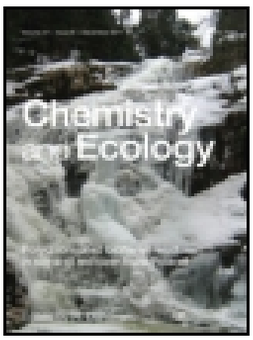

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Effect of tannins on survival and development of *Daphnia menucoensis* and the abundances of bacteria

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ABSTRACT

Polyphenols – products of organic-matter decomposition entering water bodies from autochthonous and especially allochthonous sources – affect primary producers, bacterioplankton, and zooplankton to consequently modify food webs. Cladocerans are widely used in research experiments because they constitute the most frequent prey of high-trophic-level organisms in the majority of lakes, and certain species symbolise the ecologic prototype of the generalist filter feeder. In our study area's shallow lakes, cladocerans, though generally of low abundance, do attain significantly high biomasses. We accordingly evaluated the mortality of the cladoceran *Daphnia menucoensis* Paggi, 1996 exposed to different tannin concentrations along with the bacterial abundance. In two experiments *D. menucoensis* females exposed to high tannin concentrations reproduced inefficiently, exhibited high mortality, and manifested altered behaviour, such as reduced reflexes and diminished mobility. Though tannins apparently affected algal abundances, the cladoceran herbivory strongly reduced those numbers. Positive effects on bacteria, however, were not recorded. High-allochthonous-organic-matter-containing water bodies support trophic webs because bacterioplankton provide an alternative energetic base for the zooplankton, as possibly occurs in shallow lakes of the Salado-River basin, characterised by cyclic hydrologic periods alternating between draught and flooding and intensive land use. In conclusion, tannins diminish *D. menucoensis*'s survival, locomotion, and the capacity to respond to stimuli.

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KEYWORDS
Tannins; zooplankton; *Daphnia menucoensis*; bacterioplankton

1. Introduction

Polyphenols are mainly poorly biodegradable products of the decomposition of organic matter [1] and some are known for their powerful toxic properties and solubility in water.[2] These compounds enter water bodies from both allochthonous (through leaf litter and soil) and autochthonous (derived from macrophyte and algal metabolism) sources.[3,4] The allochthonous sources – generally the most significant quantitatively – are largely dependent on the type of land use (e.g. cattle raising, agriculture).[5,6] For

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example, in water bodies of a large basin, phenolic compounds increase during flooding periods because of runoff [7] to produce variable effects on the plankton community.[8]

Furthermore, because polyphenols constitute a broad spectrum of different potentially noxious compounds that are produced and liberated by various macrophytes, the generation of those compounds is considered to be a form of allelopathy.[3,9]

Because the action of polyphenols within a water bodies affects the primary producers, thus altering the first step in food webs, these compounds at high concentrations modify the composition and abundance of the phytoplankton (e.g. the cyanobacteria, chlorophytes, and diatoms),[10] the periphyton,[11] and the macrophytes (e.g. *Potamogeton* sp. and *Elodea* sp.) [12] as well as diminish bacterioplankton diversity and abundance [13]; which effects have been suggested as a potential cause of low algal production. [14] In addition, high levels of polyphenols affect other organisms such as zooplankton (e.g. cladocerans and rotifers) [15] and other invertebrates (e.g. chironomids, copepods, and ostracods).[16] For example, those compounds inhibit the swimming or feeding of cladocerans, causing a reduction in the growth and/or reproduction of those crustaceans,[17] while other pernicious effects have been registered in *Daphnia* sp. [18] or *Ceriodaphnia* sp. [19] that likewise imply biologic detriment. Thus, the inhibitory influences of polyphenols at high concentrations have been widely recognised,[7] and there mainly by decreased abundances and biomasses of various species [15] along with the consequent modifications in the food webs.[20]

Zooplankton play an essential role in aquatic ecosystems through maintaining water transparency, decreasing the concentrations of detritus and suspended solids,[21] and an integral involvement in nutrient cycles,[22] thus constituting an essential link between the primary producers and the predators.[22] Consequently, in Pampean shallow lakes, cladocerans are the prey of the larger invertebrates (e.g. *Mesostoma* sp., *Buenoa* sp.) [23] and vertebrate predators (e.g. *Odonthestes bonariensis*; *Astyanax sp.*) [24] and in this fashion transfer energy along the food web. In shallow lakes of our study area, cladocerans are widely distributed,[15,24] generally reaching from low abundance to dominance in plankton assemblages – at up to 36% of the total zooplankton [25] – though likewise being relevant through attaining high biomasses (e.g. 405 µg dry weight per L– 456 µg dry weight per L).[15] Several members of the genus *Daphnia* have been extensively used in experimentation because they are considered as ecologic models for the generalist filter feeder.[26] In particular, *Daphnia menucoensis* was first found in arid and semiarid regions of Argentina in the temporary and semipermanent water of endorheic basins.[27] In the Pampas this species is a member of the plankton, inhabiting shallow lakes over a broad conductivity range (i.e. between 10,800 and 42,000 µS cm^{-1}).[27]

