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Metal stress in zooplankton diapause production: post-hatching response

Adriana Aránguiz-Acuña[1] · Pablo Pérez-Portilla[1]

Abstract Aquatic organisms commonly respond to harsh conditions by forming diapausing stages, which enable populations to survive adverse periods forming egg banks. Production of diapausing eggs is frequently observed in monogonont rotifers, previously changing from asexual to partial sexual reproduction (mixis). In despite that zooplankton are frequently used in ecotoxicological assessment because of their sensitivity to various toxicants and their important role in the ecosystems, toxicity evaluations often consider the directly exposed population produced by parthenogenetic reproduction, exclusively. We assessed experimentally effects of exposure to metals on mixis delay and fitness of hatchlings of the rotifer *Brachionus plicatilis* obtained from a brackish water lagoon with high metal content, especially copper. We show that sub-lethal concentrations of copper affected traits related to sexual reproduction and diapausing egg production in the rotifer. Copper addition did not delay the start of mixis, suggesting that rapid initiation of mixis is promoted in risky environments, according to the hypothesis of mixis as an escape strategy. Higher investment in mixis was obtained when individuals were exposed to metal. Addition of copper negatively affected the hatching success of diapausing eggs and performance of hatchlings. Nevertheless, these effects were greater for individuals formed in non-metal conditions, suggesting an adaptive advantage of populations from natural sediments exposed to copper. These results highlight the ecological and evolutionary consequences of the presence of metals in freshwater environments by modulating diapause adaptive efficacy and the selective process in egg banks.

Keywords Mixis ratio · Hatching success · Rotifers · Copper · Toxicity

Introduction

Environmental fluctuation, as observed in seasonal climates, may involve harsh conditions for aquatic populations. A common response in different aquatic taxa to that fluctuation is dormancy (Brock et al. 2003). Diapause is a well-defined type of dormancy in animals, in which organisms produce a resting stage during their life cycle in response to environmental signals and internal timing (Alekseev et al. 2007). Metabolic functions are minimized in resting stages, which enable populations to survive recurrent adverse periods forming resting stage banks (Marcus et al. 1994; Hairston 1998; Kotani et al. 2001; Cáceres and Tessier 2004; García-Roger et al. 2006; Lennon and Jones 2011; Nielsen et al. 2012).

Diapause is frequently observed in cladocerans and monogonont rotifers, two cyclical parthenogens. In cyclically parthenogenetic rotifers diapausing eggs hatch producing asexual (amictic) females, which produce asexual daughters and initiate parthenogenetic proliferation, allowing fast clonal growth. Under mixis (sex) stimuli, sexual reproduction is initiated with the production of sexual (mictic) females. Sexual females produce haploid eggs parthenogenetically. If these eggs are not fertilized, they develop into males; if fertilized, they develop into diploid

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diapausing eggs, restarting the cycle (Schröeder 2005; Tortajada et al. 2009) (Fig. 1). The ecological relevance of diapause is that this strategy prevents population extinction by temporal and spatial dispersion, overcoming harsh environmental conditions (Fussmann et al. 2007) and promoting dispersal and colonization of new environments by passive transport of resting stages (Hairston and Cáceres 1996; Cáceres 1997; Slusarczyk and Pietrzak 2008).

Different environmental cues are known as inducers of the sexual phase in aquatic invertebrates (Wallace et al. 2006; Gilbert 2007). In many rotifer species the sexual phase is induced by the presence of chemical cues released to the medium by the females themselves. Thus higher population density or crowding is a stimulus for mictic female production (Gilbert 2002, 2003; Stelzer and Snell 2003). Production of sexual daughters can vary, even in clones exposed to a standardized stimulus. A hypothetical mechanism for this variation is transgenerational phenotypic plasticity (Gilbert 2002, 2007), in which stem females (i.e., those hatching from diapausing eggs) and females from the next several parthenogenetic generations are less responsive than later generations to the environmental crowding cues that induce sexual reproduction, thus promoting a phase of parthenogenetic proliferation regardless of the presence of sexual reproduction induction factors (Gilbert 2002; Schröeder and Gilbert 2004). Adaptive advantage of this strategy would rest in that phenotypes with the ability to delay mixis could consistently invade populations without mixis delay because former would initiate sexual reproduction at greater population sizes, and therefore will produce many more resting eggs than phenotypes initiating mixis before at a smaller size (Serra et al. 2005). Nevertheless, evidence shows that in populations inhabiting greatly stressed ponds, either by ephemerality or by intense predation, mixis responsiveness to crowding may be observed from the stem female generation (Schröeder et al. 2007; Gilbert and Diéguez 2010).

The presence of metals in freshwater environments is increasing due to continued human population growth, industrialization, urbanization and expansion of productive activities (Landis and Yu 2005; Larios et al. 2013; Tapia and Audry 2013). Moreover, arid zones are commonly characterized by naturally high concentrations of minerals (Laurent et al. 2008). The region including Southern Peru and Northern Chile is one of the driest regions in the world, with elevated concentrations of heavy metals (lead, copper, cadmium and zinc), and metalloids (arsenic) (Pell et al. 2013), and supports intense mining operations mainly focused on copper extraction. In addition, high evaporation in the region makes continental water highly saline, and with high concentration of the metals (Díaz-Palma et al. 2012).

Tolerance to different anthropogenic pressures, specifically toxicant exposure, depends upon the conditions of parental generations. Some aquatic invertebrates produce low quality offspring when there are pollutants in the maternal environment, as result of energetically expensive mechanisms of tolerance, as repair and detoxification in the parental generation (Marshall 2008; Fernández-González et al. 2011), with reduced resource allocation to other functions (Van Straalen and Hoffmann 2000). Nevertheless, scarce and contradictory information is available on the effects of chemical exposure on hatching of dormant zooplankton eggs or fitness of hatchlings. Both reduction of hatching and hatchlings success and null effects from toxicant exposure on hatching have been observed in

