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Assimilation efficiencies of Cd and Zn in the common carp (*Cyprinus carpio*): Effects of metal concentration, temperature and prey type

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Assimilation efficiency of Cd and Zn in food of carp is affected by metal load, prey type and temperature.

Abstract

The impact of several factors on the assimilation efficiency (AE) of Cd and Zn from food in the common carp (*Cyprinus carpio*) was studied. Tested prey species were midge larvae (*Chironomus riparius*), zebra mussels (*Dreissena polymorpha*) and oligochaetes (*Tubifex tubifex*). The Cd load of the larvae did not affect the Cd AE in the carp. The Zn AE however, was negatively related to the Zn load of the prey. Food quantity and starvation of the carp did not significantly affect the Cd AE. For Zn, a significant decrease in AE was found when carp were fed *ad libitum*. Decreasing the temperature from 25 °C to 15 °C did not influence the Cd AE, while for Zn a significant decrease of the AE was measured. Carp assimilated Cd from both zebra mussels and oligochaetes with a significantly lower efficiency in comparison to the midge larvae, although Zn AEs was prey independent.

Keywords: *Cyprinus carpio*; Assimilation efficiency (AE); Heavy metals; Temperature; Food type

1. Introduction

In spite of the increased waste water treatment activities, water quality, habitats and in particular biota of certain aquatic ecosystems remain often affected by heavy metal contamination. As in these cases fish interact with heavily contaminated sediments and depend on contaminated food supply, throphic transfer along the food web can be an important route for uptake and accumulation of heavy metals (Dallinger and Kautzky, 1985; Suedel et al., 1994; Woodward et al., 1995; Besser et al., 2001; Baldisserotto et al., 2005). While the mechanisms of waterborne metal uptake and toxicity are beginning to be characterised, uptake and toxicity of food borne metals still remain a complex and further to be unravelled puzzle. The facts that dietary metal exposure can be of significant importance and that it has to be included in regulatory guidelines have become generally accepted statements, the need for a more profound mechanistic understanding of the underlying processes increases exponentially. Moreover, the dual uptake pathways associated with the continuous exposure to a polluted medium and the subsequent reliance on contaminated food items remain an ecotoxicological challenge unique to the aquatic environment.

The study of the different pieces of the dietary exposure puzzle has therefore become an important research topic. A key factor herein is the metal assimilation efficiency (AE). The metal assimilation efficiency can be defined as the fraction of the ingested metals which remains in the body after a complete emptying of the gut of undigested material and is directly proportional to dietary metal accumulation (Wang

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and Fisher, 1999; Wang and Rainbow, 2000; Zhang and Wang, 2005). The assimilation efficiency of metals is a physiological factor, which can usually be described by first-order kinetics, is element- and species-specific and can be used in kinetic models to compare and predict the importance of different uptake pathways to the overall metal accumulation in aquatic organisms (Reinfelder et al., 1998; Barata et al., 2002).

Although dietary metal assimilation is not a new research topic, the mechanistic understanding of the underlying processes that control metal absorption and assimilation in fish is still limited, and this especially under realistic natural conditions.

Natural environments are characterised by differences in metal composition and metal load of the food items present, fluctuating water temperatures, differences in food availability and food choice, to mention some of the most important factors. These factors have been shown or are likely to have an important impact on metal AE in fish (Langevoord et al., 1995; Farag et al., 1999; Clearwater et al., 2000). Moreover, several studies have investigated the concentration- and temperature-dependence of the dietary uptake of the essential metals Cu and Zn (Clearwater et al., 2000; Bury et al., 2003; Glover and Hogstrand, 2002; Glover et al., 2003, 2004). These results suggest a homeostatic regulated and temperature-dependent absorption for essential metals in fish. Due to the lack of any essential role of Cd, it is expected that its uptake and assimilation from food in fish passes off differently than that of essential metals. Although it has been demonstrated that food can be of significant importance to the overall accumulation of Cd in fish (Thomann et al., 1997; Szebedinszky et al., 2001; Franklin et al., 2005), detailed insights in the underlying absorption and transport mechanisms still mostly depend on studies performed in mammals (Zalups and Ahmad, 2003).

