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# Aquatic Toxicology



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# Effects of chronic uranium exposure on life history and physiology of *Daphnia magna* over three successive generations

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#### ABSTRACT

Daphnia magna was exposed to waterborne uranium (U) at concentrations ranging from 10 to 75  $\mu$ g L<sup>-1</sup> over three successive generations (F0, F1 and F2). Progeny was either exposed to the same concentration as mothers to test whether susceptibility to this radioelement might vary across generations or returned to a clean medium to examine their capacity to recover after parental exposure.

Maximum body burdens of 17, 32 and 54 ng U daphnid<sup>-1</sup> were measured in the different exposure conditions and converted to corresponding internal alpha dose rates. Low values of 5, 12 and 20  $\mu$ Gy h<sup>-1</sup> suggested that radiotoxicity was negligible compared to chemotoxicity. An increasing sensitivity to toxicity was shown across exposed generations with significant effects observed on life history traits and physiology as low as 10  $\mu$ g L<sup>-1</sup> and a capacity to recover partially in a clean medium after parental exposure to  $\leq 25 \,\mu$ g L<sup>-1</sup>.

Using a <sup>14</sup>C-labelled food technique, the study showed that uranium affected carbon assimilation in F0 at concentrations of 25 and 75  $\mu$ g L<sup>-1</sup> (34 and 80% reduction respectively) and as low as 10  $\mu$ g L<sup>-1</sup> in F1 and F2 (40 and 36% reduction respectively). Consequences were strong for both somatic growth and reproduction and increased in severity across generations. Maximum size was reduced by 12% at 75  $\mu$ g L<sup>-1</sup> in F0 and 23% at 25  $\mu$ g L<sup>-1</sup> in F2. Reduction in 21-day fecundity ranged from 27 to 48% respectively at 25 and 75  $\mu$ g L<sup>-1</sup> in F0 and from 43 to 71% respectively at 10 and 25  $\mu$ g L<sup>-1</sup> in F2. Growth retardation caused a delay in deposition of first brood of 1.3 days at 75  $\mu$ g L<sup>-1</sup> in F0, of 1.9 days at 25  $\mu$ g L<sup>-1</sup> in F1 and of 5 days at 25  $\mu$ g L<sup>-1</sup> in F2. Differences in respiration rates and egg dry mass between the control and exposed daphnids were mainly an indirect result of uranium effect on body size.

The observed increase in toxic effects across generations indicated the necessity of carrying out multigeneration tests to assess environmental risk of uranium in daphnids.

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# 1. Introduction

Uranium (U), a naturally occurring radioactive metal, is present in freshwaters at trace concentrations from 0.02 to  $6 \mu g L^{-1}$  and may increase up to  $2 m g L^{-1}$  in the vicinity of uraniferous sites (Bonin and Blanc, 2001; WHO, 2001). Anthropogenic activities mainly associated with the use of uranium as nuclear fuel (uranium mining, milling and refining as well as spent fuel reprocessing) contribute to uranium redistribution in freshwaters and may cause water concentrations to exceed background concentrations

\* Corresponding author. Fax: +33 4 42 19 91 43. E-mail address: frederic.alonzo@irsn.fr (F. Alonzo). (Ragnarsdottir and Charlet, 2000). As a consequence, uranium has become of increasing concern for biota protection over the past decades (Environment Canada, 2003; Sheppard et al., 2005). In fact, natural uranium causes both a chemical and radiological hazard to aquatic ecosystems, because of its toxicity as a metal and as a mixture of three alpha-emitting radioisotopes along with their progenies (<sup>234</sup>U, <sup>235</sup>U and <sup>238</sup>U, in relative abundance of 0.005, 0.720 and 99.275%; Weigel, 1986).

To address these hazards, toxicity of uranium has been intensively studied in a wide range of freshwater species, including benthic and pelagic invertebrates as well as fish. Reported effects have covered many different endpoints which concern levels of biological organisation from subcellular damages to perturbations in physiology and life history of organisms: reduced hepatic antiox-

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idant activities and increased levels of neurotoxicity biomarker in the zebrafish, Danio rerio, after 20 days of waterborne exposure to 100  $\mu$ g U L<sup>-1</sup> (Barillet et al., 2007); at an organism level in *D. rerio*, increase in mortality and a reduction in hatching success and larval growth at 250  $\mu$ g UL<sup>-1</sup> (Bourrachot et al., 2008); reduced larval survival, development time and growth in benthic insect Chironomus riparius, after 10 days at 3  $\mu$ g U g<sup>-1</sup> dry wt sediment (Dias et al., 2008). Toxicity data in cladocerans concern mainly acute exposure whereas studies on chronic toxicity remain scarce. Both acute and chronic toxicities vary among tested species (Semaan et al., 2001; Pickett et al., 1993; Kuhne et al., 2002) and have been shown to depend strongly on water conditions (Trapp, 1986; Bywater et al., 1991; Barata et al., 1999; Semaan et al., 2001; Kuhne et al., 2002). Alkalinity, hardness and pH modify uranium speciation, changing concentrations of free uranyl ion and inorganic uranium complexes (e.g., carbonate complexes) and allowing potential competition for biotic ligands with other cations such as calcium and magnesium (Markich, 2002). In Daphnia magna, water concentrations causing 50%-lethality at 48 h range from 0.39 to  $51.9 \text{ mg} \text{UL}^{-1}$  and 50%effect on 21-day reproduction has been reported to vary from 91 to  $520 \mu g U L^{-1}$  (Poston et al., 1984; Barata et al., 1998; Zeman et al., 2008). Differences in uranium toxicity resulted from changes in uranium bioavailability because water parameters varied among studies (Sheppard et al., 2005; Zeman et al., 2008).

Consequences of toxicity of uranium for the population demography were not evaluated although higher levels of organisation are ecologically more relevant than the organism level. Several concepts, such as the Scope for Growth (SFG) and the Dynamic Energy Budget (DEB) theory have been used to link effects at the individual level with population level effects (Widdows and Johnson, 1988; Kooijman, 2000). These approaches assume that toxic exposure might cause perturbations in nutrition and/or induce compensatory processes which are energetically costly (Calow and Sibly, 1990). Decrease in energy acquisition and/or increase in metabolic costs associated with toxicity come at the expense of growth and reproduction, which are fundamental processes for population dynamics. Zeman et al. (2008) addressed effects on various components of energy budget in daphnids exposed to uranium for 21 days at a controlled pH of 7 to enhance uranium bioavailability. Perturbations in physiological processes were reported at  $25 \,\mu g \, L^{-1}$  with strong consequences for SFG, body mass and fecundity. Observed damages in intestinal epithelium at  $50 \,\mu g \, L^{-1}$  indicated assimilation inhibition as a possible mode of action (Zeman, 2008).

Assessing population responses to pollutants requires examining toxic effects on a long-term basis, as a population might be exposed for a period of time which exceeds individual lifespan. This issue is of major importance because biological effects have been shown to differ from a generation to the next during exposure to the radionuclide <sup>241</sup>Americium (Am-241) and other metals (Bodar et al., 1990; Muyssen and Janssen, 2004; Pane et al., 2004; Guan and Wang, 2006; Alonzo et al., 2008). Multigenerational differences between parent and progeny included increase in sensitivity (as a result of toxicant transfer from mothers to offspring and/or exposure during early stages of development) and increase in tolerance to toxicants (on a longer term basis by adaptive responses). As observed for Am-241, an increase in sensitivity of offspring to uranium was strongly suggested by the observed reduction in egg mass above 25  $\mu$ g L<sup>-1</sup> (Zeman et al., 2008). This hypothesis remained to be investigated as effects of uranium were studied over one generation only.

In this study, *D. magna* was exposed to waterborne uranium at concentrations ranging from 10 to 75  $\mu$ g UL<sup>-1</sup> over three successive generations. The objectives were to examine: (1) how chronic uranium exposure might affect food assimilation; (2) whether uranium toxicity for *D. magna* might differ between generations. Simultaneously, recovery from uranium stress was investigated

in offspring returned to a clean medium after parental exposure.