Fig. 1 Life cycle of monogonont rotifers (from Tortajada et al. 2009). In asexual phase, asexual females produce asexual diploid eggs by parthenogenesis. When environmental stimulus induces sexual phase, sexual females are produced, which parthenogenetically produce haploid males that fertilize sexual females that develop into diploid diapausing eggs. These diapausing eggs are layered into the sediments forming diapausing egg banks
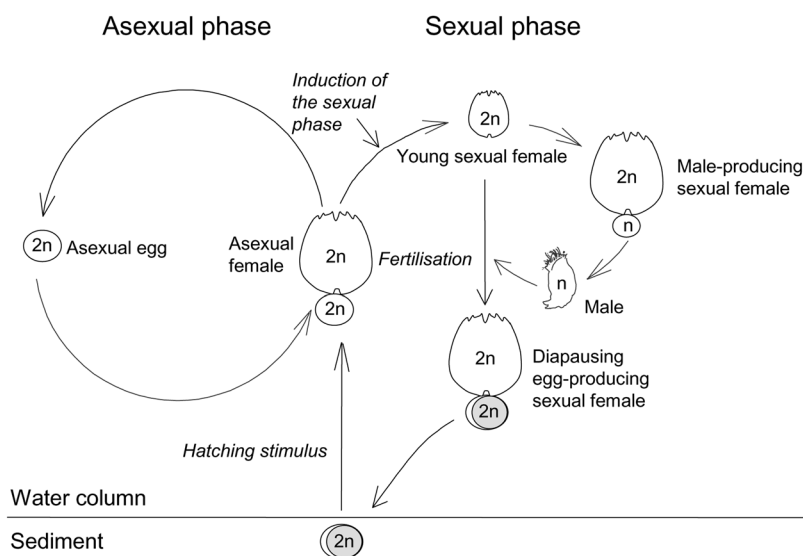
zooplankton populations from field and laboratory (Hairston et al. 1999; Kerfoot et al. 1999; Varó et al. 2006; Jiang et al. 2007; Marcial and Hagiwara 2007; Sarabia et al. 2008; Navis et al. 2013; Aránguiz-Acuña and Serra 2016). We showed that arsenic increased rotifer diapausing egg production, which suggests a strategy of escaping an adverse environment. Nevertheless, the eggs produced had low quality, and therefore the long-term effectiveness of this response remains unclear (Aránguiz-Acuña and Serra 2016).

The aim of this study is to assess the toxic transgenerational effects of exposure to sublethal concentration of metals present in lagoons nearby important mining centers on the production of diapausing eggs and performance of hatchlings rotifers from diapausing eggs produced in an environment exposed to heavy metals. We focused specifically on the propensity to sexual reproduction in the rotifer *Brachionus plicatilis* of stem females and females from the next parthenogenetic generations hatched from diapausing eggs, and fitness of hatchlings obtained from a brackish water lagoon in the Chilean Altiplano with high content of metals. We hypothesize that (1) the presence of sublethal concentration of a selected heavy metal from those present in sediments during the formation of diapausing eggs will have delayed mixis as a consequence, and (2) mothers from an environment with a selected heavy metal from those present in sediments will transfer the toxic effects to diapausing eggs, resulting in decreased hatchability and fitness of the hatchlings.

Materials and methods

Sampling site and field collection

The sampling site, Inca-Coya, Chile (22°33′S-68°59′W), is a small permanent pond located at Chiu-Chiu village, 54 km from Chuquicamata, which by the excavated volume is the largest open pit copper mine in the world. The pond is isolated from other ponds and is located near the Salado River, the main perennial tributary of the Loa River, the only one in northern Chile starting in Los Andes and flowing into the sea (Latorre et al. 2006). Both the Loa River and the Salado River are slightly alkaline (de Wet et al. 2015) and the latter is arsenic-enriched by water from the El Tatio geothermal fields, reaching levels up to 27 mg As L^{-1} (Romero et al. 2003). Mean annual precipitation is 6.1 mm at Chiu-Chiu; almost 90% of the annual rainfall occurs in summer (Latorre et al. 2006). The extremely arid conditions and high evaporation maintain high concentrations of arsenic, copper, boron, chloride, sulfate, and others throughout the Loa River course (Pell et al. 2013).

Superficial sediment samples were obtained at the center of the pond by the use of a Van Veen bottom sampler, with a grasping area of 250 cm^2. Sediment samples were kept in the dark at 4 °C until used to extract diapausing eggs. Part of the sediment sample was used to analyze its metal content in order to identify which heavy metals are mainly accumulated in Inca-Coya pond sediment. Atomic absorption spectroscopy (AAS) was used to quantify aluminum, cadmium, zinc, copper and lead, while mercury was analyzed by vapor generation AAS and arsenic by AAS with hydride generation. Although replicate samples were not obtained, and therefore there is no statistical support for sampling, the reading of several standards and certified reference material and the compliance with detection and quantification limits for each analyses, guarantee efficiency of analytical results and constitute an indicator of metals accumulated in the sediment.

Isolation of diapausing eggs and identification of rotifers

Diapausing eggs were isolated from sediment samples using a modified sucrose flotation technique (Gómez and Carvalho 2000), and were individually isolated in 96-multiwell dishes and induced to hatch in in f/2 medium-enriched saline water (Guillard and Ryther 1962), prepared with diluted artificial seawater (ASTM 2013) with 3.5 g L^{-1} salinity at 25 °C under white fluorescent constant illumination (150–170 μmol quanta m^{-2} s^{-1}). Dishes were checked every 24 h for hatchlings for up to 10 days. Clones of *Brachionus plicatilis* were obtained by asexual proliferation of individual females hatched from isolated diapausing eggs.

Species identification of the clones was performed by polymerase chain reaction (PCR). Previously, DNA extraction it was performed in 5–7 females per clone using HotSHOT method (Montero-Pau et al. 2008). A fragment of the mitochondrial gene cytochrome *c* oxidase subunit I (COI) (ca. 710) was amplified using the LCO1490 and HCO2198 primers (Folmer et al. 1994). PCR was performed in vials containing 2 μL of template DNA, 1 × PCR buffer, 0.5 μM of each primer, 1.7 mM $MgCl_2$, 0.2 mM dNTPs, and 0.5 U taq DNA polymerase (Thermo). The thermal profile consisted of a 3 min initial cycle at 94 °C, followed by 35 cycles of 94 °C for 45 s, 50 °C for 1 min, with a final extension of 72 °C for 5 min. Five microliters of the PCR product were separated by electrophoresis in a 1% agarose gel. Gels were stained with ethidium bromide and exposed to a UV transilluminator for direct DNA fragment visualization. The sequence of reverse and forward strand were edited in Chromas 2.6 program (Technelysium 2016) and then aligned in NCBI bioinformatics database.

Culture of the test species

Stock rotifer cultures from isolated clones were maintained in f/2 medium-enriched standard saline water. Cultures were fed daily with the green microalga *Pseudokirchneriella subcapitata* at a density of 1×10^6 cell mL^{-1}, which was cultured with Bold's basal medium (Borowitzka and Borowitzka 1988). The algae used as food were harvested during the exponential growth phase, centrifuged (3000 rpm for 5 min) and re-suspended in experimental f/2 medium for use. Their concentration was measured by direct counting. The medium with concentrated microalgae was renewed every 2 days.

Toxicity bioassays

Bioassays were conducted to identify the tolerance of *B. plicatilis* hatched from natural sediments to metals found in high concentrations in Inca-Coya pond sediments. Stock cultures of *B. plicatilis* were established by asexual proliferation of the isolated hatchlings from diapausing eggs obtained from Inca-Coya pond sediments. Identical bioassays were also conducted with a referential strain of *B. plicatilis* obtained from the Marine Research Center of Quintay of the Universidad Andrés Bello, in Central Chile (33°11′00″S 71°41′10″O), which has been maintained by years in standard laboratory conditions unexposed to metals previously.

