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Aquatic Toxicology 73 (2005) 155–170

AQUATIC TOXICOLOGY

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Relationship between the energy status of *Daphnia magna* and its sensitivity to environmental stress

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Received 22 December 2004; accepted 15 March 2005

Abstract

This work tested the hypothesis that animals with a high energy status are more successful in dealing with stress than animals with a low energy status. Daphnids (*Daphnia magna*) were reared for 2 weeks in four different concentrations of food. Survival was not affected by food supply, and growth and reproduction increased with increasing food ration. This increase correlated well with the energy status, as was measured by scope for growth on day 15. After 2 weeks, the daphnids in the four different food ration groups were exposed for another 2 weeks to a range of increased salinities or cadmium concentrations, while remaining in their respective food concentrations. In the salinity groups, survival, growth, or reproduction were not influenced at low salinities. Exposure to higher salinity significantly decreased survival and reproduction, but this decrease was more pronounced in the highest food concentrations. In the cadmium exposed daphnids, cadmium content increased with increasing exposure concentrations, but accumulation was independent of food rations. Cadmium exposure significantly decreased survival, growth, and reproduction and this decrease again was more pronounced with increasing food concentration. Thus, the high energy status of the daphnids from the high food concentrations at the start of the exposure did not provide an increased capacity to cope with additional stress. Instead, the sensitivity of the daphnids to stress increased with increasing food ration. This increased sensitivity is likely to be the result of a change in life history from emphasizing survival at low food supply to stressing reproduction at high food supply.

Keywords: Chronic toxicity; Salinity; Cadmium; Reproduction; Food; Energy status

1. Introduction

Organisms in the environment are continuously exposed to a wide variety of natural and anthropogenic stressors. If these stressors are present at a high enough level or for a long enough period, they will eventually have an impact on the organism's physiological integrity, thus decreasing the overall fitness. Any effector that disrupts this physiological integrity will induce defense and repair mechanisms, which depend on energy requiring processes such as active transport (e.g. exclusion of chemical stressors) and synthetic activity (e.g. synthesis of stress proteins). As such, combating stress is likely to be energetically costly for stressed organisms (Calow, 1991; Calow and Sibly, 1990; Genoni,

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doi:10.1016/j.aquatox.2005.03.006

1997; De Coen and Janssen, 2003). If, indeed, stress will increase the energy expenditure of organisms, the energy status of an organism at any given time should affect its capacity to cope with stress, too. Other things being equal, organisms with a high energy status would likely be more successful in dealing with stress than organisms with a low energy status. Because of this, there has been an increasing interest in using fitness-related parameters, such as energy reserves, condition, or growth as criteria for environmental stress (Calow and Sibly, 1990; Mayer et al., 1992; Kooijman and Bedaux, 1996; De Coen and Janssen, 2003; Smolders et al., 2003). Although these indicators are to a large extent non-specific measures of exposure, they have the advantage of incorporating a high degree of ecological relevance.

This paper describes a series of experiments that were conducted to test the hypothesis presented above. In these experiments, energy status was measured as scope for growth (SFG), which is calculated from the energy balance equation: SFG = $A - R - E$ (A = energy assimilated from food, R = energy metabolized, and E = energy lost in nitrogen excretion). SFG represents the fraction of assimilated energy that is not consumed in basal maintenance and that is therefore available for growth and reproduction (Warren and Davis, 1967; Widdows and Johnson, 1988; Smolders et al., 2003; Crowe et al., 2004). A positive SFG indicates a net energy gain, and thus indicates that there is an excess of energy available to invest in reserves, growth, and reproduction. A negative SFG indicates that the animal is depleting its energy reserves. SFG has frequently been used to study the effects of environmental conditions or pollutants on physiological condition (Widdows and Johnson, 1988; Maltby et al., 1990; Smolders et al., 2002). It has generally been found that SFG correlates well with energy reserves, growth, condition and reproduction (Beiras et al., 1994; Okumus and Stirling, 1994; Baillieul et al., 1996; Pouvreau et al., 2000; Smolders et al., 2002, 2004). Still, most validation of SFG is indirect, since only few experiments actually measured SFG and growth, condition or reproduction on the same animals. Therefore, a second aim of the experiments was to compare the estimate of growth and reproduction based on SFG-measurements with the actual growth and reproduction observed.

The experiments were conducted with the waterflea *Daphnia magna* Strauss. Being a clonal organism, *Daphnia* offers the possibility of studying physiological processes without interference of genetic variability (Barata et al., 2002; Antunes et al., 2003). The experiments comprised two parts, each lasting 2 weeks. In the first part, the overall energy status of the test organisms was manipulated by exposing it to a non-specific environmental stress, in this case food limitation. After 2 weeks, the daphnid's energy status was measured through SFG. Subsequently, the animals were subjected to different salinity or cadmium concentrations while maintaining the different food rations. Their success in coping with this second stressor, measured as survival, growth and reproduction, was then compared to their initial energy status.

Food limitation was chosen as an ecologically relevant environmental stressor because it represents the most direct way of influencing the organism's energy intake and therefore its energy status. However, it does not cause structural or functional damage to the organism. Salinity is a natural stressor, which challenges osmotic and ionic regulation. Osmo- and ion regulation are part of the normal physiological functioning and all organisms possess specialized systems to maintain cellular volume and composition in a steady state or to return them to some preferred condition after a perturbation (Kirchner, 1991; Aladin and Potts, 1995; Heugens et al., 2001). *D. magna* is essentially a freshwater organism, but it is known as a euryhaline species with a relatively high salinity tolerance (Langerspetz, 1955; Arner and Koivisto, 1993; Teschner, 1995; Schuytema et al., 1997). Cadmium on the other hand has been shown to decrease food intake in daphnids and thus affects the energy supply of the animals (Allen et al., 1995; Bodar et al., 1988; Knops et al., 2001; De Coen and Janssen, 2003). In addition to this decreased energy availability, cadmium induces toxic effects on a wide variety of biological processes by disrupting the structural and functional integrity of the organisms (Baillieul and Blust, 1999; Heugens et al., 2001; Barata et al., 2002; De Coen and Janssen, 2003).

2. Materials and methods

2.1. Culture conditions

A single clone of *D. magna* was used throughout the study. The clone was kindly provided by Prof.