# 2. Materials and methods

#### 2.1. Culture conditions

D. magna cultures were maintained in laboratory for several years in continuous parthenogenetic reproduction following OECD guideline 211 (1998). Daphnids were cultivated in freshwater artificial medium M4 (Elendt and Bias, 1990) at pH 7 over several generations so as to increase uranium bioavailability during uranium tests (Zeman et al., 2008). A pH of 7 was obtained with a modification in Cl concentration and maintained by renewing medium twice a week. Daphnids were reared at a density of 1 animal per 50 mL in 2-L bottles at  $20 \pm 1$  °C under a 16:8h light:dark photoperiod and a light intensity of  $30 \,\mu\text{E}\,\text{m}^{-2}\,\text{s}^{-1}$ . Axenic cultures of unicellular green algae Chlamydomonas reinhardtii were grown at 24°C in high salt medium (HSM, Harris, 1989) under fluorescent light with gentle shaking. Algal cultures were centrifuged, resuspended in M4-pH7 and fed to daphnids at a concentration of 80,000 cells mL<sup>-1</sup> equivalent of a daily ration of 100 µg C daphnid<sup>-1</sup>. Culture conditions met the OECD requirement of >60 neonates produced per adult over 21 days.

#### 2.2. Exposure conditions

Uranium was obtained from Sigma–Aldrich (Saint-Quentin Fallavier, France) as uranyl nitrate hexahydrate  $(UO_2(NO_3)_2, 6H_2O)$  and stored as a stock solution of  $1 \text{ g L}^{-1}$  in 0.2% HNO<sub>3</sub>. Daphnids were exposed in polycarbonate bottles at a pH of 7 to four different conditions, including three nominal U concentrations of 10, 25 and 75  $\mu$ g L<sup>-1</sup> and an unexposed control. In all conditions, NaNO<sub>3</sub> concentration was adjusted to 3.2  $\mu$ M (without consequence for survival in the control) so as to eliminate differences in NO<sub>3</sub><sup>-</sup> concentration associated with uranium spikes. The tested concentration range was selected in agreement with an EC<sub>10</sub> of 14  $\mu$ g U L<sup>-1</sup> and an EC<sub>50</sub> of 91  $\mu$ g U L<sup>-1</sup> for 21-day reproduction reported in M4-pH7 (Zeman et al., 2008).

Test and control media were renewed every day before food addition. Animals were fed daily with *C. reinhardtii* (80,000 cells mL<sup>-1</sup>) at a ration of 100  $\mu$ g C daphnid<sup>-1</sup>. Bottles were changed every week to limit U adsorption on bottle walls. Samples of freshly renewed medium were collected on a daily basis. Samples of medium after daphnid exposure were collected twice a week and filtrated on 2- $\mu$ m membranes. All water samples were stored at 4 °C in darkness until U and ion analyses. Uranium concentrations in freshly renewed medium remained within 10% of nominal concentrations. Decrease in uranium concentration after 24 h exposure never exceeded 30% of nominal concentrations.

#### 2.3. Experimental design

All generations were started with neonates of the brood 5 (within 24 h of release). Offspring were either exposed to the same concentration as their parent (Experiment A: Exposure of generations F0, F1 and F2) or returned to a clean medium to asses their capacity to recover from a parental exposure (Experiment B: Recovery of generations F'1 and F'2).

(A) Exposure experiment (generations F0, F1 and F2): Each treatment was composed of six 1-L bottles containing 20 neonates. Three bottles were used to monitor daily survival and neonate production for 23 days. The three remaining bottles were used to measure respiration rate, body length and dry mass, brood size and dry mass and uranium bioaccumulation in daphnid tissues and eggs upon deposition of broods 1, 3 and 5. Ingestion and assimilation rates were measured upon deposition of broods 1 and 5.

(*B*) Recovery experiment (generations F 1 and F 2): Each treatment was composed of five 500-mL bottles containing 10 neonates. Three bottles were used to monitor daily survival and neonate production for 23 days. The two remaining bottles were used to measure body length and dry mass, brood size and dry mass upon deposition of broods 1, 3 and 5.

#### 2.4. Ingestion and carbon assimilation rates

In generations F0, F1 and F2, ingestion and carbon assimilation rates were measured in adult daphnids upon deposition of broods 1 and 5 using the radiocarbon method described by Peters (1984) and Lampert (1987).

Radiolabelled algae were prepared by incubating 300 mL of log phase culture with 925 Bq mL<sup>-1</sup> NaH<sup>14</sup>CO<sub>3</sub> in a closed flask for 2 days. Algae were centrifuged and washed three times with M4-pH7 to remove traces of radioactivity in the solution. <sup>14</sup>C-labelled algae were adjusted to a concentration of 80,000 cells mL<sup>-1</sup> in M4-pH7 of each uranium concentration. Triplicate samples of 5 mL were collected and filtered on acetate cellulose membranes (0.2  $\mu$ m) in order to measure radioactivity in algae.

Six polycarbonate vials per exposure condition, each containing five daphnids in 70 mL of radiolabelled food, were placed on a plankton wheel (1 rpm). Daphnids were allowed to feed on labelled algae for 30 min only, to prevent losses of <sup>14</sup>C by defecation (Peters, 1984). Then, daphnids were rinsed and half were collected to measure ingestion rate, whereas the second half were transferred into vials filled with 70 mL of unlabelled food at respective uranium concentration. Daphnids were placed onto the plankton wheel for 3 additional hours, then rinsed and used to measure carbon assimilation.

Daphnid and membrane samples were placed individually in scintillation vials with 1 mL of tissue solubiliser (Soluene 350, PerkinElmer, Boston, USA) and incubated overnight at 55 °C. After solubilisation, 19 mL of scintillation cocktail (Ultima Gold XR, PerkinElmer, Boston, USA) were added and radioactivity of <sup>14</sup>C was measured with a liquid scintillation counter (Quantulus 1220, Wallac–PerkinElmer, Finland; detection limit: 30 mBq). Carbon ingestion rate ( $\mu$ g C h<sup>-1</sup> daphnid<sup>-1</sup>) was calculated by the following equation:

$$I = \frac{A1_{\text{daphnid}}}{A_{\text{algae}} \times \Delta t}$$

where  $A1_{daphnid}$  was the activity in daphnids (Bq daphnid<sup>-1</sup>) after 30 min,  $A_{algae}$  was the specific activity in algae (Bq  $\mu$ g<sup>-1</sup>C) and  $\Delta t$  = 0.5 h of incubation time.

During the 3 h-incubation, a fraction of the newly assimilated food was turned over and respired as  $^{14}CO_2$ . This fraction was taken into account by measuring dissolved  $^{14}CO_2$  in samples of filtered medium from each vial (0.2 µm acetate cellulose membrane) following Bohrer and Lampert (1988). A first 8-mL subsample was transferred into a scintillation vial containing one drop of 10 M NaOH. Another 8-mL subsample was acidified with one drop of concentrated HNO<sub>3</sub> and aerated for 10 min to remove CO<sub>2</sub>. Both vials were added with 12 mL of scintillation cocktail and counted by liquid scintillation analysis. The difference in radioactivity between the acidified and alkaline subsamples was attributed to  $^{14}CO_2$  (Bohrer and Lampert, 1988). Carbon assimilation rate (µg Ch<sup>-1</sup> daphnid<sup>-1</sup>) was calculated by the following equation:

$$A = \frac{A2_{\text{daphnid}} + A_{\text{CO}_2}}{A_{\text{algae}} \times \Delta t}$$

where  $A2_{daphnid}$  was the activity in daphnids (Bq daphnid<sup>-1</sup>) after 3 h,  $A_{CO_2}$  was the "respired" fraction of activity (Bq daphnid<sup>-1</sup>) after 3 h,  $A_{algae}$  was the specific activity in algae (Bq  $\mu$ g<sup>-1</sup> C) and  $\Delta t$  = 0.5 h of incubation time. Assimilation efficiency AE (dimensionless) was calculated as: AE = A/I.