Standard acute bioassays were conducted for 48 h following the guidelines described by ASTM (1991). Neonates (<6 h-old) were selected from each *B. plicatilis* strain (Inca-Coya and Quintay), for the bioassays. In order to identify the sensitivity of each *B. plicatilis* strain to different metals found in Inca-Coya pond sediments, toxicity bioassays were conducted with quantified metals in previous section. The range of tested nominal concentrations was selected for each toxicant based on previous studies (Queirolo et al. 2000; Aránguiz-Acuña and Serra 2016). After conducting preliminary bioassays, ultimate bioassays for both strains were conducted at 0, 0.2, 0.4, 0.6, 0.8 and 1 mg L^{-1} of $CuSO_4 \cdot 5 H_2O$. For $ZnSO_4 \cdot 7 H_2O$ and $AlCl_3 \cdot 6 H_2O$ bioassays were conducted at 0, 2, 4, 6, 8 and 10 mg L^{-1}. Finally, As_2O_3 toxicity was tested at 0, 0.0001, 0.001, 0.01, 0.1 and 1 mg L^{-1}.

In order to control for addition of sulphates and chlorides on the mortality estimation caused by exposure to metals, additional bioassays were conducted with $MgSO_4$ and $MgCl \cdot 6 H_2O$. These were performed under identical conditions and with the same concentrations of sulphates and chlorides employed in the metal test described above. The difference in the mortality of *B. plicatilis* between treatments with metal and sulphate or chloride as appropriate,

was estimated for identify toxicity of each metal on the rotifer.

Median lethal concentrations (LC50%) of metals determined in these assays were estimated using probit analysis or the trimmed Spearman–Karber method according to the established standard procedure (USEPA 2012) for each rotifer strain. *t*-tests were used to compare LC50% values between strains. From these results one metal compound and one sublethal concentration was selected to be used in following experiments.

Transgenerational effect of metals on mixis ratio

With the aim of test if sublethal metal concentration delay the initiation of mixis, clonal cultures of *B. plicatilis* were established by asexual proliferation of the isolated hatchlings from diapausing eggs obtained from Inca-Coya pond sediments. Fifteen clones were founded and kept individually in 20 mL vessels at 22 °C and 3.5 g L^{-1} salinity. These clonal linages were maintained isolated under standard conditions. Previous to experiments, multi-clonal pre-experimental populations were established by mixing 25 females of each clone, following Gabaldón et al. (2013). Multi-clonal cultures were fed with inert *P. subcapitata* in concentrations of 5×10^5 cell mL^{-1}. Using inert algae avoids the problem of growing *P. subcapitata* at salinities outside of its optimum (Gabaldón et al. 2015). To test the response of the mixis ratio to metal exposure over generations, 48 neonate females (<6 h-old) from multi-clonal pre-experimental populations were transferred individually to multiwell dishes with 100 L medium, 24 of them in f/2-enriched diluted saline water prepared as above (control) and 24 in the same medium plus the metal at the concentration selected from the toxicity bioassays (see above). Medium with algae plus metal, as appropriate, was renewed every 2 days. These individuals were our P generation. The first offspring these females produced, identified as the F_1 generation, were isolated and cultured in the same conditions as their mothers. F_2 and subsequent generations were obtained by isolating the first offspring of the preceding generations and culturing them at a population density of 1 ind mL^{-1}. In each generation the first offspring produced was recorded and used to initiate the next generation, and age of mothers at first reproduction were recorded. The same procedure was repeated until the F_{10} generation.

In generations F_4, F_6, F_8 and F_{10} all the offspring produced during the mother's life were cultured individually in the same conditions as the mother in 100 L medium in wells of multiwell plates, and complete life tables were constructed by recording the age-specific survival and reproduction of individual organisms, as above. Offspring produced were cultured until each female could be typed as amictic or mictic based on the size of the eggs they were

carrying. The proportion of mictic females (mixis ratio) of the offspring was determined in each final population.

Transfer of toxic effects on hatching proportion and fitness of hatchlings

Three 250 mL containers were established from multi-clonal pre-experimental populations. These populations were cultured for at least ten generations to control for maternal effects. Every day the medium was renewed and microalga *P. subcapitata* was provided at 5×10^5 cells mL^{-1}. The experimental cultures were allowed to grow and produce diapausing eggs for 3 weeks. Diapausing eggs produced were retired every 2 days and stored in the dark at 4 °C. After a period of 1 month of obligated dormancy (Hagiwara and Hino 1989), diapausing eggs were individually isolated in 96-multiwell dishes and induced to hatch in standard saline water at 25 °C under constant illumination. Ninety-six diapausing eggs were induced in control medium and ninety-six diapausing eggs were induced in medium with the metal at the concentration selected from toxicity bioassays (see above). Diapausing eggs were checked every 24 h, and hatchings were recorded for 10 days. Additionally, diapausing eggs isolated from pond sediments were directly placed in multiwell plates for hatching under the same conditions as above.

Hatchlings from both control culture and sediments were individually cultured at the same metal condition as hatching, in multiwell dishes with 100 L of food suspension of *P. subcapitata* at density of 5×10^5 cells mL^{-1} in f/2-enriched saline water. Complete life tables were constructed by recording the age-specific survival and reproduction of individual organisms of *B. plicatilis* from birth to death. Survival of individuals was checked every 24 h; offspring were counted and discarded and the medium renewed. Age-specific fertility (m_x, computed using number of live offspring at each age x) and proportion of survivors to a given age (l_x) were estimated. The finite population growth rate (λ) was calculated using the discrete Euler-Lotka equation:

$$1 = \sum \lambda^{-x} l_x m_x$$

We calculated 95% confidence intervals for λ by 5000 bootstrap re-samplings.

Statistical analysis

To test if sublethal concentration of copper produced delayed mixis, total offspring per generations F_1 to F_{10} and age at first reproduction per generations were analyzed by ANCOVAs, with treatments of copper exposure as factor and generation as covariate. Mixis ratio of generations F_4, F_6, F_8 and F_{10} generation was analyzed by GLM with

binomial distribution; the logit link function was used to determine if they varied significantly across generations or among treatments of copper exposure.

To test if hatchability of diapausing eggs and fitness of the hatchlings were affected by exposure to sublethal concentration of copper, Generalized Linear Models (GLM) were used with Poisson distribution and log link function for hatching counts, with medium where diapausing eggs were obtained (control culture or sediment), and exposure to metal for diapausing eggs hatching (with/without) as factors.

Differences in growth rate of hatchlings between treatments (cultures/sediments) were tested by generating pseudo-samples by means of a permutation test of individual life tables, using $\alpha = 0.05$. All the statistical analyses were implemented in R v.3.2.2 statistical software (R Core Team 2015).

Results

Toxicity bioassays

Metal content in sediments is shown in Table 1. Bioassays with two *B. plicatilis* strains were performed to assess the tolerance to copper, zinc, aluminum and arsenic. Figure 2 shows the results of bioassays with two *B. plicatilis* strains. Higher tolerance to all tested metals was observed in the strain from Inca-Coya pond (from sediments exposed to metals) than in the strain from Quintay Culture Center (unexposed to metals). The lowest tolerance was found for copper, and this metal was selected as the toxicant for the following experiments; it was added in a concentration of 0.2 mg CuSO4·5·H$_2$O·L^{-1}, corresponding to LC10% of the strain from Inca-Coya pond.