C.R. Janssen of the Environmental Toxicology Research Group at the University of Ghent (Belgium), and originated from a small pond near Antwerp. The clone has been reared in our laboratory since 1991. The daphnids were cultured at a constant temperature of $20 \pm 1\,^{\circ}C$ and a 14:10 light:dark cycle. Artificial freshwater was used in both daphnid cultures and the experiments (composition [concentrations in g/l]: KCl: 0.0030; $CaCl_2 \cdot 2H_2O$: 0.014; $NaHCO_3$: 0.0328; $MgSO_4 \cdot 7H_2O$: 0.0518; CaO: 0.0238). The pH of this water was 8.0 ± 1. The animals were fed a diet of the unicellular green algae *Selenastrum capricornutum* Printz (8×105 cells/ml). The algae were obtained from the Institute of Freshwater Ecology (Windermere, UK). The daphnid medium was enriched with 1.5 ml/l of a vitamin-mix (concentrations in mg/l): biotin: 5; thiamine: 100; vitamin B12: 100; inositol: 90; nicotinic acid: 50; Ca-panthothenate: 250; pyridoxine: 100 (after Goulden et al., 1982). SeO_2 was added to achieve a concentration of 2 μg Se per liter culture medium (USEPA, 1994). The medium was changed daily and the aquaria were cleaned with denatured ethanol to remove bacteria and fungi attached to the walls. All aquaria were rinsed with tap water and culture medium before use. Food concentrations in cultures and experiments were checked with a Coulter Counter (Model ZF; Coulter Electronics Ltd., Harpenden, UK)

2.2. Experimental design

The experimental design is graphically illustrated in Fig. 1. Each experiment consisted of two parts, each lasting for 2 weeks. The first part aimed at the induction of groups of daphnids with different physiological conditions. Four groups of 200 neonate daphnids were assigned to different concentrations of *S. capricornutum* (100, 200, 400, and 800×10^3 cells/ml) for 2 weeks. The animals were kept in 5 l aquaria containing 3 l of artificial fresh water with the respective food concentrations, enriched with 1.5 ml/l of the vitamin mix. Every day, the media were changed, survival was monitored and neonates were removed and stored for subsequent counting. This first part of the experiment resulted in four groups with different physiological conditions, induced by the differences in food rations. On day 15, physiological conditions were measured as SFG in five samples of five animals per treatment.

In the second part of the experiment, the remaining daphnids from each of the four food rations were exposed to the same range of either increased salinities or cadmium (as cadmium nitrate). From each food ration, six groups of 20 daphnids were randomly selected and transferred to each of six salinity (0.06, 2, 4, 6, 8, and 10‰) or cadmium (1, 2.5, 5, and 10×10^{-8} mol l^{-1} Cd^{2+} and two control groups: one with and one without NTA [nitrillotriacetate, see below]) made up in 800 ml polypropylene beakers containing 500 ml of the respective media. The freshwater used for the cadmium exposure had a constant salinity of 0.06‰. The food concentrations were the same as in the first part of the experiment. Every day, the media was changed, survival was monitored and neonates were removed and stored for later counting. After 2 weeks of exposure to the different salinities, the experiment was terminated.

The selection of the salinity range (0.06–10‰) was based on preliminary acute toxicity tests and previous experiments, and were expected to span the range from no mortality to heavy mortality. The media were prepared by adding commercial sea salt (hw-Meersalz, Wiengandt GmbH, Krefeld, Germany) to our artificial freshwater. The salinity of the artificial freshwater in the control aquaria, without the addition of sea salt, was 0.06‰. Salinity in the different exposure aquaria was checked with a refractometer (Atago Co., Ltd., Tokyo, Japan), food concentrations were checked with a Coulter Counter (Model ZF, Coulter Electronics, Luton, UK).

The experimental cadmium concentrations were based on preliminary 96 h acute toxicity tests, and again were expected to span from no to heavy mortality. The free cadmium ion concentrations were 1, 2.5, 5, and 10×10^{-8} mol l^{-1} Cd^{2+}. These concentrations were obtained by addition of the right amount of the metal ion buffer Na_3NTA to total cadmium concentrations that were 100 times higher than the free cadmium concentrations (Perrin and Dempsey, 1974). NTA did not affect the daphnids and therefore only one control was considered henceforth. In such metal ion buffered systems, free cadmium lost from solution through adsorption on surfaces of the vials, algae or daphnids, or through uptake by algae and daphnids is replaced by cadmium ions released from the Cd-NTA pool. The chemical speciation was calculated with a speciation model previously developed as an adaptation of the program COMPLEX (Ginzburg, 1976). Two

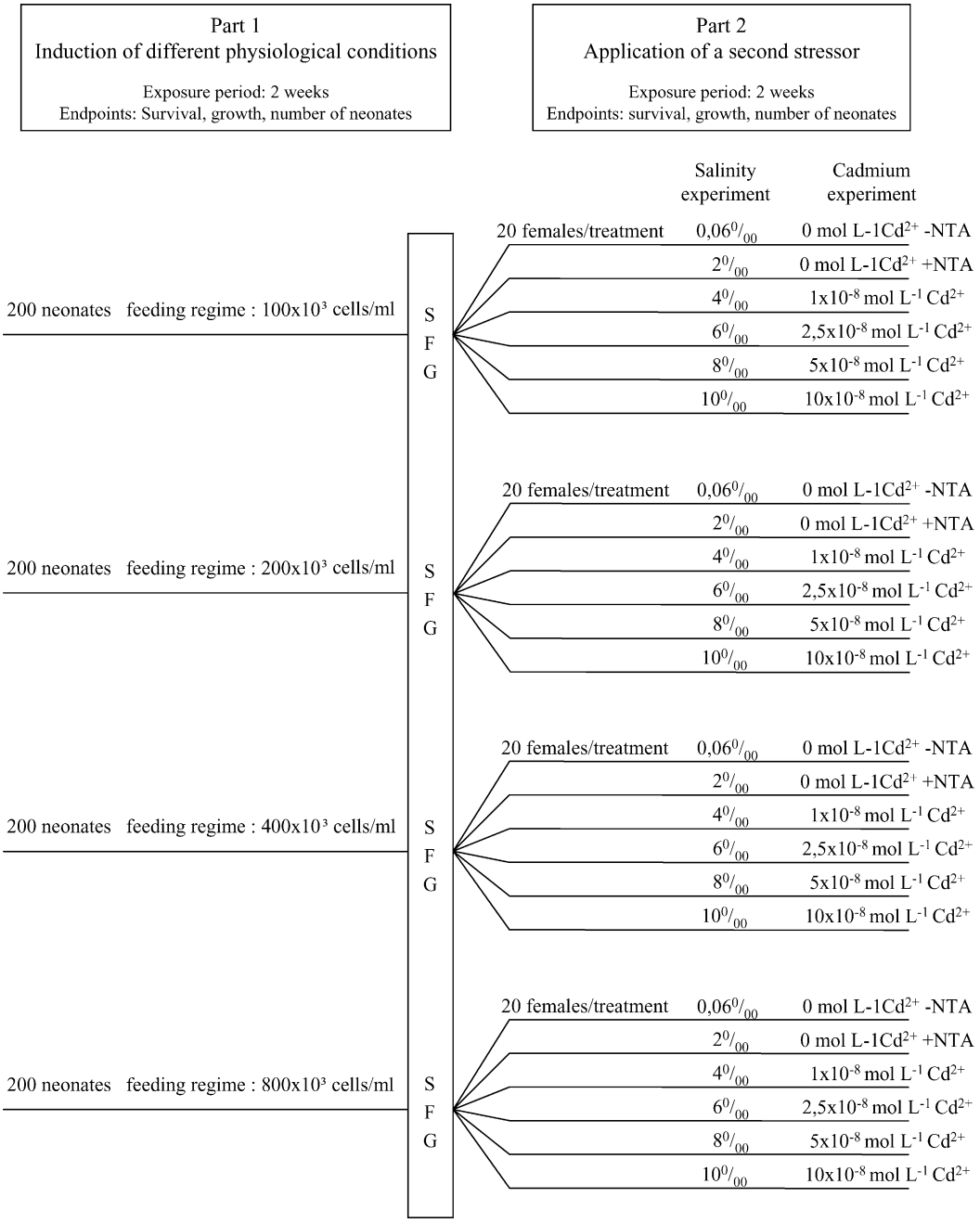

Fig. 1. The experimental design. Four groups of 200 neonate daphnids were reared in different cadmium concentrations to induce different physiological conditions. SFG was measured on day 15. Thereafter, each cadmium group was subdivided in groups of 20 daphnids that were exposed to the same range of increased salinity for another 2 weeks.

