RESISTANCE OF CLADOCERAN SPECIES TO TOXIC *MICROCYSTIS*

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ABSTRACT

In the last decades, eutrophic ecosystems have shown an increasing number of toxic cyanobacterial blooms. The morphology, chemical composition and toxicity of cyanobacteria negatively interfere with feeding, nutrition, survival, reproduction and growth of Cladocera species. In the present study, the acute toxic effects of *Microcystis aeruginosa* on *Daphnia magna*, *D. pulex*, *D. longispina*, *D. pulicaria* and *Ceriodaphnia pulchella*, isolated from three Portuguese lakes with different cyanobacteria abundance, were determined. The sensitivity of these zooplankters, expressed as 48hr-LC_{50} values, was analysed having in mind their previous contact with cyanobacteria in natural conditions. A direct relationship between cladoceran sensitivity and previous contact with cyanobacteria could not be established.

Keywords - Cladocera, *Microcystis,* acute toxicity, cyanobacteria resistance.

RESUMEN

En los últimos años los ecosistemas eutróficos han mostrado un número creciente de blooms de cianobacterias tóxicas. La morfología, composición química y toxicidad de las cianobacterias interfieren negativamente con la alimentación, nutrición, supervivencia, reproducción y crecimiento de las especies de Cladóceros. En este estudio se ha determinado el efecto agudo de *Microcystis aeruginosa* sobre poblaciones de *Daphnia magna, D.pulex, D. longispina, D. pulicaria y Ceriodaphnia pulchella* aisladas de tres lagos portugueses con diferente abundancia de cianobacterias. En este estudio se ha determinado la sensibilidad de estas especies del zooplancton, expresada como valores de 48h-LV50, teniendo presente su contacto previo con las cianobacterias en su ecosistema natural. No se pudo establecer una relación entre la sensibilidad de los cladóceros con el contacto previo con las cianobacterias.

Palabras clave: Cladocera, *Microcystis,* toxicidad aguda, resistencia a las cianobacterias.

INTRODUCTION

The cyanobacterium *Microcystis aeruginosa* is very frequently associated with toxic blooms in Portuguese freshwaters, and microcystin-LR (MCYST-LR) is the most common hepatotoxin (Vasconcelos, 1994; Vasconcelos *et al.,* 1996).

In natural environments and in laboratory studies, growth, reproduction and survival of cladocerans are negatively affected by cyanobacteria populations due to toxicity, poor nutritional value and physical interference by large colonies or filaments with feeding (Hanazato, 1995; Hietala *et al.,* 1997). Cladocerg exposed to cyanobacteria cells or toxins show different sensitivities depending on species (Gilbert, 1990; DeMott *et al.,* 1991), clones (Hietala *et al.,* 1996; 1997) and also on the exposure characteristics, intact cells of cyanobacteria usually being more toxic than cell extracts or purified toxins (DeMott *et al.,* 1991; Hanazato, 1995). The different sensitivity of species and clones to the harmful effects of toxic cyanobacteria lead to the hypothesis that in natural environments these cyanobacteria exert a selective pressure favouring the most resistant strains and species of zooplankters due to physiological and behavioural adaptations (Snell, 1980; DeMott *et al.,* 1991; Hietala *et al.,* 1997).

Keeping in mind the eventual development of resistance to cyanobacteria during the study, we hypothesized that cladocerans from lakes where cyanobacteria are frequent would be tolerant to their toxins than those collected in lakes where cyanobacteria are scarce. To test this hypothesis, we evaluated the acute toxicity of an extract of *M. aeruginosa* to juveniles of *Daphnia magna, D. pulex, D. pulicaria, D. longispina* and *Ceriodaphnia pulchella*. Juveniles were obtained in the laboratory from females collected in three Portuguese lakes during periods of varying cyanobacteria abundance. Based upon the 48 hr-LC_{50} values for the *M. aeruginosa* extract, the sensitivity of the different cladoceran species and clones was analysed and related to the cyanobacteria abundance lake of origin.

