Uptake, distribution, and bioaccumulation of copper in the freshwater mussel *Anodonta anatina*

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Copper (Cu) is present in aquatic ecosystems in dissolved form, associated with suspended food particles, and as insoluble sulfide in the sediment. Due to its wide technical use and its presence in municipal and industrial waste waters, levels in rivers and sediments may be elevated. The aims of this study are to assess the relative importance of copper uptake by a typical freshwater mussel (*Anodonta anatina*), its distribution, accumulation among the mussel organs, and elimination. Using the stable isotope $^{63}$Cu as tracer, the mussels are exposed via the water (0.3 mol L\textsuperscript{-1} Cu) or via the food (1.5 mg L\textsuperscript{-1} Cu-loaded algae, equivalent to 0.06 mol L\textsuperscript{-1} Cu) for 24 days. The levels of exogenous and total Cu increase in all body compartments. Relative increases are highest in the digestive gland, followed by mantle and gills. Upon depuration for 12 days, $^{63}$Cu is quickly but not completely eliminated.

**Keywords**: copper; *Anodonta anatina*; bioaccumulation; elimination; water; freshwater bivalve

**Introduction**

Metals of technical importance are found in the environment at increasing concentrations resulting from mining and metallurgic activities, due to their wide technical use, or due to emissions from corrosion and from fossil fuel combustion. Therefore, many metals show the tendency to increase over natural levels and to accumulate in soil and aquatic compartments, especially in river sediments. Copper (Cu) is one of them; due to its use as fungicide, in the building sector as roofing material, for overland high-voltage power lines, and many other electrotechnical applications it may be present in municipal and industrial waste waters. Apparently, Cu is also spread in the environment as nonpoint source pollutant and can attain elevated levels in declining freshwater pearl mussels (Frank and Gerstmann 2007), sedentary animals living at the interface of free-flowing water and sediments of mountain streams. In water, copper can exist in dissolved form or associated with dissolved organic carbon and with suspended food particles (Vinot and Pihan 2005). In sediments it may be present as insoluble sulfide or dissolved in the interstitial water (Besser, Ingersoll, and Giesty 1996). Copper is essential to mussels up to 10 $\mu$mol kg\textsuperscript{-1} (0.6 mg kg\textsuperscript{-1}) body weight (Julshamn et al. 2001), as part of the oxygen-binding site in
hemocyanin (Birge and Black 1979) and as cofactor of the prosthetic groups of enzymes such as of cytochrome-c oxidase, tyrosinase, dopamine β-hydroxylase, alcohol dehydrogenase, prolyl and lysyl oxidase, or others involved in growth regulation and development (Amiard-Triquet et al. 2006; Company et al. 2008). At higher concentrations, Cu is toxic to mussels, resulting in altered calcium (Ca) homeostasis of blood cells (Viarengo et al. 1994); the 96-hour LC50 for mollusks ranges between 6 and 30 μmol L⁻¹ (0.4–2 mg L⁻¹) (Crompton 1998).

Copper can be taken up by freshwater mussels with the water or the food. The route of uptake influences the distribution of the metal in the various organs, determines the dynamics of Cu-bioaccumulation and elimination, and has consequences on the pathophysiology of copper in the mussels (Croteau and Luoma 2005). In this work, duck mussels (*Anodonta anatina*) are used as model species to study the toxicological relevance of copper uptake via both pathways. The stable isotope ⁶³Cu is used as tracer to follow its distribution within the mussel and its elimination upon depuration.

**Materials and methods**

**Algal food preparation**

Algae (*Parachlorella kessleri*) are used as food for the mussels and, when grown at a ⁶³Cu-concentration of 5.9 μmol L⁻¹ (Nugroho and Frank 2010), for one experimental group as Cu-exposure source. Algae are grown in modified K-medium (Kuhl and Lorenzen 1964) for 7 days to produce normal or copper-loaded algae. Freeze-dried normal and copper-loaded algae contain 0.01 mmol kg⁻¹ Cu (0.6 mg kg⁻¹ Cu) and 40 mmol kg⁻¹ Cu (2.4 mg kg⁻¹ Cu) dry weight (dw).