experiments were run in parallel. One served for the measurement of survival, growth and reproduction, the other served for the analysis of cadmium accumulation by the daphnids. The cadmium content of five individual daphnids per treatment was measured after 1 and 2 weeks of exposure. The daphnids were allowed to swim for approximately 10 min in a 1 mM solution of 8-hydroxyquinoline-5-sulfonic acid to remove cadmium bound to the external surfaces of the daphnids (Blust et al., 1992). The medium also contained 8×10^5 cells/ml *S. capricornutum* to clear the guts from cadmium loaded algae or medium. Thereafter, the animals were rinsed with deionised water, transferred individually in polypropylene sample cups and dried overnight in an oven at 80 °C. When dry, their body weight was determined individually to the nearest μg on a Cahn Model 4100 electrobalance (Cahn/Ventron Corp. Paramount, CA). Thereafter, they were processed for cadmium analysis using graphite furnace atomic absorption spectrometry. Water samples were acidified to 1% HNO_3 and preserved at −20 °C until analysis. Tissue samples (daphnids) were digested in a microwave oven after addition of concentrated nitric acid. The dissolved samples were diluted with deionised water to a 10% nitric acid solution. To each sample 200 μg $(NH_4)_2HPO_4$ and 10 μg $Mg(NO_3)_2$ were added as matrix modifier (Merck, supra pure) (Blust et al., 1988). Calibration series were made up with commercial standard solutions (Merck, Cadmium standard solution 1000 mg/l in 0.5 mol l^{-1} HNO_3) and diluted with Milli-Q deionised water.

2.3. Growth and reproduction

Body size was measured on a randomly selected sample of daphnids three times per week. Sample size per treatment was 25 animals in the first 2 weeks of the experiments and 5 in the second part of the experiment. The daphnids were placed on a microscope slide and viewed through a microscope. Carapace length was determined as the distance from the top of the eye to the base of the caudal spine.

The net reproduction rate R_0 was calculated as $\sum l_x m_x$, where l_x is the proportion of individuals surviving to age x (in days), and m_x is the number of juveniles per surviving female between age x and $x+1$ (Stearns, 1992; De Coen and Janssen, 2003; Antunes et al., 2004). R_0 was calculated for both parts of the experiment. To avoid including neonates that were born in the second part of the experiment but that were still formed during the first part of the experiment, R_0 for the second part of the experiment included only neonates collected after day 6.

2.4. Scope for growth

Respiration and assimilation were determined on the same animals. Excretion was not measured since its contribution to the total energy budget generally is negligible (Baillieul et al., 1996; Smolders et al., 2002). Five daphnids were placed into 25 ml of ^{14}C-labeled algae of the respective concentrations, and were allowed to feed. Radiolabeled food was prepared by incubating 400 ml of algae with 100 mCi $NaH^{14}CO_3$ for 2 days. After 45 min, they were rinsed and transferred into glass-stoppered vials containing 22 ml of non-labeled food (depuration medium). After 3 h, the vials were opened, the daphnids were removed, rinsed, killed with a few drops of ethanol, and samples for determination of $^{14}CO_2$ and oxygen were taken. Five vials per diet were used. Vials containing the respective test media without the presence of daphnids were included to correct for algal oxygen production. *Daphnia* oxygen consumption was measured in a 10 ml subsample using the Winkler method (APHA, 1989). The energy value of the algae was determined by bomb calorimetry (IKA C400 Adiabatic calorimeter) to be 2.202 kJ/g. The dry weight of the algae was determined on a Cahn Model 4100 electrobalance and was 19.5 pg/cell. For the conversion of oxygen uptake to energy consumption, an oxyjoule equivalent of 21 kJ/l O_2 was used (Elliot and Davison, 1975).

The killed daphnids were dried overnight at 55 °C and their body weight was determined to the nearest μg on a Cahn Model 4100 electrobalance (Cahn/Ventron Corp., Paramount, CA, USA). One ml of tissue solubiliser (Soluene 350, Canberra Packard, Meriden, CT, USA) was added to the dried tissue, the samples were shaken and incubated overnight at 55 °C. After solubilisation, 9 ml of scintillation cocktail (Hionic-fluor, Canberra Packard Meriden, CT, USA) was added, the sample was left to stabilize for a minimum of 12 h and counted in a liquid scintillation counter (Packard Tri-Carb 1900 TR, Canberra Packard, Meriden, CT, USA). During the incubation time, part of the newly assimilated food is turned over and respired as $^{14}CO_2$ (Bohrer

and Lampert, 1988). To account for this, radioactive $^{14}CO_2$ was measured. A sample of 4 ml depuration medium was filtered over a 0.2 mm membrane filter. One ml of the filtrate was transferred into a scintillation vial containing one drop of 10 mol L^{-1} NaOH, and 9 ml of Hionic-fluor was added. The rest of the filtrate was acidified with one drop of concentrated HNO_3 and aerated for 10 min to remove CO2. Thereafter, 1 ml was pipetted into a scintillation vial and counted with 9 ml of Hionic-fluor. The difference in counts between the acidified and alkaline vial was attributed to $^{14}CO_2$ (Bohrer and Lampert, 1988).

2.5. Statistics

Differences in body size and energetics on day 15 were tested using ANOVA followed by a Duncan's multiple range post hoc test. Growth rates in the second part of the experiment were analyzed by comparing body size (pooled over time) of different groups measured during the second part of the experiments, using ANCOVA with time as covariant.

3. Results

3.1. Part 1: induction of different physiological conditions

There was no significant impact of differences in food ration on *Daphnia* mortality (data not shown). Growth rates however, increased significantly with increasing food ration (Fig. 2A). On day 15, all treatments differed significantly in average body size ($p < 0.001$) and body size increased with increasing food ration. Also neonate production increased with increasing ration, resulting in an increasing net reproduction rate (Fig. 2B; $r^2 = 0.98$, $p < 0.01$). Offspring were produced in all ration groups. When physiological energetics was plotted against food rations, it was observed that assimilation, respiration, and SFG all increased hyperbolically with increasing food rations (Fig. 2C). Assimilation was higher than respiration in all treatments, resulting in a positive SFG for all food rations. The relationship between reproduction and SFG is shown in Fig. 2D. Net reproduction growth R_0 increased exponentially with increasing SFG ($r^2 = 0.98$, $p < 0.05$).

