

Toxic effects of *Pseudanabaena tenuis* (Cyanobacteria) on the cladocerans *Daphnia magna* and *Ceriodaphnia dubia*

Efectos tóxicos de *Pseudanabaena tenuis* (Cyanobacteria) en los cladóceros *Daphnia magna* y *Ceriodaphnia dubia*

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ABSTRACT

Some cyanobacteria can produce toxins that affect the aquatic biota and represent a human health risk. The cyanobacterium *Pseudanabaena tenuis* was isolated from the Valle de Bravo dam, and cultured in the laboratory under controlled conditions. We determined the acute toxic effects and performed a chronic (consumption) test in the cladocerans *Daphnia magna* (a reference test organism) and *Ceriodaphnia dubia* (a cosmopolitan species). To determine acute toxicity, three exposure ways were assayed: a) cell-free culture medium, b) crude cell extracts of the cyanobacterium after lysing, and c) aqueous extracts of *P. tenuis* phycobiliproteins. On the other hand, both cladocerans were fed *P. tenuis*, assessing the effects on survival and reproduction. For comparison, a control culture of both cladocerans was fed the green microalga *Pseudokirchneriella subcapitata*. Exposure to the cell-free culture medium did not produce any mortality in either cladoceran, but the aqueous and crude extracts generated acute toxicity. *D. magna* and *C. dubia* were negatively affected when fed *P. tenuis*, since their survival, total progeny, average number of neonates per clutch, and the number of clutches decreased. *C. dubia* was more sensitive than *D. magna*, both in the acute toxicity tests and to the effects of *P. tenuis* consumption. Although most of the blooms around the world are dominated by cyanobacteria of the genus *Microcystis*, attention should be given to other species, such as *P. tenuis*, because, frequently, it is not recognized that smaller cyanobacteria could exceed the larger species in terms of biomass, and produce noxious biological effects

Key words: Cyanobacteria, eutrophication, cyanotoxins, cladoceran, zooplankton.

RESUMEN

Algunas cianobacterias producen toxinas que afectan la biota acuática y representan un riesgo para la salud humana. La cianobacteria *Pseudanabaena tenuis* fue aislada del embalse Valle de Bravo y cultivada en el laboratorio. Se determinaron los efectos tóxicos agudos (por exposición) y se evaluó la toxicidad crónica (por consumo), empleando los cladóceros *Daphnia magna* (organismo de referencia) y *Ceriodaphnia dubia* (especie cosmopolita). Para determinar la toxicidad aguda se ensayaron tres formas de exposición: a) medio de cultivo libre de células, b) extractos crudos de la cianobacteria después del lisado de las células, y c) extractos acuosos de las ficobiliproteínas. Por otra parte, ambos

cladóceros fueron alimentados con *P. tenuis*, evaluando los efectos sobre la sobrevivencia y la reproducción; como comparación se utilizaron cultivos control alimentados con la microalga verde *Pseudokirchneriella subcapitata*. La exposición al medio de cultivo libre de células no produjo mortalidad en ninguno de los cladóceros, pero los extractos crudos y los extractos acuosos generaron toxicidad aguda. Adicionalmente la sobrevivencia y la reproducción de ambos cladóceros se afectó negativamente cuando se alimentaron con *P. tenuis*. *C. dubia* fue más sensible que *D. magna*, tanto en las pruebas de toxicidad aguda como en las pruebas de consumo con *P. tenuis*. Aunque en la mayoría de los florecimientos registrados en todo el mundo domina la cianobacteria *Microcystis*, se debe poner atención a especies como *P. tenuis*, que pueden ser importantes en términos de biomasa y de los efectos biológicos que pudieran generar.

Palabras clave: Cianobacteria, eutroficación, cianotoxinas, cladóceros, zooplancton.

INTRODUCTION

Cyanobacteria are cosmopolitan organisms distributed in terrestrial, fresh and marine water environments, and are very common in phytoplankton communities. Under specific environmental conditions, such as high water column stability, high temperature, and low N:P ratio, massive growth of some bloom-forming species is fostered (Roset *et al.*, 2001). These blooms are frequent in eutrophicated water bodies worldwide, representing an increasing ecotoxicological problem in fresh and marine water environments (Vardaka *et al.*, 2005; Barbosa *et al.*, 2006). Cyanobacterial blooms represent serious problems for water quality, including pH alterations, reduction of dissolved oxygen, foam production, foul smell, and unpleasant taste of the water (Codd, 2000).