Several studies performed in the Salado-River basin and in shallow lakes associated with this river have shown that polyphenol concentrations are widely variable though always highly elevated. For example, polyphenol concentrations varies in San Miguel del Monte Lake from 1.5 to 4.6 mg L^{-1},[28] in Lacombe Lake from 3.7 to 10.1 mg L^{-1},[29] while in the Salado River the mean value was 3 mg L^{-1} in the lower sector of the basin [cf. reference 6, for more details]. Thus, in view of the markedly annual fluctuations of polyphenols in water bodies located in the Salado-River basin and the effects of those compounds in high concentrations on different organisms of the food web, the aim of this work was (a) to evaluate the possible negative effects of tannins on the survival of the

cladoceran *D. menucoensis* and on the abundance of bacteria and (b) to determine the lethal doses for the cladoceran over a short response time under experimental conditions. The specific hypotheses were that (1) the mortality of *D. menucoensis* would be higher under conditions of high tannin concentrations, (2) the *D. menucoensis* populations would be inefficient in reproduction under that form of chemical stress, and (3) the abundance of the bacteria would increase in the presence of high tannin levels.

2. Materials and methods

The studies were carried out in the laboratory to determine the mortality *versus* survival of *D. menucoensis* under the experimental conditions imposed. The experiments were thus designed to create a microcosm complementing the conditions in nature, but with the advantage that the simplified environment offered the possibility to discriminate parameters affecting those organisms that might have otherwise been confounded or not readily separated or distinguished. The adult *D. menucoensis* females required were obtained from axenic crops that our research group has maintained successfully for months in aquariums at 5,000 μS cm^{-1} conductivity and an optimal temperature range (between 18°C and 22°C). The tap water used in the experiments has a conductivity of 650 μS cm^{-1}, a pH value of 7.7, a hardness of 120 mg L^{-1}, a concentration of soluble reactive phosphorous of 20 μg L^{-1} and nitrates of 1.54 mg L^{-1} (analysed according to [30]).

2.1. Effects of tannins on abundances of D. menucoensis, algae and bacteria

To evaluate how different tannin concentrations affected the mortality *versus* survival of the cladoceran with respect to exposure time (days), we conducted an experiment. The experimental design consisted of four treatment groups ($n = 16$ for each) with different tannin concentrations: (1) a low concentration (2.5 mg L^{-1}), (2) a medium concentration (5.0 mg L^{-1}), (3) a high concentration (7.5 mg L^{-1}), and (4) controls without extra tannins added (0.5 mg L^{-1}, the endogenous levels within the tap water used). These tannin concentrations were chosen because, as mentioned above, the levels are representative of the values typically found in pampean shallow lakes. All the experimental units (EUs) consisted in transparent glass containers closed with a plastic cap (3 L) containing a hole just large enough for the entry of a glass pipette connected to an aeration system for maintaining a constant flow of oxygen. In each EU, 2.5 L of tap water (see above) without any organisms was introduced after increasing the conductivity to 5,000 μS cm^{-1} with added sodium chloride (the optimum level; cf. the previous section). Tannin concentration was measured for application to the experimental and control treatments (cf. Groups 1–4 above) and to estimate the necessary concentrations for treatments. Next, 60 parthenogenetic females of *D. menucoensis* without eggs (before the first offspring) were introduced into each EU to give a density of 24 ind L^{-1}. Furthermore, to avoid mortality as a result of stress conditions, all the EUs remained 24 h in a period of acclimation under the same conditions. On the next day, the EUs were randomly assigned to different treatments and the corresponding levels of tannins, as tannic acid (Biopack™), were added to reach the desired tannin concentrations indicated above. The EUs were then distributed among five aquariums (length: 100 cm; height: 30 cm; width: 50 cm) filled with water up to 3/4 capacity. Each aquarium was equipped with heaters and a bubbling system to maintain

a constant range of temperature that was optimal for cladocerans, and each EU was finally connected to its individual aeration system.