3.2. Part 2a: exposure to increased salinities

3.2.1. Survival

Mortality increased with increasing salinity at all food rations (Fig. 3). An increase in salinity to 10‰ was lethal in all food concentrations and resulted in 100% mortality within 4 days. The toxicity of 8‰ was comparable in 100×10^3, 200×10^3, and 800×10^3 cells/ml, causing 50% mortality between 4 and 6 days. In the 400×10^3 cells/ml group, mortality was somewhat delayed in the 8‰ treatment (50% mortality occurring between 8 and 10 days), but did not differ from other rations at the end of the exposure period of 14 days. Mortality in the 6‰ treatments increased with increasing ration, while mortality in the freshwater controls and the 2 and 4‰ salinity groups was generally low (<20%) and independent of food ration.

3.2.2. Growth

At the end of the first part of the experiment, body size in the different treatments increased with increasing food ration (Fig. 2A). This pattern was maintained during the second part of the experiment (Fig. 4A). Daphnids continued to grow in all rations, and growth rates increased with increasing ration. However, daphnids exposed to 8‰ salinity showed a significantly lower growth in all food concentrations compared to the other salinities ($p < 0.01$). Exposure to salinities of 0.06–6‰ did not affect growth rates in any ration group, except in the highest food concentrations where growth rates were also significantly reduced in the 6‰ treatment ($p < 0.01$).

3.2.3. Reproduction

The number of neonates increased with increasing food ration (Fig. 5A), but also the effect of salinity on reproduction increased with increasing ration. In the 100×10^3 and 200×10^3 cells/ml groups, production of neonates was affected by salinity only at 8‰ salinity. In the 400×10^3 cells/ml group, the pattern was similar except for a small peak at 2‰. In the 800×10^3 cells/ml group, there was also a slightly increased production of neonates at 2‰, but there was already a strong reduction in reproduction at 6‰, where R_0 was not different from the net reproduction rate in the 400×10^3 cells/ml group. At 8‰ finally, the number of neonates produced was low in all ration groups, and was no longer related to differences in food ration.

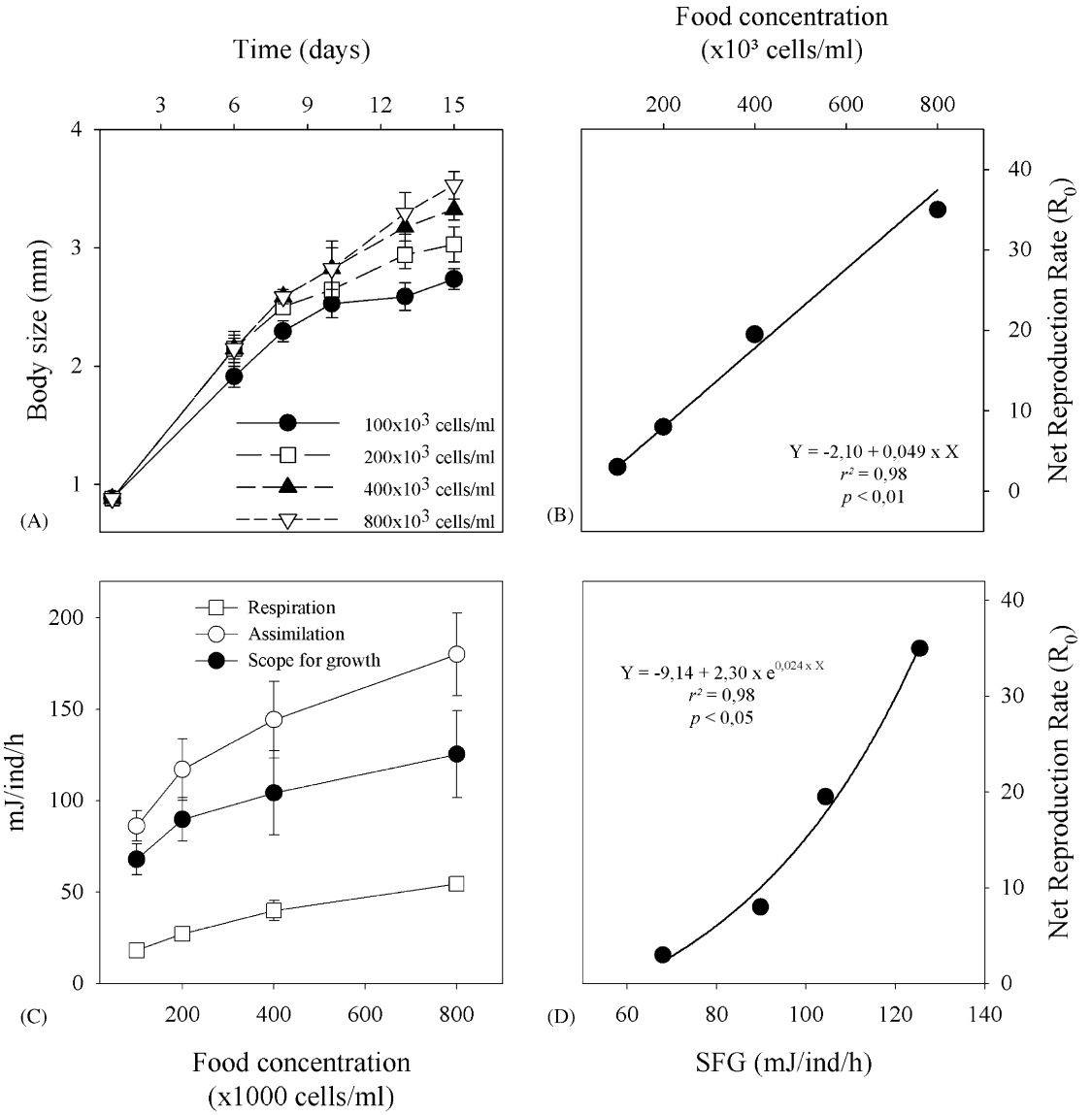

Fig. 2. Results of the first part of the experiment: daphnids reared for 2 weeks in different concentrations of food. (A) Growth ($n = 25$; means ± S.D.). (B) Net reproduction rate (R_0). (C) Physiological energetics measured on day 15 ($n = 5$; means ± S.D.). Data points average five samples of five pooled daphnids. (D) Relationship between net reproduction rate and scope for growth.