During blooms, the structure of the zooplanktonic community is altered (Nagle & Paul, 1999; Nogueira *et al.*, 2006), because some cyanobacteria are able to produce a large variety of bioactive metabolites (Burja *et al.*, 2001), among them, potent toxins, known as cyanotoxins, are released into the environment once the bloom has developed (Carmichael, 1994). These toxins are classified, depending on their effects on mammals, in neurotoxins, hepatotoxins, and dermatotoxins. The most common cyanotoxins are microcystins, cylindrospermopsins, anatoxins, lyngbyatoxins, nodularins, and saxitoxins (Codd, 2000; Ouellette & Wilhelm, 2003; van Apeldoorn, 2007).

In case of excessive cyanobacterial growth, such as bloom formation, these organisms are responsible for diseases and mortality in both humans and animals (van Apeldoorn *et al.*, 2007). *Microcystis*, *Anabaena*, *Oscillatoria*, *Aphanizomenon*, and *Cylindrospermopsis* are the most common cyanobacterial genera giving rise to blooms (Moustaka-Gouni *et al.*, 2006).

In aquatic systems, blooms of cyanobacteria can affect the organisms inhabiting them (Barbosa *et al.*, 2006). The toxic effect is exerted through direct exposure to the metabolites released into the water column during the collapse of these blooms (Carmichael, 1994; Moustaka-Gouni *et al.*, 2006; Leflaive & Ten-Hage, 2007), or cyanobacteria can also be ingested by the zooplankton, producing negative effects on its survival and development.

In particular, filter-feeding cladocerans are potential consumers of planktonic cyanobacteria; cladocerans are fundamental in the planktonic structure of freshwater systems, and play an important role in the transfer of energy, stability, productivity, and increase diversity of trophic chains (Ghadouani *et al.*, 2004).

The objective of this study was to evaluate the effect of the metabolites present in the cyanobacterium *Pseudanabaena tenuis* Kopee on two zooplankton organisms, *Daphnia magna* Straus, 1820 (international and national reference species for toxicological standards) and *Ceriodaphnia dubia* (Richard, 1894) (a cosmopolitan species widely distributed in Mexico, Alonso, 1996), by simulating the lysis of a massive bloom and by direct ingestion of cyanobacterial cells. This study is relevant, because attention on the nociceptive effects of blooms is focused usually on *Microcystis* and on the production of microcystins, without considering other poorly-known cyanobacteria that could be present in blooms. Other cyanobacteria different from *Microcystis* could represent a risk for environmental and human health, because they can produce cyanotoxins and metabolites with noxious biological activity, besides they can become the dominant taxon, as has been seasonally observed in some dams that supply drinking water to Mexico City.

MATERIAL AND METHODS

The filamentous cyanobacterium *Pseudanabaena tenuis* Kopee 1924 was isolated from a bloom in the "Valle de Bravo" dam in the state of Mexico (19° 21' 30" N and 100° 11' 00" W), during a sampling performed in January 2005 (Carrera-Ramírez, 2005). This strain was deposited in the Laboratorio de Fisiología Vegetal of the Escuela Nacional de Ciencias Biológicas (ENCB). The cladocerans *D. magna* and *C. dubia* were obtained from the cladocerans collection of the Laboratorio de Hidrobiología Experimental of ENCB; in particular, *C. dubia* was isolated from the "Valle de Bravo" dam 8 years ago and has been maintained under controlled conditions since that time.

Pseudanabaena tenuis was cultured in BG-11 medium (Rippka, 1988), incubated at constant temperature (25 °C), under

continuous aeration, and fixed illumination provided by “day-light” fluorescent lamps ($54 \mu\text{mol photons m}^{-2} \text{s}^{-1}$), with a 12:12 photoperiod (light:darkness). Cultures in the exponential growth phase (ca. 15 days of propagation) were used in all experiments. It should be noted that, because of the high content of phycoerythrin in *P. tenuis*, the color of these cultures was brown, different from the blue-green color frequently observed for most cyanobacterial cultures.

Daphnia magna and *C. dubia* were cultured in reconstituted hard water ($160\text{--}180 \text{ mg L}^{-1}$ as CaCO_3 ; U.S. Environmental Protection Agency, 2002), at 25°C with a 16:8 photoperiod (light:darkness). Both species were fed the microalga *Pseudokirchneriella subcapitata* (Korshikov) Hindák (Chlorophyceae) at a $1,300,000 \text{ cells mL}^{-1}$ concentration. Controlled batches of reproducers of known age were established to obtain neonates (offspring of less than 24 h of age) that were used as test organisms in all the experiments.

