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The effect of calcium concentration on the calcification of *Daphnia magna*

Abstract—Soft waters are characterized by low Ca concentrations, and the distribution and relative success of Ca-demanding invertebrates could be limited by low Ca in extreme softwater localities. A further Ca depletion caused by reversed acidification could thus seriously affect freshwater crustaceans. Experimental studies on the calcification and Ca content of *Daphnia magna* clearly suggested the potential of a Ca limitation. Saturated calcification was reached at Ca concentrations >0.13 mM. Individuals reared in media with lower Ca concentrations were unable to compensate by increasing the period of postmolt Ca uptake and thus had a lower specific Ca content. Specific Ca content decreased from 4.2% to 1% of dry weight over the range 0.25–0.013 mM Ca. Even at the low Ca concentrations, only 10% of total Ca was reclaimed upon molting, the rest was lost with the old exuviae (~40%) or to the ambient medium (~50%). This incomplete calcification under low ambient Ca concentrations could represent a competitive drawback in Ca-poor waters.

Ca is an essential element to crustaceans and other groups of animals with a calcified exoskeleton. While Ca demands are easily satisfied in marine environments and hardwater lakes, Ca deficiency may limit the success of some zooplankton species in softwater lakes. These lakes may have a scattered distribution depending on local geology, or they may cover larger areas like the Canadian Shield lakes (Neary and Dillon 1988; Yan et al. 1989) and major parts of Scandinavia. In a national survey of 1,500 Norwegian lakes, the median Ca concentration was 0.025 mM Ca (Skjelkvåle et al. 1997). In another survey of 346 Norwegian lakes where acidified and high-altitude lakes were omitted, less than 25% of the lakes had Ca concentrations >0.13 mM, and more than half had Ca concentrations <0.063 mM Ca (Hessen et al. 1995b). In addition, reduction in the long-term inputs of anthropogenic SO_4^{2-} has led to a decrease in runoff sulfate that is matched by an equal decline in water Ca concentration (Likens et al. 1996). Because of the reduced mineral weathering rates at reduced sulfate deposition, the depletion of Ca will be long-lasting. Reduced water Ca concentrations

may thus add to the prevailing Ca deficiency in softwater localities.

The major sources of Ca for crustaceans are water and food, with ionic Ca in water being the most important source of Ca for zooplankton (Cowgill et al. 1986) as for crayfish (Hessen et al. 1991). Various species have a variable Ca content, ranging from 2.8 to 7.7% of dry weight in *Daphnia* spp. (Cowgill 1976; Havas 1985; Yan et al. 1989) to as low as 0.06% in copepods (Yan et al. 1989). Most of the Ca is probably associated with carbonate and phosphate minerals in the carapace (Stevenson 1985), and thus at least some proportion of this body-Ca must be regenerated after each molt.

It has been observed that the Ca content in *D. magna* is affected by the chemical composition of the medium (Cowgill et al. 1986), and this apparent adjustment to low Ca may also imply that some of the more heavily calcified cladocerans may suffer from Ca deficiency in softwater lakes. Recent studies have shown that low Ca concentrations affect both survival and reproduction in *Daphnia* spp. (Hamza et al. 1998; D. O. Hessen et al. in press). Low Ca concentrations may hence constrain the distribution of certain branchiopods, and this will also affect community structure. The latter is supported by Tessier and Horwitz (1990), who observed a transition from large crustaceans to small rotifers with decreasing water hardness, and related this to Ca deficiency in the crustaceans. Further, Hessen et al. (1995a) showed a clear connection between lake-specific Ca concentration and the occurrence of *Daphnia* spp.

Many crustaceans store a proportion of the Ca withdrawn from the old exoskeleton before ecdysis. The degree of Ca storage and storage site is, however, highly variable between different taxa (Greenaway 1985). Little information is available on Ca storage in zooplankton. However, it is suggested that freshwater ostracods, due to their small size, have no Ca storage mechanisms but rather depend on rapid Ca uptake (Turpen and Angell 1971).

The aim of this study was to evaluate the role of water Ca concentration on the Ca content and calcification of *D.*

magna. By using ^{45}Ca as a tracer, it was possible to assess the calcification on an individual basis. The relationship between individual Ca content and dry weight was also investigated. At last, the potential for Ca storage between molts and its possible interaction with Ca concentration was studied using the ^{45}Ca tracer.