3.3. Part 2b: exposure to increased cadmium concentrations

3.3.1. Cadmium analysis

The nominal amount of cadmium added to the treatment aquaria was 100 times higher than the respective free cadmium ion concentrations (1, 2.5, 5, and 10×10^{-8} mol l^{-1} Cd^{2+}). The measured cadmium concentrations in the different treatments were 8.5×10^{-7}, 2.1×10^{-6}, 4.2×10^{-6}, and 8.7×10^{-6} mol l^{-1} Cd_T, respectively. The calculated resulting free cadmium ion concentrations were approximately 85% of the respective nominal values, but the proportions between the concentrations were unchanged compared to the nom-

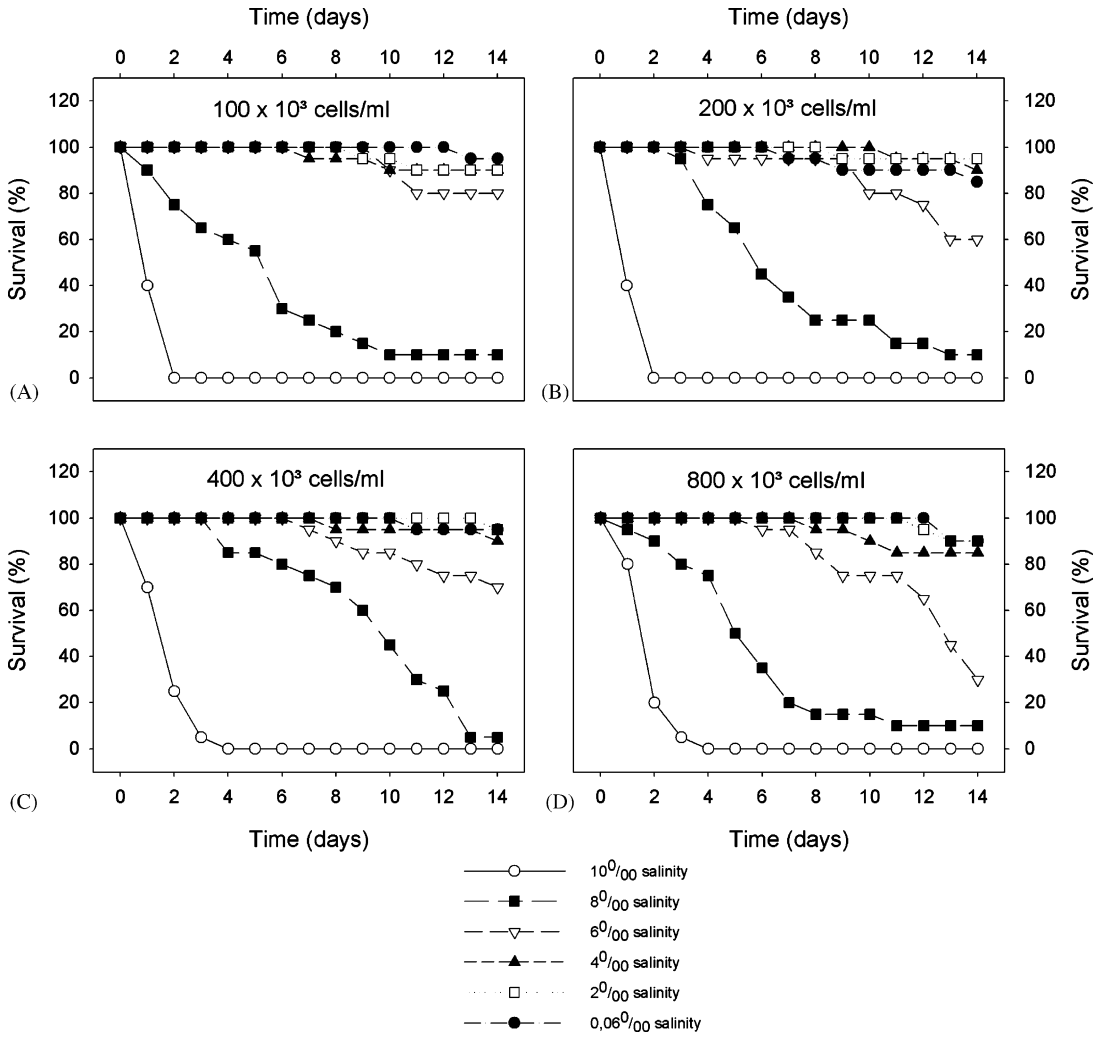

Fig. 3. Survival in the second part of the experiment. The daphnids from the first part of the experiment were exposed to a range of increased salinities while remaining in their respective food concentrations. The four graphs represent the four food concentrations; the survival curves refer to the different salinities. Each treatment started with 20 daphnids from the corresponding food concentration in the first part of the experiment.

inal concentrations (lowest concentration times 2.5, 5, and 10, respectively).

3.3.2. Cadmium accumulation

The cadmium content of the daphnids increased with increasing cadmium exposure concentration and with time, but was independent of food concentration (Fig. 6). Both after 1 and 2 weeks of exposure, the cadmium content of the daphnids did not differ between the food concentrations. Due to the high mortality in the 10×10^{-8} mol l^{-1} Cd^{2+} group, no data on cadmium content are available after 14 days of exposure.

3.3.3. Survival

Mortality increased with increasing cadmium concentration in all food concentrations (Fig. 7). Within each cadmium concentration, mortality also increased with increasing food ration. There was no mortality in the control and 1×10^{-8} mol l^{-1} Cd^{2+} treatment. Exposure to 2.5×10^{-8} mol l^{-1} Cd^{2+} did not affect survival in the two lowest food concentrations, but

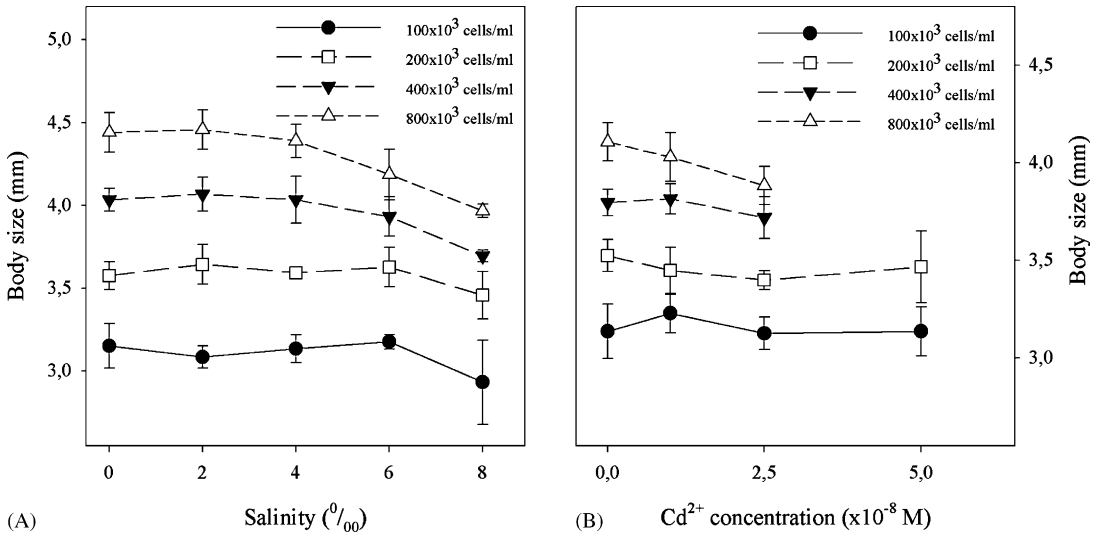

Fig. 4. Body size ($n = 5$; means ± S.D.) in the different food concentrations on the last day of the experiment, plotted vs. (A) salinity and (B) cadmium. Missing points correspond to treatments in which no daphnid survived to the end of the experiment.

decreased survival with about 40 and 80% in the 400 and 800 × 10^3 cells/ml groups respectively. Also exposure to 5×10^{-8} mol l^{-1} Cd^{2+} resulted in limited mortality in the two lowest food rations, but resulted in 100% mortality in the two highest food rations. Exposure to 10×10^{-8} mol l^{-1} Cd^{2+} resulted in total mortality in all food concentrations.

