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EFFECTS OF CHRONIC WATERBORNE NICKEL EXPOSURE ON TWO SUCCESSIVE GENERATIONS OF *DAPHNIA MAGNA*

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Abstract—In a 21-d chronic toxicity test in which an F_0 generation of *Daphnia magna* were exposed to waterborne Ni, the no-observable-effect concentration (for survival, reproduction, and growth) was 42 μg Ni L^{-1}, or 58% of the measured 21-d median lethal concentration (LC50) of 71.9 μg Ni L^{-1} (95% confidence interval, 56.5–95.0). Chronic exposure to 85 μg Ni L^{-1} caused marked decreases in survival, reproduction, and growth in F_0 animals. In the F_1 generation (daphnids born of mothers from the chronically exposed F_0 generation), animals chronically exposed to 42 μg Ni L^{-1} for 11 d weighed significantly less (20%) than controls, indicating increased sensitivity of F_1 animals. Additionally, in this successive generation, significant decreases in whole-body levels of metabolites occurred following exposure to both 42 μg Ni L^{-1} (decreased glycogen and adenosine triphosphate [ATP]) and 21 μg Ni L^{-1} (decreased ATP). No significant changes were observed in whole-body total lipid, total protein, and lactate levels at any concentration. Whereas F_1 neonates with mothers that were exposed to 21 μg Ni L^{-1} showed increased resistance to acute Ni challenge, as measured by a significant (83%) increase in the acute (48-h) LC50, F_1 neonates with mothers that were exposed to 42 μg Ni L^{-1} were no more tolerant of acute Ni challenge than control animals were. Nickel accumulations in F_1 animals chronically exposed to 21 and 42 μg Ni L^{-1} were 11- and 18-fold, respectively, above control counterparts. The data presented suggest that chronic Ni exposure to two successive generations of *D. magna* lowered the overall energy state in the second generation. Whereas the quantity of neonates produced was not affected, the quality was; thus, environmentally meaningful criteria for regulating waterborne Ni concentrations in freshwater require consideration of possible multigenerational effects.

Keywords—Nickel   *Daphnia magna*   Chronic   Waterborne   Successive generations

INTRODUCTION

There have been relatively few studies to date investigating the effects of chronic waterborne Ni exposure in fish [1,2] or *Daphnia* [3–6]. Whereas Munzinger and Monicelli [4,5] investigated the effects of chronic Ni exposure on survival, growth, and reproduction of *Daphnia*, these studies did not evaluate the physiological costs associated with chronic exposure. Biesinger and Christensen [6] added a metabolic component and reported declines in whole-body total protein and glutamic oxalacetic transaminase activity in chronically Ni-exposed *D. magna*. Additionally, our laboratory has recently shown that whole-body hemoglobin and oxygen consumption rates are markedly decreased in *D. magna* following chronic waterborne Ni exposure [7]. The latter two studies, however, although physiologically based, were conducted on only one generation of *D. magna* that was chronically exposed to waterborne Ni.

The central hypothesis underlying the present investigation was formulated from two observations. First, Munzinger [3] showed that survival and morphometrics of successive generations of *Daphnia* were increasingly susceptible to chronic waterborne Ni exposure. Additionally, metabolic parameters appear to be more sensitive than other indicators to chronic Ni exposure [7]. We therefore hypothesized that specific metabolic indicators would be highly sensitive to chronic Ni exposure across successive generations at low Ni concentrations within the range of environmental relevance.

The primary objective of the present study, therefore, was to evaluate the effects of chronic Ni exposure on the metabolic state of a generation (F_1) of *Daphnia* with mothers (F_0 generation) that were also chronically exposed to Ni. Accordingly, whole-body levels of total lipid, total protein, glycogen, lactate, and adenosine triphosphate (ATP) were analyzed in F_1 daphnids following 11 d of exposure to the same Ni concentration to which their respective mothers (F_0 generation) were exposed. Additionally, potential acclimation to chronic waterborne Ni exposure over two successive generations was investigated by running acute toxicity tests (48-h median lethal concentration [LC50]) on F_1 *Daphnia* neonates.