**Isotopic Cu stock solution preparation and labware**

A ⁶³Cu stock solution (3.1 mmol L⁻¹, equivalent to 200 mg L⁻¹) is prepared by dissolving 25 mg isotopically enriched (99%) ⁶³Cu oxide (Euriso-top, Saarbrücken, Germany) in 1 mL suprapur HNO₃ (69%, Carl Roth, Karlsruhe, Germany) in a 100 mL glass beaker; 85 mL bidistilled water are added, and the pH of the solution is adjusted to 7.0 with aqueous ammonia (25%, VWR, Darmstadt, Germany). The solution is transferred to a 100 mL polypropylene (PP) volumetric flask which is filled to the mark with bidistilled water. Glassware and plastic equipments used for analytical purposes are rinsed twice with half-concentrated HNO₃ (65%; Sigma-Aldrich, Munich, Germany), and deionized and bidistilled water.

**Organisms**

About 70 duck mussels (*A. anatina*) (ZOO-Erlebnis Online Shop, Grossefehn, Germany) with shell lengths of 10–12 cm and weights of 100–200 g are brought to the laboratory in pond water. The mussels are brushed with dilute KMnO₄ solution (0.1 mg L⁻¹), rinsed with tap water, and placed in 38 L aerated tap water in 45-L glass aquaria at dim light for 7 days. During this period they are not fed; every day, half of the water is exchanged. Then the mussels are marked, weighed, and the shell lengths are measured. They are fed with freeze-dried Cu-free algae, 1.0 mg L⁻¹ per day, and acclimatized for further 7 days to laboratory conditions at a temperature of 17 ± 1°C with a photoperiod of 12 h light per day, a photon flux of 13–19 μmol m⁻² s⁻¹, in 38 L artificial pond water (APW) at pH 7.0 ± 0.3
(Ngo, Gerstmann, and Frank 2011) in 45-L glass aquaria covered with transparent polypropylene lids. The aquaria are equipped with inner bio-filters and stainless steel aeration tubes. Eight kilograms glass beads are used as substrate. Two-third of the water is exchanged every two days; a complete change is conducted on every sixth day.

**Experimental design**

Of these mussels, 63 are selected to match in size and divided into three groups consisting of 21 mussels each. They are placed in three 45-L aquaria containing 38 L artificial pond water (APW). Two-third of the water is exchanged every second day; a complete change is conducted on every sixth days. A control group (1) is kept in APW. Another group (2) is exposed to 0.3 μmol L⁻¹ (20 μg L⁻¹) ⁶³Cu in the water using the ⁶³Cu stock solution; after each water change, the concentration is re-adjusted by adding appropriate volumes of the stock solution. A third group (3) receives daily 1.5 mg L⁻¹ freeze-dried ⁶³Cu-loaded algae for 24 days, equivalent to a nominal copper concentration of 0.06 μmol L⁻¹ (3.6 μg L⁻¹).

The mussels in the control and the exposure groups are fed with algae in amounts adjusted to their actual number. For 18 mussels, 1.5 mg L⁻¹ of freeze-dried Cu-free (groups 1 and 2) or ⁶³Cu-loaded algae (group 3) are given per day. When the number of mussels is less than 18, 1.0 mg L⁻¹ of freeze-dried Cu-free or ⁶³Cu-loaded algae are given daily (Ngo, Gerstmann, and Frank 2011) corresponding to a nominal concentration of 0.04 μmol L⁻¹ (group 3). On day 24, the six mussels remaining in each group are transferred to APW-filled aquaria for 12 days of depuration, fed with 1.0 mg L⁻¹ of freeze-dried Cu-free algae per day.