# 2.5. Respiration rate

On deposition of broods 1, 3 and 5, three daphnids per condition were placed individually into respiration chambers containing 1 mL of the test medium, maintained at 20 °C. The decrease in oxygen partial pressure associated with respiration was recorded for 40–60 min using the Unisense microrespiration system (Unisense S/A, Arhus, Denmark). Sensor signal was calibrated using vigorously bubbled clean medium (100% of O<sub>2</sub>-saturation) and a solution of sodium ascorbate (0.1 M) in NaOH (0.1 M) (0% of O<sub>2</sub>-saturation). The percentage saturation was converted into oxygen concentration using an equilibrium concentration in water of 282.3  $\mu$ mol O<sub>2</sub> L<sup>-1</sup> (20 °C, 1 atm). Oxygen consumption rates *R* ( $\mu$ mol O<sub>2</sub> daphnid<sup>-1</sup> h<sup>-1</sup>) were calculated as:

$$R = \frac{[O_2]_0 \times (1 - \exp^{-k \times \Delta t}) \times V}{\Delta t}$$

where  $[O_2]_0$  was the oxygen concentration  $(\mu \text{mol } L^{-1})$  measured at t=0, V the volume (L) of medium in the respiration chamber,  $\Delta t=1$  h and k was the consumption coefficient (h<sup>-1</sup>) obtained by fitting exponential models to observed oxygen concentrations:  $[O_2]_t = [O_2]_0 \times \exp^{-k \times t}$ .

# 2.6. Body length and dry mass

Body length and dry mass were measured in each condition in neonates (<24 h) upon start of a new generation and in adult daphnids within 24 h of deposition of broods 1, 3 and 5. Five replicate samples composed of 5–8 neonates or individual adults (including those used for respirometry in F0, F1 and F2) were also collected. Samples were rinsed with ultra-pure water and body length was measured from the apex of the helmet to the base of the tail spine under a binocular microscope equipped with a micrometer. In adults, eggs were carefully dissected out from the brood pouch and counted. Neonates, dissected females and pooled eggs were transferred into pre-weighed aluminium pans. Samples were dried for 24 h at 55 °C, cooled in a desiccator and weighed immediately with an SE2 ultra-microbalance (Sartorius AG, Göttingen, Germany) at a precision of 0.1  $\mu$ g.

#### 2.7. Uranium and major ion analyses

Water samples were acidified with 0.2 mL HNO<sub>3</sub> (69%) prior to the quantification of U and major cations by inductively coupled plasma-atomic emission spectrometry (ICP-AES Optima 4300DV, PerkinElmer, Welleslay, MA, USA; detection limit of  $10 \,\mu g \, L^{-1}$  and 0.5 mg  $L^{-1}$  for U and cations respectively). Major anions were quantified by ion chromatography (ILC DX-120, Dionex, detection limit of  $50 \,\mu g \, L^{-1}$ ).

Dry daphnid, egg and neonate samples were mineralized on sand bath (90 °C) until evaporation in 1 mL of HNO<sub>3</sub> (69%) and 1 mL of H<sub>2</sub>O<sub>2</sub> (30%) successively. Mineralised samples were taken in 10 mL of HNO<sub>3</sub> ultra pure 2% (v/v) and diluted before analysis by inductively coupled plasma-mass spectrometry (ICP-MS 7500Cx, Agilent Technologies, Tokyo, Japan; detection limit ~ 0.11 ng UL<sup>-1</sup>).

Table 1

List of equations and parameters used to describe daphnid physiology and life history in agreement with the DEB theory (Kooijman, 2000; Kooijman and Bedaux, 1996).

States variables (units)	Equations		Parameters, definitions (units)
Body length (mm)	$BL_t = BL_m - (BL_m - BL_b) \cdot \exp(-\gamma \cdot t)$	(1)	$BL_m$ , Maximum body length (mm); $BL_b$ , body length at birth (mm); $\gamma$ , von Bertalanffy growth rate (d <sup>-1</sup> )
Body dry mass (µg)	$BW_t = \delta \cdot BL_t^3$	(2)	$\delta$ form coefficient (µg DW mm <sup>-3</sup> )
Ingestion rate ( $\mu gCdaphnid^{-1}h^{-1})$	$I_t = \alpha \cdot BL_t^2$	(3)	$BL_t$ , Body length at time t (mm); $\alpha$ , surface specific ingestion rate ( $\mu$ g C mm <sup>-2</sup> h <sup>-1</sup> )
Assimilation rate ( $\mu gCdaphnid^{-1}h^{-1})$	$A_t = \varepsilon \cdot BL_t^2$	(4)	$\epsilon$ , Surface specific assimilation rate $(\mu gCmm^{-2}h^{-1})$
Respiration rate ( $\mu g O_2 daphnid^{-1} h^{-1}$ )	$M_t = r_1 \cdot BL_t^2 + r_2 \cdot BL_t^3$	(5)	$r_1$ , Surface specific respiration rate ( $\mu g O_2 mm^{-2} h^{-1}$ ); $r_2$ , volume specific respiration rate ( $\mu g O_2 mm^{-3} h^{-1}$ )
Egg dry mass (µg)	$EW_t = \beta \cdot BL_t + \omega$	(6)	$eta$ (µg DW mm $^{-1}$ ), $\omega$ (µg DW), slope and intercept of the linear regression
Internal [U] in daphnid (ng U $\mu L^{-1})$	$\frac{d}{dt}C_t = C_{ext} \cdot \frac{k_1}{BL_t} - C_t \cdot \left(\frac{k_2}{BL_t} + \frac{d}{dt} \ln BL_t^3\right)$	)(7)	$C_{ext}$ , External [U] ( $\mu$ gUL <sup>-1</sup> ); $k_1$ , accumulation rate (d <sup>-1</sup> ); $k_2$ , elimination rate (d <sup>-1</sup> )

#### 2.8. Bioaccumulation and calculation of dose rates

Uranium was considered to be homogenously distributed in daphnid tissues. Internal uranium concentration  $C_t$  (ng U  $\mu$ L<sup>-1</sup>) was calculated as:

$$C_t = \frac{Q_t}{V_t}$$

where  $Q_t$  is the amount of bioaccumulated uranium measured per daphnid (ng U daphnid<sup>-1</sup>) and  $V_t = 0.04\pi$ .  $BL_t^3$  is the estimated body volume ( $\mu$ L) assuming an ellipsoid shape with constant axial ratio of 1:0.6:0.4 (Alonzo et al., 2008).

Bioaccumulation factor (BAF) was defined at equilibrium as the amount of uranium in daphnids ( $\mu$ g kg<sup>-1</sup> fresh weight, hereafter "fw"; calculated assuming that daphnid dry weight represents 10% of fresh weight) relative to the concentration in the medium ( $\mu$ g L<sup>-1</sup>). Values at equilibrium were calculated, using the coefficients  $k_1$  and  $k_2$  of the fitted biokinetic model (Table 1).

Grams of U were converted to activities (Bq) based on depleted uranium specific activity of  $1.43 \times 10^4$  Bq g<sup>-1</sup> U measured in the stock solution. Delivered dose rates *DR* (mGy h<sup>-1</sup>) were obtained from the following equation:

$$DR = \sum_{i} DCC_{i} \cdot [activity]_{i}$$

where  $DCC_i$  (mGy h<sup>-1</sup> Bq<sup>-1</sup> mL) are dose conversion coefficients calculated for uranium in the medium and in daphnid tissues (Table 2) by EDEN-v2 software (Beaugelin-Seiller et al., 2006), [*activity*]<sub>*i*</sub> are volume-specific activities (Bq mL<sup>-1</sup>) in daphnid tissues and in the medium. Uranium daughters down to <sup>231</sup>Thorium were accounted for in the dose calculations.

#### Table 2

Dose conversion coefficients (*DCC*) for depleted uranium in daphnid tissues and in the medium calculated in relation to body length (mm) and used to convert volume-specific activities ( $Bq mL^{-1}$ ) to dose rates (mGy  $h^{-1}$ ) delivered to daphnids.