Materials and methods—All the experiments were performed with females of a laboratory clone of *D. magna*, fed a diet of the green alga *Selenastrum capricornutum* reared on Guillard's WC medium (Guillard and Lorenzen 1972). To avoid Ca contamination from algal medium to the zooplankton culture and ensure similar food quality during the experiments, a dense culture of *Selenastrum* was washed twice with Ca-free medium and frozen in small batches at -20°C . The stock culture of *D. magna* was reared in the artificial Elendt M7 medium. All experiments were performed at constant temperature ($18 \pm 1^\circ\text{C}$) in a thermostatically controlled room at dim artificial room light. At these conditions, maturation is reached at about 10 d when sufficient food and Ca concentrations are provided. Intermolt periods of *D. magna* range from 2 d in the early juveniles to 4–5 d in adults, irrespective of medium Ca concentration (Hessen et al. in press).

The Elendt M7 medium was made by adding salts and vitamins to distilled water, according to the OECD guideline (OECD 1996). The medium had a Ca concentration of 2.0 mM Ca, and pH was adjusted to 7.8 ± 0.2 by addition of HCl or NaOH. The test media were made by adding less $\text{CaCl}_2 \cdot \text{H}_2\text{O}$, while all other elements were kept constant. pH was adjusted to 7.8 the same way as in the original medium. Manipulating the Ca concentrations of the Elendt M7 medium in the range 0.013–0.25 mM Ca only yielded minor differences in ionic strength ($50\text{--}55 \times 10^{-4}\text{ M}$) and conductivity ($221\text{--}273\ \mu\text{S cm}^{-1}$). Thus, effects caused by changes in ionic strength or osmoregulation were presumably small.

Ca content: Neonate *D. magna* were allowed to grow for 10 d in test media with nominal Ca concentrations of 0.013, 0.025, 0.13, and 0.25 mM Ca (measured Ca concentrations were identical). Each medium was represented by three beakers with 500 ml medium, each beaker containing 10 neonates. Neonates (0–24 h old) from the stock culture were rinsed in Ca-free medium before being transferred to the beakers. The animals were fed with a *Selenastrum* culture every third day, giving a final concentration of 0.033 mM C in the beakers. This food concentration was chosen to approach incipient limiting level (Lampert 1987). At the end of the experiment, some of the animals contained their first clutch of eggs, and these were included when the animal dry weight was measured.

The animals were rinsed in water from a milli-Q reagent water system (Millipore), placed on preweighed aluminum sheets and dried at 60°C for 24 h. The animals were cooled in a desiccator before dry weights were recorded to the nearest 0.1 μg with a Mettler ME 30 electrobalance. Each sample consisted of 10 animals. Digestion was performed in 20 ml 10% HNO_3 at 120°C for 30 min. The samples were filtered through a Millipore filter (0.45 μm , type HAWP) prewashed in 10% HNO_3 solution. Ca content was analyzed on a flame

atomic absorption spectrophotometer (Varian SpectrAA 10). A nitrous oxide/acetylene flame was used, and 200 μl LaCl_3 was added to each 20-ml sample to avoid chemical interference.

Calcification: In order to assess the calcification of *D. magna* on an individual basis, tracer experiments with ^{45}Ca were performed. Adult *D. magna* were reared individually until molting. After molting, they were rinsed in Ca-free medium and placed individually in beakers containing 50 ml medium labeled with a ^{45}Ca isotope (Amersham, CES 3, 200 $\text{mCi}\cdot\text{mmol}^{-1}\text{ Ca}$). The test media had nominal Ca concentrations of 0.013, 0.025, 0.13, 0.25, or 0.50 mM Ca (measured Ca concentrations were 0.012, 0.022, 0.12, 0.25, and 0.51 mM). The animals were fed with a *Selenastrum* culture, giving a final concentration of 0.011 mM C in the beakers. This food concentration was chosen to slightly exceed threshold food level (Lampert and Muck 1985), thus securing survival while not strongly encouraging reproduction. The production of eggs or neonates could constitute a problem in calculating adult calcification, as the specific Ca content of *D. magna* is higher in neonates than in adults (Porcella et al. 1969).