Acute Toxicity Assays. To assess the toxic effects of different exposure ways on both cladocerans, we performed three types of acute toxicity assays (48 h): a) using the culture medium in which *P. tenuis* had been grown, but free of biomass, which was completely separated by filtration at the end of the exponential growth phase; b) using the crude extract of the cell content obtained by cellular lysis; and c) using the aqueous extract of phycobiliproteins from *P. tenuis*, obtained from crude extracts.

The purpose of the first way of exposure was to determine whether the exudates or the extracellular metabolites from whole cells could cause mortality; the second condition would emulate the effect of the release of cellular contents as a consequence of the collapse of the bloom; whereas the third condition was aimed at evaluating the possible toxic effects of phycobiliproteins, typical pigments from cyanobacteria, which are also released during cellular lysis.

For the cell-free medium, the culture was pre-filtered with Wathman N° 3 paper and then through microfiltration using a $0.45\text{-}\mu\text{m}$ pore nitrocellulose membrane mesh.

To attain cell lysis, 127 mg of cultured biomass (wet weight) was subjected to three continuous freezing (-20°C) and thawing (room temperature) cycles (Kós *et al.*, 1995; Chorus & Bartram, 1999); cells rupture was confirmed through observation of fresh samples under an optical microscope. Separation of the crude extract from the cell debris was achieved by filtration through nitrocellulose membranes ($0.45 \mu\text{m}$).

For phycobiliproteins extraction (phycoerythrin, phycocyanin, and allophycocyanin), 127 mg of biomass (wet weight) were resuspended in 100 mL of sodium phosphate buffer (0.1 M, pH 7) and cell rupture was performed as described above. Then, 1% streptomycin sulfate (w/v) was added to precipitate cell debris

containing chlorophyll (Bermejo *et al.*, 2002) and to obtain an extract mainly composed by phycobiliproteins; this extract was maintained for 24 h at 4°C and centrifuged at 3,500 rpm during 45 min at 4°C . The final concentration of the aqueous extract corresponded to 1.27 mg of wet *P. tenuis* biomass per milliliter. The concentration of phycobiliproteins present in the concentrated aqueous extract and in the used dilution was determined by absorbance measures at 565, 620, and 650 nm in a Lambda 19-UV/VIS/NIR Perkin Elmer spectrophotometer, according to the procedure established by Bermejo *et al.* (2002).

To determine acute toxicity in the three conditions, we applied the test procedure established by the U. S. Environmental Protection Agency (2002). Reconstituted hard-water was used as dilution water. Mortality was recorded at 24 and 48 h, and with the 48 h results, we determined the median lethal concentration (LC_{50}) by the probit method (Stephan, 1977).

Chronic (Consumption) Test. *Daphnia magna* and *C. dubia* were fed three *P. tenuis* concentrations (dry weight): 2 (Pt1), 4 (Pt2), and 8 mg L^{-1} (Pt3) during 23 days. We assessed the effects on survival and on the main reproductive parameters (total progeny, mean neonates per clutch, number of clutches, age at first reproduction, and inter-clutch time) of both cladocerans. Results were compared with those obtained in a control series fed the green microalgae *Pseudokirchneriella subcapitata* (reference diet) at the same concentrations (Ps1, Ps2, and Ps3). To quantify the *P. tenuis* diet, it was necessary to develop an absorbance-dry weight calibration curve, because the size of the filaments is variable and, hence, it is difficult to quantify in a Neubauer chamber. Additionally, the size of the filaments (trichomes) was determined measuring 100 filaments with a micrometric ruler under a phase contrast microscope (40X).

Chronic consumption assays were performed in individually cultured organisms, in 100-mL containers with 80 mL of test volume, using reconstituted hard water as culture medium. Each experiment had ten replicates. Experiments were started with neonates and, during 23 days, observations were made on the survival and reproduction of organisms of all replicates. Once reproduction had started, the progeny was separated, counted, and discarded. The culture medium and food were exchanged every 48 h. Experiments were performed at constant temperature (25°C) and with a 16:8 (light:darkness) photoperiod in an environmental chamber.

At the end of the experiment, surviving *D. magna* and *C. dubia* females were measured to assess possible effects of the food on their size.

To know the protein supply of the used diets, we determined the amount of total soluble protein of *P. tenuis* and *P. subcapitata* by the Bradford method (Bradford, 1976).

The Kaplan-Meier function was used to determine significant differences in the survival of organisms subjected to the diverse treatments, using chi square as statistical test. Kruskal-Wallis test was used to establish the presence of possible differences in the total number of neonates, total number of clutches, age at first reproduction, and inter-clutch time. The average clutch size and the size of the adult females at the end of the assay were compared with a one-way analysis of variance (ANOVA). Finally, Student's t-test was used to compare the amount of total protein between the cyanobacteria and the green microalgae. Tukey's multiple comparison test (*post hoc* comparisons) was used for all parametric analyses to determine the groups with significant differences ($p < 0.05$). Statistica ver. 7.0 software was used for all analyses.