Another point of interest is the fact that several studies on dietary metal accumulation have been performed using laboratory-prepared diets supplemented with metals (Harrison and Curtis, 1992; Lanno et al., 1987; Handy, 1993; Franklin et al., 2005). Therefore, much of these data lead to contradictory conclusions, especially when comparing them with studies using metal-contaminated invertebrates (Clearwater et al., 2002). These results raise the need for insights into the impact of food type and metal composition on the subsequent efficiency of metal assimilation in fish under more natural conditions. It has indeed been demonstrated that fish assimilate Cd with different efficiencies depending on several factors such as the Cd speciation in the food and the food choice (Langevoord et al., 1995; Farag et al., 1999).

In this work we experimentally studied the effect of some environmentally relevant factors, namely metal load of the food, food availability, water temperature, and food type, which can have an important impact on Cd and Zn assimilation efficiencies in the common carp (*Cyprinus carpio*). Food items were exposed to Cd and Zn simultaneously as is often the fact under natural conditions (Baudrimont et al., 1999; Andres et al., 2000; Bervoets et al., 2005). The food items used were the midge larvae *Chironomus riparius*, the zebra mussel *Dreissena polymorpha* and the oligochaete *Tubifex tubifex*, which form an important part of the diet of the common carp (Sibbing, 1988; Tucker et al., 1996; García-Berthou, 2001). The use of gamma-emitting isotopes allowed repeated measurements of the same individual and the use of environmentally realistic metal concentrations.

2. General materials and methods

2.1. Acclimation conditions

Common carp (*Cyprinus carpio*) were obtained from the fish hatchery at the Agriculture University of Wageningen (The Netherlands). They were maintained at the University of Antwerp in 300-l tanks filled with softened tap water (25 ± 1 °C, pH 7.3 ± 0.2, $CaCO_3$ 86.8 ± 1.0 mg/l). Two weeks before the start of the experiments the carp (body weight 1–5 g) were transferred in aquaria in a climate chamber filled with reconstituted fresh water according to the OECD test guidelines ($CaCl_2 \cdot 2H_2O$: 2 mM, $MgSO_4 \cdot 7H_2O$: 500 μM, $NaHCO_3$: 771 μM, KCl: 77.1 μM, hardness: 250 expressed as mg/l $CaCO_3$) (Organisation for Economic Cooperation and Development, 1993) at 25 ± 1 °C or 15 ± 1 °C, depending on the experiment. The water was filtered with trickling filters and the levels of NH_4^+, NO_2^-, and NO_3^- in the water were maintained below 0.1 mg/l, 0.1 mg/l, and 20 mg/l, respectively. All salts used were of analytical grade and supplied by Merck (Darmstadt, Germany).The fish were fed daily with frozen midge larvae (*Chironomus* sp.) at 8% of their body weight, but starved 48 h before use in the experimental conditions unless stated differently.

Egg ropes of the midge were obtained from a controlled laboratory culture at the Royal Belgian Institute for Natural Sciences (KBIN, Brussels, Belgium). The larvae were cultured in plastic 10-l aquaria containing reconstituted fresh water (composition as described for carp acclimation conditions) and a paper towel substrate at 25 ± 1 °C and a 12/12 h light/dark regime. They were fed with a suspension of ground commercial food (TetraMIN®, Melle, Germany) (Bervoets et al., 2003). The levels of NH_4^+, NO_2^-, and NO_3^- in the water were maintained below 0.1 mg/l, 0.1 mg/l, and 20 mg/l respectively.

The zebra mussels (*Dreissena polymorpha*) were collected at the drinking water basin of the Antwerp Drinking Water Company (AWW, Duffel) and the oligochaetes (*Tubifex tubifex*) were obtained from a local aquarium shop. The animals were acclimatised in the lab at 25 ± 1 °C to reconstituted fresh water (composition as described for carp acclimation conditions) in plastic 10-l aquaria for 14 days with a 12/12 h light/dark regime. The water was filtered with trickling filters and the levels of NH_4^+, NO_2^-, and NO_3^- in the water were maintained below 0.1 mg/l, 0.1 mg/l, and 20 mg/l respectively. The worms were fed a suspension of finely ground TetraMin flakes (Tetra Werke, Germany) (Steen Redeker et al., 2004). Zebra mussel were fed yeast cells (20,000 cells/l; Lansy PZ, INVE) every 2 days (Voets et al., 2004).