Twenty individuals were used for each Ca concentration, and five individuals were removed for scintillation counting each day for 4 d, i.e., the entire intermolt period. The individual lengths were measured from the top of the head to the base of the tail spine, and dry weights were calculated using the equation of Bottrell et al. (1976), assuming a fixed length–weight ratio. A test of this assumption has shown that *D. magna* had a constant length–weight ratio when reared in medium with 0.025, 0.13, and 0.25 mM Ca, but animals reared in medium with 0.013 mM Ca had a somewhat higher length–weight ratio (Hessen et al. in press). The linear regression should thus produce reliable data on Ca accumulation as % of dry weight for animals reared in medium with $\geq 0.025\text{ mM Ca}$. However, there might be an overestimation of dry weight and hence an underestimation of weight-specific Ca content in animals reared in the lowest Ca concentration (0.013 mM Ca), compared to those reared in 0.025 mM Ca.

The animals were rinsed in Ca-free medium and dried on a filter paper before being digested in 500 μl 10% HNO_3 at 120°C for 30 min. Fifteen milliliters scintillation cocktail (Ultima Gold, Packard) was added to 200 μl of the sample. Initial activity in the media, as well as the samples of digested animals were counted at the ^{14}C window settings in a 1500 Tri-carb Liquid Scintillation Counter. Due to lack of internal standards, activities were recorded as counts per minute (CPM). This would still allow for comparison among treatments. The counting efficiency was above 94% for all samples.

The ratio of the CPM in the animal to the actual amount of ^{45}Ca in the animal was assumed to be equal to the ratio of measured CPM in the medium to the actual amount of ^{45}Ca isotope in the medium [$^{45}\text{Ca}_m$]. Calcification was calculated as total postmolt Ca uptake:

$$[\text{Ca}_\Delta] = \frac{[^{45}\text{Ca}_a][\text{Ca}_m]}{[^{45}\text{Ca}_m]}$$

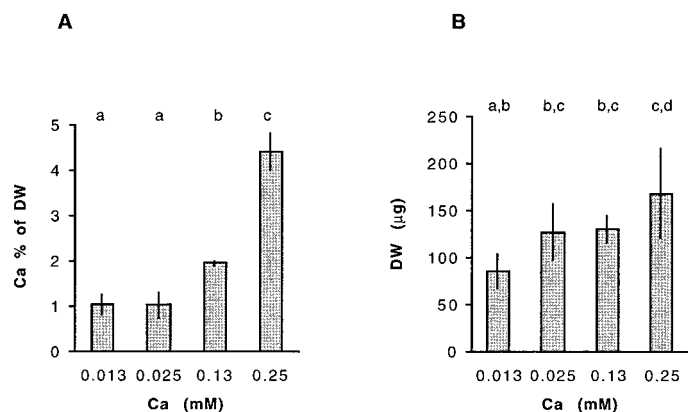


Fig. 1. (A) Mean Ca % of dry weight (\pm SD) and (B) mean individual dry weight (\pm SD) in *D. magna* incubated for 10 d in medium with different Ca concentrations (nominal values). $n = 3$ samples, each containing 10 animals for each concentration. Different letters above figure bars denote significant ($P < 0.05$) differences between treatments (Dunnnett's test).

where Ca_A = total Ca uptake per animal, $^{45}Ca_a$ = ^{45}Ca in the animal, Ca_m = total Ca concentration in the medium, and $^{45}Ca_m$ = ^{45}Ca in the medium.

When assessing the relationship between individual Ca content and dry weight, one regression line was fitted to the calcification data from animals reared in media with the highest Ca concentrations (0.13, 0.25, and 0.50 mM Ca) and another to the data from those reared in media with 0.013 and 0.025 mM Ca. This was done because animals reared in media with 0.013 and 0.025 mM Ca were considerably less calcified than those reared in media with higher Ca concentrations (Fig. 2). To investigate further the relationship between Ca content and animal size, regressions of individual Ca content and surface area were performed, realizing that Ca is mainly associated with the carapace. Surface area was calculated from dry weight by the regression: $SA = 2.54 + 0.05DW$ ($R^2 = 0.86$; slope \pm 95% confidence interval [CI] = 0.05 ± 0.005 ; $F = 294$; $P < 0.0001$; $n = 50$), where SA = surface area (mm^2) and DW = dry weight (μg). The relationship between surface area and dry weight was derived by P. Færøvig et al. (unpubl. data) using an ellipsoid model based on maximum animal width and length ($SA = \pi$ -width-length).