Actual Cu concentrations in the water including the suspended algae in each group are determined every second day. On the control group, Cu concentrations in the APW during experiment are below detection limit. For the experiment involving Cu exposure via the water, after exchange of water the concentration is adjusted to 0.32 ± 0.006 μmol L⁻¹, which falls to 0.03 ± 0.01 μmol L⁻¹ within the next 2 days. By the food pathway (group 3), the nominal Cu concentration in the beginning and after each water exchange is 0.07 ± 0.01 μmol L⁻¹, falling to below detection limit within the next 2 days.

For sampling, three mussels of each group are taken for analysis at days 0, 6, 12, 18, and 24 (exposure), and at days 30 and 36 (depuration). The mussels are anaesthetized with an aqueous 2-phenoxyethanol solution (4 mL L⁻¹) for 30 min. Hemolymph (HML) and extrapallial fluid (EPF) are withdrawn using 5 mL syringes with 0.55 × 25 mm needles (B. Braun, Melsungen, Germany), transferred into 2-mL microtubes, and kept at −80°C. The mussels are dissected on ice into gills, mantle, kidney, digestive gland, foot, adductors, and intestines; the remainder is collected in a combined sample (GHL), i.e., gonads, heart, and labial palps. The tissues are washed twice with bidistilled water, dried using filter paper, placed in 15 mL polypropylene (PP) tubes of known weights, weighed to obtain the wet weights (ww), and lyophilized. After lyophilization, the tubes are weighed again for dry weights (dw). Tissue fractions and body fluids of the nine mussels taken at day 0 are used to calculate the respective percentages relative to the total weight of soft body (twsb).

**Metal analyses**

Each lyophilized tissue fraction of about 10–100 mg is placed in a 55 mL borosilicate glass tubes. 5 mL of a mixture (4 + 1) of suprapure concentrated HNO₃
(65%, Merck, Darmstadt, Germany) and suprapure concentrated HCl (30%, Merck, Darmstadt, Germany) are added to each tube. The tubes are kept in an oven at 40°C for 1 h and at 95°C for 3 h. The digested samples are diluted with bidistilled water to 10 mL and filtered through 0.45 μm cellulose syringe filters (Carl Roth, Karlruhe, Germany). For the determination of Cu in HML and EPF, 0.4–1 mL of each are acidified with 0.5 mL suprapure concentrated HNO3 in PP tubes, diluted to 10 mL with bidistilled water, and filtered through 0.45 μm cellulose syringe filters. Total Cu and its isotopes 62Cu and 65Cu are determined by inductively-coupled plasma mass spectrometry (ICP-MS, Agilent 7500ce, Cetac ASX-510, Agilent Technologies, Waldbronn, Germany). The detection limits for total Cu is 0.02 μmol L−1 for isotopic Cu 0.01 μmol L−1.

Total copper in each tissue fraction is calculated in μmol kg−1 ww by multiplying the analytical data with the ratio of ww versus dw. The concentration of exogenous copper C_{63Cu} is calculated as C_{63Cu} = 2.34 × C_{65Cu}, the concentration of endogenous copper as 3.33 × C_{65Cu}, considering the natural relative abundances of 69% 63Cu and 31% 65Cu. For body fluids, the Cu concentrations are given in μmol L−1. Total and exogenous Cu-pools in the tissue fractions and body fluids are calculated in mmol kg−1 twsb by multiplying the concentration data with the weight fraction of the respective organ or body fluid.

**Statistical data analyses**

Data are transformed to log units before statistical analysis for homogeneity of variance and normality. The data for total Cu are statistically evaluated by two-way analysis of variance (ANOVA) considering exposure time and Cu exposure pathways as independent variables; if significant differences are found, those between exposure times are tested by the Dunnett multiple comparison tests, between exposure pathways and controls using the Duncan multiple comparison tests. To assess the differences in exogenous Cu between exposure pathways, the independent t-test is performed.