Transgenerational effect of metals on reproduction and mixis ratio

Exposure of asexual females to sublethal copper concentrations did not have statistically significant effect on total offspring (ANCOVA, $p = 0.105$) or on its age at first reproduction (ANCOVA, $p = 0.841$). Nevertheless, there was a tendency to increase total offspring (ANCOVA, $p = 0.002$) and age at first reproduction (ANCOVA, $p = 0.003$) over ten generations from hatching from diapausing eggs. By contrast, mixis ratio increased as a result of exposure to copper sublethal concentrations (GLM, Chi square $p = 0.029$), although did not show a significant relationship with the generation in which mixis ratio was measured (Fig. 3).

Table 1 Content of metals in sediment of Inca Coya pond, in Chiu-Chiu, Chile

Parameter	Concentration (mg Kg^{-1})
Aluminum	2884
Arsenic	788
Cadmium	1.82
Zinc	47
Copper	394
Mercury	0.026
Lead	27

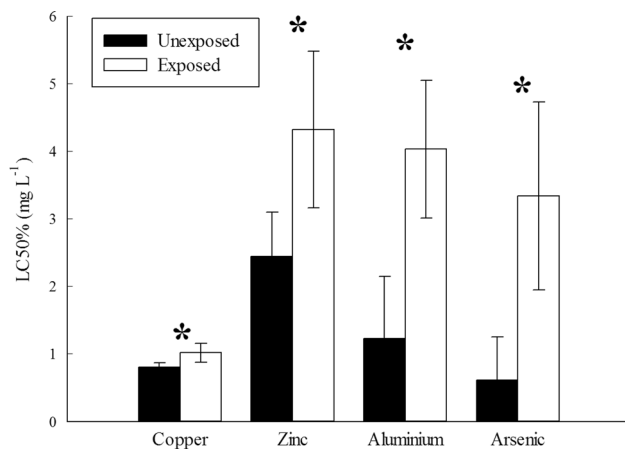The metals were analyzed by atomic absorption spectrophotometry (AAS)

Fig. 2 Median lethal concentration (LC50%) of the population for two strains of *Brachionus plicatilis* rotifer: one from Quintay Culture Center (*white columns*) and another from Inca-Coya pond in Chiu-Chiu (*black columns*). The strain from Quintay Culture Center has been unexposed to metals by years, while Inca-Coya pond has high metals concentrations in sediments (see Table 1). Both strains were exposed to different metals. Mean values and 95% CI are shown. Asterisks show significant differences (*t*-test, $p < 0.05$)

Hatching proportion and fitness of hatchlings

Diapausing eggs produced by *B. plicatilis* sexual females had a significantly decreased hatching success when were exposed to a sublethal concentration of copper (GLM, Chi square $p < 0.001$). More interesting, interaction was observed between mother environment (control culture of laboratory or sediment from Inca-Coya pond) and diapausing eggs hatching medium (with addition of sublethal copper concentration or control without metals) (Chi square $p < 0.001$). The addition of copper in the hatching medium had lower negative effect for eggs isolated from the field than for eggs produced in the laboratory, which were more affected (Fig. 4).
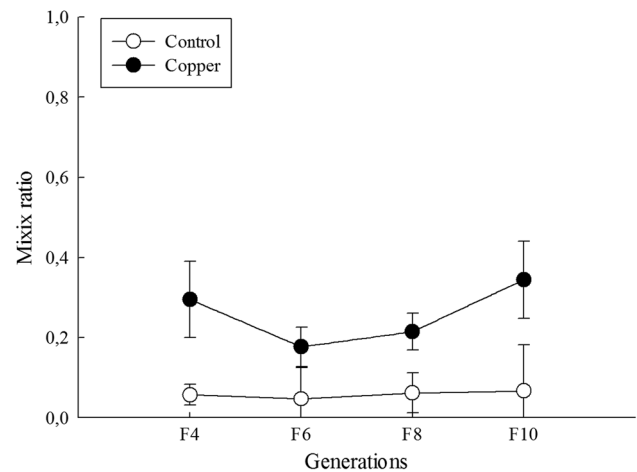

Fig. 3 Mixis ratio of *Brachionus plicatilis* produced in generations F_4, F_6, F_8 and F_{10}, after exposure to sublethal copper concentrations, and a control unexposed to copper (mean ± 95 % CI)

Patterns for population growth rates were qualitatively similar to those in hatching success. A negative effect of copper was observed in population growth rates both in hatchlings from Inca-Coya diapausing eggs as laboratory diapausing eggs, but this effect is weaker in clones from eggs isolated in the field than those clones hatched from eggs produced in the laboratory (Fig. 5).

Discussion

The rotifer *B. plicatilis* from Inca-Coya pond in northern Chile showed great resistance to tested heavy metals in toxicity bioassays. It was more resistant to the different heavy metals tested than individuals of the same species obtained from standard cultures where exposure to metals were much lower or null (as the strain from Marine Research Center of Quintay). *B. plicatilis* strain from Inca-Coya showed acute toxicity on the half of the population after 2 days of exposure to copper at 1 mg L^{-1}, while other authors have reported early full mortality of experimental populations of *B. plicatilis* at 0.5 mg L^{-1} (Luna-Andrade et al. 2002), and strong effect on population density of the closely related *B. rotundiformis* even at 0.03125 mg L^{-1} of the same compound, $CuSO_4$ (Gama-Flores et al. 2005). Moreover, the addition of copper did not affect critical reproductive parameters, such as total offspring produced or the age at first reproduction, of populations initiated from diapausing eggs from Inca-Coya pond. This is not surprising because water bodies of this region have a long history of exposure to metals (Dittmar 2004). Nevertheless, traits related to sexual reproduction and diapause egg production were affected by copper exposure, with increased investment in mixis when neonate individuals were exposed to the
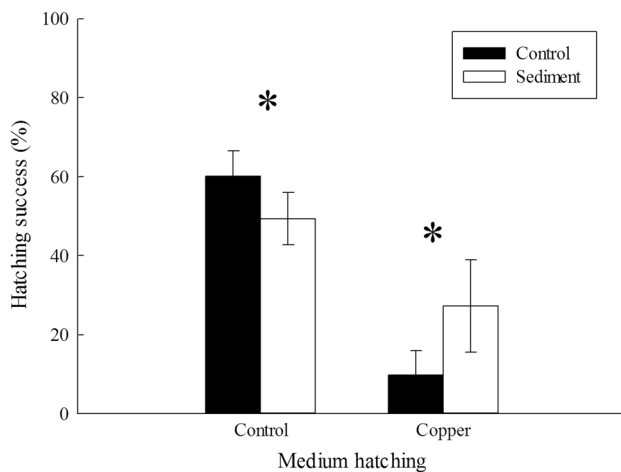
Fig. 4 Hatching proportion (mean values ± 95% CI) of diapausing eggs produced by *Brachionus plicatilis* in control conditions (*black columns*) and from Inca-Coya pond sediments (*white columns*). Diapausing eggs were induced to hatch in two media: control and exposed to sublethal copper concentrations

Fig. 5 Population growth rates (mean values ± 95% CI) of *Brachionus plicatilis* individuals hatched from diapausing eggs produced in control conditions and from sediments from Inca-Coya pond, and hatched in fresh medium unexposed to copper or in medium exposed to copper (significant differences between treatments, 5000 randomizations, $\alpha = 0.05$)

metal along its life cycle. This result is in agreement with reports showing that the sexual phase in rotifers may be more sensitive to exposure to environmental toxicants than the asexual phase (Snell and Carmona 1995; Snell et al. 1999; Preston and Snell 2001; Marcial et al. 2005, Aránguiz-Acuña and Serra 2016).