Ca storage: To study the Ca metabolism during molting, and the extent to which *Daphnia* spp. reclaim Ca before shedding the exuviae, adult *D. magna* were rinsed in Ca-free medium and transferred to beakers containing 50 ml medium immediately after molting. The media were labeled with a ^{45}Ca isotope as in the calcification experiment and the nominal Ca concentrations were 0.013, 0.025, 0.13, 0.25, and 2.0 mM Ca (measured Ca concentrations were 0.012, 0.022, 0.12, 0.25, and 2.13 mM). Seven individuals were used as replicates at each Ca concentration. The animals were fed with a *Selenastrum* culture, giving a final concentration of 0.011 mM C in the beakers, as in the calcification experiment. Immediately before molting, i.e., after 4 d, the animals were rinsed and transferred to 10-ml vials containing

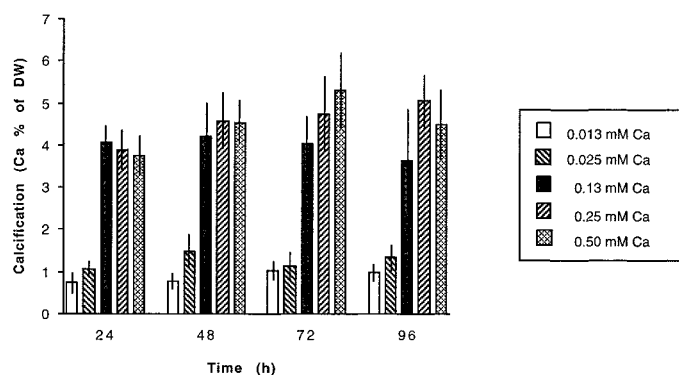


Fig. 2. Calcification (Ca % of dry weight \pm SD) in *D. magna* reared in medium with different Ca concentrations (nominal values). $n = 5$ for each concentration and time.

medium with corresponding concentrations of cold Ca, where they were allowed to complete molting. The animals and the shed exuviae were separated and rinsed in Ca-free medium before digestion. The ^{45}Ca activity was counted in samples containing digested animals, digested exuviae, and in the medium in which the animals completed molting. Digestion and scintillation counting were performed as in the calcification experiment.

Statistical analysis: All statistics were calculated with the JMP 3.2.2 computer program (SAS Institute Inc. 1997). In the Ca content and Ca storage experiments, one-way analyses of variance (ANOVA) were performed to estimate the effect of the Ca concentration in the rearing medium and the response variables could not be assumed, the rearing medium Ca concentration was used as an ordinal variable. Dunnnett's test and Tukey-Kramer HSD were performed to test for significant differences between groups. An analysis of covariance (ANCOVA) was performed in the calcification experiment. Ca concentration in the rearing medium and time after molting were used as ordinal variables in this analysis, while individual dry weight was used as a continuous variable. Linear regressions were used to estimate the relationship between individual Ca content and dry weight and surface area.

Results—Ca content: Mean specific Ca content (as % of dry weight) was more than four times higher in individuals reared in medium with 0.25 mM Ca than in individuals reared in medium with 0.013 mM Ca (Fig. 1A). A Dunnnett's test showed that the Ca concentration in the medium had a significant effect on the Ca content of the animals, except between individuals reared in medium with the two lowest Ca concentrations (0.013 and 0.025 mM). A one-way ANOVA testing the effect of medium Ca concentration on animal Ca content gave $F_{[3,7]} = 87.22$; $P < 0.0001$.

Individuals reared in medium with low Ca concentrations also tended to have a lower dry weight than those reared with higher Ca concentrations (Fig. 1B). However, this effect was statistically significant (Dunnnett's test) only when comparing animals reared in the lowest and the highest Ca con-

Table 1. ANCOVA model testing the significance of medium Ca concentration, time postmolt, and individual dry weight (DW) on the calcification of *D. magna* (as $\mu\text{g Ca}$). The model was $\text{Ca content} = a(\text{Ca}) + b(\text{Time}) + c(\text{DW}) + \epsilon$. There were no significant interaction effects. Included are effect tests of each variable. Parameter estimates and P -values of the categories of each variable are also presented. Hence, Ca (0.013–0.025 mM) is a comparison of the calcification of animals reared in 0.013 and 0.025 mM. Whole-model test: $F_{[8, 91]} = 95.78$; $P < 0.0001$; $\text{MS}_{\text{error}} = 0.3312$.