RESULTS

Acute Toxicity Tests. No lethal effects (48 h) were recorded in any of the assays performed in the cell-free culture medium, and

the physical aspect of the organisms was completely normal, as well as their mobility and locomotion capacity.

When the crude extract was evaluated (total cell content), mortality was recorded in both cladocerans. *D. magna* neonates showed a lower sensitivity than *C. dubia*, obtaining an average 48-h LC₅₀ of 130.6 mg L⁻¹. For *C. dubia*, mortality recorded at 48 h of exposure yielded an LC₅₀ of 37.1 mg L⁻¹.

On the other hand, when exposing the organisms to different concentrations of the aqueous phycobiliproteins extract, acute toxic effects were observed in *D. magna* since the first 24-h exposure in all assayed concentrations; the average 48-h LC₅₀ was 14.1 mg L⁻¹. In *C. dubia* neonates the percentage of mortality was low at 24 h of exposure and the LC₅₀ at 48 h was 13.7 mg L⁻¹, a similar value to that recorded for *D. magna*.

Chronic (Consumption) Test. The width of *P. tenuis* filaments was of 1.9 µm and the length varied from 11.7 µm to 133.2

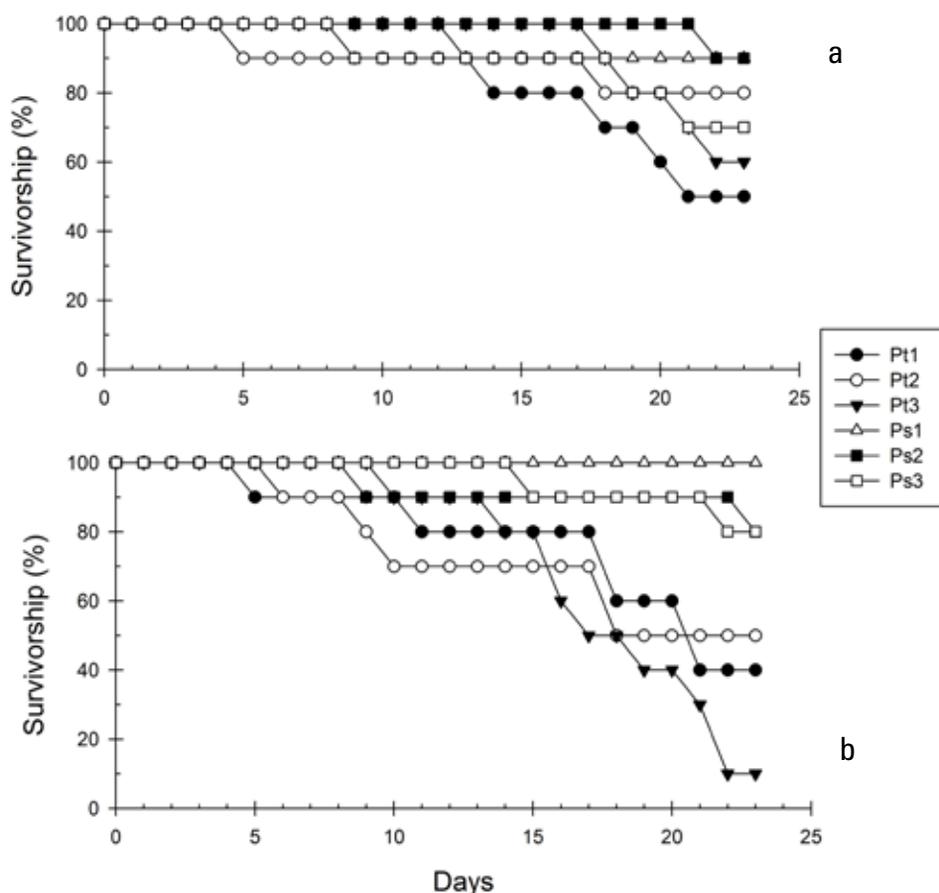


Figure 1 a-b. Survival curves for *Daphnia magna* (a) and *Ceriodaphnia dubia* (b) fed on different concentrations of the cyanobacterium *Pseudanabaena tenuis* (Pt) and the green microalgae *Pseudokirchneriella subcapitata* (Ps).