3.3.4. Growth

At the end of the first part of the experiment, daphnid body size was increased with increasing food rations. This pattern was maintained during the second part of the experiment. Body size was elevated at increasing food rations (Fig. 4B). While cadmium exposure did not show an impact on *Daphnia* growth at the two low-

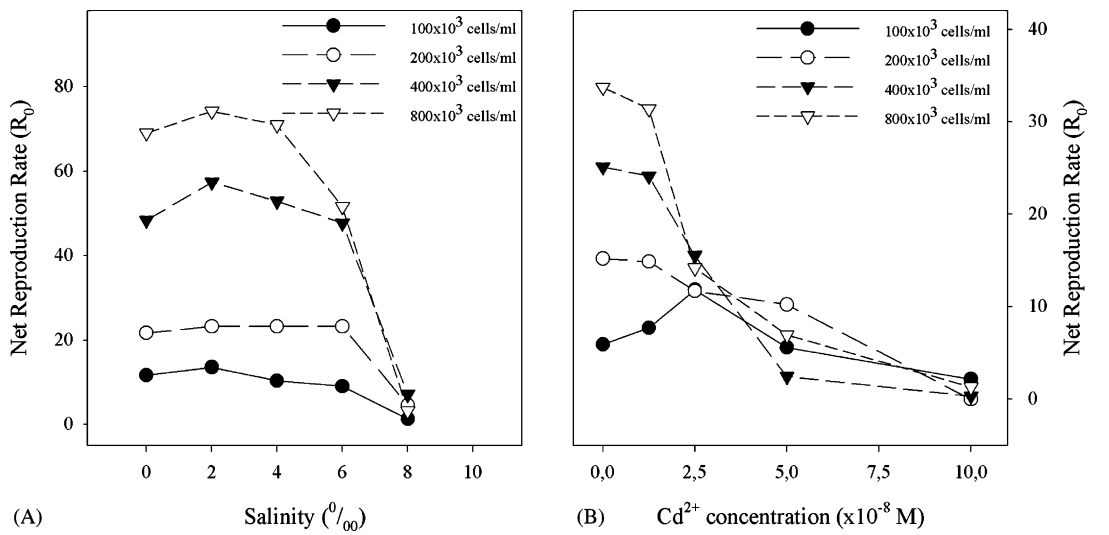

Fig. 5. Net reproduction rate in the second part of the experiment. R_0 in the different food concentrations was plotted vs. (A) salinity and (B) cadmium.

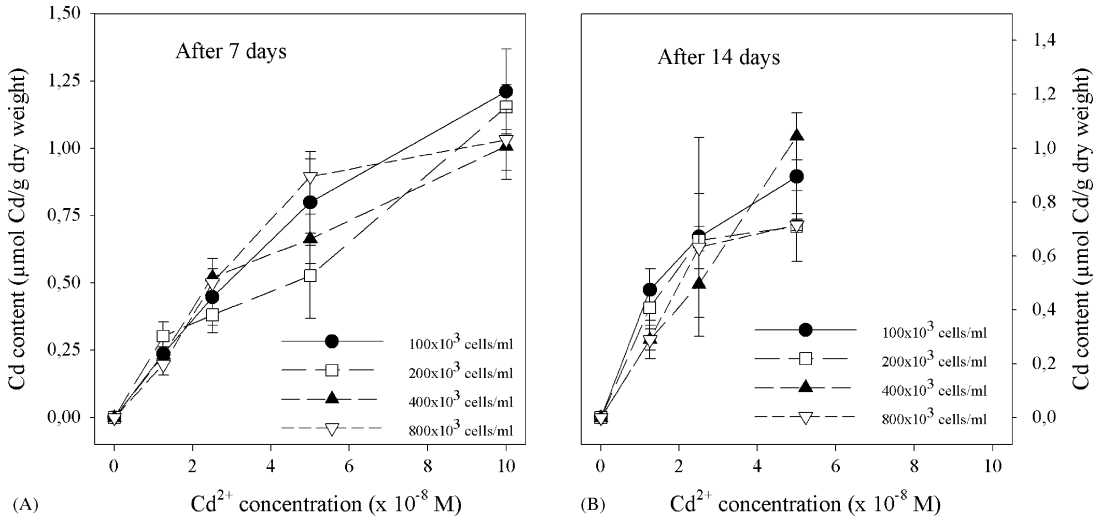

Fig. 6. Cadmium content ($n = 5$; mean ± S.D. of the daphnids after 1 and 2 weeks of exposure to cadmium. The different curves refer to the food concentrations. No daphnids survived to the end of week 2 in the highest cadmium concentration.

est food levels, body size was significantly decreased during cadmium exposure for the two highest food rations ($p < 0.05$). Therefore, the growth inhibiting effect of cadmium again was most obvious in the two highest food concentrations.

3.3.5. Reproduction

As in the first part of the experiment, net reproduction rate (R_0) in the control increased with increasing ration (Fig. 5B). Exposure to cadmium generally decreased production of neonates, but as with survival and growth, the detrimental effect of cadmium was most important at high food rations. Between 1×10^{-8} and 2.5×10^{-8} mol l^{-1} Cd^{2+}, net reproduction rate decreased strongly in the two highest rations to the level of the lower rations. In the lower food concentrations, R_0 remained unchanged by exposure to cadmium concentrations up to 5×10^{-8} mol l^{-1} Cd^{2+}. In the lowest cadmium concentration, R_0 still increased with increasing ration, as in the control. As a result, R_0 was similar in all rations at cadmium concentrations higher than 1×10^{-8} mol l^{-1} Cd^{2+}.

4. Discussion

The experiment was set up to test whether daphnids with a high energy status are more successful in coping with an additional stressor than daphnids with a low energy status. In the first part of the experiment, rearing daphnids for 2 weeks in different amounts of food generated differences in energy status. Food limitation did not affect mortality, which was low in all food rations. Growth and reproduction however increased with increasing food ration, showing that energy status differed between rations. Assimilation, respiration and SFG all increased with food ration and SFG was positive in all treatments, indicating a positive energy budget in all groups. However, there was no linear increase in SFG at the higher food concentrations. This is partly due to the fact that the highest food concentration was above the incipient limiting level (ILL), the food concentration above which the dimensions of the gut limit further uptake of food, regardless of the available food supply (Lampert, 1987). Below the ILL, the ingestion rate increases without particle interfereing with the feeding process. Above the ILL, the particles interfere with the feeding process so that filtration rates decrease as ingestion rates decline. Offspring were produced in all food rations, confirming that even the lowest food ration was more than adequate to sustain normal metabolism in the daphnids. The high correlation between SFG and reproduction indicates that SFG is a good indicator of the general physiological condition of daphnids.