The experiment, lasting 32 days, was monitored daily to ensure proper functioning. Every other day the physical–chemical parameters temperature, pH, conductivity, and dissolved-oxygen concentration were measured with a CONSORT multimeter and recorded; and every three or four days water samples (6 mL) were removed to measure tannin concentration, with tannic acid being added when necessary to maintain the initial concentrations. Finally, in order to avoid the mortality of *D. menucoensis* females from inanition, every other day food in the form of axenic crops of the green alga *Monoraphidium minutum* (20 mL containing 100 nephelometric turbidity units) was added [31] with a final abundance in each EU of 0.9×10^5 ind mL^{-1}.

To evaluate the effect of exposure time to tannins, 16 EUs corresponding to the 4 treatment protocols ($n = 4$ per treatment: low, medium, and high levels of tannins plus the control) were removed for assay in each of 4 different time points: at 4 days from the start of the experiment; at 8 days; at 16 days; and 32 days (the end of the experiment). At each time point, water samples were withdrawn from each EU for determination of tannin levels and enumeration of bacteria and phytoplankton. The remaining water was passed through a plankton net of 35-μm mesh size for storage of *D. menucoensis* individuals in 5% (v/v) aqueous formaldehyde and then counted exhaustively under a stereoscopic microscope.

To determine tannin concentrations, samples of 6 mL of water were removed and stored in the refrigerator in the dark until the time of analysis (maximum 48 h after the extraction). Tannin concentration was measured spectrophotometrically at 275 nm since we had previously done a comparison between this measurement and the Folin–Ciocalteu method [30] and obtained straight-line regressions with both that were in excellent agreement.

To measure the concentration of bacteria, 10 mL of the samples were placed in vials with 5% (v/v) aqueous formaldehyde and maintained in the dark until the time of analysis. For bacterial counting, 0.5 mL were stained with 0.5 mL of a solution of DAPI solution (0.0 l μg mL^{-1}, final concentration) for 30 min according to a procedure modified from Porter and Feig.[32] The samples were next filtered under low suction onto black 0.2-μm–pore-size Nucleopore™ membranes and the latter transferred to slides for bacterial enumeration within 16 randomly chosen fields under fluorescence microscopy at 100× magnification through the use of a digital programme (Image Pro Plus version 4.5 CD-ROM Serial Number 41N45000-31526). To assess phytoplankton concentrations, samples (15 mL) were removed and stored in acetic-acid–Lugol's solution for counting by means of digital images obtained at 40× in an inverted microscope after Ferrando et al.[31]

To test the effects of tannin concentration and time as input factors on the abundances of cladocerans, algae, or bacteria, two-way ANOVAS were performed for each response variable.[33] Then the corresponding *post-hoc* Tukey tests [33] were performed when differences occurred. Thereafter, the normality and homoscedasticity of data were evaluated through the Shapiro–Wilk and Cochran tests, respectively [33] and were used to consider other sources of variations. When an assumption could not be fulfilled, common transformations were used. If the data transformation did not compensate for a departure from assumptions, the ANOVA was carried out on the untransformed data and we

considered the difference in the data to be significant if $p < .005$, thus reducing the likelihood of committing a Type-I error.[33,34]

2.2. Effects of lethal concentration 50

LC50 is the concentration of a compound that is lethal to 50% of a cohort within a specified exposure time. To evaluate the LC50 tannin concentration, we performed an experiment involving different concentrations of tannins. 10 females of *D. menucoensis* from the same crops cited in the first section above were added to each of 21 Nessler colour-compacting cylinders containing 50 mL of water at a conductivity of 5,000 µS cm^{-1}. Then tannins were introduced to elevate the concentrations in order to give 7 different exposures (7.5; 15; 22.5; 30; 37.5; 45 and 52.5 mg L^{-1}; $n = 3$ per treatment). After 48 h, the individuals were removed and counted under the stereoscopic microscope. The null hypothesis of no relationship between organisms remaining alive and tannin concentrations was analysed by lineal regression type I.[33]

3. Results

3.1. Effects of tannins on abundance of D. menucoensis, algae and bacteria

Table 1 demonstrates that the conditions of the experimental environment (i.e. temperature, conductivity, and dissolved-oxygen concentration) remained constant during the experiment.

Measurements of the tannin concentrations during the experiment (Figure 1) indicated that, though the values *in situ* were always lower than the concentrations added on Day 0, the levels increased in all three treatment groups (high, medium and low concentrations), reaching a plateau after either two days in low concentration treatments (with the lowest concentration) or the first week in the other two treatment groups. These steady-state

Table 1. Mean values and standard deviation (in brackets) of physicochemical parameters (dissolved oxygen: saturation percentage, temperature: °C, conductivity: (µS cm^{-1}) recorded during the experiments (T1: 4 days, T2: 8 days, T3: 16 days, T4: 32 days) about the effects of different taninn concentrations on abundance of *D. menucoensis*.