Variable	df	F	$P_{(\text{effect test})}$	Parameter estimate	$P_{(\text{category})}$
Ca (0.013–0.025 mM)	4	184.07	<0.0001	0.394	0.0333
Ca (0.025–0.13 mM)				2.669	<0.0001
Ca (0.13–0.25 mM)				0.415	0.0256
Ca (0.25–0.50 mM)				0.056	0.7591
Time (24–48 h)	3	4.85	0.0035	0.530	0.0016
Time (48–72 h)				–0.001	0.9971
Time (72–96 h)				–0.086	0.5986
DW (μg)	1	18.76	<0.0001	0.014	<0.0001

centrations (0.013 and 0.25 mM), probably because of the small sample size in this experiment. A one-way ANOVA testing the effect of medium Ca concentration on animal dry weight gave $F_{[3,7]} = 3.26$; $P = 0.0897$; $\text{MS}_{\text{error}} = 1,143.07$.

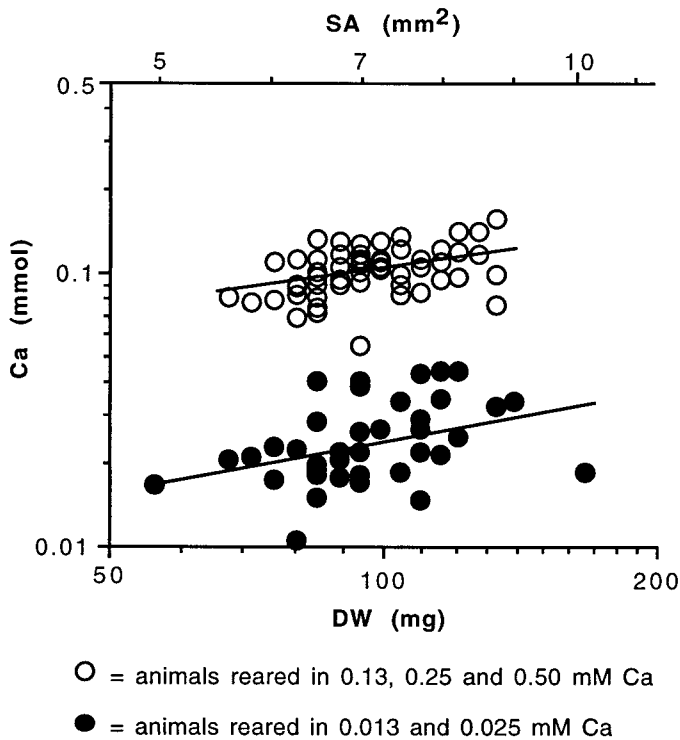


Fig. 3. Regressions of individual Ca content to dry weight (DW) and carapace surface area (SA) in *D. magna*. The regression line (slope \pm 95% CI) of Ca to DW was $\log(\text{Ca}) = -0.41 + 0.52 (\pm 0.29) \log(\text{DW})$ ($R^2 = 0.18$; $F = 12.75$; $P < 0.001$; $n = 60$; lack of fit: $F = 0.45$; $P = 0.935$) for animals reared in the highest Ca concentrations and $\log(\text{Ca}) = -1.25 + 0.62 (\pm 0.49) \log(\text{DW})$ ($R^2 = 0.15$; $F = 6.69$; $P = 0.014$; $n = 40$; lack of fit: $F = 0.45$; $P = 0.940$) for animals reared in the lowest Ca concentrations. The regression of Ca to SA was $\log(\text{Ca}) = -0.06 + 0.79 (\pm 0.45) \log(\text{SA})$ for animals reared in the highest Ca concentrations, and $\log(\text{Ca}) = -0.82 + 0.95 (\pm 0.76) \log(\text{SA})$ for animals reared in the lowest Ca concentrations. The statistics of the Ca to SA regressions were identical to the Ca to DW statistics.

Calcification: The Ca accumulation (as Ca % of dry weight) during the molting cycle was more than four times higher in individuals reared in medium with ≥ 0.13 mM Ca than in individuals reared in medium with 0.013 and 0.025 mM Ca (Fig. 2). An ANCOVA model showed that both medium Ca concentration, time postmolt, and individual dry weight significantly affected the calcification of *D. magna* (Table 1). However, the amount of accumulated Ca of animals reared in any of the Ca concentrations did not change significantly after 48 h postmolt (Fig. 2; Table 1), and apparently most of the calcification was completed shortly after molting.