**Results**

Exposure of *A. anatina* to Cu via the water results in rapid increases (Figure 1) of the concentrations of total Cu (solid lines) in the hemolymph (HML) and the extrapallial fluid (EPF) within the first 12 days, followed by slower increases until concentrations of 0.38 μmol L−1 are reached at day 24, about the 2.5-fold of control level. From the food, increases are more moderate, reaching about 0.25 μmol L−1, the 1.7-fold of controls. In respect to exogenous Cu, the concentrations in both body fluids (dotted lines) increase similarly upon exposure via the water and the food within the first 6 days although the nominal concentration per liter water volume in food is considerably lower. Later on, exposure via the water entails faster uptake, especially in the HML between days 6–12 to reach 0.14 μmol L−1, continuing until 0.17 μmol L−1 at day 24. Overall, increases during the first days are faster for the EPF than for the HML.

Within the 12 days of depuration, total Cu concentrations decline rapidly in EPF and in HML, in HML of animals having received the metal by the water pathway to about 50% over control; when having been exposed via the food, the Cu concentrations decline almost fully back to control values. For exogenous Cu, the concentrations in the HML and EPF of water- and food-exposed animals decline in similar relative rates. At the end of the depuration, the fraction of exogenous Cu, i.e., the excess of 63Cu over the natural
The abundance of this isotope, represents between 10% (food pathway) and up to 30% (water pathway) of total copper.

In the organ and tissue fractions (Figures 2 and 3), concentrations of endogenous Cu at day 0 are highest in the kidney and the digestive gland (63 and 58 μmol kg⁻¹ ww). In the other organs, initial Cu levels are much lower, i.e., in mantle, intestines (both 15 μmol kg⁻¹ ww), gills, and foot (both 12 μmol kg⁻¹ ww). The mixed fraction of the gonads, heart, and labial palps (GHL) (20 μmol kg⁻¹ ww) shows a fairly high initial copper level although nothing can be said about the distribution between the tissues contained in it. The adductors have the lowest concentration (7 μmol kg⁻¹ ww), but this is still much higher than in HML and EPF (Figure 1, 0.17 μmol L⁻¹).

The development of the total copper concentrations is quite diverse for the various tissues/organisms over time (Figures 2 and 3; solid lines) in relative and absolute terms. Upon uptake via the water, strongest relative increases are seen for the gills, the mantle, and the digestive gland, especially within the first 6 days. When ⁶³Cu is administered via the food, an almost equal increase of ⁶³Cu as via water is found for the digestive gland, although its nominal initial concentration is only a fifth of the concentration in the water in dissolved form. For other organs, uptake from food leads to moderate rise in the mantle, kidney, intestines, and GHL, almost none in the gills, adductors, and foot. In the digestive gland, highest concentrations, i.e., 120–140 μmol kg⁻¹, are reached within 24 days irrespective of exposure pathway. For other organs, exposure via water results in peak concentrations in the gills of 75 μmol kg⁻¹ (6.5–fold relative to control), 70 μmol kg⁻¹ in the mantle (4.2-fold), and 70 μmol kg⁻¹ in the mixed fraction GHL (3.5-fold); moderate to low relative increases are seen in the foot, intestines, adductors, and kidney (2.8-, 1.7-, 1.6-, and 1.4-fold). Upon depuration, Cu concentrations fall immediately and strongly in most
organs, except for the mantle and the intestines; for these even further increases are observed within the first 6 days of depuration.

In respect to exogenous $^{63}\text{Cu}$ (Figures 2 and 3; dotted lines), exposure to $^{63}\text{Cu}$ via water leads to rapid increases in the gills, mantle, digestive gland, and GHL within the first 6 days. In some organs, i.e., digestive gland, gills, and mantle, the increases continue until day 24 to reach a maxima of about 50 μmol kg$^{-1}$ ww. Exogenous copper in the kidney,
foot, intestines, and GHL shows maximum concentrations at day 12, followed by declines until the end of exposure. Via the food, exogenous $^{63}$Cu initially increases in the digestive gland as fast as via the water, followed by slight further increase to reach a maximum of 20 $\mu$mol kg$^{-1}$ ww on day 24. In the gills, mantle, adductors, and foot, after slight increases during the first 12 days of exposure, exogenous Cu remains relatively unchanged until the end of the experiment.