In the maternal (F_0) generation, the effects of Ni exposure on survival, growth, and reproduction were measured using a 21-d chronic toxicity test. The measured concentrations of dissolved Ni chosen for the present study were 21, 42, and 85 μg Ni L^{-1}. All are environmentally realistic in contaminated freshwater systems, with the lowest (21 μg Ni L^{-1}) being far less than 1% of that needed to induce acute toxicity in teleosts [8,9] and only slightly above the upper range of Ni levels found in uncontaminated freshwaters (~0–15 μg Ni L^{-1}) [10–12].

MATERIALS AND METHODS

Daphnia magna *culture*

Colonies of adult gravid *D. magna* were obtained from Aquatic Research Organisms (Hampton, NH, USA). Once in the laboratory, daphnids were cultured in dechlorinated Ottawa (ON, Canada) city tap water, the composition of which was as follows: Na $\cong$ 400 μM, Cl $\cong$ 300 μM, Ca $\cong$ 250 μM, Mg

$\cong$ 85 μM, Ni $\cong$ 0.017 μM (1 μg L^{-1}), total organic carbon $\cong$ 3.6 mg L^{-1}, total hardness $\cong$ 45 mg L^{-1} (as CaCO$_3$), and pH 7.3–7.6. All culturing and experimentation were conducted in a room with the temperature controlled at 20.5 $\pm$ 1.5°C. Photoperiod was fixed at 16:8-h light:dark.

Before any experimentation, daphnids were cultured in glass beakers using a static renewal system with 25 ml of water per animal and feeding and replacement of solutions every second or third day. *Daphnia magna* were fed 4 ml of commercially purchased YCT (a slurry of yeast, cerophyll, and trout chow; Aquatic Research Organisms) and 12 ml of algae per 800 ml of solution. The algae consisted of a 3:1 ratio of *Selenastrum capricornutum* to *Chlorella* (at a total concentration of 3.5 $\times$ 10^6 cells ml^{-1}). Solutions were not aerated, because aeration drives *Daphnia* to the water surface [13]. Rather, beakers were left uncovered to allow for atmospheric equilibration of oxygen, with oxygen saturation at approximately 70 %. Only frequently reproducing colonies (>15 neonates per female per 3 d) were used, and at no time were ephipia present in a colony. Culturing protocols for individual experiments are described below.

Twenty-one day chronic toxicity test and LC50 determination (F$_0$ generation)

A 21-d chronic toxicity test assessing survival, growth, and reproduction was run on neonates (age, <24 h) following the standard U.S. Environmental Protection Agency protocol for static renewal [14], and employing measured Ni concentrations of 0, 21, 42, and 85 μg Ni L^{-1}. An additional concentration of 247 μg Ni L^{-1} was included initially, but 100% mortality occurred before the onset of any reproduction in this treatment (data not shown). Water samples were taken on the first day and then on every fifth day thereafter, filtered (pore size, 0.45 μm), acidified with trace metal–grade HNO$_3$, and analyzed for dissolved Ni by inductively coupled plasma-mass spectrometry (Sciex Elan 6100 DRC; Perkin-Elmer, Wellesley, MA, USA) using certified standards (PlasmaCal; SCP Science, Baie D'Urfé, QC, Canada).

The intrinsic rate of population increase (*r*) for each cohort was calculated from the following formula [15]:

$$1 = l_t m_t e^{-rt} \qquad (1)$$

where l_t is the proportion of female survivors of age *t*, m_t is the mean number of progeny per female at age *t*, *t* is the age in days, and *r* is the intrinsic rate of population increase. The exponent *r* is estimated by iteration until a value is found so that the calculated value of $l_t m_t e^{-rt}$ summed over 21 d is equal to 1.

At the end of the 21-d test, all surviving animals were removed from solution by plastic transfer pipettes, blotted dry on No. 1 Whatman filter paper (Clifton, NJ, USA), placed on pieces of aluminum foil, and weighed to the nearest 0.01 mg.