μm , with a mean of $58.2 \mu\text{m}$ (± 10.6 , $p = 0.05$). Both cladocerans consumed *P. tenuis* filaments as confirmed by the reddish color observed in the digestive tract under the microscope, this reddish color is related with the digestion process of the filaments, which allowed for the release of phycoerythrin from cyanobacteria, contrasting with the green color in the control series fed *P. subcapitata*.

The survival curves for *D. magna* with the three concentrations tested indicate that despite the mortality observed with treatments Pt1 and Pt3, there was no significant difference between the Pt and Ps diets ($\chi^2 = 5.45$, $p = 0.36$; Fig. 1a). For *C. dubia*, survival of females fed *P. subcapitata* was higher, and in organisms fed Ps1 no mortality occurred during the experimental period. Organisms fed *P. tenuis* showed a higher mortality starting at day 5, and the lowest survival at the end of the assay was recorded in those fed Pt3 (10%), with significant statistical differences between the Pt and Ps diets ($\chi^2 = 17.5$; $p = 0.003$; Fig. 1b).

For *D. magna*, the highest average value of total progeny was recorded with treatment Ps2 ($\bar{x} = 189$ neonates), whereas the lowest was recorded with Pt1 ($\bar{x} = 37$ neonates; Fig. 2). The largest clutch size was recorded with treatments Ps2 and Ps3 ($\bar{x} = 27$ and 26 neonates, respectively), and the lowest clutch size was observed with treatment Pt2 ($\bar{x} = 8$ neonates; Fig. 2). Age at first reproduction ranged from 9.5 to 10.8 days in females fed *P. tenuis*, which was higher than that recorded in organisms fed microalgae (approximately 7 days; Fig. 2). The diet affected also the number of clutches per female, corresponding the highest value to females fed *P. subcapitata* (5-6 clutches), whereas females fed cyanobacteria had an average of 3.8 clutches. The inter-clutch time ranged from 1.4 to 1.8 days with both diets. An additional effect observed in *D. magna* females fed *P. tenuis* was the presence of dead or affected neonates, as well as abortions (premature release of embryos).

The body size of *D. magna* females at the end of the assay was significantly larger in those fed microalgae ($\bar{x} = 5.5$ mm) than in females fed *P. tenuis* ($\bar{x} = 4.4$ mm) ($F = 25.7$, $p < 0.05$).

Regarding *C. dubia*, significant differences were found in all reproductive parameters (total progeny, size of clutch, age at first reproduction, and number of clutches) between those fed cyanobacteria and those fed microalgae (Fig. 3). However, the inter-clutch time was similar with all treatments (between 1.5 and 1.6 days), except for treatment Pt1 that showed a higher value ($\bar{x} = 2.35$ days; Fig. 3). The body size of *C. dubia* adults was higher in organisms fed *P. subcapitata* ($\bar{x} = 1.03$ mm), as compared to those fed *P. tenuis* ($\bar{x} = 0.83$ mm), revealing statistically significant differences between both diets ($F = 17.0$, $p < 0.05$).

The amount of total soluble protein was slightly higher in *P. tenuis* than in *P. subcapitata* ($0.8191 \mu\text{g mL}^{-1}$ and $0.7299 \mu\text{g mL}^{-1}$,

respectively). Student's t-test revealed that these amounts differ significantly ($t = -3.10$, $p < 0.006$), meaning that cyanobacteria represent a better source of protein than microalgae.

DISCUSSION

We determined that *P. tenuis* contains intracellular compounds that generate acute toxicity in *D. magna* and *C. dubia* neonates; however these biomolecules were not released to the medium in active growing cultures. This is a relevant fact because the Valle de Bravo dam, from which these cyanobacteria were isolated, supplies drinking water to Mexico City, and hosts diverse recreational activities (Ramírez *et al.*, 2004). Unfortunately, toxicological studies on cyanobacteria have focused mainly on the effect of the most common cyanotoxins, *i.e.*, microcystins, ignoring the impact exerted by other bioactive compounds that have not been studied because they are not toxic for humans (Leflaive & Ten-Hage, 2007).

Phycobiliproteins have been attributed antioxidant, antitumor, anti-inflammatory, and neuroprotective pharmacological properties (Sekar & Chandramohan, 2007). Allophycocyanin purified from *Spirulina platensis* inhibited the enterovirus 71, inducing apoptosis at concentrations that were not toxic for the host cells (Shih *et al.*, 2003). Liu *et al.* (2000) demonstrated that the phycocyanin extracted also from *S. platensis* inhibits growth of human leukemia K562 cells. Other studies demonstrated that phycoerythrin inhibits growth of hepatic carcinoma cells (Huang *et al.*, 2002).