B. plicatilis from Inca-Coya pond did not delay sexual phase initiation, i.e. appearance of males and sexual females into the experimental population, after diapausing egg hatched with or without addition of copper, and therefore there is no evidence of a transgenerational plasticity effect produced by copper exposure on mixis ratio. We observed propensity to mixis in our experimental population exposed to copper from the first generation analyzed (F_4). We obtained similar results in a previous study with *Brachionus calyciflorus* exposed to arsenic, with mixis ratios around 0.8 in the F_2 generation (Aránguiz-Acuña and Serra 2016). Our results suggest that early initiation of mixis is promoted in risky environments, as found in previous studies, both due to physical and chemical stress driven by ponds that are extremely temporary (Schröeder et al. 2007; Gilbert and Diéguez 2010) and to ecological stress driven by competition and predation (Gilbert and Diéguez 2010; Aránguiz-Acuña and Ramos-Jiliberto 2014). Thus our results are in accordance with the hypothesis of mixis as an escape strategy to overcome harsh environmental conditions.

Copper negatively affects the hatching success of diapausing eggs and fitness of hatchlings shown by eggs formed under control culture conditions and eggs obtained from natural sediments in highly polluted media. Nevertheless, the reduction in hatching success was greater in diapausing eggs produced in control cultures than in those
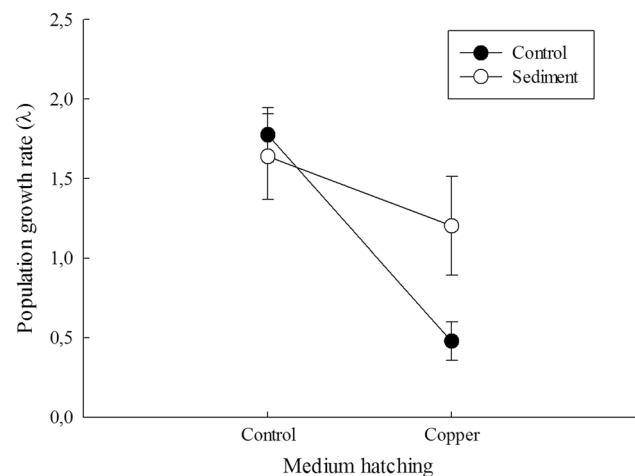
from sediments. Also, population growth rates showed a greater decrease in fitness of populations proceeding from control cultures, where growth rates indicated population decline ($\lambda < 1$), while populations hatched from diapausing eggs from sediments were able to grow ($\lambda > 1$). These results show that the conditions where diapausing eggs were formed affected hatching success and hatchlings fitness. It is suggested that post-hatching developmental performance could be affected by historical chemical exposure by two plausible mechanisms: by conditions experienced by previous generations which may alter the fitness of the offspring and by a direct toxic effect on the diapausing eggs (Rogalski 2015). About the first mechanism, multigenerational exposure to metals could generate bioaccumulation in active rotifers (Alvarado-Flores et al. 2012; Aránguiz-Acuña and Serra 2016; Woelfl et al. 2016) and may transfer this metal to their progeny, not only inheriting morphological abnormalities (Alvarado-Flores et al. 2015) but also with associated fitness costs. About the second mechanism, diapausing eggs could bioaccumulate metals directly from the environment, as has been observed in *Daphnia* ephippia (Wyn et al. 2007; Navis et al. 2015). Both mechanisms of toxicity transmission are plausible to affect the success of hatchlings.

These results suggest (1) that mothers could transfer metal tolerance to offspring through the diapause period, and (2) local adaptation of populations to the presence of metals. Strong evidence shows that sexual reproduction strategies play a key role in local adaptation of rotifers to harsh environments (Campillo et al. 2011; Scheuerl and Stelzer 2013). The prediction is rapid loss of metal resistance when they are removed, if resistance carries a cost

(Turko et al. 2016). It may be hypothesized that evolution to metal resistance has an energetic cost, which would be shown in a non-adaptive environment, i.e. in a metals-free environment, in this case. Evidence for evolution and fitness costs of metal tolerance have been previously observed in *Daphnia* (Agra et al. 2010, 2011; Rogalski 2015) as in other invertebrates (Posthuma et al. 1993; Gardeström et al. 2008). Van Straalen and Hoffmann (2000) propose two mechanisms to explain the costs of metal tolerance: (1) the trade-off hypothesis, which postulates that tolerance mechanisms could be energetically expensive, with reduced resource allocation to other functions, and (2) the metal deficiency hypothesis, which propose lower efficiency of essential metal uptake or utilization, when tolerance mechanisms are displayed, which would reduce individual fitness in an unpolluted environment. These two hypotheses may explain the results observed in this study, where *B. plicatilis* from natural sediments showed better performance in metal conditions—suggesting potential adaptation to metal exposure, while organisms unexposed to metals for some generations rapidly could lost resistance and their performance was diminished when exposed to metals.

Our results highlight how metal exposure may affect hatching success and the performance of the rotifer populations emerging from diapausing egg bank. Thus a possible consequence of elevated metal concentrations is the alteration of the structure and diversity of egg banks by toxicant-induced selection of resistant phenotypes, both in the water column and in sediment, which may have great impact on the genetic variability of populations and on the sensitivity to novel stressors (Medina et al. 2007; Gardeström et al. 2008; Agra et al. 2010). Additionally, although we selected only one metal to test our hypotheses, we know that combinations of metals, as occurs in the Inca-Coya pond and probably in many other water bodies in arid zones, produce complex interactive effects (Xu et al. 2015) with unpredictable results based on knowledge of the effects of single metals.

Ecotoxicological long-term and evolutionary consequences of the presence of toxicants on production and maintenance of diapausing are not fully understood. The existence of diapausing egg banks creates a coupling between selective forces operating in benthic and pelagic habitats; selection pressures during diapause will determine the successful development of eggs, and this selection, in turn, will shape the community composition and genetic structure of emerging populations in the water column (Hairston et al. 1996; Aparici et al. 2001; Gyllström and Hansson 2004). Additionally, the consequences of differences in timing and the investment in sexual reproduction affect not only fitness in cyclical parthenogenetic populations (Aparici et al. 1996; Serra et al. 2004), but also ecological interactions of competition and coexistence (Montero-Pau and Serra 2011; Aránguiz-Acuña and

Ramos-Jiliberto 2014; Aránguiz-Acuña et al. 2015; Gabaldón et al. 2015), as well as clonal selection, genetic diversity and adaptive traits (Gómez and Carvalho 2000; Serra and Snell 2009), playing an important role in shaping the diversity and dynamics of current and future zooplankton communities (Brendonck and De Meester 2003). Paleolimnological studies, in which analyses of structure of diapausing egg banks are main tools, may complement limnological long-term records, providing evidence for temporal patterns of zooplankton response to changes in water quality. Changes in rotifer community diversity have been described based in the record of diapausing eggs in a lake, which had a long episode of copper and ammonia pollution coupled with lake acidification (Piscia et al. 2016). Moreover, recent evidence shows that historical metal contamination of natural lakes not only affected negatively hatching rates of diapausing *Daphnia* eggs (Rogalski 2015), but also rapid adaptation of post-diapausing populations to the presence or absence of metals in the environment (Turko et al. 2016).