A chronic, 21-d LC50 value was determined using the Probit method [16]. The data used for this calculation were mortality at 21 d in each of the exposure concentrations (including the highest concentration of 247 μg Ni L^{-1}, causing complete mortality) and mean measured dissolved Ni concentrations of each treatment.

Growth, Ni accumulation, acclimation, and metabolism of F$_1$ D. magna

Neonates from the first two broods produced by each reproducing individual of the F$_0$ generation were culled (*n* $\cong$ 300) and cultured en masse in 4-L glass beakers with 3.5 L of exposure solution with the same concentration as that to which their mothers were exposed. For the control and for 21 and 42 μg Ni L^{-1} treatments, all neonates were culled on day 8 (onset of reproduction) through day 11. No mortalities occurred in the F$_0$ generation in any of these three treatments through the first 11 d, whereas F$_0$ animals exposed to 85 μg Ni L^{-1} experienced 30% mortality by day 11 (see *Results and Discussion*). Additionally, the mean time to first brood in this treatment (85 μg Ni L^{-1}) was 12.4 d, prohibiting the culling of large numbers of F$_1$ neonates within the first 11 d of exposure (see *Results and Discussion*). For these reasons, the F$_1$ generation consisted only of animals from the control and the 21 and 42 μg Ni L^{-1} treatments.

Daily, 30% of the mass culture media was replaced, and food was added at the concentration described above. The appropriate amount of Ni was added from a concentrated stock of NiCl$_2$·6H$_2$0 to maintain steady concentrations of 0, 21, and 42 μg Ni L^{-1} over the 11-d exposure period. Water samples were taken and analyzed for Ni as described above. All F$_2$ generation neonates born during the 11-d exposure of F$_1$ animals were discarded daily.

Following 11 d of Ni exposure, wet weight of individual F$_1$ animals (*n* = 10) was determined as described above for the F$_0$ generation.

Whole-body Ni burden was measured in F$_1$ animals at day 11 by removing *D. magna* in groups of four (*n* = 5) and drying overnight at 60°C. Once dried, animals were weighed to the nearest 0.001 mg. Groups of four daphnids were then transferred to plastic vials, and 50 μl of concentrated (70%) trace metal–grade HNO$_3$ were added. Vials were placed back in the oven overnight at 60°C. The digest was then diluted with deionized water and analyzed for Ni content by inductively coupled plasma-mass spectrometry as described above.

Acute (48-h) toxicity tests on F$_1$ neonates were run on the 16th day of the 21-d chronic toxicity test to examine for possible acclimation effects of previous maternal Ni exposure. Neonates from each concentration were taken at random from all those produced on that day by the F$_0$ generation. During the test, dead or completely immobilized animals were removed daily from beakers, and the 48-h LC50 and 95% confidence interval were calculated as described above using either the Probit method [16] or the Stephen method [17].

On day 11, daphnids from the F$_1$ generation were analyzed for whole-body concentrations of the following five metabolites: total lipid, total protein, glycogen, lactate, and ATP. Groups of 20 animals (*n* = 5 groups from each concentration) were initially removed from the control and the 21 and 42 μg Ni L^{-1} solutions and weighed as described above. Daphnids were then transferred to plastic assay tubes, immediately frozen in liquid nitrogen, and stored at −80°C until later biochemical analysis. Pools of 20 animals were then thawed in 600 μl of ice-cold 8% HClO$_4$ in 12- $\times$ 75-mm borosilicate test tubes and homogenized with a tissue homogenizer for 30 s. Next, 175 μl of the homogenate were removed and frozen at −20°C for later analysis of glycogen. To the remaining homogenate was added 1 ml of chloroform for lipid extraction. The homogenate was then vortexed vigorously and centrifuged at 100 *g* for 3 min. Using drawn glass Pasteur pipettes, the organic layer was removed and transferred to glass vials, sealed, and stored at −20°C until later analysis of total lipid. The remaining aqueous extract was vortexed, transferred to plastic assay tubes, and centrifuged at 5,000 *g* for 4 min, after

which the supernatant was transferred to a second set of plastic assay tubes and the pellet stored at $-20°C$ for later analysis of total protein. The supernatant was then neutralized with 3 M K_2CO_3, vortexed, and centrifuged at 5,000 g for 4 min. The neutralized extract was analyzed immediately for ATP, and the remainder was stored at $-20°C$ for later analysis of lactate.