However, we determined in the acute toxicity assays that the crude extract was less toxic than the aqueous extract with phycobiliproteins to both cladocerans. It is remarkable that when exposed to the crude extract, *C. dubia* neonates were more sensitive than *D. magna* neonates, but both cladocerans showed a similar, higher sensitivity to the aqueous extract of phycobiliproteins. Concerning this result, it is possible that remnants of the streptomycin sulfate could modify the toxic effects of phycobiliproteins, because this antibiotic was not eliminated, since the procedure to purify the phycobiliproteins extract does not consider this situation. To this respect, Isidori *et al.* (2005) have documented that this antibiotic produces moderately toxic effects on rotifers and cladocerans, but Taub *et al.* (1983) reported variable effects, depending on the species of cladoceran, because, in some cases, the effects stimulated population growth. Despite this situation, we have evidences that phycobilins can exert toxic effects in *D. magna* and *C. dubia* (unpublished data), as was confirmed in the consumption assays.

Among the few studies on the toxicity of the *Pseudanabaena* genus is that of Oudra *et al.* (2002), who evaluated the toxic effect of several cyanobacterial strains (among them, *Pseudanabaena mucicola* (Naumann et Huber-Pestalozzi) Schwabe) through bio-

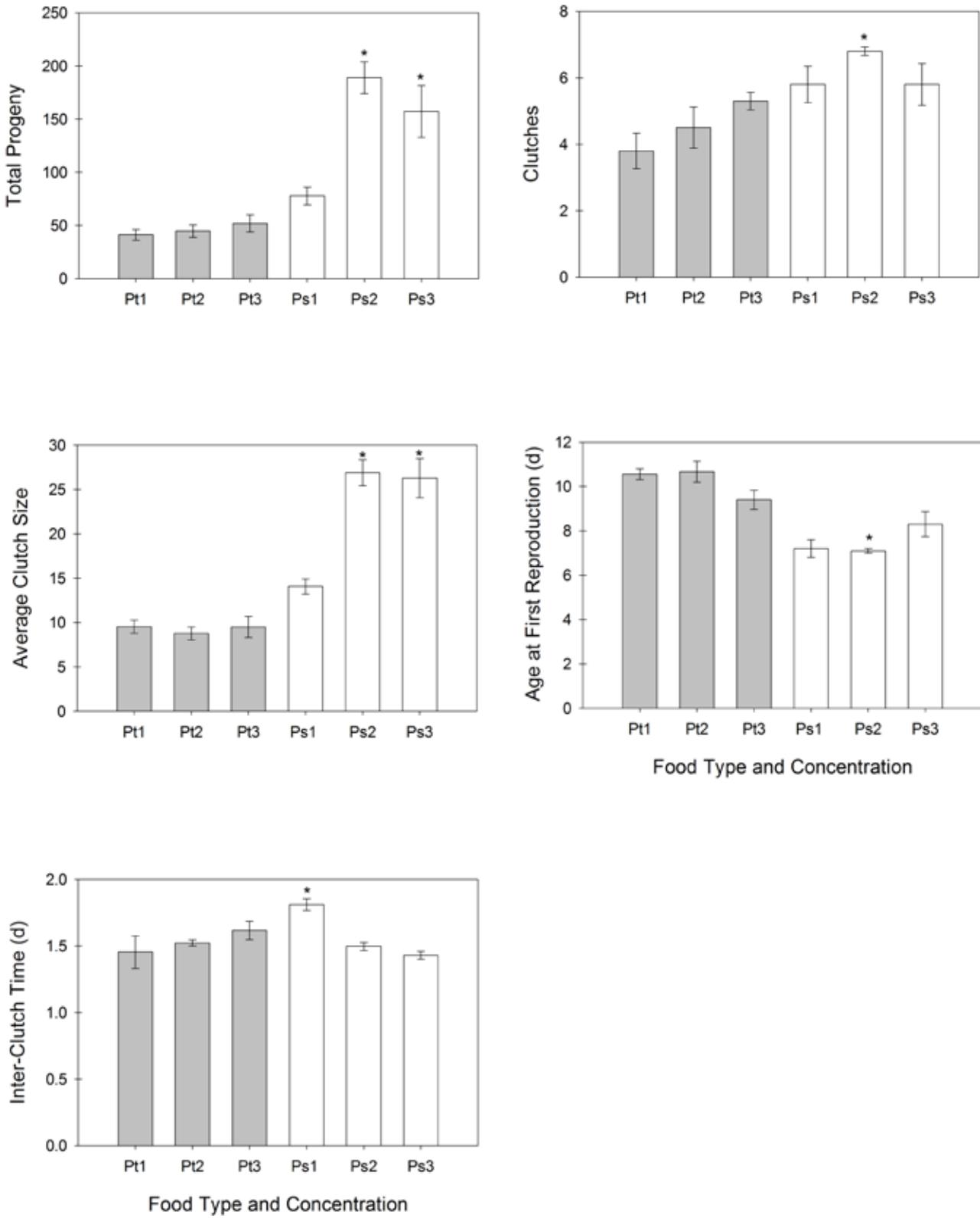


Figure 2. Average values for total offspring, clutch size, inter-clutch time, number of clutches, and age at first reproduction \pm standard error, for *Daphnia magna* fed on different concentrations of *Pseudanabaena tenuis* (Pt) and *Pseudokirchneriella subcapitata* (Ps). * Indicates significant differences ($p < 0.05$) among diets.

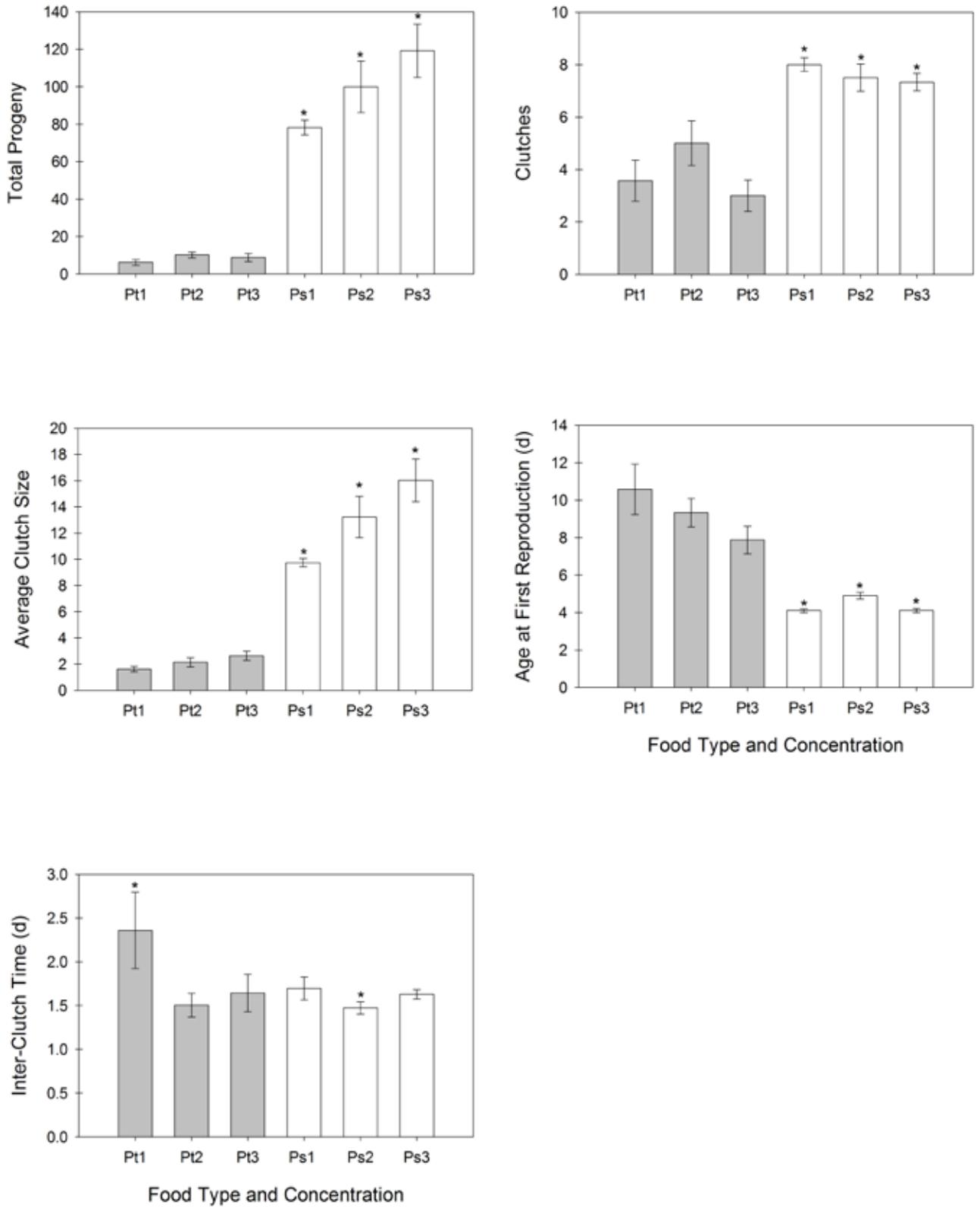


Figure 3. Average values for total offspring, clutch size, inter-clutch time, number of clutches, and age at first reproduction \pm standard error, for *Ceriodaphnia dubia* fed on different concentrations of *Pseudanabaena tenuis* (Pt) and *Pseudokirchneriella subcapitata* (Ps). * Indicates significant differences ($p < 0.05$) among diets.