Body length (mm)	$DCC$ (mGy $h^{-1}$ Bq $^{-1}$ mL)	
	Daphnid tissues	Medium
1.0	$2.64\times10^{-2}$	$13.6\times10^{-4}$
2.0	$2.70  imes 10^{-2}$	$6.84\times10^{-4}$
3.0	$2.72  imes 10^{-2}$	$4.61\times10^{-4}$
4.0	$2.73 \times 10^{-2}$	$3.46\times10^{-4}$
5.0	$2.74  imes 10^{-2}$	$2.77\times10^{-4}$

#### 2.9. Statistical analyses

Effects of uranium exposure on body length, body dry mass, ingestion rate, assimilation rate, respiration rate, egg dry mass and bioaccumulation were tested after accounting for the coincident influence of body length and/or time (Table 1). Used relationships conformed to the DEB theory (Kooiiman, 2000). The analytical solution of the differential equation for bioaccumulation  $O_t$  was solved algebraically with Mathematica 6 (Wolfram Research Inc., Champaign, USA). Statistical analyses were conducted with the statistical computing software R (R Development Core Team, 2006), linear and non-linear models being fitted with routines Im and glm following the least-squares criterion. Errors normality assumption was tested with statistics of Shapiro-Wilk, Lilliefors, Anderson-Darling, Cramer-von Mises, Shapiro-Francia and Jargue-Bera. Errors homoscedasticity assumption was tested with statistics of Levene, Bartlett and Filgner-Killeen. In case of heteroscedasticity, sandwich covariance matrix estimators, provided by the R package sandwich, were used for hypothesis testing of linear models (Zeileis, 2006). Finally, multiple comparison tests were achieved with the sequential Holm procedure (Holm, 1979), to ensure 5% error of first kind.

# 3. Results

# 3.1. Survival

Survival of daphnids in generation F0 was not affected by uranium exposure whatever the tested concentration. In F1 and F2, daphnids exposed to  $10 \,\mu g \, L^{-1}$  or recovering from a parental exposure to this concentration exhibited a very low mortality, never exceeding 3% after a 23-day period.

A slight mortality was also observed in F1 after 15 days at  $25 \,\mu g \, L^{-1}$  and reaching 15% on day 23 (Fig. 1A). Comparable effects were observed at this concentration among F'1, F2 and F'2 generations, with mortality ranging from 3 to 7% after 23 days in offspring exposed or recovering from parental exposure.

This contrasted with generation F1 exposed to  $75 \,\mu g \, L^{-1}$  (Fig. 1A), which showed a mortality of 52% after 4 days of exposure and 100% on day 16. A strong mortality was similarly observed on day 11 in F'1 offspring transferred to clean medium after a parental exposure to  $75 \,\mu g \, L^{-1}$  (Fig. 1B). No F2 and F'2 generations could be studied at  $75 \,\mu g \, L^{-1}$ , in absence of reproduction in F1 at this concentration.



Fig. 1. Daphnid survival in the successive generations exposed to uranium concentrations of 0, 10, 25 and 75 µg L<sup>-1</sup> (F1 and F2) and in recovering generations (F'1 and F'2).

#### 3.2. Somatic growth in length and mass

Uranium exposure induced a reduction in body length with increasing concentration (Fig. 2 and Table 3). Observation suggested that the effect on somatic growth in length might increase over generations. This reduction was significant in F0 at a concentration as low as  $10 \,\mu g \, L^{-1}$  as shown by fitting von Bertalanffy curves (Table 1–Eq. (1)). At this concentration, fits yielded a reduction in maximum length  $L_m$  of 6% compared to the control in F0 ( $p \leq 0.05$ ). This reduction in body length remained very slight, although statistically significant, in F1 and F2 exposed to  $10 \,\mu g \, L^{-1}$  as well as in F'1 and F'2 recovering from parental exposure. Growth

inhibition was strong at  $25 \,\mu g L^{-1}$  with maximum length reduced by 9, 16 and 23% compared to the control in F0, F1 and F2 respectively. At 75  $\mu g L^{-1}$ ,  $L_m$  was reduced by 11% in F0 and 44% in F1 compared to the control. This strong effect was also observed in F'1, showing that offspring returned to a clean medium did not recover after parental exposure.

Body dry mass was strongly affected by uranium exposure mainly as a result of the reduction in size observed with increasing concentration (Fig. 3 and Table 3). Furthermore, body dry mass as a function of body length (Table 1–Eq. (2)) significantly differed in F0 at concentrations  $\geq 25~\mu g\,L^{-1}$  and in F1 at concentrations  $\geq 10~\mu g\,L^{-1}$  compared to the control. In fact, at equal body length,



**Fig. 2.** Daphnid body length (mm) in relation to age in the successive generations exposed to uranium concentrations of 0, 10, 25 and 75 µg L<sup>-1</sup> (F0, F1 and F2) and in recovering generations (F'1 and F'2). Statistics: \*\*\*p < 0.001, \*\*p < 0.05.



**Fig. 3.** Daphnid body dry mass ( $\mu$ g) in relation to body length (mm) in the successive generations exposed to uranium concentrations of 0, 10, 25 and 75  $\mu$ g L<sup>-1</sup> (F0, F1 and F2) and in recovering generations (F'1 and F'2). Statistics: ""p < 0.001, "p < 0.01.

daphnid dry mass was 22% smaller at 25 and 75  $\mu$ g L<sup>-1</sup> than in the control in both F0 and F1 and 16% smaller at 10  $\mu$ g L<sup>-1</sup> than in the control in F1. No significant difference in body dry mass between

the control and exposed daphnids was observed in F2 while offspring returned to a clean medium exhibited significantly greater body mass than the control in F'2.



Fig. 4. Daphnid ingestion rate (µg C daphnid<sup>-1</sup> h<sup>-1</sup>) in relation to body length (mm) in the successive generations exposed to uranium concentrations of 0, 10, 25 and 75 µg L<sup>-1</sup> (F0, F1 and F2). Statistics: \*\*\* p < 0.001.

o a clean medi	um (F'1 and F'2	) (statistics: *	"p < 0.001, "p	i < 0.01, <sup>*</sup> <i>p</i> ·	< 0.05	·									
Generation	$\left[ U\right] \left( \mu g  L^{-1}\right)$	Body length	l			Body dry mass		Egg dry mass		-	C ingestion		C assimilation	O <sub>2</sub> consumption	
		$BL_{b}$ (mm)	$BL_m (mm)$	$\gamma \left( \mathrm{d}^{-1}  ight)$	р	$\delta (\mu gmm^{-3})$	р	$\beta$ (µg mm <sup>-1</sup> )	$(\mathfrak{pd}) \omega$	d d	$\chi (\mu g h^{-1} mm^{-2})$	d	$\varepsilon (\mu g h^{-1} m m^{-2}) p$	$r_1 (\mu g h^{-1} mm^{-2})$	$r_2 (\mu g h^{-1} mm^{-3}) p$
FO	0	0.99	4.42	0.13		3.92		3.03	-3.57		0.20		0.21	0.033	0.035
	10	0.99	4.18	0.14	*	4.27		3.88	-5.75	-	0.17		0.19	0.104	0.025
	25	0.99	4.05	0.14	* **	3.06	***	3.05	-3.75	-	0.19		0.14 **	0.125	0.016
	75	1.00	3.92	0.14	***	3.04	***	2.01	-0.91	-	0.08	*	0.04	0.120	-0.008
F1	0	1.07	4.20	0.14		4.85		2.76	-1.37	-	0.32		0.26	0.219	-0.008
	10	1.02	4.30	0.11	:	4.10	***	1.44	3.02	-	0.23		0.16	0.443	-0.071
	25	1.01	3.55	0.16	***	3.78	***	2.11	-0.03	-	0.21	***	0.16	0.231	-0.034
	75	0.93	2.34	0.12	÷	3.61	t	3.32	-3.41						
F2	0	1.02	4.45	0.12		4.63		2.29	-0.68		0.37		0.31	0.146	0.011
	10	0.99	4.34	0.11	*	4.84		1.63	1.25	-	0.27	Ŧ	0.20	0.121	0.349
	25	0.96	3.46	0.13	÷	4.45		1.83	0.92	-	0.18	Ŧ	0.14 ***	0.591	-0.122
F'1	From 10	1.04	4.28	0.10	÷	5.34		4.43	-6.65						
	From 25	1.01	4.22	0.12		4.57		2.39	-0.72						
	From 75	Failed <sup>a</sup>				3.28									
F′2	From 10	0.96	4.60	0.08	***	5.27	:	2.91	-2.47						
	From 25	0.98	4.03	0.10	÷	5.56	:	3.02	-3.05						