MATERIAL AND METHODS

The Cladocera used to obtain laboratory cultures were collected from three lakes with different cyanobacteria densities: Vela, Viriato and Mira lakes.

Lake Vela is located in the Central-West coast of Portugal and has been classified as "eutrophic" since the sixties (Nauwerck, 1960). In this lake, high nutrient concentrations coming from boundary decomposing vegetation as well as from fertilizers used in neighbouring agricultural fields were found. The zooplankton community of Vela is dominated by rotifers and high cyanobacteria densities are frequent in this lake (Barros, 1994). In winter and spring, the phytoplankton is dominated by green algae and *D. longispina* is the dominant Cladocera. In early summer, green algae and diatoms are replaced by cyanobacteria. In the cladoceran assemblage, *D. longispina* is replaced by C. *pulchella* and *Bosmina longirostris* until late autumm (Barros, 1994).

Lake Viriato is located in the Natural Park of Serra da Estrela at an altitude of 1560 m above sea level. Barros et al. (2000) found *D. pulicaria* and several Chydoridae species were the dominant Cladocera. Cyanobacteria were never found in the phytoplankton community (Barros, personal observation).

Lake Mira, also located on the Central-West coast of Portugal, is used for recreational purposes and presents, heavy cyanobacteria blooms of *Microcystis aeruginosa* and *Anabaena* spp. throughout the year, albeit more frequently in summer, (Vasconcelos, 1995; Barros *et al.*, 1998). In this lake high nutrient concentrations were also recorded, with high loads from watershed run-off, which is dominated by crop fields. Rotifers and *Bosmina longirostris* dominate the zooplankton community. *D. pulex* and C. *pulchella* are present in spring and are replaced by *Bosmina longirostris* when cyanobacteria densities increase (Barros *et al.*, 1995).

Females of *Ceriodaphnia pulchella* and *D. longispina* were isolated from lake Vela Using a plankton net (55 mm pore) (Barros, 1994), *D. pulex* and *D. pulicaria* were isolated from Lakes Mira and Viriato, respectively. The isolation of *D. longispina* isolation coincided with the presence of cyanobacteria (49.6% of total phytoplankton) although *Chroococcus* was the dominant genus. C. *pulchella* was isolated in the presence of a high density of *M. aeruginosa*. *D. pulex* was isolated from lake Mira during the initial phase of a bloom of *M. aeruginosa* (Barros *et al.*, 1995). The clones used in experiments were isolated in the laboratory from single egg-carrying females collected in the field. Isolated females were kept in individual culture until the release of juveniles from the brood chamber. These offspring were then used to initiate laboratory cultures. Juveniles from (i) three females of C. *pulchella* (clones A, B, C), (ii) three females of *D. longispina* (clones A, B, C), (iii) one female of *D. pulex* and (iv) one female of *D. pulicaria* were selected. Individuals of *D. magna* (clone A sensus Baird *et al.*, 1989) from a laboratory culture initiated in 1990 at the Laboratory of Ecotoxicology from the "*Znstituto do Ambiente e Vida*" at Coimbra University were used as standard organism. Cladocera identification was made under an optical microscope, using identification keys by Armengol (1978) and Amoros (1984).

Animals were kept in M4 culture medium (Elendt and Bias, 1990), fed daily with the green

algae *Chlorella vulgaris* under a photoperiod of 16 hours light: 8 hours dark, at 20±1°C.