Adenosine triphosphate concentration was determined using a hexokinase/glucose-6-phosphate dehydrogenase/NADP enzyme system (Sigma-Aldrich, St. Louis, MO, USA). Lactate concentration of neutralized extracts was measured enzymatically (L-lactate dehydrogenase/NADH; Sigma-Aldrich). Total lipid content of the organically extracted fraction was determined by the sulphophosphovanillin method [18] against cholesterol standards (Sigma-Aldrich). To measure glycogen concentration, 175 μl of $HClO_4$ homogenate extracts were neutralized with 3 M K_2CO_3 and vortexed vigorously. Glycogen was then converted enzymatically to free glucose by a technique modified from that described by Bergmeyer [19] using amyloglucosidase in a sodium acetate buffer. Free glucose was then analyzed using the hexokinase method and Sigma-Aldrich reagents and standards. The glucose concentration of the neutralized aqueous extract was also measured using the hexokinase method with Sigma-Aldrich reagents and standards and then subtracted from the glycogen-derived free glucose to yield a glycogen concentration expressed in glucosyl units per wet weight of tissue. The glucose concentration of the neutralized extract was very low and not significantly different between treatments (data not shown). A 0.5 M potassium hydroxide solution was added to the protein pellet, followed by vortexing and centrifugation at 5,000 g for 5 min. The supernatant was then analyzed for total protein using Bradford reagent and bovine serum albumin standards (Sigma-Aldrich).

All metabolite concentrations are expressed on a whole-body, wet-weight basis. All metabolite concentrations were multiplied by 1.25 to correct for fluid present in the carapace [20].

Statistical analysis

Data are presented as the mean ± one standard error. Experimental means were compared to control means using a one-way analysis of variance with Bonferroni's post-hoc multiple-comparison test.

RESULTS AND DISCUSSION

Survival, reproduction, and growth of F_0 generation D. magna

Survival patterns of *D. magna* exposed chronically to four concentrations of waterborne Ni (0, 21, 42, and 85 μg Ni L^{-1}) are given in Figure 1. No effect on survival was observed in either the control or 21 μg Ni/L treatments, 10% mortality occurred at 42 μg Ni L^{-1}, and survival dropped linearly with time at 85 μg Ni L^{-1} to only 30% of control levels by the end of the 21-d test (Fig. 1). The chronic (21-d) LC50 was 71.9 μg Ni L^{-1} (95% confidence interval, 56.5–95.0).

In water of the same hardness as that of the present study (45 mg L^{-1} [as $CaCO_3$]), Biesinger and Christensen [6] reported a 21-d LC50 of 130 μg Ni L^{-1}. This value is considerably higher than that reported here (71.9 μg Ni L^{-1}). Caution should be taken, however, in comparing the two results, because the Ni concentrations reported by Biesinger and Christensen appear to be nominal. It has been well documented that hardness protects against Ni toxicity [21]. Accordingly, a 21-
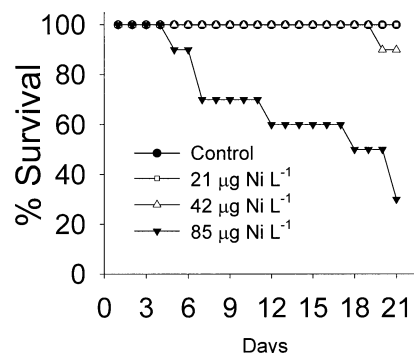

Fig. 1. Time course of survival of F_0 *Daphnia magna* during a 21-d chronic toxicity test. Exposure concentrations were 0 (Control), 21, 42, and 85 μg Ni L^{-1}.

d LC50 reported by Enserink et al. [22] in very hard water (225 mg L^{-1} [as $CaCO_3$]) was much higher (360 μg Ni L^{-1}).