Parameters of growth in body length and body dry mass, ingestion, assimilation, respiration and egg dry mass fitted for different exposure conditions in three successive generations (F0, F1 and F2) and in generations returned

**Table 3** 

# 3.3. Ingestion, assimilation and respiration

Ingestion and assimilation rates were described as linear functions of square body length (Table 1–Eqs. (3) and (4)). Because uranium strongly affected daphnid size, increasing concentration resulted in a strong reduction in nutrition (Fig. 4). Relationships fitted in exposed daphnids were compared to the control in order to take the indirect effect on size into account. Surface-specific ingestion rates  $\alpha$  (Table 3) did not differ from the control in FO at concentrations  $\leq 25 \,\mu g \, L^{-1}$  suggesting that the reduced ingestion was only a consequence of the observed reduction in daphnid size. The values of  $\alpha$  were significantly smaller than the control at a concentration of 75  $\mu g \, L^{-1}$  in F0 and at concentrations  $\geq 10 \,\mu g \, L^{-1}$ in F1 and F2, suggesting that uranium directly affected ingestion, independent of body length.

Surface specific assimilation rates  $\varepsilon$  were calculated and showed a significant difference from the control at concentration  $\geq 25 \ \mu g L^{-1}$  in F0 and as low as concentrations  $\geq 10 \ \mu g L^{-1}$  in F1 and F2. Assimilation efficiency was reduced in F0 at  $25 \ \mu g L^{-1}$  (74%) and 75  $\ \mu g L^{-1}$  (57%) compared to the control (80–100%) and in F1 at deposition of brood 5, e.g., after 24 days of exposure to  $10 \ \mu g L^{-1}$  (60%) and 25  $\ \mu g L^{-1}$  (57%).

Respiration rates, measured in F0, F1 and F2, were described as functions of square and cubic body length (Table 1–Eq. (5)). Relationships fitted for exposed daphnids did not significantly differ from the control at any uranium concentration and in any generation, due to a high individual variability. As a consequence, the observed reduction in respiration rates with increasing concentration (as an example in F0, from 2.77–3.69 to  $2.01-2.54 \,\mu g O_2 \, daphnid^{-1} h^{-1}$  respectively in the control and at  $75 \,\mu g \, L^{-1}$  at deposition of brood 5) was mainly a result of the reduction in body size induced by uranium (in the example, from 4.0 to  $3.7 \, \text{mm}$ ).

# 3.4. Reproduction

<sup>a</sup> General linear model failed using R.

Reproduction closely followed moulting cycles with brood deposition occurring every 3 days in the control within a few hours of cuticle renewal. Uranium exposure induced perturbations in reproduction causing delay in brood release, reduction in fecundity and mass invested per egg with increasing concentration (Figs. 5 and 6).

In F0, average delays in brood release were greatest at 75  $\mu$ g L<sup>-1</sup>, ranging from 1.3 to 2.5 days at deposition of broods 1 and 4, respectively. Furthermore, fecundity was reduced by 48% compared to the control. The vast majority of daphnids exposed to 75  $\mu$ g L<sup>-1</sup> did not reproduce in F1, possibly because daphnids did not reach the required size for reproduction. Very few abortive eggs were produced in this condition. Delays in F0 exposed to 25  $\mu$ g L<sup>-1</sup> ranged from 0.7 to 1.9 days at deposition of broods 1 and 4, respectively. Delays increased over the course of generations reaching 1.9 and 5.0 days in F2 at deposition of broods 1 and 4, respectively. Fecundity decreased in parallel over the course of generations, with reduction in average brood size of 25 and 42% in F1 and F2, respectively. The only effect on reproduction observed at 10  $\mu$ g L<sup>-1</sup> was a reduction in fecundity of 20% in F2.

Dry mass invested per egg increased linearly with mother body length. No significant difference in this relationship was observed between the control and exposed daphnids (Fig. 6). Consequently, the decrease in egg dry mass observed between exposure conditions was a result of the decrease in size observed with increasing uranium concentration.

Returning offspring to a clean medium improved reproduction only after parental exposure to  $25 \,\mu g \, L^{-1}$ . This improvement mainly concerned fecundity, with a neonate production 28% greater in F'1 than in the control F1 and not significantly different in F'2



**Fig. 5.** Cumulated number of neonates produced per daphnid over four broods in relation to age in the successive generations exposed to uranium concentrations of 0, 10, 25 and 75 μg L<sup>-1</sup> (F0, F1 and F2) and in recovering generations (F'1 and F'2). Statistics: <sup>\*\*\*</sup> *p* < 0.001, <sup>\*\*</sup> *p* < 0.01.

compared to the control F2. Delays in brood release remained of the same magnitude in both F'1 and F'2 as in exposed parents at  $25 \,\mu g \, L^{-1}$ . Coping with exposure to  $10 \,\mu g \, L^{-1}$  might have metabolic costs which become visible only once offspring are returned to a clean medium. In fact, delays of 1–2.3 days in brood release were observed after parental exposure to  $10 \,\mu g \, L^{-1}$  although no significant delay was detected in daphnids exposed to  $10 \,\mu g \, L^{-1}$ .

# 3.5. Bioaccumulation and dose rates

Uranium amounts in freshly deposited eggs were below quantification limit of ICP-MS, in every exposure condition and generation. In agreement with the biokinetic model (Table 1–Eq. (7)), uranium content in daphnids increased with age and with increasing concentration in the medium (Fig. 7), reaching average maxima



Fig. 6. Dry mass per egg (µg) in relation to body length (mm) in the successive generations exposed to uranium concentrations of 0, 10, 25 and 75 µg L<sup>-1</sup> (F0, F1 and F2) and in recovering generations (F'1 and F'2).



**Fig. 7.** Uranium bioaccumulation in daphnids (ng U daphnid<sup>-1</sup>) in relation to age in the successive generations exposed uranium concentrations of 0, 10, 25 and 75 µg L<sup>-1</sup> (F0, F1 and F2). Lines represent to fitted curves.

of  $17 \pm 1$ ,  $32 \pm 6$  and  $54 \pm 13$  ng U daphnid<sup>-1</sup> after 20 days of exposure to 10, 25 and 75 µg L<sup>-1</sup>, respectively. However, no difference in the amount of bioaccumulated uranium was observed between exposures at 25 and 75 µg L<sup>-1</sup> in F1, due to the strong inhibition in somatic growth observed at the highest concentration in F1. Best fits (least sums of squares) were obtained with accumulation and elimination rates differing between exposure conditions, with  $k_1 = 0.223 d^{-1}$  and  $k_2 = 1.327 d^{-1}$  at concentrations  $\leq 25 \mu g L^{-1}$  and  $k_1 = 0.043 d^{-1}$  and  $k_2 = 0.126 d^{-1}$  at 75 µg L<sup>-1</sup> independent of the generation.

Bioconcentration factors based on measured burdens ranged from 417 to 870 L kg<sup>-1</sup> fw at 25  $\mu$ g L<sup>-1</sup> and from 348 to 658 L kg<sup>-1</sup> fw at 75  $\mu$ g L<sup>-1</sup>. However, measurements were not performed at equilibrium. Bioaccumulation factors at equilibrium predicted from  $k_1$  and  $k_2$  reached 690 and 1410 L kg<sup>-1</sup> fw at  $\leq$ 25 and 75  $\mu$ g L<sup>-1</sup>, respectively.