Although our results were obtained using diapausing eggs from surface sediments, and therefore recent, which do not allow demonstrating evolution in a natural egg bank, we show clear phenotypic modification of a rotifer population mediated by diapause production. It has been proven that rotifers evolve rapidly to changing environmental conditions (Fussmann 2011; Declerck et al. 2015), even in laboratory conditions (Fussmann et al. 2003) and that propensity to mixis is under strong selection. The concentrations of metals in sediments from Inca-Coya pond are in the range of those found in mining-impacted water bodies, with the exception of copper that far exceeds the concentrations observed in sediments of polluted lagoons (Marín-Guirao et al. 2008; Karstens et al. 2016). This high concentration of metals suggests that strong environment pressures could be operating on aquatic populations of water bodies of arid zones, that may have relevant consequences on clonal diversity of inhabitant rotifer populations both at short-term and long-term through on modification on structure and function of diapausing egg bank.

Compliance with ethical standards

Conflict of interest The authors declare that they have no competing interests.

Ethical approval All applicable international, national, and/or institutional guidelines for the care and use of animals were followed.

References

Agra AR, Guilhermino L, Soares AMVM, Barata C (2010) Genetic costs of tolerance to metals in *Daphnia longispina* populations historically exposed to copper mine drainage. Environ Toxicol Chem 29:939–946

Agra AR, Soares AMVM, Barata C (2011) Life-history consequences of adaptation to pollution. "*Daphnia longispina* clones historically exposed to copper". Ecotoxicology 20:552–562

Alekseev VR, De Stasio BT, Gilbert JJ (2007) Diapause in aquatic invertebrates: theory and human use. Springer, The Netherlands, Monographiae Biologicae, Vol 84

Alvarado-Flores J, Rico-Martínez R, Ventura-Juárez J, Silva-Briano M, Rubio-Franchini I (2012) Bioconcentration and localization of lead in the freshwater rotifer *Brachionus calyciflorus* Pallas 1677 (Rotifera: Monogononta). Aquat Toxicol 109:127–132

Alvarado-Flores J, Rico-Martínez R, Adabache-Ortíz A, Silva-Briano M (2015) Morphological alterations in the freshwater rotifer *Brachionus calyciflorus* Pallas 1766 (Rotifera: Monogononta) caused by vinclozolin chronic exposure. Ecotoxicology 24:915–925

Aparici E, Carmona MJ, Serra M (1996) Polymorphism in bisexual reproductive patterns of cyclical parthenogens. A simulation approach using a rotifer growth model. Ecol Model 88:133–142

Aparici E, Carmona MJ, Serra M (2001) Variability for mixis initiation in *Brachionus plicatilis*. Hydrobiologia 446/447:45–50

Aránguiz-Acuña A, Ramos-Jiliberto R (2014) Diapause may promote coexistence of zooplankton competitors. J Plankton Res 36 (4):978–988

Aránguiz-Acuña A, Ramos-Jiliberto R, Serra M (2015) Zooplankton competition promotes trade-offs affecting diapause in rotifers. Oecologia 177:273–279

Aránguiz-Acuña A, Serra M (2016) Diapause as escape strategy to exposure to toxicants: response of *Brachionus calyciforus* to arsenic. Ecotoxicology 25:708–719

ASTM (1991) A standard practice for performing acute toxicity tests using rotifers in the genus *Brachionus*. American Society of Testing and Materials, E1440(11.04):1210–1216

ASTM (2013) Standard practice for the preparation of substitute ocean water. ASTM International, West Conshohocken

Borowitzka MA, Borowitzka LJ (1988) Micro-algal biotechnology. Cambridge University Press, Cambridge

Brendonck L, De Meester L (2003) Egg banks in freshwater zooplankton: evolutionary and ecological archives in the sediment. Hydrobiologia 491:65–84

Brock MA, Nielsen DL, Shiel RJ, Green JD, Langley JD (2003) Drought and aquatic community resilience: the role of eggs and seeds in sediments of temporary wetlands. Freshwater Biol 48:1207–1218

Cáceres C (1997) Temporal variation, dormancy, and coexistence: a field test of the storage effect. Ecology 94:9171–9175

Cáceres CE, Tessier AJ (2004) To sink or swim: variable diapause strategies among *Daphnia* species. Limnol Oceanogr 49:1333–1340

Campillo S, García-Roger EM, Carmona MJ, Serra M (2011) Local adaptation in rotifer populations. Evol Ecol 25:933–947

de Wet CB, Godfrey L, de Wet AP (2015) Sedimentology and stable isotopes from a lacustrine-to-palustrine limestone deposited in an arid setting, climatic and tectonic factors: Miocene–Pliocene Opache formation, Atacama Desert, Chile. Palaeogeogr Palaeoclimatol Palaeocl 426:46–67

Declerck SAJ, Malo A, Diehl S, Waasdorp D, Lemmen KD, Proios K, Papakostas S (2015) Rapid adaptation of herbivore consumers to nutrient limitation: eco-evolutionary feedbacks to population demography and resource control. Ecol Lett 18:553–562

Díaz-Palma P, Stegen S, Queirolo F, Arias D, Araya S (2012) Biochemical profile of halophilous microalgae strains from high-andean extreme ecosystems (NE-Chile) using methodological validation approaches. J Biosci Bioeng 113:730–736

Dittmar T (2004) Hydrochemical process controlling arsenic and heavy metal contamination in the Elqui river system (Chile). Sci Total Environ 325:193–207

Fernández-González MA, González-Barrientos J, Carter MJ, Ramos-Jiliberto R (2011) Parent-to-offspring transfer of sublethal effects of copper exposure: metabolic rate and life-history traits of *Daphnia*. Rev Chil Hist Nat 84:195–201

Folmer O, Black M, Hoen W, Lutz R, Vrijenhoek R (1994) DNA primers for amplification of mitochondrial cytochrome *c* oxidase subunit I from diverse metazoan invertebrates. Mol Ecol 10:1743–1750

Fussmann GF, Ellner SP, Hairston Jr NG (2003) Evolution as a critical component of plankton dynamics. Proc R Soc Lond B Biol Sci 270:1015–1022

Fussmann GF, Kramer G, Labib M (2007) Incomplete induction of mixis in *Brachionus calyciflorus*: patterns of reproduction at the individual level. Hydrobiologia 593:111–119