Given a measured acute (48-h) LC50 of 1,068 μg Ni L^{-1} (see below) and a measured chronic (21-d) LC50 of 71.9 μg Ni L^{-1}, the resultant acute to chronic ratio (ACR) for Ni in moderately soft water (45 mg L^{-1} [as $CaCO_3$]) was 15. Although the species mean ACR for Ni in *D. magna*, as given by the U.S. Environmental Protection Agency [21], is considerably higher (ACR = 27), it should be noted that two of the three literature ACR values used to generate this species mean ACR were 14 and 17, which is in very good agreement with the value generated in the present study. The ACR value for Ni in *D. magna* generated in the present study is roughly an order of magnitude greater than that for Cu in *D. magna* (ACR = 2.4 [23]), lower than that for Cd in *D. magna* (ACR = 104.3 [24]), and most similar to that of Pb in *D. magna* (ACR = 18 [25]).

In the F_0 generation, no effects were observed at the two lower Ni concentrations (21 and 42 μg Ni L^{-1}) on five reproductive parameters measured over the 21-d period, including mean number of neonates per brood, mean number of broods per reproducing animal, neonates produced per breeding animal, time to first brood, and the intrinsic rate of population increase (r) (Table 1). All these indices, however, were strongly affected by exposure to 85 μg Ni L^{-1}, being reduced by 36, 32, 57, 42, and 45%, respectively.

Growth of F_0 daphnids at the two lower Ni concentrations (21 and 42 μg Ni L^{-1}), expressed as individual wet weight at the end of the 21-d test, was not significantly different from control levels (Fig. 2). Body weight of animals surviving exposure to 85 μg Ni L^{-1}, however, was severely impacted, being only approximately 40% that of control animals (data not shown). Note that survival in this treatment was only 30% at the end of the 21-d period (Fig. 1).

For the three parameters of survival, reproduction, and growth measured during the 21-d chronic toxicity test, we therefore observed a very clear break between a no-observable-effect concentration (NOEC) of 42 μg Ni L^{-1} and observed effects at 85 μg Ni L^{-1} (Fig. 1 and Table 1). Similar results were given by Biesinger and Christensen [6], who reported 16% and 50% reproductive impairment levels at 30 and 95 μg Ni L^{-1} (apparent nominal concentrations), respectively, for *D. magna* using a similar experimental design and water hardness. The 16% level was considered to be a safe level by Biesinger and Christensen [6]; anything lower did not differ from the normal variation in control treatments. Accordingly,

Table 1. Reproductive parameters of F_0 (maternal) *Daphnia magna* exposed to waterborne Ni for 21 d[a]

Treatment	No. of neonates per brood	No. of broods per reproducing animal	No. of neonates produced per breeding animal in 21 d	Time to first brood (d)	Intrinsic rate of population increase (r) over 21 d
Control	26.88 ± 1.82 A	5.00 ± 0.19 A	134.38 ± 4.64 A	8.75 ± 0.25 A	0.385
21 μg Ni L^{-1}	28.55 ± 1.96 A	5.30 ± 0.15 A	135.40 ± 8.16 A	8.00 ± 0.00 A	0.430
42 μg Ni L^{-1}	29.68 ± 1.82 AB	5.00 ± 0.21 A	148.40 ± 3.25 A	8.40 ± 0.22 A	0.413
85 μg Ni L^{-1}	17.18 ± 1.98 AC	3.40 ± 0.24 B	58.40 ± 5.52 B	12.40 ± 0.81 B	0.210

[a] Data are presented as the mean ± one standard error ($n = 5$–10). Values not sharing the same letter are significantly different.

those authors reported a hardness-dependent (45 mg L^{-1} [as CaCO$_3$]) safe concentration for *Daphnia* of 30 μg Ni L^{-1}.