Average dose rates were calculated in each exposure condition based on uranium uptake predicted by the biokinetic model, yielding maxima of 5, 12 and  $20 \,\mu \text{Gy} \, \text{h}^{-1}$  at 10, 25 and 75  $\mu \text{g} \, \text{L}^{-1}$  respectively. Alpha particles from uranium in daphnid tissues contributed >99.9% to total dose rates whereas beta-and gamma-emissions from uranium in daphnids or in the water remained well below 0.1% of total dose rates.

# 4. Discussion

The low penetrating power of alpha particles, which are attenuated by water and do not pass through exoskeletons of aquatic organisms (Whicker and Schultz, 1982) resulted in minimal radiological hazard of external exposure to uranium. Levels of internally accumulated uranium were used to calculate bioaccumulation factors (BAF) and address the associated radiological hazard (Blaylock et al., 1993; Whicker and Schultz, 1982). Values of BAF estimated at equilibrium were from 690 to  $1410 L kg^{-1}$  fw at 25 and 75  $\mu g L^{-1}$ but showed some variability among sampled daphnids. BAF values for uranium in freshwater invertebrates were recently reviewed in the literature and ranged over five orders of magnitude (from 3.6 to  $60,000 \text{ Lkg}^{-1}$  fw) with a mean of  $170 \text{ Lkg}^{-1}$  fw (IAEA, 2009). Internal dose rates were evaluated based on maximal average burdens observed at the different concentrations independent of the generation. Maximum values obtained in the different exposure conditions ranged from 5 to  $20 \,\mu\text{Gy}\,h^{-1}$ . These values were of the same order of magnitude as those found by Zeman et al. (2008) under similar exposure conditions. In Alonzo et al. (2008), slight reduction in growth with no effect on ingestion and fecundity rates was observed after exposure of several generations to internal alpha delivered by exposure to Am-241 at a 15-fold higher dose rate  $(300 \,\mu\text{Gy}\,h^{-1})$  than in the present study. Thus, effects of uranium in daphnids were only attributable to chemical toxicity, confirming that chemotoxicity of depleted uranium is of much greater concern in comparison to its radiotoxicity, in agreement with Miller et al. (2002), Sheppard et al. (2005) and Mathews et al. (2009).

In D. magna exposed to uranium, structural damages on intestinal epithelium were observed at 50  $\mu$ g L<sup>-1</sup> suggesting that uranium might cause some functional perturbations in assimilation (Zeman, 2008). This hypothesis was confirmed in this study with a significant reduction in surface specific assimilation rates detected at 25 and 75  $\mu$ gL<sup>-1</sup> in F0 and at concentrations as low as 10  $\mu$ gL<sup>-1</sup> in F1 and F2. Metabolic rates are known to vary with organism size. Observed respiration rates were affected only through the reduction in size induced by uranium, since no significant difference in oxygen consumption was visible between the control and exposed daphnids of equal size. Baillieul et al. (2005) similarly found that cadmium mainly affected energy budget through a reduction in assimilation, whereas respiration showed little response to this metal. Other studies similarly showed that stress affects energy intake to a greater extent than energy consumption in a wide range of species including Gammarus pulex (Maltby et al., 1990), D. magna (Baird et al., 1990; Barber et al., 1990) and Dreissena polymorpha (Schneider et al., 1998; Smolders et al., 2002).

In the present study, observed effects on growth and reproduction were in good agreement with predictions of net production and DEB theories (Nisbet et al., 2004; Kooijman, 2000), in case of a reduced energy intake, due to low food availability or exposure to toxicants. In fact, daphnids exposed to uranium showed reduced fecundity, body size and mass. Furthermore, a delay in the release of the first brood was observed at 75  $\mu$ g L<sup>-1</sup> in F0 and at 25  $\mu$ g L<sup>-1</sup> in F1 and F2. This observation might partly result from growth retardation under toxic stress (Reynaldi et al., 2006) because daphnids need to reach a size threshold in order to mature. It might also be caused by the reduced size at birth observed at  $\geq 25 \,\mu g L^{-1}$  as small neonates are known to grow smaller, reach maturity later and produce fewer eggs than large neonates (Ebert, 1991, 1992, 1994). At  $10 \,\mu g \, L^{-1}$  the slight reduction in size observed in F2 caused only a small reduction in fecundity and no delay in reproduction. Age and size at maturity are among key life history traits in *Daphnia* (Ebert, 1992), delay in first reproduction having critical consequences for population dynamics (Alonzo et al., 2008).

In our study, increasing uranium concentration was shown to induce a reduction in egg mass in agreement with Zeman et al. (2008). This observation was mainly a consequence of the reduced size of mothers, in agreement with Tessier and Consolatti (1989). Small egg mass might be a good indicator of a strong and irreversible reduction in offspring viability at 75  $\mu$ g L<sup>-1</sup>, as suggested by the very strong mortality observed in the following generation either under the same uranium exposure or after neonates were

returned to a clean medium. Similarly, neonates of small size or born from eggs of small mass showed a greater sensitivity to toxic effects after parental exposure to Am-241 contamination, external gamma irradiation and cadmium (Alonzo et al., 2008; Gilbin et al., 2008; Enserink et al., 1990). Those results highlight the necessity to consider offspring quality as an additional endpoint in chronic 21day tests (Hammers-Wirtz and Ratte, 2000). A reduction in energy reserves deposited in eggs, as reflected in egg mass, might explain the increased offspring sensitivity to uranium. This observation might also result from an exposure of eggs in the brood pouch, during 3 days of embryogenesis. A direct exposure of embryos in the brood chamber to environmental pollutants, such as aniline derivatives, was suggested by Abe et al. (2001) on the basis that daphnids actively exchange the fluid in the brood chamber for environmental water in order to support embryonic oxygen demand (Kobayashi et al., 1987). In our results, the absence of uranium in freshly laid eggs, even at the highest tested concentration, suggested that ovaries acted as a barrier against a direct maternal transfer of uranium. Further experimental tests are required to investigate uranium contamination during embryonic stage.

In this study, effects of uranium were shown to increase in severity across exposed generations. This result might differ depending on which brood number is used to start subsequent exposed generations. Using 5th brood in our experiments allowed uranium to accumulate in mothers and potentially increased effects across generations whereas 3rd brood is often regarded as the fittest offspring in D. magna. At  $75\,\mu g\,L^{-1},$  sublethal effects on reproduction and growth were observed in F0 whereas daphnids did not survive in F1. Like Brennan et al. (2006) observed with daphnids exposed to four environmental oestrogens for two generations, results with uranium suggested that a weakening of the offspring might occur in the first generation leading to a higher susceptibility to lethal effects in the progeny. Toxicity of uranium for reproduction also increased across generations at 10 and 25  $\mu$ g L<sup>-1</sup>, causing delay in brood deposition and reduction in number of eggs per brood. Similar observations were reported in the third generation exposed to internal alpha irradiation at  $15 \,\mathrm{mGy}\,\mathrm{h}^{-1}$  (Alonzo et al., 2008) or to  $50 \text{ mg L}^{-1}$  estrogen diethylstilbestrol (Baldwin et al., 1995). Daphnid reproductive capacity mainly declined across generations exposed to alpha irradiation at 0.3 and 1.5 mGy h<sup>-1</sup>, through a decreased proportion of breeding daphnids (Alonzo et al., 2008).

The observed increase in toxic effects of uranium across generations contrasted with results obtained during long-term exposure to other stable metals such as cadmium, nickel and copper. In fact, D. magna exposed to sublethal concentrations of copper for 12 successive generations or cadmium for three generations was shown to become more resistant to acute toxicity (LeBlanc, 1982; Bodar et al., 1990). D. magna exposed to  $160 \,\mu g \, L^{-1}$  nickel for seven successive generations showed an increase in intrinsic rate of population growth despite decreased mean life span and body length of primiparous daphnids (Münzinger, 1990). These results suggested the development of an adaptive response which was shown to involve the induction of metallothionein-like protein (MTLP) (Stuhlbacher et al., 1992; Guan and Wang, 2004; Bodar et al., 1990). This mechanism controlling the homeostasis of essential metals and sequestering toxic metals in the cell might protect organisms against chemotoxicity but not radiotoxicity. In our study, toxic resistance was not observed over three exposed generations suggesting that uranium might not induce MTLP or might induce MTLP after a longer multigeneration exposure, or that MTLP does not protect against uranium chemotoxicity.