The parameter estimates from the ANCOVA model showed that animals reared in media with lower Ca concentrations were significantly less calcified than the animals reared in medium with higher Ca concentrations (Table 1). This effect was evident at all Ca concentrations except at 0.50 mM Ca, where the animal calcification was not different from that of animals reared in medium with 0.25 mM Ca. The amount of postmolt Ca accumulation calculated in this experiment did not differ significantly from the Ca content measured in the former experiment (two-tailed t -test, $P > 0.12$). This indicates that *D. magna* stored only a minor amount of Ca during molting.

There was a significant positive correlation between calculated individual Ca content and dry weight (Fig. 3). In spite of a pronounced scatter, the slope of the regression for animals reared in media with 0.13, 0.25, and 0.50 mM Ca was significantly smaller than 1 (slope \pm 95%, CI = 0.52 ± 0.29). This indicates a negative allometric relationship where the total Ca content increased at a slower rate than individual dry weight, and thus the specific Ca content decreased with size. The slope of the regression for animals reared in media with 0.013 and 0.025 mM Ca showed the same trend but was not significantly smaller than 1. One reason for this could be an overestimation of the dry weight

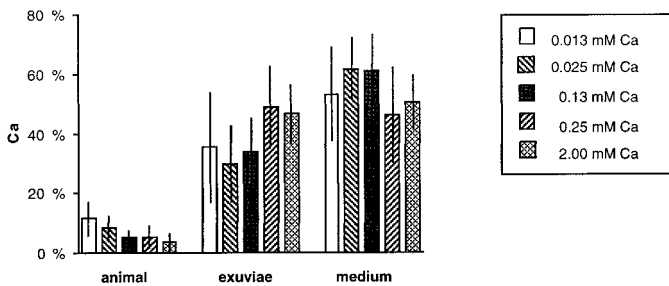


Fig. 4. The proportion (\pm SD) of ^{45}Ca isotope in shed exuviae, rearing medium, and animals after molting in *D. magna* reared in medium with different Ca concentrations (nominal values). $n = 7$ for each Ca concentration.

of animals reared in 0.013 mM Ca, as mentioned above, and hence a greater scatter in the regression in Fig. 3.

Because a linear transformation was used to convert dry weight to surface area, the statistics of Ca content to surface area was essentially identical to the statistics of Ca content to dry weight (subject to rounding error). However, the slopes of the Ca content to surface area regressions were not significantly different from 1 (Fig. 3).

Ca storage: Only 10% or less of total body Ca was stored in *D. magna* during molting. There was a tendency toward higher activities in the animals reared in media with low Ca concentrations than in those reared in high Ca concentrations, i.e., a higher proportion of Ca being reclaimed at low ambient Ca. A one-way ANOVA yielded a significant effect of Ca concentration in the medium during intermolt on the proportion of ^{45}Ca in the postmolt animals ($F_{[4,30]} = 5.11$; $P = 0.0029$). Additionally, a Tukey–Kramer HSD test showed significant differences in animal ^{45}Ca concentration between animals reared in medium with 0.013 mM Ca and animals reared in medium with ≥ 0.13 mM Ca. However, the variation within the replicates was considerable, partly because of differences in individual size, so the apparent differences between Ca allocation patterns should be judged with some caution.

There was no clear correlation between the Ca concentration in the medium during intermolt and the proportion of the ^{45}Ca isotope in the medium and the exuviae shed at the end of the experiment (Fig. 4). The leakage of ^{45}Ca from the animals to the medium during molting was considerable (46–62%), and this effect was consistent at all Ca concentrations. The somewhat imprecise term leakage is used because the present techniques were not able to distinguish between excretion and passive diffusion.

Discussion—Ca content: We observed a significant reduction of the Ca content in *D. magna* reared in medium with low Ca concentrations. In addition, the dry weight of individuals reared in medium with 0.013 mM Ca was only half the weight of individuals reared in medium with 0.25 mM Ca. Lynch et al. (1986) found that the biomass of the carapace constitutes only 5–10% of the total biomass in *Daphnia pulex*. Assuming a similar relationship in *D. magna*, the decreased dry weight at lower ambient Ca concentrations must

reflect both a decrease in carapace weight and a smaller animal size. The latter is supported by Hessen et al. (in press) who showed that the negative effect of low Ca concentrations on growth and weight gain in *D. magna* was most pronounced at the juvenile stages and at high food concentrations.