assays in mice. These authors found that *P. mucicola* produces microcystins at a concentration of $19 \mu\text{g g}^{-1}$, an amount much lower than that recorded for the *Microcystis* strain they used ($600 \mu\text{g g}^{-1}$), but surprisingly *P. mucicola* was more toxic ($\text{LD}_{50} = 28 \text{ mg kg}^{-1}$) than *Microcystis* ($\text{LD}_{50} = 33 \text{ mg kg}^{-1}$), suggesting that the toxicity of *P. mucicola* is not only due to the presence of cyanotoxins but also to other intracellular metabolites that exert a toxic effect, as could be inferred from our results.

When cladocerans were fed *P. tenuis*, survival, growth, and reproduction decreased, which agrees with other studies performed on the interaction of cyanobacteria-zooplankton, which demonstrated that the exposure to cyanobacterial extracts, purified toxins, or cells (as feeding source) exerts negative effects on herbivorous organisms (Nogueira *et al.*, 2006). This can be explained by the fact that cyanobacteria are deficient in essential nutrients, many species have a colonial morphology or are filamentous, making their consumption difficult (Porter & McDonough, 1984), aside that several species produce toxic metabolites (including cyanotoxins).

The nutritional value of cyanobacteria for the zooplankton is a controversial issue, since some studies have demonstrated that they are a low nutritional quality food, mainly due to the lack of some essential nutrients needed for growth (DeMott, 1999; Ghadouani *et al.*, 2004; Nogueira *et al.*, 2006). However, other studies have demonstrated that some zooplankton species develop adequately when fed non-toxic cyanobacterial strains (Ferrão-Filho *et al.*, 2000; Kurmayer, 2001).

It has been argued that the filamentous morphology of cyanobacteria could interfere with the filtration mechanisms of the zooplankton; however, in this work we observed that both cladocerans were able to consume the filaments of *P. tenuis*.

Wilson *et al.* (2006) suggest that the filaments of some cyanobacteria reduce fecundity in large cladocerans (such as *Daphnia*), as compared to small cladocerans (such as *Ceriodaphnia*), this could explain the decrease in the predominance of large cladocerans in water bodies where blooms develop. Notwithstanding, we observed that *C. dubia* (the smaller species) was the most affected in both survival and reproductive parameters.

Our results suggest that the negative effects on survival and reproduction of *D. magna* and *C. dubia* were caused by the toxic metabolites or biomolecules released by cell rupture during digestion, and not by the nutritional deficiency or the difficulty in ingesting the filaments (Okumura *et al.*, 2007). The protein content of *P. tenuis* was slightly higher than that of *P. subcapitata* and its average size was similar to that of large size Chlorophycean frequently used as food for cladocerans, such as *Ankistrodesmus falcatus* ($45 \mu\text{m}$ length). The presence of dead offspring, as well as the abortions in *D. magna* fed *P. tenuis* can be considered as responses to the toxic effect of cyanobacteria. Notwithstanding, *C.*

dubia was most affected, as shown by the fact that most females did not reproduce and many did not survive.

Studies on *P. tenuis* metabolites and their interaction with the zooplankton are scarce. Frequently, it is ignored that small species of cyanobacteria could exceed the larger species in terms of their biomass and biological activity, and, therefore, the major role they play in the ecosystems dynamics is missed (Acinas *et al.*, 2009). In this study, we found evidences of the negative effect on the reproduction and survival of the studied cladocerans, although it is necessary to continue the study on *P. tenuis* metabolites and their effects on aquatic systems used as water sources for human consumption. The presence of *P. tenuis* in Valle de Bravo dam, which is a source for water supply for Mexico City, has also been reported previously (Carrera-Ramírez, 2005), and its toxigenic potential to the aquatic biota is now confirmed with this study.

Because of the poisoning incidents with animals and humans, the effects of many cyanotoxins have been investigated mainly in mammals, documenting increases in their effects on target cells and organs; whereas in herbivorous zooplankton the available information is still insufficient. Hence, it is important to make more detailed studies on all bioactive compounds with toxic characteristics produced by cyanobacteria, to better evaluate their effects on aquatic biota. This study evidences the negative effect that the exposure to metabolites and the consumption of these cyanobacteria exert on important zooplankton organisms, such as cladocerans.

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