Continuously changing conditions in ecosystems require taking an interest in recovery ability of organisms after a stress situation, which determines the population resistance to episodes of toxic stress in the environment. This study showed that once returned to a clean medium, progeny did not survive after parental exposure to 75  $\mu$ g L<sup>-1</sup> uranium suggesting irreversible effects at this concentration whereas effects on survival and reproduction decreased after parental exposure to  $\leq$ 25  $\mu$ g L<sup>-1</sup>. A similar increase in reproduction rates was observed in *D. magna* whose parents were previously exposed to diazinon below 0.5 ng L<sup>-1</sup> (Sanchez et al., 1999). Daphnids returned to a clean medium after parental exposure to uranium  $\leq$ 25  $\mu$ g L<sup>-1</sup> exhibited a greater body mass than the control. This difference was more complex to interpret and might involve some compensatory process such as additional energy storage in F2 to counterbalance effects of uranium toxicity. This process might also take place in daphnids returned to a clean medium despite the absence of toxicity and resulted in an increased body mass compared to the control.

# 5. Conclusion

In *D. magna*, uranium affects carbon assimilation with strong consequences for somatic growth and reproduction. Moreover, increased effects on life history traits and physiology were observed across exposed generations as low as  $10 \,\mu g \, L^{-1}$ , with potential capacity to recover in a clean medium after exposure to  $\leq 25 \,\mu g \, L^{-1}$ . Results presented in this study illustrate the necessity of carrying out multigeneration tests or examining offspring quality to assess environmental risk of waterborne uranium and other pollutants and suggest that chronic toxicity measured over one generation of *Daphnia* (21-day tests) might lead to underestimating risk.

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#### References

- Abe, T., Saito, H., Niikura, Y., Shigeoka, T., Nakano, Y., 2001. Embryonic development assay with *Daphnia magna*: application to toxicity of aniline derivatives. Chemosphere 45, 487–495.
- Alonzo, F., Gilbin, R., Zeman, F.A., Garnier-Laplace, J., 2008. Increased effects of internal alpha irradiation in *Daphnia magna* after chronic exposure over three successive generations. Aquatic Toxicology 87, 146–156.
- Baillieul, M., Smolders, R., Blust, R., 2005. The effect of environmental stress on absolute and mass-specific scope for growth in *Daphnia magna* Strauss. Comparative Biochemistry and Physiology C 140, 364–373.
- Baird, I., Barber, D.J., Calow, P., 1990. Clonal variation in general responses of Daphnia magna Straus to toxic stress. I. Chronic life-history effects. Functional Ecology 4, 399–407.
- Baldwin, W.S., Milam, D.L., Leblanc, G.A., 1995. Physiological and biochemical perturbations in *Daphnia magna* following exposure to the model environmental estrogen diethylstilbestrol. Environmental Toxicology and Chemistry 14, 945–952.
- Barata, C., Baird, D.J., Markich, S.J., 1998. Influence of genetic and environmental factors on the tolerance of *Daphnia magna* Straus to essential and non-essential metals. Aquatic Toxicology 42, 115–137.
- Barata, C., Baird, D.J., Markich, S.J., 1999. Comparing metal toxicity among Daphnia magna clones: an approach using concentration-time-response surfaces. Archives of Environmental Contamination and Toxicology 37, 326–331.
- Barber, I., Baird, D.J., Calow, P., 1990. Clonal variation in general responses of Daphnia magna Straus to toxic stress. II. Physiological effects. Functional Ecology 4, 409–414.
- Barillet, S., Adam, C., Palluel, O., Devaux, A., 2007. Bioaccumulation, oxidative stress, and neurotoxicity in *Danio rerio* exposed to different isotopic compositions of uranium. Environmental Toxicology and Chemistry 26, 497–505.
- Beaugelin-Seiller, K., Jasserand, F., Garnier-Laplace, J., Gariel, J.C., 2006. Modeling radiological dose in non-human species: principles, computerization, and application. Health Physics 90, 485–493.
- Blaylock, B.G., Frank, M.L., O'Neal, B.R., 1993. Methodology for estimating radiation dose rates to freshwater biota exposed to radionuclides in the environment. In: Methodology for Estimating Radiation Dose Rates to Freshwater Biota Exposed to Radionuclides in the Environment.
- Bodar, C.W.M., Van der Sluis, I., Van Montfort, J.C.P., Voogt, P.A., Zandee, D.I., 1990. Cadmium resistance in *Daphnia magna*. Aquatic Toxicology 16, 33–40.
- Bohrer, R.N., Lampert, W., 1988. Simultaneous measurement of the effect of food concentration on assimilation and respiration in *Daphnia magna* Straus. Functional Ecology 2, 463–471.

- Bonin, B., Blanc, P.L., 2001. L'uranium dans le milieu naturel, des origines jusqu'à la mine. In: Métivier, H. (Ed.), L'Uranium de l'Environnement à l'Homme. EDP Sciences, Les Ulis, pp. 7–41.
- Bourrachot, S., Simon, O., Gilbin, R., 2008. The effects of waterborne uranium on the hatching success, development, and survival of early life stages of zebrafish (*Danio rerio*). Aquatic Toxicology 90, 29–36.
- Brennan, S.J., Brougham, C.A., Roche, J.J., Fogarty, A.M., 2006. Multi-generational effects of four selected environmental oestrogens on *Daphnia magna*. Chemosphere 64, 49–55.
- Bywater, J.F., Banaczkowski, R., Bailey, M., 1991. Sensitivity to uranium of six species of tropical freshwater fishes and four species of cladocerans from Northern Australia. Environmental Toxicology and Chemistry 10, 1449–1458.
- Calow, P., Sibly, R.M., 1990. A physiological basis of population processes: ecotoxicological implications. Functional Ecology 4, 283–288.
- Dias, V., Vasseur, C., Bonzom, J.M., 2008. Exposure of *Chironomus riparius* larvae to uranium: effects on survival, development time, growth, and mouthpart deformities. Chemosphere 71, 574–581.
- Ebert, D., 1991. The effect of size at birth, maturation threshold and genetic differences on the life-history of Daphnia magna. Oecologia 86, 243–250.
- Ebert, D., 1992. A food-independent maturation threshold and size at maturity in Daphnia magna. Limnology & Oceanography 37, 878–881.
- Ebert, D., 1994. A maturation size threshold and phenotypic plasticity of age and size at maturity in *Daphnia magna*. Oikos 69, 309–317.
- Environnement Canada, 2003. Liste des substances d'intérêt prioritaire Rapport d'évaluation – Rejets de radionucléides des installations nucléaires (effets sur les espèces autres que l'être humain), Gouvernement du canada.
- Elendt, B.P., Bias, W.R., 1990. Trace nutrient deficiency in Daphnia magna cultured in standard medium for toxicity testing. Effects of the optimization of culture conditions on life history parameters of D. magna. Water Research 24, 1157– 1167.
- Enserink, L., Luttmer, W., Maas-Diepeveen, H., 1990. Reproductive strategy of *Daphnia magna* affects the sensitivity of its progeny in acute toxicity tests. Aquatic Toxicology 17, 15–26.
- Gilbin, R., Alonzo, F., Garnier-Laplace, J., 2008. Effects of chronic external gamma irradiation on growth and reproductive success of *Daphnia magna*. Journal of Environmental Radioactivity 99, 134–145.
- Guan, R., Wang, W.X., 2004. Cd and Zn uptake kinetics in Daphnia magna in relation to Cd exposure history. Environmental Science and Technology 38, 6051–6058.
- Guan, R., Wang, W.X., 2006. Multigenerational cadmium acclimation and biokinetics in Daphnia magna. Environmental Pollution 141, 343–352.
- Harris, E.H., 1989. The Chlamydomonas Sourcebook: A Comprehensive Guide to Biology and Laboratory Use. Academic Press, Inc.
- Hammers-Wirtz, M., Ratte, H.T., 2000. Offspring fitness in Daphnia: Is the Daphnia reproduction test appropriate for extrapolating effects on the population level? Environmental Toxicology and Chemistry 19, 1856–1866.
- Holm, S., 1979. A simple sequentially rejective multiple test procedure. Scandinavian Journal of Statistics 6, 65–70.
- IAEA, 2009. Quantification of Radionuclide Transfer in Terrestrial and Freshwater Environments for Radiological Assessments. IAEA-TECDOC.
- Kobayashi, M., Hayakawa, F., Ninomiya, M., 1987. Hatchability and hemoglobin of Daphnia magna embryo. Physiological Zoology 60, 507–512.
- Kooijman, S.A.L.M., 2000. Dynamic Energy and Mass Budgets in Biological Systems. 2nd ed.
- Kooijman, S.A.L.M., Bedaux, J.J.M., 1996. The Analysis of Aquatic Toxicity Data. VU University Press, Amsterdam, p. 149.
- Kuhne, W.W., Caldwell, C.A., Gould, W.R., Fresquez, P.R., Finger, S., 2002. Effects of depleted uranium on the health and survival of *Ceriodaphnia dubia* and *Hyalella* azteca. Environmental Toxicology and Chemistry 21, 2198–2203.
- Lampert, W., 1987. Feeding and nutrition in Daphnia. Memorie dell'Istituto Italiano di Idrobiologia 45, 143–192.
- LeBlanc, G.A., 1982. Laboratory investigation into the development of resistance of *Daphnia magna* (Straus) to environmental pollutants. Environmental Pollution Series A: Ecological and Biological 27, 309–322.
- Maltby, L., Naylor, C., Calow, P., 1990. Effect of stress on a freshwater benthic detritivore: scope for growth in *Gammarus pulex*. Ecotoxicology and Environmental Safety 19, 285–291.
- Markich, S.J., 2002. Uranium speciation and bioavailability in aquatic systems: an overview. The Scientific World Journal 2, 707–729.
- Mathews, T., Beaugelin-Seiller, K., Garnier-Laplace, J., Gilbin, R., Adam, C., Della-Vedova, C., 2009. A probabilistic assessment of the chemical and radiological risks of chronic exposure to uranium in freshwater ecosystems. Environmental Science and Technology 43, 6684–6690.