The acute toxicity tests were done with two strains of *M. aeruginosa,* strain IZANCYA7 isolated from lake Vela and strain IZANCYA2 isolated from lake Mira (Vasconcelos *et al.,* 1995). IZANCYA2 produces MCYST-LR (94.5%), MCYST-LA (4.7%) and [D-Asp^3]MCYST-LR (0.8%), while IZANCYA7 only produces MCYST-LR (Vasconcelos *et al.,* 1995). IZANCYA7 was used to prepare the toxin extract in the tests with *D. longispina, D. magna* and *C. pulchella* and IZANCYA2 was used with *D. pulex* and *D. pulicaria.* Acute toxicity tests were done using an aqueous extract of lyophilisized cells of *M. aeruginosa* suspended in M4 culture medium and sonicated. The resulting homogenate was centrifuged and the supernatant used in the toxicity tests. Extract concentration was expressed as the weight of lyophilisized cells used in its preparation (mg ml^{-1} extract volume). Animals used in toxicity tests were juveniles of the third brood, less than 24 hours old, obtained in the laboratory from females kept in individual beakers. During tests, animals were starved and the culture medium was not renewed. Five extract concentrations and a control treatment made with M4 culture medium were used. Each treatment was applied in four replicate beakers, each containing five individuals in 100 ml of test solution. The criterion for toxic effect was lack of movement of the juveniles under bright light exposure. The acute toxicity data are presented as 48hr-LC_{50}, calculated by probit analysis (Finney, 1971).

RESULTS AND DISCUSSION

The 48hr-LC_{50} values and confidence limits obtained for the species mentioned above are shown in figure 1.

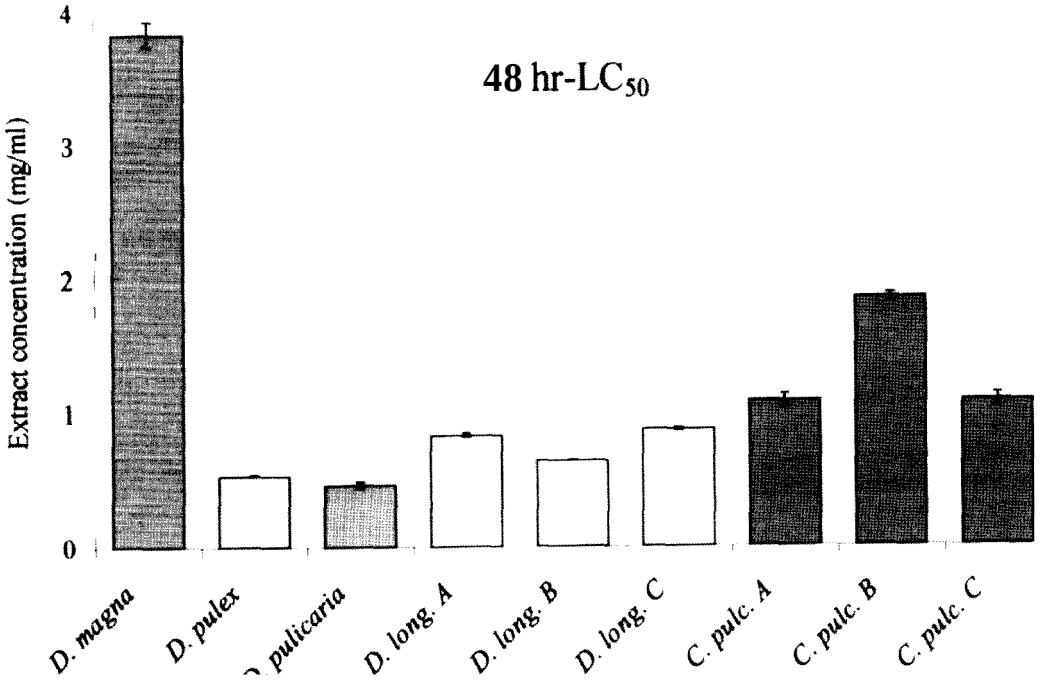

Figure 1. 48h-LC_{50} values for *Daphnia magna, D. pulex, D. pulicaria, D. longispina* and *Ceriodaphnia pulchella* exposed to different concentrations of a toxic extract of *Microcystis aeruginosa* (the bars represent 95% confidence limits). *Valores de 48h-LC50 para* Daphnia magna, D. pulicaria, D. longispina *y* Ceriodaphnia pulchella *expuestos a diferentes concentraciones de un extracto tóxico de* Microcytis aeruginosa *(las barrus representan lo límites del 95% del intervalo de condifidencia).*