This safe concentration agrees reasonably well with our data from F_0 daphnids exposed to waterborne Ni given that both studies examined only the effects of Ni exposure to one generation of *D. magna*. The NOEC, however, drops from 42 to 21 μg Ni L^{-1} (Fig. 2) when considering the growth of F_0 and F_1 daphnids, respectively. Whereas F_0 daphnids exposed for 21 d to 42 μg Ni L^{-1} were the same weight as controls, F_1 animals exposed for 11 d to 42 μg Ni L^{-1} weighed significantly (20%) less than control daphnids (Fig. 2). This shift in the NOEC points out a discrepancy between quality and quantity. Based on just quantity (reproductive output calculated solely on the number of neonates produced; i.e., all columns in Table 1), F_0 animals exposed to 42 μg Ni L^{-1} were just as successful as control animals, but their offspring (F_1 animals) were significantly smaller (20%) than their control cohorts (Fig. 2). Indeed, Munzinger [3] found that over seven generations of *Daphnia* exposed to waterborne Ni, the intrinsic rate of population increase (r) increased but the length of progeny decreased with successive generations.

These results also demonstrate the increased sensitivity of a successive generation of *D. magna* with respect to this one parameter (growth). These results should serve as a possible caution against using results from experiments conducted with only one generation of *Daphnia* to determine environmental criteria for waterborne Ni.

Nickel accumulation, acclimation, and metabolism of F_1 generation D. magna

The severe effects of exposure to 85 μg Ni L^{-1} on the survival and reproduction of the F_0 generation prohibited the
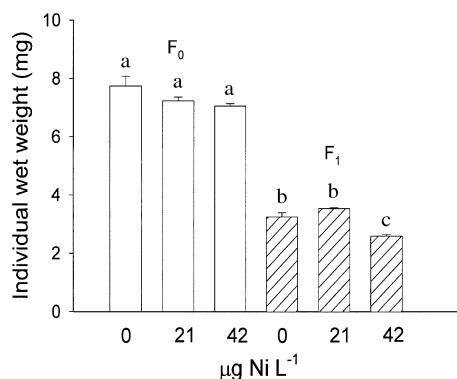

Fig. 2. Wet weights of F_0 and F_1 generation *Daphnia magna*. Data are presented as the mean ± one standard error ($n = 7$–10). The F_0 weights were taken at the end of a 21-d bioassay; the F_1 animals were weighed after 11 d of exposure. Bars not sharing the same letter are significantly different.

inclusion of F_1 daphnids from this concentration in the following data set. Essentially, too few neonates were produced, too few neonates survived, and too little overall biomass was available to analyze survival, growth, Ni accumulation, and metabolic parameters in the F_1 generation (see *Materials and Methods*).

Nickel accumulation, expressed as micrograms of Ni per gram of *D. magna* dry weight, increased significantly in a concentration-dependent manner in F_1 daphnids. The concentration of Ni in the whole body of F_1 *D. magna* was 4.83 ± 1.06 ($n = 5$), 51.50 ± 1.76 ($n = 5$), and 87.58 ± 5.60 ($n = 5$) μg g^{-1} (dry wt) for the control, 21 μg Ni L^{-1}, and 42 μg Ni L^{-1} exposures, respectively. Interestingly, however, acclimation to waterborne Ni via previous maternal exposure did not confer resistance to F_1 animals in a concentration-dependent manner. Although maternal exposure to 21 μg Ni L^{-1} resulted in a significant 1.8-fold increase in the 48-h LC50 value, neonates maternally pre-exposed to 42 μg Ni L^{-1} were no more resistant than control daphnids to acute Ni challenge, because the 48-h LC50 values for these two groups of daphnids were essentially identical. The 48-h LC50 values for F_1 neonates with mothers that were exposed to 0, 21, and 42 μg Ni L^{-1} were 1,068 (95% confidence interval, 818–1,486;), 1,955 (95% confidence interval, 1,654–2,374), and 1,086 (95% confidence interval, 803–1,469) μg Ni L^{-1}, respectively.