Porcella et al. (1969) reported that the specific Ca content of *D. magna* (as % Ca of DW) decrease as the animals grow larger, and this is supported by the present study (Fig. 3). Thus, the observed decrease in specific Ca content of the smaller individuals reared at low Ca media probably reflects a thinner and less calcified carapace, because the Ca in crustaceans is mainly associated with the carapace (Stevenson 1985). In the wild a less calcified carapace may, in turn, lead to an increasing vulnerability to invertebrate predators. It is well documented that crayfish are more susceptible to predation by fish during early postmolt when the carapace is still soft (Stein 1977).

This study supports the conclusion of previous studies that Ca content in *D. magna* is determined by the chemical composition of the water (Cowgill et al. 1986). *D. magna* reared at 0.063 mM Ca are reported to have a Ca content in the range 2.8–5.4% of dry weight (Havas 1985), increasing to 7.7% of dry weight at 0.11 mM Ca (Cowgill 1976), which agrees well with our results. Studies of freshwater decapods also confirm a correlation between water Ca and animal Ca content (France 1987; Jussila 1997).

Calcification: *D. magna* reared in medium with < 0.13 mM Ca were significantly less calcified than those reared at higher Ca concentrations. A saturation level at which the ambient Ca concentration was sufficient to allow *D. magna* a saturated calcification of the the exoskeleton was apparently reached at Ca concentrations between 0.13 and 0.25 mM, as also indicated by Marshall et al. (1964).

Most of the calcification in *D. magna* was completed during the first 48 h after molting, in line with Porcella et al. (1969). The individuals reared in media with low Ca concentrations (0.013 and 0.025 mM) were unable to compensate for the lower Ca^{2+} uptake rate in early postmolt by prolonging the period of Ca uptake. Consequently, these individuals completed the molting cycle with a considerably lower level of Ca. This is in support of Wheatly and Gannon (1993), who examined the effect of external electrolytes on postmolt calcification in the freshwater crayfish *Procambarus clarkii*. After being reared in Ca-free medium, the crayfish commenced Ca uptake when Ca was reintroduced 5 days postmolt, but only at rates insufficient to complete calcification of the exoskeleton. Wheatly and Gannon (1993) thus suggested that there is a restricted time window for calcification during the initial postmolt period and that this cannot be compensated for by an elongated time of calcification.

The present study suggested that the carapace-specific Ca content decrease with increasing individual size, as also observed by Porcella et al. (1969). The main reason for this is probably that the surface:volume ratio decrease with increasing size. However, Lynch et al. (1986) reported a strong positive allometry of molt weight:total body weight in *D. pulex* (slope = 1.47), indicating that increasing size was accompanied by a relative increase in carapace dry weight. The

present study thus suggests that the carapace calcium content per surface area remains constant, even if the dry weight of the carapace increases with increasing animal size. Corresponding observations are made in several studies on calcification in freshwater crayfish, where an increase in exoskeleton carapace density with age/growth was noted while calcium levels remained relatively constant (Greenaway 1974; Huner and Lindqvist 1985). However, other studies have presented evidence for an increase in mineralization with size (Mills and Lake 1976; Wheatly and Ayers 1995).

Ca storage: The leakage of Ca from *D. magna* to the medium during molting constituted about 50% of total body Ca. About 40% of total body Ca was lost with the shed exuviae, while only 10% or less was reclaimed by the animals. This is in agreement with our previous observation that the intermolt Ca content was not significantly different from the amount of postmolt Ca accumulation. This sloppiness in the Ca metabolism supports Porcella et al. (1969), who found that at least 85% of the total Ca in *D. magna* was lost during molting.

Freshwater crustaceans may store a variable proportion of the Ca withdrawn from the exoskeleton before ecdysis, and the storage site is often some part of the digestive tract. Decapods may store some Ca in the hepatopancreas or in gastroliths (Greenaway 1985; Wheatly and Ayers 1995), while amphipods may store calcareous concretions in the gut and posterior ceca (Neville 1975). As the major problem in Ca storage is simply one of space (Greenaway 1985), Turpen and Angell (1971) suggested that freshwater ostracods have no Ca storage mechanisms but rather have evolved cellular mechanisms for the rapid uptake and deposition of Ca. Considering the small size of the digestive tract, Turpen and Angell (1971) doubted that significant amounts of Ca could be stored in ostracods, and the same argument would hold for *Daphnia* spp.