Results of toxicity tests suggest the existence of inter- and intraspecific differences of sensitivity among the cladoceran species (Fig. 1). *D. rnagna* was the less sensitive species to the *M. aeruginosa* extract, followed by C. *pulchella* < *D. longispina* < *D. pulex* < *D. pulicaria*. Differences of sensitivity between Cladocera species and clones had already been found in previous works (Lampert, 1981; 1982; Gilbert, 1990; Hietala *et al.*, 1995; 1997). In the present study, *Daphniu* species also showed different sensitivites to the cyanobacteria extract. *D. magna* was the most resistant species and *D. pulicaria* the most sensitive. *D. pulicaria* was isolated from a lake where cyanobacteria are practically absent. Its greater sensitivity may be due to the lack of previous selective pressures for resistance to cyanobacteria toxicity. C. *pulchella* was the most resistant among species isolated from the lakes where cyanobacteria were present. In Lake Vela, this small species replaces *D. longispina* when cyanobacteria becomes dominant, perhaps because it coexists better with cyanobacteria than *Daphnia*, and this is probably due to its smaller size. Smaller species are usually more tolerant to cyanobacteria than larger ones, because physical interference with colonies and filaments is reduced. *D. pulex* although isolated from a lake with heavy blooms of *M. aeruginosu*, was less resistant than *D. longispina*. *D. pulex* and *D. longispina* showed differences in survivorship under acute exposure to toxic *M. aeruginosa* (Hietala *et al.*, 1996). *D. longispina* is more resistant than *D. pulex*, with a three fold difference in EC_{50} values. Differences found are related to their size and to evolutionary history. *D. longispina* is smaller and occurs in lakes where cyanobacterial blooms are more common than in small ponds where *D. pulex* is usually found (Hietala *et al.*, 1996). In lake Vela, cyanobacteria abundance allows survival and reproduction of the most tolerant *D. longispina* and *C. pulchella* individuals. In Lake Mira, *M. aeruginosa* densities are greater and blooms persistent almost all over the year. Blooms probably act as a strong stress factor against *D. pulex*. Resistant individuals of *D. pulex* only coexist with *M. aeruginosa* during a short period of the year (one or two months). *Bosmina longirostris* dominates the cladoceran community almost throughout the year. This small Cladocera is more resistant to cyanobacteria than *Daphnia* since it can avoid ingestion of toxic cells more easily and is more tolerant to those ingested (Fulton, 1988; DeMott and Kerfoot, 1982).

D. magna juveniles, although obtained from females maintained in laboratory culture for many generations, were the most resistant to the toxic extract. The environmental conditions prior and during tests influenced the measured sensitivity of tested organisms (Baird *et al.*, 1989; Soares *et al.*, 1991). The clone of *D. magna* used has been in laboratory culture since 1990, and is probably better adapted than the other species tested to the experimental conditions used in tests. This may be explain the highest resistance to the toxic extract.

It should be emphasized that the Cladocera species used in the tests differed in terms of size. Size is sometimes related to cladoceran sensitivity through mechanical interference of cyanobacteria with animal feeding and filtration behaviour (Haney, 1987). However, in our case, this hypothesis can be discarded because an aqueous extract was used. In some cases, differences of sensitivity between clones were found to be larger than between species. For example, the difference in 48h-LC_{50} between clone C of *D. longispina* and clone **A** of C. *pulchella* was smaller than that between clones **A** and B of C. *pulchella* (Fig. 1). Moreover, we must realize that a high number of other environmental variables are very likely involved in species sensitivity, calling for further experimental research.

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