Mechanistic reasons for this discrepancy (increased tolerance at 21 μg Ni L^{-1} but none at 42 μg Ni L^{-1}) are unknown, though it is of interest to consider that the very different results of the acute toxicity tests may be caused by markedly unequal whole-body Ni burdens in the two neonate cohorts. Whereas the initial (neonatal) whole-body Ni concentrations of these daphnids are unknown, the marked difference in burdens at 11 d (see above) suggests that the F_1 animals exposed to 42 μg Ni L^{-1} also carried a higher neonatal Ni burden. It is possible that a critical burden was exceeded only by F_1 neonates exposed to 42 μg Ni L^{-1} and that resistance to acute Ni challenge depends on whole-body levels less than this critical amount. A whole-body Ni burden greater than the critical amount may be incompatible with other physiological mechanisms conferring resistance to acute challenge. It is noteworthy that the 11-d Ni burden of F_1 neonates reared at 42 μg Ni L^{-1} was very high, being 70 to 75% of a maximal, critical Ni burden in adult *D. magna* exposed to 685 μg Ni L^{-1}, which is an acutely lethal concentration [7].

Metabolically, whole-body concentrations of total lipid, total protein, and lactate expressed on a wet-weight basis were not significantly different in any of the treatments in the F_1 generation after 11 d of exposure (Fig. 3A). Whole-body levels of glycogen (Fig. 3B) and ATP (Fig. 3A) expressed on a wet-weight basis, however, were decreased in a concentration-de-
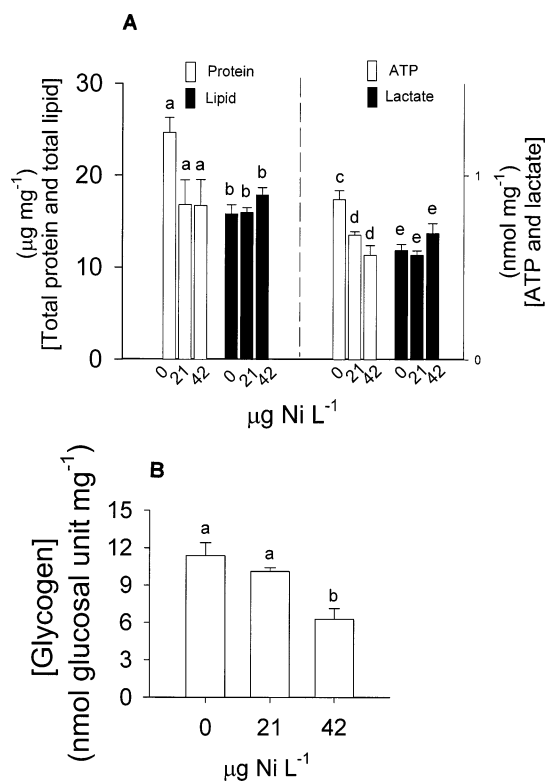
Fig. 3. Whole-body metabolite concentrations of F_1 *Daphnia magna* following 11 d of exposure. (**A**) Total protein, total lipid, adenosine triphosphate (ATP), and lactate. (**B**) Glycogen. Data are presented as the mean ± one standard error ($n = 5$). Note that metabolite concentrations are expressed on a wet-weight basis. Bars not sharing the same letter are significantly different.

pendent fashion. Percentage decreases from control levels of these two metabolic parameters at 21 and 42 µg Ni L^{-1} were as follows: [glycogen], 11 and 45%, respectively; and [ATP], 22 and 35%, respectively (Fig. 3B). The decreases were statistically significant at both Ni concentrations for [ATP] and significant only at 42 µg Ni L^{-1} for [glycogen].

The amount of stored lipid in *Daphnia* is an indicator of feeding success [26]. It was therefore surprising to observe no change in whole-body total lipid concentration with Ni exposure in F_1 daphnids (Fig. 3) given our observations that chronic Ni exposure reduced feeding rates in *D. magna* (data not shown). Stored lipid levels, however, in breeding daphnids are intricately linked to stages of the molt cycle, increasing through the egg stages as lipid is progressively accumulated (assuming no food limitation) and transferred to the ovaries. Once a brood of neonates is released and a new brood of eggs laid in the brood chamber, lipid reserves reach their lowest level, and the cycle of lipid accumulation (and egg development) begins again [26]. If Ni impacted the reproductive cycle or output of F_1 animals, there may have been confounding effects on lipid content responses. Indeed, 16% of the intermolt biomass increase in *Daphnia* is attributable to lipid accumulation [27].