Treatment		T1	T2	T3	T4
Control	pH	7.9 (0.2)	8.1 (0.2)	8.1 (0.2)	8.2 (0.1)
	Dissolved oxygen	80.5 (2.7)	81.2 (3.5)	84.7 (8.8)	86.2 (8.1)
	Temperature	22.3 (2.3)	22.2 (2.2)	21.7 (2.7)	21.8 (2.7)
	Conductivity	5779 (199)	6010 (105)	5658 (297)	5512 (297)
Low concentration (2.5 mg L^{-1})	pH	8.0 (0.2)	8.0 (0.2)	8.1 (0.2)	8.1 (0.1)
	Dissolved oxygen	81.4 (2.7)	78.7 (3.3)	85.3 (8.3)	86.5 (8.4)
	Temperature	22.1 (2.4)	21.6 (2.8)	21.8 (2.3)	22.0 (2.5)
	Conductivity	5808 (276)	5822 (145)	5673 (296)	5466 (294)
Medium concentration (5 mg L^{-1})	pH	7.9 (0.2)	8.0 (0.2)	8.0 (0.2)	8.1 (0.1)
	Dissolved oxygen	80.1 (4.1)	78.9 (3.9)	82.9 (6.3)	87.8 (8.2)
	Temperature	22.6 (2.1)	21.6 (2.7)	21.4 (2.9)	22.2 (2.1)
	Conductivity	5913 (198)	5891 (202)	5578 (265)	5488 (303)
High concentration (7.5 mg L^{-1})	pH	7.8 (0.1)	7.9 (0.2)	8.0 (0.2)	8.1 (0.1)
	Dissolved oxygen	79.5 (4.3)	77.5 (4.6)	83.4 (6.0)	85.8 (8.3)
	Temperature	22.4 (2.2)	22.0 (2.3)	21.3 (2.9)	21.8 (2.3)
	Conductivity	5920 (255)	5878 (169)	5694 (291)	5566 (321)

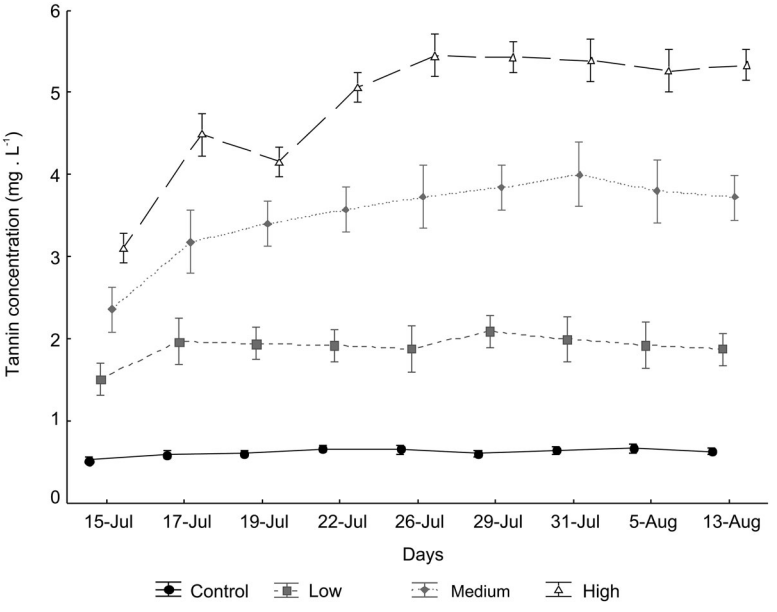

Figure 1. Tannin concentrations in the four treatment groups throughout the experimental period. Vertical bars denote 0.95 confidence intervals. Treatments: controls (0.5 mg L^{-1}); low concentrations (2.5 mg L^{-1}); medium concentrations (5.0 mg L^{-1}); and high concentrations (7.5 mg L^{-1}).

levels thereafter, though less than the concentrations added, were nevertheless markedly different, thus enabling valid conclusions to be drawn from the experimental results.

The abundance of *D. menucoensis* (Figure 2) evidenced interactions between time and tannin concentration ($F_{9,\ 47} = 7.66$ $p < .001$). After an initial decrease under the four experimental conditions, the abundance of all four groups had begun to increase at 8 days, but by 16 days a clear separation occurred among the groups, with the two exposed to the higher tannin concentrations (5 and 7.5 mg L^{-1}) undergoing a marked decline in abundance, thus indicating the occurrence of mortality. The group exposed to the lowest tannin concentration (2.5 mg L^{-1}), however, continued to increase in abundance along with the controls. Nevertheless, by 32 days, the abundance of even that treatment group had likewise dropped to values intermediate between those of the other two treatment groups and the controls, with the latter having reached a plateau relative to the previous value at 16 days.