Salinity decreased survival, growth and reproduction at salinity levels of 6‰ or higher, which is comparable to the LC_{50} for salinity of 6.6 g/l reported by Schuytema et al. (1997). A salinity of 4‰ or lower had no significant effect on survival, growth or reproduction, but above a certain threshold salinity, physiological condition declined rapidly. In the present experiment, the decline in reproduction was always

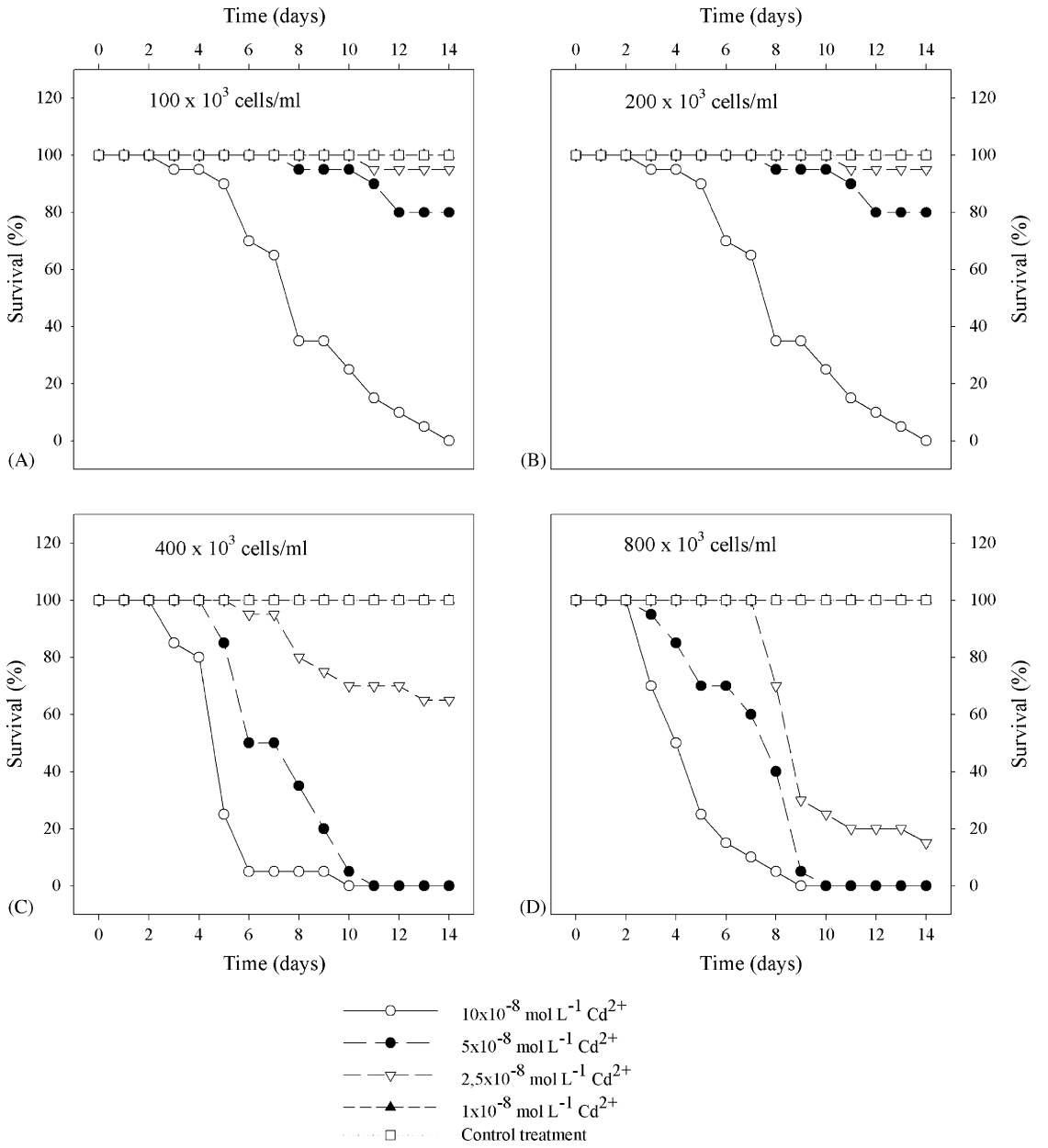

Fig. 7. Survival in the second part of the experiment. The daphnids from the first part of the experiment were exposed to a range of cadmium concentrations while remaining in their respective food concentrations. The four graphs represent the four food concentrations; the survival curves refer to the different Cd^{2+} treatments. Each treatment started with 20 daphnids from the corresponding food concentration in the first part of the experiment.

accompanied by the occurrence of mortality; there were no treatments in which salinity decreased reproduction without decreasing survival. This indicates that there is only a very small difference between non-lethal and lethal effects of ion and osmoregulatory stress on *D. magna*. Growth and reproduction were more or less constant between 0.06 and 6‰ in all except the highest food ration. In this highest food ration of 800×10^3 cells/ml, reproduction at 6‰ was decreased to the level of the 400×10^3 cells/ml group. At 8‰, reproduction was strongly reduced in all food ration groups and did not differ much between treatments. As such, the threshold above which growth and reproduction decreased, was higher than 6‰ in the three lower food concentrations, but was between 4 and 6‰ in the highest food concentration. Thus, the effect of salinity was highest in the highest food concentration. On the other hand, although reproduction in the highest food ration was decreased at 6‰, R_0 was still higher than in the other rations. Therefore, based only on the values of R_0 at each salinity, the daphnids from the high rations were still more successful under stress than those from the lower rations.

This apparently contradictory response indicates that there may be two different aspects of "being successful in coping with stress". On the one hand, reproduction increased with increasing food concentration, which could lead to the conclusion that the daphnids that were offered the highest food ration were more successful in coping with increased salinity. On the other hand however, the salinity-induced decrease in reproduction was more pronounced in the highest food rations, which could also lead to the conclusion that the daphnids in the high food rations were more susceptible to salinity stress. This is also illustrated by the observation that mortality in the 6‰ treatment increased with increasing food ration. Of course, due to the effect of the different food rations, reproduction in the lower rations could not have decreased as much as in the high rations, but it could have fallen to zero at lower salinities, which did not happen. In contrast, reproduction was virtually unaffected by salinity up to 6‰ in the low ration groups.