During depuration, in the gills, mantle, and digestive gland, the levels of exogenous copper drops within the first 6 days by 85, 70, and 60%. For animals having received the metal via food, similar patterns of decrease are observed for the kidney and GHL, only the levels being lower, i.e. about a third.

The isotope ratios of $^{63}$Cu/$^{65}$Cu and its deviation from the natural ratio (2.33) are also monitored (Figure 4). Complementary to Figures 2 and 3, this allows to follow the movement of exogenous Cu within the body. When $^{63}$Cu is administered via water, the relative abundance of $^{63}$Cu in the body fluids HML and EPF rise up to 4.0 at day 12, then remain constant. Upon depuration, the ratio declines to about 3.0. In the kidney, GHL, foot, and intestines, peaks of $^{63}$Cu are reached at day 12, while in the adductors, digestive gland, mantle, and gills, maximum isotope ratios are found at the end of exposure at day 24. During depuration, the relative abundance of $^{63}$Cu in all organs declines, but not

Figure 3. Concentrations of total (solid lines; ⋆ = via water, ■ = via food; ▲ = control) and exogenous (dotted lines; ◆ = via water, □ = via food) Cu in the GHL (gonads, heart, and labial palps), intestines, foot, and adductors of A. anatina during Cu exposure via water and food and during depuration. Significant differences in comparison to control within each group are indicated by *. The same letters indicate that differences of Cu concentrations are not significant among groups at each time sampling (day (d)) while the different letter indicate $P<0.05$. Significant differences between concentrations of exogenous Cu via food or water are indicated by +. Total and exogenous Cu are calculated by multiplication of the analytical data with the ratio of dry weight versus wet weight.
totally back to the natural ratio remaining about 25–45% higher than before exposure. When $^{63}\text{Cu}$ is administered via food, the increase in the isotope ratio is pronounced for the digestive gland, while all the organs show only small increases.

Calculating the Cu-pools in the body compartments gives interesting insights (Figure 5). Although HML and EPF together constitute about 70% (33 ± 5% and 37 ± 4%) of the total soft body volume (Figure 5, A), both are insignificant as Cu-pools. The mantle, the gills, and the intestines are the largest solid organs; together they represent about 18% (6.4 ± 0.5, 5.9 ± 0.6, and 5.4 ± 0.8%) twb. Smaller body fractions are the adductors (2.9 ± 0.2% twb), the digestive gland (2.8 ± 0.3% twb), the foot (2.2 ± 0.4% twb), the mixed fraction GHL (3.9 ± 0.6% twb), and the kidney (0.5 ± 0.06% twb). In the beginning (Figure 5, B), the total pool of Cu (endogenous Cu) is about 6 μmol kg$^{-1}$ twb, the largest being in the digestive gland, followed by the mantle, gills, intestines, and GHL (Figure 5, B). Upon exposure via water (W), the total Cu-pools increases, continuing until day 24 to reach a maximum of 25 μmol kg$^{-1}$ twb, i.e., the four-fold of the initial pool size; uptake via the food (F) entails a total Cu-pool of only 9 μmol kg$^{-1}$ twb at day 24, i.e., slightly less than double the control, the largest pool being in the digestive gland. The exogenous $^{63}\text{Cu}$-pool increases in parallel to total Cu-pool upon exposure via the water, reaching a maximum of about 12 μmol kg$^{-1}$ twb at day 24. For the food pathway, it increases only slightly, the maximum level being at about 1.5 μmol kg$^{-1}$ twb (Figure 5, C). During the 12 days of depuration, all the pools are rapidly emptied, particularly the gills. The mantle and the digestive gland retain the Cu-pools relatively long (as also reflected in Figures 2 and 3), in the latter most tenaciously. Similar patterns are found for exogenous Cu.