During the quantitative analysis of the results, we observed the presence of males and resting eggs in the treatments. With respect to the abundance of males, ANOVA indicated no effects of time or tannins (interaction: $F_{9,\ 47} = 0.42$, $p = .91$; time: $F_{3,\ 47} = 1.89$, $p = .14$; tannin: $F_{3,\ 47} = 0.98$, $p = .4$), whereas the abundance of resting eggs was significantly different over time ($F_{3,\ 47} = 4.79$, $p < .01$), having increased by 32 days relative to the values at 4 and 8 days (Figure 3).

The bacterial abundances were not different between treatments with respect to the main parameters (interaction: $F_{9,\ 32} = 0.75$, $p = .65$; time: $F_{3,\ 32} = 1.08$, $p = .37$; tannin: $F_{3,\ 32} = 1.59$, $p = .21$; Figure 4).

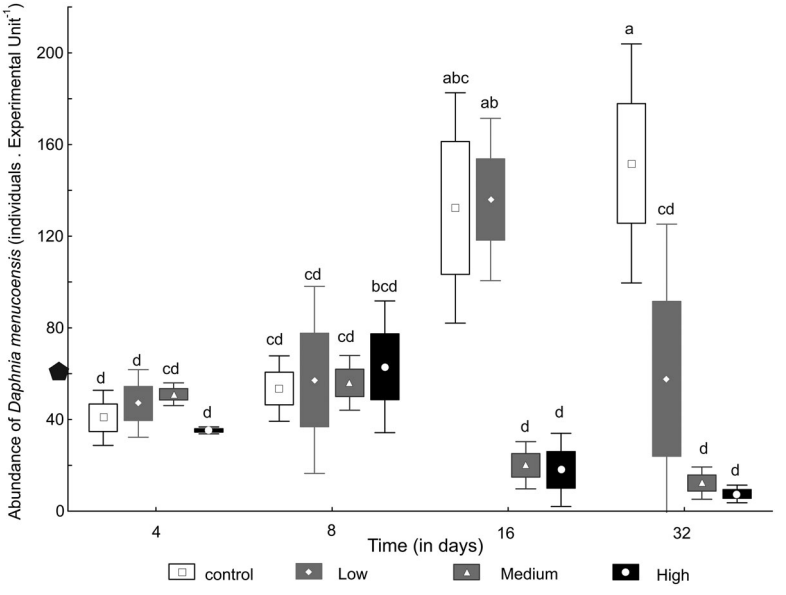

Figure 2. Abundance of *D. menucoensis* exposed to different tannin concentrations over time. Treatments: controls (0.5 mg L^{-1}); low concentrations (2.5 mg L^{-1}); medium concentrations (5.0 mg L^{-1}); and high concentrations (7.5 mg L^{-1}) at 4 days, 8 days, 16 days, and 32 days. The small symbols within the boxes denote the means, the boxes' limits are the standard errors of the mean, and the error bars represent nonoutlier ranges. Different letters denote statistically significant differences between treatments. The pentagon in the ordinate indicates the initial density of the cladocerans.

The abundance of the alga *M. minutum* showed interactions between parameters (after square-root transformation; $F_{9,\ 48} = 4.41$ $p < .001$) with the values exhibiting an inverse pattern with respect to the cladoceran abundance (Figure 5). For example, the highest algal abundance occurred at 16 days in the groups exposed to the medium and high tannin concentrations (5 and 7.5 mg L^{-1}).

3.2. Effects of lethal concentration 50

The calculated tannin concentration for 50% mortality (LC50) was 36.4 mg L^{-1} (Figure 6). The experiments showed that after 48 h approximately 50% of the individuals survived exposure to a tannin concentration of 30 mg L^{-1}, although with evident sublethal effects (i.e. a reduction in mobility and a slow reaction to stimuli). After exposure to a tannin concentration of 45 mg L^{-1}, a nearly 100% mortality of females occurred ($r^2 = 0.83$; $p < .01$).