In a previous report, daphnids from different food rations were exposed to only one increased salinity (7‰; Baillieul et al., 1996). The results were comparable to those in the 8‰ treatment in the present experiment, where survival, growth and reproduction were decreased but there was no significant difference between ration groups. The lack of any clear effect at 7‰ may be due to acclimation processes. Preliminary reports have shown that exposure to even low salinities (2‰) initially decreased growth and reproduction. Since growth and reproduction did not differ between the low salinities in any food ration, this indicates that the daphnids not only had successfully acclimated to increased salinity, but showed "accelerated" growth and reproduction. This agrees with reports on the beneficial effects of low salinity over freshwater (Arner and Koivisto, 1993; Teschner, 1995). In our study, salinity was increased by addition of sea salt, which also resulted in increased calcium concentrations. Most of the calcium in daphnids is located in the exoskeleton that is shed with each moult. Since daphnids do not have a carry-over reservoir for calcium at moulting, each moult-period will be accompanied by a considerable loss of calcium (Porcella et al., 1969; Hessen and Rukke, 2000; Waervagen et al., 2002). The experiments were carried out in soft water (0.52 mM Ca, Hardness 73 mg $CaCO_3$) and this low calcium concentration may have been limiting reproduction in freshwater. Cowgill and Milazzo (1991) showed that addition of $CaCl_2$ indeed increased neonate production. Arner and Koivisto (1993) noted an increased neonate production at low salinity (4‰, diluted seawater) compared to the control, which is in agreement with our own data on 2 and 4‰ (Fig. 5). Thus, increased growth and reproduction after acclimation may have compensated for the initial decrease.

As expected, cadmium decreased survival, growth, and reproduction in all rations and these toxic effects increased with increasing cadmium concentration. However, cadmium toxicity also increased with increasing food concentration. At the end of the experiment, mortality in the 2.5×10^{-8} mol l^{-1} Cd^{2+} treatments was marginal in the two lowest rations but substantial in the two highest rations. Also in the 5×10^{-8} mol l^{-1} Cd^{2+} treatments, mortality was low in the lowest two rations, but increased to 100% in the two highest rations. Growth decreased with increasing cadmium concentration, but did so increasingly with increasing food ration. Net reproduction rate was only slightly affected by cadmium in the lower food concentrations, but in the two highest rations, R_0 decreased substantially to the level of the lower rations at 2.5×10^{-8} mol l^{-1}

Cd^{2+}. Thus, the high-food daphnids appeared more sensitive to cadmium stress than the low-food daphnids. Similar results have been reported by other authors for different cladocerans. Daphnids in control treatments grew larger and produced more neonates in a high-, compared to a low-food environment, but the high-food animals were more sensitive to DTPA (Diethylene-triamine pentaacetic acid; Van Dam et al., 1995), lindane (Antunes et al., 2004), endosulfan (Barry, 1996) or chromium (Gorbi et al., 2002). Cadmium decreased body size in *D. magna* at food concentration of 1.5 mg C/l, but not at 0 or 0.5 mg C/l (Barber et al., 1994) and also Winner et al. (1977) and Patterson et al. (1992) reported an increased sensitivity to toxic stress of what were, based on fecundity, considered the 'high-quality' adults.

Cadmium may adsorb to the external surfaces of algae, thereby decreasing the concentration of cadmium in the soluble phase. Alternatively, once ingested, the metals associated with the algae may form an alternative route of uptake besides uptake of cadmium from the aqueous phase (Taylor et al., 1998; Gorbi et al., 2002). As such, the different concentrations of algae could have altered the bioavailability and the uptake route of the cadmium, and thus the dose to which the daphnids were exposed. The algae did however not change the concentration of cadmium in solution. Thus, the uptake of cadmium from solutions should have been unaffected by the concentration of algae. Since the cadmium body load did not differ between food concentrations, uptake of cadmium via food was small. Differences in response to cadmium in our experiments were therefore due to differences in the physiology and reproductive strategy of the daphnids and not to differences in dose or uptake route. As such, the results of the Cd-exposure experiment are similar to those of the salinity experiment. In both experiments, the energy status of the daphnids increased with increasing ration at the start of the second part of the experiment, and in both experiments, the high-food daphnids were more susceptible to stress. Thus the high energy status of the high-food daphnids did not increase their capacity to deal with stress, but instead rendered them more sensitive. Since the same results were found with stressors as different as increased salinity and cadmium, the results suggest that this response was not characteristic for one specific type of stress, but may be of a more general nature.

Daphnids from the high food concentrations, and thus the daphnids that had the highest energy status at the start of the salinity or cadmium exposure, were not necessarily more successful in dealing with a subsequent stressor. At the stress levels that were tolerated in all food rations, production increased with increasing food ration, but the daphnids from the high rations could not tolerate higher stress levels than daphnids from the low rations. In fact, the high-ration daphnids were in some aspects more sensitive to increases in salinity or cadmium than the lowration animals. This increased sensitivity was not observed in changes in growth, and the impact of salinity or cadmium was independent of the food levels. Hence, mechanisms such as dilution by growth (for cadmium) or differential sensitivity to stressors for fast- and slow growing organisms did not appear to play a major role in describing the observed effects. Effects on mortality or reproduction of the daphnids however were clearly dependent on food rations for both salinity and cadmium as an environmental stressor. A mechanism that could explain this increased sensitivity of the high-food daphnids is based on the generation of differential sensitivity towards toxicants through a ration-dependent change in life history (Porter et al., 1983; Martinez-Jeronimo et al., 1994; Barry, 1996; Glazier, 1998; Hanazato, 1998). The highfood environment allows high reproduction and well-fed daphnids may allocate a large proportion of their energy to reproduction. This increased allocation to reproduction may be at the expense of allocation to self-maintenance, and thus stress resistance. If daphnids from the high rations invest more energy in reproduction at the expense of survival, longevity itself becomes less important since the high number of offspring already assures the success of the population. In a low-food environment, reproduction is necessarily low. The daphnids may have to increase their allocation to self-maintenance in order to increase their longevity and thus their lifetime reproduction. This increased allocation to self-maintenance may then increase their stress resistance. Experimental data, indeed, suggest that daphnids can change from a survival-based life history in low food supply to a reproduction-based life history in high food supply (Porter et al., 1983; Martinez-Jeronimo et al., 1994). Daphnids in a low-food environment produce small broods of large neonates, whereas daphnids in a high-food environment produce

large broods of small neonates (Enserink et al., 1990; Gliwicz and Guisande, 1992; Ebert, 1993; Enserink et al., 1995; Cleuvers et al., 1997; Coors et al., 2004). The large neonates contain a higher amount of lipids (Enserink et al., 1993) and are assumed to be of higher quality than small neonates. Several authors report a general increased survival, and an increased resistance to starvation (Gliwicz and Guisande, 1992; Enserink et al., 1995; Knops et al., 2001; Coors et al., 2004) or toxic stress (Baird et al., 1989; Enserink et al., 1990) of the larger neonates, supporting this hypothesis. Thus, though food levels have a significant impact on daphnids' growth, development and reproduction, it does not automatically make them more resistant towards subsequent environmental stressors.

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