Although not statistically significant, whole-body protein concentrations were reduced by approximately 33% following 11 d of Ni exposure to both 21 and 42 µg Ni L^{-1} (Fig. 3). Similarly, following chronic (21-d) exposure of one generation of *D. magna* to a higher level of waterborne Ni (125 µg Ni L^{-1}; apparent nominal concentration), Biesinger and Christensen [6] found that whole-body protein was decreased. Addi-

tionally, when we exposed one generation of *D. magna* chronically (14 d) to a higher concentration of waterborne Ni (131 µg Ni L^{-1}), a 68% drop occurred in whole-body hemoglobin concentration [7]. Possible effects of Ni exposure on protein synthesis and metabolism should be investigated in future studies.

In *Daphnia*, glycogen is generally considered to be the most prominent, labile fuel source [27]. In the present study, glycogen was reduced in a concentration-dependent manner in Ni-exposed F_1 daphnids (Fig. 3B). A simple anaerobic conversion of glycogen stores to lactate in the face of any possible Ni-induced oxygen limitation did not occur given that whole-body lactate concentration was not increased in F_1 *Daphnia* (Fig. 3A). Additionally, although waterborne Ni is a respiratory toxicant in the rainbow trout, we do not believe that Ni limits gas exchange in *Daphnia* (for discussion, see Pane et al. [7]) and, therefore, would not expect anaerobic conversion of glycogen to lactate. Acute Ni-induced decreases in liver glycogen have been documented in two fish species [28,29], putatively caused by increased catecholamine release by stressed fish.

An overall concentration-dependent drop in the energy state of F_1 animals was further evidenced by significantly reduced ATP levels (Fig. 3). Interestingly, both acute and chronic exposure of one generation of *D. magna* to waterborne Ni caused a time-dependent decrease in whole-body Mg^{2+} concentration because of an inhibition of unidirectional Mg^{2+} uptake [7]. The magnesium ion is a cofactor for ATP on a 1:1 molar basis [30]. The molar ATP loss in F_1 animals exposed to 42 µg Ni L^{-1} for 11 d was 250 nmol/g wet tissue (Fig. 3B), and the molar Mg^{2+} loss in one generation of *Daphnia* following 14 d of exposure to 131 µg Ni L^{-1} was 1,000 nmol/g wet tissue [7]. Comparison across different generations and Ni concentrations is complicated, but it is interesting to speculate whether chronic impairment of magnesium homeostasis is related to overall reduction of energy charge (ATP levels) and metabolic health of Ni-exposed *Daphnia*. Adaptive downregulation of metabolic rate below the standard rate (metabolic depression) is a common response to a stressor in fish [31] and may similarly be applicable to daphnids under chronic toxicant stress. Seemingly, the data patterns of the present study suggest as much with respect to chronic Ni exposure.

The importance of looking at NOECs across two successive generations of *D. magna* is again highlighted by the metabolic data regarding F_1 animals (Fig. 3). Although the original NOEC level of F_0 animals was 42 µg Ni L^{-1} for survival, growth, and reproductive parameters (Figs. 1 and 2 and Table 1), a significant negative effect was observed on whole-body ATP and glycogen in the F_1 generation exposed to 42 µg Ni L^{-1} (Fig. 3). Furthermore, with respect to all nonmetabolic parameters measured, no effects were observed in either the F_0 or F_1 generation caused by exposure to 21 µg Ni L^{-1}. However, one metabolic parameter, whole-body ATP, was significantly affected in F_1 animals exposed to 21 µg Ni L^{-1} (Fig. 3B). These data indicate that certain metabolic parameters are more sensitive indicators of Ni exposure than survival, reproduction, and growth and, again, emphasize the value of exposing multiple generations of *Daphnia* to a waterborne toxicant such as Ni.

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