4. Discussion

Our results have demonstrated that high tannin concentrations have negative effects on the cladoceran *D. menucoensis*. Females exposed to tannin concentrations of 5 and 7.5 mg L^{-1} were less efficient in reproducing and manifested a high rate of mortality even after a few hours of exposure, with other behavioural effects also becoming evident,

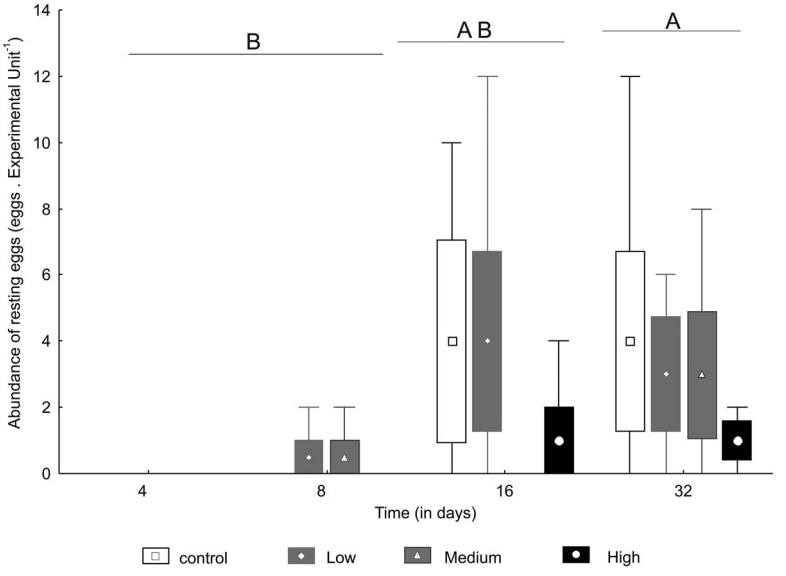

Figure 3. Abundance of *D. menucoensis* resting eggs exposed to different tannin concentrations over time. Treatments: controls (0.5 mg L^{-1}); low concentrations (2.5 mg L^{-1}); medium concentrations (5.0 mg L^{-1}); and high concentrations (7.5 mg L^{-1}) at 4 days, 8 days, 16 days, and 32 days. The small symbols within the boxes denote the means, the boxes' limits are the standard errors of the mean, and the error bars represent nonoutlier ranges. The horizontal straight lines above the boxes denote statistically indistinguishable values between the indicated treatment groups and capital letters denoted differences between treatments groups.

such as sluggish reflexes and diminished mobility. Likewise, the number of resting eggs varied during the course of the experiment, though generally increasing with time, and attained high levels by the end of the experiment.

The effect of tannins on the abundance of *M. minutum* was less evident, especially since the cladoceran herbivory strongly reduced the abundance of those algae, whereas, contrary to our initial hypothesis, no positive effects on the bacteria were recorded.

Phenolic compounds, such as tannin, and other substances play a significant ecologic role in aquatic systems because those compounds affect the humification processes that insure the stability of the global cycling of energy and organic materials [35,36; also cf. the Introduction] and affect different species assemblages [for a review cf. 37]. For example, polyphenols may attenuate the digestive enzymes of herbivores [38]; inhibit the production and export of algal exoenzymes; act on processes such as cell division, respiration, and photosynthesis [39]; change membrane permeability in bacteria; and as a result inhibit the biochemistry, physiology, and behaviour of a wide spectrum of organisms – ranging from fish [40] to bacteria.[41]

Consistent with these previously indicated interactions, our experimental results demonstrated that polyphenols – and here specifically tannins – affected the development and reproduction of the cladoceran *D. menucoensis*. At high tannin concentrations, after 8 days of exposure, the number of females decreased from mortality, and the females remaining alive lost their reproductive efficiency. Analogous results on other cladocerans had been observed by other authors as detailed in reports published worldwide. For

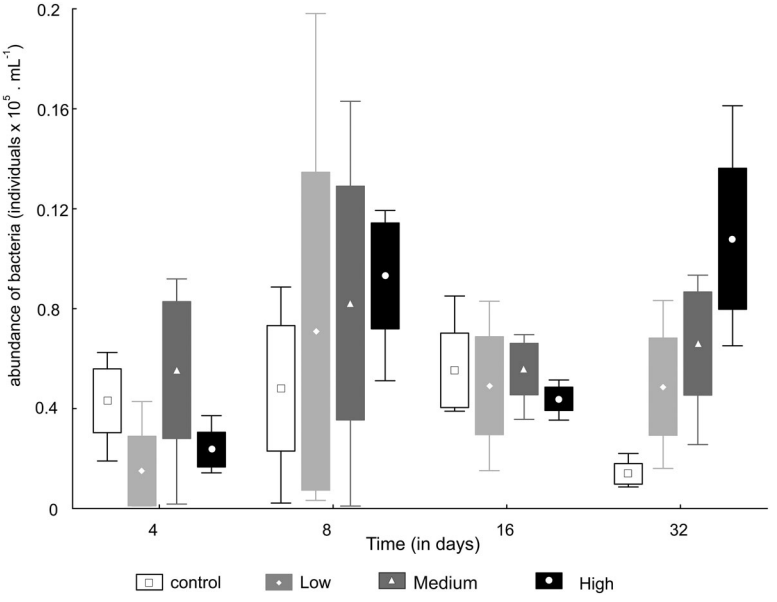

Figure 4. Abundance of bacteria exposed to different tannin concentrations over time. Treatments: controls (0.5 mg L^{-1}); low concentrations (2.5 mg L^{-1}); medium concentrations (5.0 mg L^{-1}); and high concentrations (7.5 mg L^{-1}) at 4 days, 8 days, 16 days, and 32 days. The small symbols within the boxes denote the means, the boxes' limits are the standard errors of the mean, and the error bars represent nonoutlier ranges.