**Discussion**

The experiments show that $^{63}\text{Cu}$ is highly available to *A. anatina* (Figures 2 and 3, a and b), both in water-dissolved form or from $^{63}\text{Cu}$-loaded algae. Calculation of Cu speciation
in artificial pond water (APW) at a pH of 7.0 and at 17°C shows that the metal is completely in the free Cu$^{2+}$ ionic form, ready for uptake (Gustaffsson 2010). By the food pathway, the low nominal Cu concentration in the APW may be the main factor responsible for the low Cu accumulation in the mussel in absolute terms, but in relative terms it is obviously even more efficient.

During the 24 days of water-borne Cu exposure, exogenous $^{63}$Cu levels in the organs increase differently, strongest in the gills to represent about 70% of total Cu (Figures 2 and 3, a and b). There is evidence of mobilization and re-distribution of endogenous Cu among the organs derived from the time pattern of the $^{63}$Cu/$^{65}$Cu isotope ratio (Figure 4). The ratios are highest in the gills, mantle, and digestive gland (both pathways) at the sixth day of exposure, showing that the exogenous $^{63}$Cu is initially taken up into these organs. The peaks of highest ratios at day 12 in the kidney, GHL, foot, and intestines indicate that these organs first receive fairly high amounts of exogenous Cu but – as the exposure continues – become recipients of endogenous copper mobilized from the other organs, presumably mobilized by exogenous $^{63}$Cu. Later, exogenous and mobilized endogenous Cu is mainly stored in the digestive gland, gills, adductors, and mantle, the latter serving as transient recipient even beyond the exposure phase. The observation of copper being particularly strongly retained in the mantle is noteworthy as it is one of the most important organs for regulating the calcium household and for building the protective shell of the bivalve (Lopes-Lima et al. 2008).

In the body fluids, the isotope ratio remains relatively constant at about 4.0 during days 12–24, reflecting the roles of HML and EPF as transitory exchange and transport compartments, being small as pools (Figure 5). Upon depuration the isotope ratios tend to fall strongly, indicating that a large fraction of exogenous $^{63}$Cu remains in a relatively easily exchangeable form while the endogenous Cu is more tenaciously retained.
Nevertheless, at the end of the depuration, the relative abundance of $^{63}$Cu taken up via the water pathway is between 25% and 45% higher than in the beginning in various body compartments, indicating that about a third of the functional Cu pool has been exchanged for exogenous $^{63}$Cu.

Distribution of Cu in the mussel’s body allows to assess the relative importance of the various copper pools (Figure 6). From the water it is mainly compartmentalized into the mantle (30%), the gills (24%), and the digestive gland (22%), altogether three quarters of the total Cu-pool. The former two organs have large surface areas and interact directly with the water coming into mantle cavity during filtration (Marigómez et al. 2002); the digestive gland is the major receiving organ for the hemolymph pathway. The mantle has a high secretor epithelium lined with acid mucopolysaccharides for digestion of trapped small particles (Machado 2011). By the food pathway, the digestive gland and the intestines are the major Cu-recipients. In addition, the former organ secretes high amount of digestive mucus to facilitate Cu storage (Machado 2011). High Cu levels in GHL suggest a role of the heart as ion recipient and its close anatomical relation to the intestines and the kidney (Gosling 2003; Machado 2011).
In relation to the organ pools of *A. anatina*, the size of the respective volumes is not directly related to Cu burden (Figure 5, A, B, and C). Binding to specific compounds and compartmentalization within the organs, and physiological and metabolic functions of the organs may play some roles (Otchere 2003). In any case, the organs which serve as the primary sites for uptake, i.e., gills, mantle, and digestive gland, tend to concentrate the copper.