- Miller, A.C., Stewart, M., Brooks, K., Shi, L., Page, N., 2002. Depleted uraniumcatalyzed oxidative DNA damage: absence of significant alpha particle decay. Journal of Inorganic Biochemistry 91, 246–252.
- Münzinger, A., 1990. Effects of nickel on Daphnia magna during chronic exposure and alterations in the toxicity to generations pre-exposed to nickel. Water Research 24, 845–852.
- Muyssen, B.T.A., Janssen, C.R., 2004. Multi-generation cadmium acclimation and tolerance in *Daphnia magna* Straus. Environmental Pollution 130, 309–316.
- Nisbet, R.M., McCauley, E., Gurney, W.S.C., Murdoch, W.W., Wood, S.N., 2004. Formulating and testing a partially specified dynamic energy budget model. Ecology 85, 3132–3139.
- OECD, 1998. *Daphnia magna* Reproduction Test, Guidelines for Testing of Chemicals, Rep. No. 211. Organisation for Economic Cooperation and Development, Paris, France.
- Pane, E.F., McGeer, J.C., Wood, C.M., 2004. Effects of chronic waterborne nickel exposure on two successive generations of *Daphnia magna*. Environmental Toxicology and Chemistry 23, 1051–1056.
- Peters, R.H., 1984. Methods for the study of feeding, grazing and assimilation by zooplankton. In: A Manual on Methods for the Assessment of Secondary Productivity in Fresh Waters, pp. 336–412.
- Pickett, J.B., Specht, W.L., Keyes, J.L., 1993. Acute and Chronic Toxicity of Uranium Compounds to Ceriodaphnia dubia. Savannah River, Aiken, South Carolina, p. 57.
- Poston, T.M., Hanf Jr., R.W., Simmons, M.A., 1984. Toxicity of uranium to Daphnia magna. Water, Air, and Soil Pollution 22, 289–298.
- Ragnarsdottir, K.V., Charlet, L., 2000. Uranium behaviour in natural environments. In: Environmental Mineralogy: Microbial Interactions, Antropogenic Influences, Contaminated Lands and Waste Management, pp. 333–377.
- Reynaldi, S., Duquesne, S., Jung, K., Liess, M., 2006. Linking feeding activity and maturation of *Daphnia magna* following short-term exposure to fenvalerate. Environmental Toxicology and Chemistry 25, 1826–1830.
- Sanchez, M., Ferrando, M.D., Sancho, E., Andreu, E., 1999. Assessment of the toxicity of a pesticide with a two-generation reproduction test using *Daphnia magna*. Comparative Biochemistry and Physiology—C Pharmacology Toxicology and Endocrinology 124, 247–252.
- Schneider, D.W., Madon, S.P., Stoeckel, J.A., Sparks, R.E., 1998. Seston quality controls zebra mussel (*Dreissena polymorpha*) energetics in turbid rivers. Oecologia 117, 331–341.
- Semaan, M., Holdway, D.A., Van Dam, R.A., 2001. Comparative sensitivity of three populations of the cladoceran *Moinodaphnia macleayi* to acute and chronic uranium exposure. Environmental Toxicology 16, 365–376.
- Sheppard, S.C., Sheppard, M.I., Gallerand, M.O., Sanipelli, B., 2005. Derivation of ecotoxicity thresholds for uranium. Journal of Environmental Radioactivity 79, 55–83.
- Smolders, R., Bervoets, L., Blust, R., 2002. Transplanted zebra mussels (*Dreissena poly-morpha*) as active biomonitors in an effluent-dominated river. Environmental Toxicology and Chemistry 21, 1889–1896.
- Stuhlbacher, A., Bradley, M.C., Naylor, C., Calow, P., 1992. Induction of cadmium tolerance in two clones of *Daphnia magna* straus. Comparative Biochemistry and Physiology—C Pharmacology, Toxicology and Endocrinology 101, 571–577.
- Tessier, A.J., Consolatti, N.L., 1989. Variation in offspring size in *Daphnia* and consequences for individual fitness. Oikos 56, 269–276.
- Trapp, K.E., 1986. Acute Toxicity of uranium to waterfleas (Daphnia pulex) and bluegill (Lepomis macrochirus). Environmental and Chemical Sciences. Savannah River, Aiken, South Carolina (Report ESC-SR-30).
- Weigel, F., 1986. Uranium. In: Katz, J.J., Seaborg, G.T., Morss, L.R. (Eds.), The Chemistry of the Actinide Elements, 2nd edition. Chapman and Hall, London, New York (Chapter 5).
- Whicker, F.W., Schultz, V., 1982. Radioecology: Nuclear Energy and the Environment, vol. 1. CRC, Boca Raton, FL, USA.
- Widdows, J., Johnson, D., 1988. Physiological energetics of Mytilus edulis: scope for growth. Marine Ecology Progress Series 46, 113–121.
- World Health Organisation, 2001. Depleted Uranium, Sources, Exposure and Health Effects. WHO/SDE/PHE/01.1. Geneva, Switzerland.
- Zeileis, A., 2006. Object-oriented computation of sandwich estimators. Journal of Statistical Software 16, 1–16.
- Zeman, F., 2008. Toxicité d'un mélange binaire sur la daphnie, Daphnia magna. Etude des effets biologiques de l'uranium et du sélénium seuls et en mélange. Thesis (IRSN-2008/103), Univ. Montpellier II, p. 200.
- Zeman, F.A., Gilbin, R., Alonzo, F., Lecomte-Pradines, C., Garnier-Laplace, J., Aliaume, C., 2008. Effects of waterborne uranium on survival, growth, reproduction and physiological processes of the freshwater cladoceran *Daphnia magna*. Aquatic Toxicology 86, 370–378.