example, concentrations of humic substances similar to those used by us (i.e. 2.15 and 10.8 mg L^{-1}) affected the life-history patterns of *Daphnia magna* with respect to a delay in maturation, a reduced lifespan of the females to 28%, and a decrease in the number of offspring to 30% [42], thus proving to be harmful and eventually toxic.[43] Whereas other results indicated that humic substances at low concentrations had the potential to expand the lifespan of *D. magna*, those same findings also demonstrated that the number of offspring was reduced.[44] Similar negative results were also obtained with the cladoceran *Ceriodaphnia dubia* [19] or with *Latonopsis australis* [45] although positive effects of polyphenols were, in fact, reported for specific clones of *Moina macrocopa* [46,47] and for *Daphnia magna* at low polyphenol concentrations.[48] Humic substances therefore have been shown to alter the lifespan of the cladocerans. The direction and intensity of the above-mentioned effects are clearly characteristic of certain species, and in some instances, even gender-specific.[42,43] Our results further indicated that exposure of females to high tannin concentrations also had evident sublethal effects, as manifested in a reduction in mobility and sluggish reaction to stimuli. Similarly, toxic effects, such as lethality and teratogenicity, upon contact with natural organic matter, [49] have been observed in noncrustacean freshwater biota such as snails or fish embryos, while morphologic abnormalities of the antennae in *D. magna* were registered after exposure to the solvent acetone.[50] Information on the toxicity of polyphenols – and specifically tannins – on lethality and teratogenicity in cladocerans is scarce.

In addition, other investigations [cf. 42] have shown that the stress produced by humic substances could possibly cause an increase in the number of males of *D. magna* so as to

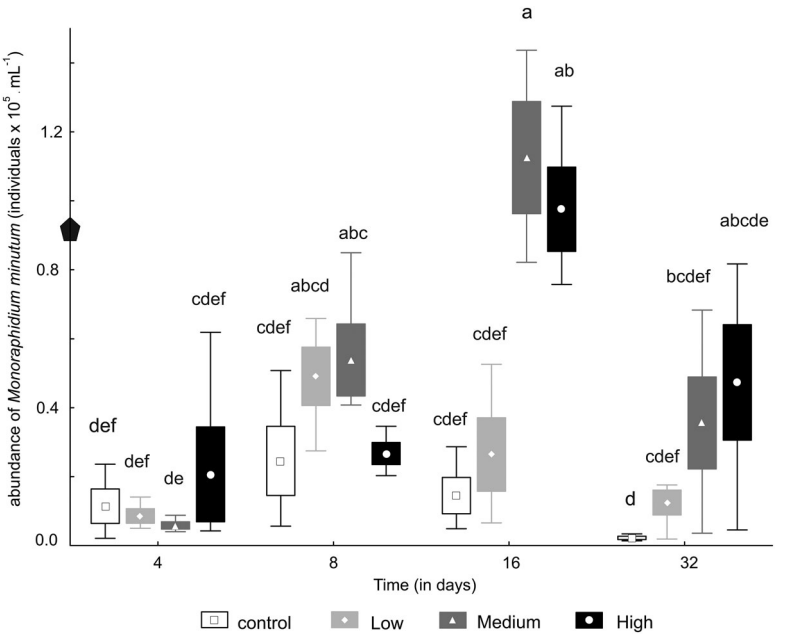

Figure 5. Abundance of algae exposed to different tannin concentrations over time. Treatments: controls (0.5 mg L^{-1}); low concentrations (2.5 mg L^{-1}); medium concentrations (5.0 mg L^{-1}); and high concentrations (7.5 mg L^{-1}) at 4 days, 8 days, 16 days, and 32 days. The small symbols within the boxes denote the means, the boxes' limits are the standard errors of the mean, and the error bars represent nonoutlier ranges. Data are shown before transformations. Different letters denote statistically significant differences between treatments. The pentagon in the ordinate indicates the algae density added as food.