During depuration, Cu is eliminated fairly fast from the body (Figures 2 and 3, a and b), due to the large differences in gradient Cu concentration between the mussel and APW. Han et al. (1993) reported that the initial rapid elimination can be caused by desorption of loosely bound, unassimilated copper, whereas slower elimination reflects the loss from pools (endogenous Cu) where copper is more tightly bound to tissue components. Rapid elimination is also observed in the gills and digestive gland of the marine clam *R. decussatus* within the first 10 days of depuration (Serafim and Bebianno 2009). In respect to Cu elimination from the digestive gland, the level in *A. anatina* drops to about 60% over control within 6 days (Figures 2 and 3, a). A similar pattern is observed in the marine mussels *Mytilus galloprovincialis* exposed to Cu at 0.63 μmol L⁻¹ (40 μg L⁻¹) via water for 3 days (Viarengo et al. 1981). This confirms that the digestive gland is the main organ for metal elimination in bivalves (Marigómez et al. 2002). According to Marigómez et al. (2002), the release of metals from mussel body can occur via the digestive tract as a component of feces or via the kidney together with excretory concretions as a component of urine (Figure 6).

Copper accumulation in *A. anatina* during exposure via water or food represents two different processes, i.e., bioconcentration (water) and biomagnification (food). Calculation of the bioconcentration and biomagnification levels allows to assess the relative importance of exposure via water or food. Bioconcentration can be expressed as enrichment factor (EF), i.e., the ratio of the concentration of exogenous Cu kg⁻¹ twsb (Figure 5, C) to the concentration in the water. Biomagnification is normally assessed as transfer factor (TF). The enrichment factor in the mussel at the end of the exposure (day 24) is about 43, by the food pathway a TF of 25 is reached (Cu concentration in the APW-added algal food is equivalent to 0.06 μmol L⁻¹, exogenous Cu-pools in the mussel = 1.5 μmol kg⁻¹ twsb; Figure 5, C). Thus, exposure via water is more effective from this point of view. In respect to biomagnification, the TF is lower than the EF for algae which is about 400-fold (Nugroho and Frank 2010), indicating only weak biomagnification of copper along the food chain from the algae to the mussel. Overall, distribution and accumulation of copper in *A. anatina* are the results of exposure time, exposure pathways, and physiological functions of the respective organs. Food uptake is more efficient taking the five-fold lower nominal concentration of copper in these experiments into consideration.

These experiments will help understand the risks associated with copper exposure of freshwater mussels. Copper accumulation may promote the situation of metabolic acidosis leading to the dissolution of CaCO₃ deposits, inducing the increase of Ca concentration in the EPF (Antunes et al. 2002; Faubel et al. 2008; Lopes-Lima et al. 2008). Interference with Ca homeostasis by the inhibition of Ca-ATPase by Cu (Santini et al. 2011) may lead to physiological stress. These factors together with the involvement of copper in the formation of reactive oxygen species (Company et al. 2008) may be a contributory factor in the overall Europe-wide observed decline of freshwater bivalves.

**Conclusions**

Exposure of *A. anatina* to Cu via the water or via the food leads to enrichment of the transition metal in the mussel. Copper is mainly stored in the digestive gland, gills,
and mantle. The digestive gland and the kidney are the main organs for accumulation and elimination, although the latter represents only a small Cu-pool. Distribution and accumulation of copper are the results of exposure time, exposure pathways, and physiological functions of organ. Upon depuration, A. anatina eliminates Cu from the body quickly but not completely. Elevated exposure to the transition metal is suggested to be a main stress factor responsible for reduced viability of fresh water mussel populations.

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References


Han, B.-C., W.-L. Jeng, Y.-N. Tsai, and M.-S. Jeng. 1993. Depuration of copper and zinc by green oysters and blue mussels of Taiwan. Environmental Pollution 82: 93–7.