Fussmann GF (2011) Rotifers: excellent subjects for the study of macro- and microevolutionary change. Hydrobiologia 662:11–18

Gabaldón C, Montero-Pau J, Serra M, Carmona MJ (2013) Morphological similarity and ecological overlap in two rotifer species. PLoS One 8(2):e57087

Gabaldón C, Carmona MJ, Montero-Pau J, Serra M (2015) Long-term competitive dynamics of two cryptic species: diapause and fluctuating conditions. PLoS One 10(4):e0124406

Gama-Flores JL, Sarma SSS, Nandini S (2005) Interaction among copper toxicity, temperature and salinity on the population dynamics of *Brachionus rotundiformis* (Rotifera). Hydrobiologia 546:559–568

García-Roger EM, Carmona MJ, Serra M (2006) Patterns in rotifer diapausing egg banks: density and viability. J Exp Mar Biol Ecol 336:198–210

Gardeström J, Dahl U, Kotsalainen O, Maxson A, Elfwing T, Grahn M, Bengtsson B-E, Breitholtz M (2008) Evidence of population genetic effects of long-term exposure to contaminated sediments. A multi-endpoint study with copepods. Aquat Toxicol 86:426–436

Gilbert JJ (2002) Endogenous regulation of environmentally induced sexuality in a rotifer: a multigenerational parental effect induced by fertilization. Freshw Biol 47:1633–1641

Gilbert JJ (2003) Specificity of crowding response that induce sexuality in the rotifer *Brachionus*. Limnol Oceanogr 48:1297–1303

Gilbert JJ (2007) Induction of mictic females in the rotifer *Brachionus*: oocytes of amictic females respond individually to population-density signal only during oogenesis shortly before oviposition. Freshw Biol 52:1417–1426

Gilbert JJ, Diéguez MC (2010) Low crowding threshold for induction of sexual reproduction and diapause in a Patagonian rotifer. Freshw Biol 55:1705–1718

Gómez A, Carvalho GR (2000) Sex, parthenogenesis and genetic structure of rotifers microsatellite analysis of contemporary and resting egg bank populations. Mol Ecol 9:203–214

Guillard RRL, Ryther JH (1962) Studies of marine diatoms. I. *Cyclotella nana* Hustedt and *Detonula confervacea* Gran. Can J Microbiol 8:229–239

Gyllström M, Hansson L-A (2004) Dormancy in freshwater zooplankton: Induction, termination and the importance of benthic-pelagic coupling. Aquat Sci 66:274–295

Hagiwara A, Hino A (1989) Effect of incubation and preservation on resting egg hatching and mixis in the derived clones of the rotifer *Brachionus plicatilis*. Hydrobiologia 186:415–421

Hairston Jr NG (1998) Time travelers: what's timely in diapause research? Arch Hydrobiol 52:1–15

Hairston Jr NG, Cáceres C (1996) Distribution of crustacean diapause: micro- and macroevolutionary pattern and process. Hydrobiologia 320:27–44

Hairston Jr NG, Ellner S, Kearns CM (1996) Overlapping generations: the storage effect and the maintenance of biotic diversity. In: Rhodes Jr OE, Chessner RK & Smith MH (eds) Population dynamics in ecological space and time. University of Chicago Press, Illinois, pp 109–145

Hairston Jr NG, Perry LJ, Bohonak AJ, Fellows MQ, Kearns CM, Engstrom DM (1999) Population biology of a failed invasion: paleolimnology of *Daphnia exilis* in upstate New York. Limol Oceanogr 44:477–486

Jiang X, Wang G, Li S, He J (2007) Heavy metal exposure reduces hatching success of *Acartia pacifica* resting eggs in sediment. J Environ Sci 19(6):733–737

Karstens S, Buczko U, Jurasinski G, Peticzka R, Glatzel S (2016) Impact of adjacent land use on costal wetland sediments. Sci Total Environ 550:337–348

Kerfoot WC, Robbins JA, Weider LJ (1999) A new approach to historical reconstruction: Combining descriptive and experimental paleolimnology. Limnol Oceanogr 44:1232–1247

Kotani T, Ozaki M, Matsuoka K, Snell TW, Hagiwara A (2001) Reproductive isolation among geographically and temporally isolated marine *Brachionus* strains. Hydrobiologia 446/447:283–290

Landis W, Yu M (2005) Introduction to environmental toxicology: impacts of chemicals upon ecological systems, 3rd edn. CRC Press, Boca Raton

Larios R, Fernández-Martínez R, Silva V, Rucandio I (2013) Chemical availability of arsenic and heavy metals in sediments from abandoned cinnabar mine tailings. Environ Earth Sci 68:535–546

Latorre C, Betancourt JL, Arroyo MTK (2006) Late Quaternary vegetation and climate history of a perennial river canyon in the Río Salado basin (22°S) of Northern Chile. Quat Res 65:450–466

Laurent B, Marticorena B, Bergametti G, León JF, Mahowald NM (2008) Modeling mineral dust emissions from the Sahara desert using new surface properties and soil database. J Geophys Res 113:D14218

Lennon JT, Jones SE (2011) Microbial seed banks: the ecological and evolutionary implications of dormancy. Nature 9:119–130

Luna-Andrade A, Aguilar-Durán R, Nandini S, Sarma SSS (2002) Combined effects of copper and microalgal (*Tetraselmis suecica*) concentrations on the population growth of *Brachionus plicatilis* Müller (Rotifera). Water Air Soil Pollut 141:143–153

Marcus NH, Lutz R, Burnett W, Cable P (1994) Age, viability and vertical distribution of zooplankton resting eggs from an anoxic basin: evidence of an egg bank. Limnol Oceanogr 39:154–158

Marín-Guirao L, Lloret J, Marin A (2008) Carbon and nitrogen stable isotopes and metal concentration in food webs from a mining-impacted coastal lagoon. Sci Total Environ 393:118–130

Marshall DJ (2008) Transgenerational plasticity in the sea: context-dependent maternal effects across the life history. Ecology 89:418–427

Marcial HS, Hagiwara A, Snell TW (2005) Effect of some pesticides on reproduction of rotifer *Brachionus plicatilis* Müller. Hydrobiologia 546:569–575

Marcial HS, Hagiwara A (2007) Effect of diazinon on life stages and resting egg hatchability of rotifer *Brachionus plicatilis*. Hydrobiologia 593(1):219–225

Medina MH, Correa JA, Barata C (2007) Micro-evolution due to pollution: Possible consequences for ecosystem responses to toxic stress. Chemosphere 67:2105–2114

Montero-Pau J, Gómez A, Muñoz J (2008) Application of an inexpensive and high-throughput genomic DNA extraction method for the molecular ecology of zooplanktonic diapausing eggs. Limnol Oceanogr 6:218–222

Montero-Pau J, Serra M (2011) Life-Cycle switching and coexistence of species with no niche differentiation. PLoS One 6:e20314

Navis S, Waterkeyn A, Voet T, De Meester L, Brendonck L (2013) Pesticide exposure impacts not only hatching of dormant eggs, but also hatchling survival and performance in the water flea *Daphnia magna*. Ecotoxicology 22:803–814