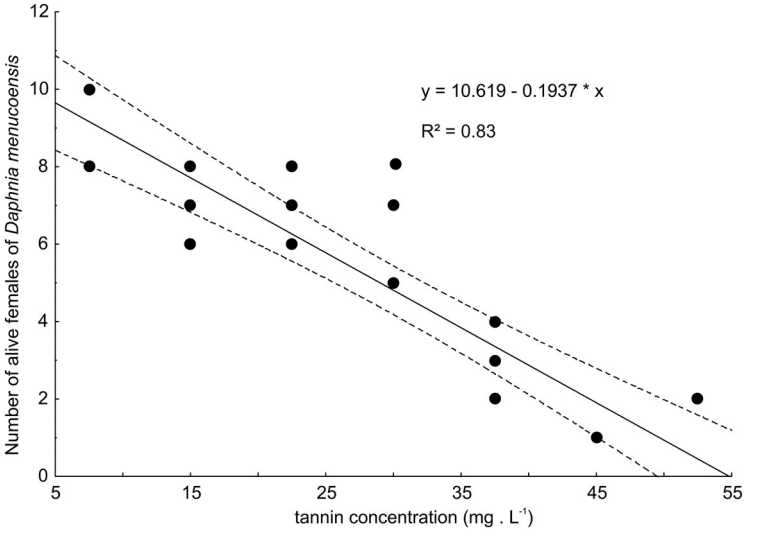

Figure 6. Number of live females of *D. menucoensis* as a function of the tannin concentrations. The solid line indicates the best fit to the data and the dotted lines the 95%-confidence limits.

result in resting eggs.[51] Accordingly, we found *D. menucoensis* males present at the end of all the treatments in our experiments, though we recorded no significant differences in the number of males during the course of the experiment or with respect to the presence or absence of tannins. Nevertheless, we did observe a slight tendency towards an increase in male abundance at 16 days. Moreover, consistent with this tendency of the males, we found a higher production of resting eggs towards the end of the experiments in the treatments at all tannin concentrations. This observation would indicate that the population had been subjected to stress factors such as crowding in the EU or certain tannin concentrations since, in the face of unfavourable circumstances, cladocerans produce resting eggs for the purpose of species perpetuation.[42]

Among the freshwater zooplankton, the cladocerans comprise the most efficient filter feeders in the pelagic community. Individuals of *Daphnia*, in particular, are large-bodied zooplankters whose herbivory on phytoplankton can modulate trophic cascades. In the present experiments, algal abundance was reduced through the cladoceran herbivory, dropping markedly under conditions where *D. menucoensis* reached high densities. Nevertheless, polyphenols might have possibly also had negative effects on algal abundances because in the treatments where the density of *D. menucoensis* was minimal – and there, specifically at 16 days – the algae had not continued to grow or reproduce, with the algal abundance recorded being similar to the number routinely added on every second day. Nevertheless, other conditions that were not analysed, such as the nutrient levels or light intensity, could have influenced algal growth in addition to the effects of polyphenols. Likewise, although no differences were found in the abundance of bacteria, at the end of experiments (Day 32) the bacteria exhibited a tendency to be less abundant at low tannins concentrations (e.g. without tannins in the control and at 2.5 mg L^{-1}) than at high concentrations (such as 5 and 7 mg L^{-1}). This pattern could be related to specific tannin effects; alternatively, if this result is analysed with reference to the abundances of algae and *D. menucoensis*, the low bacterial abundance could possibly be caused by a consumption by the cladocerans since during this time at control and low tannin concentrations the algal abundances also passed through a trough whereas the cladocerans exhibited their highest density (compare Figures 2, 4, and 5).

As mentioned in the Introduction, the organic-matter content of water bodies is principally of allochthonous origin, with the input occurring in accordance with land use, the basin size, and the onset of periods of flooding. [cf. 6] High levels of allochthonous organic matter in water bodies support trophic webs because bacterioplankton provide an alternative base for the energetic and nutritional support of the consumers – mainly the microzooplankton (i.e. the flagellates) [for a review also cf. 52], though also the macrozooplankton grazers (i.e. the cladocerans). Such a situation can occur in the shallow lakes of the Salado-River basin, [cf. 6] where the water bodies are under cyclic hydrologic periods with an alternation between intervals of draught and flooding, and where the land uses are intensive. In this regard, the information on bacterial loops and trophic webs in relation to variations in the origin of organic matter in the Salado-River basin is scarce.

In conclusion, our work demonstrates that polyphenols – in the form of tannins – reduce the survival of the cladoceran *D. menucoensis*, even over short time periods, and also compromise the locomotion of those individuals along with their capacity to respond to stimuli, whose impairments imply that their abilities as filter feeders become

diminished. Negative effects of tannins on algal abundance, however, were less evident and would require additional investigation.

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Disclosure statement

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