio Grande, Brasil

Delaney JM, Wilkins RM (1995) Toxicity of microcystin-LR, isolated from Microcystis aeruginosa, against various insect species. Toxicon 33(6):771–778

DeMott WR, Zhang Q, Carmichael WW (1991) Effects of toxic cyanobacteria and purified toxins on the survival and feeding of a copepod and three species of Daphnia. Limnol Oceanogr 36(7): 1346–1357

Depledge M (1989) The rational basis for detection of the early effects oh marine pollutants using physiological indicators. Ambio 18(5): 301–302

Falconer IR, Yeung DSK (1992) Cytoskeletal changes in hepatocytes induced by Microcystis toxin and their relation to hyperphosphorylation of cell proteins. Chem Biol Interact 81:181–196

Freedman JH, Ciriolo MR, Peisach J (1989) The role of glutathione in copper metabolism and toxicity. J Biol Chem 264:5598–5605

Gallagher EP, Canada AT, DiGiulio RT (1992) The protective role of glutathione in chlorothalonil-induced toxicity to channel catfish. Aquat Toxicol 23:155–168

Hamilton MA, Russo RC, Thurton RV (1977) Trimmed Spearman Karber method for estimating median lethal concentrations in toxicity biossays. Environ Sci Technol 11:714–719

Hietala J, Laurenmaatta C, Walls M (1997) Life history responses of Daphnia clones to toxic Microcystis at different food levels. J Plankton Res 19(7):917–926

Mackintosh C, Mackintosh RW (1994) The inhibition of protein

phosphatases by toxins: implications for health and an extremely sensitive and rapid bioassay for toxin detection. In: Codd GA, Jeries TM, Keevil CW, Potter E (eds) Detection methods for cyanobacterial toxins. Royal Society of Chemistry, Cambridge, 90–99

Mañé-Garzon F (1949) Un nuevo tanaidaceo ciego del sud America, Kalliapseudes schubartii, nov. sp. Comun Zool Museo Hist Nat Montevideo 3(52):1–6

Maracine M, Segner H (1998) Cytoxicity of metals in isolated fish cells: importance of the cellular glutathione status. Comp Biochem Physiol 120A:83–88

Matthiensen A (1996) Ocorrência, distribuição e toxicidade de Microcystis aeruginosa (Kütz. Emend. Elekin) no Estuário da Lagoa do Patos, RS. Dissertação de Mestrado, Fundação Universidade Federal do Rio Grande, Rio Grande, Brasil

McLaughin PA (1980) Comparative morphology of recent crustacea. W. H. Freeman, San Francisco, CA

Metcalf JS, Beattie KA, Pflugmacher S, Codd GA (2000) Immunocrossreactivity and toxicity assessment of conjugation products of the cyanobacterial toxin, microcystin-LR. FEMS Microbiol Lett 189:155–158

Nery LEM, Santos EA (1993) Carbohydrate metabolism during osmoregulation in Chasmagnatus granulata Dana, 1851 (Crustácea, Decapoda). Comp Biochem Physiol 106B:747–753

Pflugmacher S, Wiegand C, Oberemm A, Beattie KA, Krause E, Codd GA, Steinberg CEW (1998) Identification of an enzymatically formed glutathione conjugate of the cyanobacterial hepatotoxin microcystin-LR: the first step of detoxication. Biochim Biophys Acta 1425:527–533

Pinho GLL (2000) Efeito da amônia na composição orgânica de pós-larvas do camarão-rosa Farfantepeneaus paulensis (Pérez-Farfante, 1967) (Crustacea:Decapoda). Trabalho de Graduação, Fundação Universidade federal do Rio Grande, Rio Grande, Brasil

Rippka R, Deruelles J, Waterbury JB, Herdman M, Stanier RY (1979) Generic assignments, strain histories and properties of pure cultures of cyanobacteria. J Gen Microbiol 111:1–61

Runnegar MTC, Berndt N, Kaplowitz N (1995) Microcystin uptake and inhibition of protein phosphatases: Effects of chemoprotectants and self-inhibition in relation to known hepatic transports. Toxic Appl Pharmacol 134:264–272

Salomon PS, Yunes JS, Parise M, Cousin JCB (1996) Toxicidade de um extrato de *Microcystis aeruginosa* da Lagoa dos Patos sobre camundongos e suas alterações sobre o tecido hepático. Vittale 8:23–32

Sivonen K, Jones G (1999) Cyanobacterial toxins. In: Chorus I, Bartram J (eds) Toxic Cyanobacteria in water. A guide to their public health consequences, monitoring and management. E & FN Spon, New York, London, pp 41–111

Strickland JDM, Parsons TR (1972) A practical handbook of seawater analysis. Fish Res Board Can Bull 167:311

Takenaka S (2001) Covalent glutathione to cyanobacterial hepatotoxin microcystin LR by F344 rat cytosolic and microsomal glutathione S-transferases. Environ Toxicol Pharmacol 9:135–139

Toivola DM, Eriksson JE (1999) Toxins affecting cell signalling and alteration of cytoskeletal structure. Toxicol In Vitro 13:521–530

Vasconcelos VM, Oliveira S, Teles FO (2001) Impact of a toxic and non-toxic strain of *Microcystis aeruginosa* on the crayfish *Procambarus clarkii*. Toxicon 39:1461–1470

Vinagre TM, Alciati JC, Regoli F, Bochetti R, Yunes JS, Bianchini A, Monserrat JM (2003) Effect of microcystin on ion regulation and antioxidant system in gills of the estuarine crab *Cahasmagnathus granulata* (Decapoda, grapsidae). Comp Biochem Physiol C 135: 67–75

Yogui GT (1999) Considerações biótica e abiótica sobre a toxicidade de Microcystis aeruginosa Kutzing (Cianobacteria) no estuário da Lagoa dos Patos, RS. Trabalho de Graduação, Fundação Universidade Federal do Rio Grande, Rio Grande, Brasil

Yunes JS (2000) Ecotoxicologia em Lagoas Costeiras—Florações de Cianobactérias Tóxicas na laguna dos Patos. In: Esteves FA, Lacerda LD (eds) Ecologia de Restingas e lagoas Costeiras. NUPEM/UFRJ. Macaé, Rio de Janeiro, pp 231–260

Yunes JS, Odebrecht C, Niencheski LFH, Codd GA (1992) Efeito do balanço de nutrientes e de fatores fisicos na ocorrência de florações de cianobactérias. Paper presented at 2nd Enc Ecotoxicol, Rio Grande, Brasil

Yunes JS, Niencheski LFH, Salomon PS, Parise M, Beattie KA, Raggett SL, Codd GA (1996) Toxic blooms of cyanobacteria in the Patos Lagoon estuary, southern Brazil. J Aquat Ecosyst Health 5:223–229

Yunes JS, Niencheski LFH, Salomon PS, Parise M, Beattie KA, Raggett SL, Codd GA (1998) Effect of nutrient balance and physical factors on blooms of toxic cyanobacteria in the Patos Lagoon, southern Brazil. Verh Internat Verein Limnol 26:1796–1800

Zamboni AJ (2000) Caracterização ecotoxicológica do estuário da Lagoa dos Patos no entorno de Rio Grande, RS: Fundamentos e técnicas. Tese de Doutorado. Escola de Engenharia de São Carlos/USP, São Carlos, Brasil