Navis S, Waterkeyn A, De Meester L, Vanermen G, Brendonck L (2015) Timing matters: Sensitivity of *Daphnia magna* dormant eggs to fenoxycarb exposure depends on embryonic developmental stage. Aquat Toxicol 159:176–183

Nielsen DL, Smith D, Petrie R (2012) Resting egg banks can facilitate recovery of zooplankton communities after extended exposure to saline conditions. Freshw Biol 57:1306–1314

Pell A, Márquez A, López-Sánchez JF, Rubio R, Barbero M, Stegen S, Queirolo F, Díaz-Palma P (2013) Ocurrence of arsenic species in algae and freshwater plants of an extreme arid region in northern Chile, the Loa River Basin. Chemosphere 90:556–564

Piscia R, Tabozzi S, Bettinetti R, Nevalainen L, Manca MM (2016) Unexpected increases in rotifer resting egg abundances during the period of contamination of Lake Orta. J Limnol 75(s2):76–85

Posthuma L, Hogervorst RF, Joosse ENG, Van Straalen NM (1993) Genetic variation and covariation for characteristics associated with cadmium tolerance in natural populations of the springtail *Orchesella cincta*. Evolution 47:619–631

Preston BJ, Snell TW (2001) Direct and indirect effects of sublethal toxicant exposure on population dynamics of freshwater rotifers: a modeling approach. Aquat Toxicol 52:87–99

Queirolo F, Stegen S, Mondaca J, Cortés R, Rojas R, Contreras C, Munoz L, Schwuger MJ, Ostapczuk P (2000) Total arsenic, lead, cadmium, copper, and zinc in some salt rivers in the northern Andes of Antofagasta, Chile. Sci Total Environ 255:85–95

R Core Team (2015) R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, http://www.R-project.org/

Rogalski MA (2015) Tainted resurrection: metal pollution is linked with reduced hatching and high juveniles mortality in *Daphnia* egg banks. Ecology 96:1166–1173

Romero L, Alonso H, Campano P, Fanfani L, Cidu R, Dadea C et al. (2003) Arsenic enrichment in waters and sediments of the Río Loa (Second Region, Chile). Appl Geochem 18:1399–1416

Sarabia R, Ramo JD, Varó I, Díaz-Mayans J, Torreblanca A (2008) Sublethal zinc exposure has a detrimental effect on reproductive performance but not on the cyst hatching success of *Artemia parthenogenetica*. Sci Total Environ 398:48–52

Scheuerl T, Stelzer C-P (2013) Patterns and dynamics of rapid local adaptation and sex in varying habitat type in rotifers. Ecol Evol 3:4253–4264

Schröeder T (2005) Diapause in monogonont rotifers. Hydrobiologia 546:291–306

Schröeder T, Gilbert JJ (2004) Transgenerational plasticity for sexual reproduction and diapause in the life cycle of monogonont rotifers: intraclonal, intraspecific and interspecific variation in the response to crowding. Funct Ecol 18:458–466

Schröeder T, Howard S, Arroyo ML, Walsh EJ (2007) Sexual reproduction and diapause of *Hexarthra* sp. (Rotifera) in short-lived ponds in the Chihuahuan Desert. Freshw Biol 52:1033–1042

Serra M, Snell TW, King CE (2004) The timing of sex in cyclically parthenogenetic rotifers. In: Moya A & Font E (eds) Evolution: from molecules to ecosystems. Oxford University Press, London, pp 135–146

Serra M, Snell TW, Gilbert JJ (2005) Delayed mixis in rotifers: an adaptive response to the effects of density dependent sex on population growth. J Plankton Res 27:37–45

Serra M, Snell TW (2009) Sex Loss in Monogonont Rotifers. In: Schön I, Martens K & van Dijk P (eds) Lost sex: the evolutionary biology of parthenogenesis. Springer, The Netherlands, pp 281–294

Slusarczyk M, Pietrzak B (2008) To sink or float: the fate of dormant offspring is determined by maternal bahaviour in *Daphnia*. Freshw Biol 53:569–576

Snell TW, Carmona MJ (1995) Comparative toxicant sensitivity of sexual and asexual reproduction in the rotifer *Brachionus calyciflorus*. Environ Toxicol Chem 14:415–420

Snell TW, Serra M, Carmona MJ (1999) Toxicity and sexual reproduction in rotifers: reduced resting egg production and heterozygosity loss. In: Forbes VE (ed) Genetics and Ecotoxicology. Taylor & Francis, Oxfordshire, United Kingdom. pp 169–185

Stelzer CP, Snell TW (2003) Induction of sexual reproduction in *Brachionus plicatilis* (Monogononta, Rotifera) by a density dependent chemical cue. Limnol Oceanogr 48:939–943

Tapia J, Audry S (2013) Control of early diagenesis processes on trace metal (Cu, Zn, Cd, Pb and U) and metalloid (As, Sb) behaviors in mining- and smelting-impacted lacustrine environments of the Bolivian Altiplano. Appl Geochem 31:60–78

Technelysium (2016) DNA Sequencing Software. http://technelysium. com.au/wp/chromas

Tortajada AM, Carmona MJ, Serra M (2009) Does haplodiploidy purge inbreeding depression in rotifer populations?. PLoS One 4: e8195

Turko P, Sigg L, Hollender J, Spaak P (2016) Rapid evolutionary loss of metal resistance revealed by hatching decades-old eggs. Evolution 70:398–407

USEPA (2012) Methods for measuring the acute toxicity of effluents and receiving waters to freshwater and marine organisms, 5th edn. U.S. Environmental Protection Agency, Washington, DC

Van Straalen NM, Hoffmann A (2000) Review of experimental evidence of physiological costs of tolerance to toxicants. In: Kammenga J & Laskowski R (eds) Demography in ecotoxicology. Wiley, Chichester, pp 147–161

Varó I, Amat F, Navarro JC, Barreda M, Pitarch E, Serrano R (2006) Assessment of the efficacy of *Artemia* sp. (Crustacea) cysts chorion as barrier to chlorpyrifos (organophosphorus pesticide) exposure. Effect on hatching and survival. Sci Total Environ 366:148–153

Wallace RL, Snell TW, Ricci C, Nogrady T (2006) Rotifera 1: biology, ecology and systematics. Backhuys, Leiden

Woelfl S, Óvari M, Nimptsch J, Neu TR, Mages M (2016) Determination of trace elements in freshwater rotifers and ciliates by total reflection X-ray fluorescence spectrometry. Spectrochim Acta B 116:28–33

Wyn B, Sweetman JN, Laevitt PR, Donald DB (2007) Historical metal concentrations in lacustrine food webs revealed using fossil ephippia from *Daphnia*. Ecol Appl 17:754–764

Xu X-P, Xi Y-L, Huang L, Xiang X-L (2015) Effects of multi-metal (Cu, Zn, Cd, Cr, and Mn) mixtures on the reproduction of freshwater rotifer *Brachionus calyciflorus*. Bull Environ Contam Toxicol 95:714–720