Turpen and Angell (1971) suggested that a more efficient reclaiming of Ca could occur when ambient Ca is insufficient. The present study only gives limited support to this suggestion. Although a somewhat larger percentage of total Ca was stored at very low ambient Ca, this was not sufficient to ensure a complete calcification of the carapace. Because low Ca apparently may pose constraints on growth and calcification in *D. magna*, it is somewhat puzzling that most of the body Ca simply is lost, even at very low ambient Ca concentrations. The only crustaceans who reclaim a major part of total body Ca during molting are terrestrial species, being even more limited in ambient Ca availability. These species have evolved completely different strategies of Ca reclaiming, like eating their exuviae or the special biphasic molt in isopods.

This study clearly suggests a potential for Ca limitation in *D. magna*. The high Ca demands of this species should indicate a strong competitive drawback in Ca-poor waters, either directly via growth limitation or indirectly via increased predation risks. Setting 0.013 mM Ca as a lower threshold, Ca limitation would exclude this species from more than 25% of Norwegian lakes. Apparently saturation is reached at ambient Ca concentrations >0.13 mM, comprising less than 15% of Norwegian lakes. *D. magna* is typ-

ically a coastal rock pool form in Scandinavia and may thus have adjusted to high Ca levels. The high Ca content seems to be a generic property of *Daphnia* spp., however (Cowgill 1976; Havas 1985; Yan et al. 1989), and further studies should pursue the potential role of Ca limitation on more typical lake forms of this genus.

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Major flux of terrigenous dissolved organic matter through the Arctic Ocean

Abstract—High-latitude rivers supply the Arctic Ocean with a disproportionately large share of global riverine discharge and terrigenous dissolved organic matter (DOM). We used the abundance of lignin, a macromolecule unique to vascular plants, and stable carbon isotope ratios ($\delta^{13}\text{C}$) to trace the high molecular weight fraction of terrigenous DOM in major water masses of the Arctic Ocean. Lignin oxidation products in ultrafiltered DOM (UDOM; >1,000 Da) from Arctic rivers were depleted in syringyl relative to vanillyl phenols ($S/V = 0.3–0.5$) compared to UDOM in temperate and tropical rivers ($S/V = 0.5–1.2$), indicating that gymnosperm vegetation is a major source of terrigenous UDOM to the Arctic Ocean. High concentrations of lignin oxidation products (83–320 ng L⁻¹) and a depletion of ^{13}C ($\delta^{13}\text{C} = -23.0$ to -21.9) in UDOM throughout the surface Arctic Ocean indicate that terrigenous UDOM accounts for a much greater fraction of the UDOM in the surface Arctic (5–33%) than in the Pacific and Atlantic oceans (0.7–2.4%). In contrast, UDOM in deep water from the Arctic Ocean as well as waters from throughout the Greenland Gyre had relatively low concentrations of lignin oxidation products (24–45 ng L⁻¹) and was enriched in ^{13}C ($\delta^{13}\text{C} = -21.0$ to -20.8). Terrigenous UDOM has a relatively short

residence ($\sim 1–6$ yr) in surface polar waters prior to export to the north Atlantic Ocean. Assuming that the bulk of Arctic-derived DOM is compositionally similar to the UDOM fraction, we estimate that 12–41% of terrigenous DOM (2.9–10.3 Tg C yr⁻¹) discharged by rivers to the Arctic Ocean is exported to the North Atlantic via surface waters of the East Greenland Current. It appears very little terrigenous DOM from the Arctic is incorporated into North Atlantic Deep Water and distributed globally via deep thermohaline circulation.

The Arctic Ocean receives $\sim 10\%$ of the freshwater and dissolved organic matter (DOM) supplied globally by rivers (Aagaard and Carmack 1989; Gordeev et al. 1996; Macdonald et al. 1998; Anderson et al. 1998), yet it represents only 1% of the global ocean volume (Menard and Smith 1966). Thus, the flux of terrigenous DOM to the Arctic Ocean is much greater on a volume basis than corresponding fluxes to the Atlantic, Indian, and Pacific oceans. Furthermore, the supply of terrigenous DOM to the Arctic Ocean may be