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Tissue distributions and seasonal dynamics of the hepatotoxic microcystins-LR and -RR in two freshwater shrimps, *Palaemon modestus* and *Macrobrachium nipponensis*, from a large shallow, eutrophic lake of the subtropical China

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Abstract

So far no information is available on microcystin (MC) contents in shrimps, prawns or crayfish from natural freshwaters. Tissue distributions and seasonal dynamics of the hepatotoxic MC-LR and -RR in two freshwater shrimps, *Palaemon modestus* and *Macrobrachium nipponensis* were studied monthly (during June–November, 2003) in a Chinese lake containing toxic cyanobacterial blooms. The shrimps *P. modestus* and *M. nipponensis* accumulated high MCs not only in the hepatopancreas (mean 4.29 and 0.53 μg g^{-1} DW, respectively) but also in the gonad (mean 1.17 and 0.48 μg g^{-1} DW, respectively), and the crayfish *Procambarus clarkii* accumulated as much as 0.93 μg g^{-1} DW in the gonad. This indicates that gonads of these invertebrates are the second important target organ of MCs. *P. modestus* apparently accumulated more MCs in their organs than *M. nipponensis*, which might be a reflection of their difference in trophic niche. Eggs of the shrimps accumulated 8.4% (*M. nipponensis*, 0.27 μg g^{-1} DW) and 29.0% (*P. modestus*, 2.34 μg g^{-1} DW) of total toxin burden, indicating that MCs had been transferred into offspring from their adults. Among the shrimp muscle samples analyzed, 31% were above the provisional WHO TDI level, suggesting the risk of consuming shrimps in Lake Chaohu.

Keywords: Microcystin-RR and -LR; Shrimps; Crayfish; Hepatopancreas; Gonad and egg; Muscle; Human consumption; Lake Chaohu

1. Introduction

Serious eutrophication accompanied with the presence of massive cyanobacterial blooms and the associated cyanotoxins has been documented in many inland waters worldwide (Paerl et al., 2001). Among cyanotoxins, the hepatotoxic microcystins (MCs) are considered to be one of the most dangerous groups (Carmichael, 1997; Chorus and Bartram, 1999), because they are highly toxic to mammals, e.g. the LD_{50} of MC-LR i.p. or i.v. in mice and rats ranged between 36 and 122 μg kg^{-1}, comparable to the toxicity of the chemical organophosphate nerve reagents (Dawson, 1998). The exposure to MCs has been implicated in acute death of terrestrial animals and through haemodialysis also caused death of humans (Carmichael et al., 2001; Azevedo et al., 2002). The long-term exposure to MCs is related to chronic human intoxication such as primary liver cancer (Yu, 1989, 1995). Now, MCs are of great concern to public due to their potential risk to human health.

Since it is a common belief that human exposure to MCs is mainly through drinking water and recreation, numerous researches have focused on intracellular and extracellular

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MCs of phytoplankton in the water (Chorus and Bartram, 1999); and also as freshwater products are rarely used for human consumption in western countries, little information in the scientific literatures is available on contamination of microcystins in aquatic animals of natural waters. There have been only occasional reports on microcystin contents in edible wild aquatic animals such as fish in a Brazil lagoon (Magalhàes et al., 2001) and an Egyptian fish farm (Mohamed et al., 2003). MC contents were also measured occasionally for mussels in Lake Suwa (Japan) (Watanabe et al., 1997; Yokoyama and Park, 2002) and snails in several Canadian lakes (Kotak et al., 1996) and Lake Biwa (Japan) (Ozawa et al., 2003) because of the concern that MCs may be transported to terrestrial food web (Prepas et al., 1997; Ozawa et al., 2003).

However, so far no information is available on MC contents in shrimps, prawns or crayfish collected from natural freshwater environments. There are some immersion bioassay studies on the toxicity (indicated by LC_{50} or EC_{50}) of MCs on brine shrimp (*Artemia salina*) (Kiviranta et al., 1991; Delaney and Wilkins, 1995; Metcalf et al., 2002; Sabour et al., 2002) or fairy shrimp (*Thamnocephalus platyurus*) (Blom et al., 2001; Keil et al., 2002). In a laboratory experiment, toxin accumulation and depuration kinetics were studied by injecting prawn with MC-LR at a dose of 86 ng g^{-1} body weight, and maximums in the hepatopancreas and muscle reached 130 and 5 ng g^{-1}, respectively, while the majority of MC-LR in the hepatopancreas and muscle was depurated within a few hours (Kankaanpää et al., 2004).

There were only a few experimental studies on MC accumulation in crayfish. In laboratory, 12 signal crayfish (*Pacifastacus leniusculus*) (collected from a crayfish farm in southern Sweden) were fed with a toxic strain of *Planktothrix agardhii* with a MC content of 3.6 mg g^{-1} DW, and MCs were detected in the hepatopancreas of six of 12 of the animals, but the amounts of MCs accumulated in the hepatopancreas could not be quantified accurately because of interference from other components (Liras et al., 1998). In the laboratory, the crayfish *Procambarus clarkii* were fed with toxic *Microcystis aeruginosa* strain containing a MC content of 2.3 mg g^{-1} DW, and their whole body accumulated up to 2.9 μg MC g^{-1} DW (determined by ELISA method) at the end of an uptake period (2 weeks) with 53, 38 and less than 0.1% of the MC in the intestine, hepatopancreas and muscle (edible part), respectively (Vasconcelos et al., 2001).

In China, freshwater shrimps are commercially important because they are widely used for human consumption. They are not only cultured in ponds but also abundantly present in many natural freshwater lakes. However, during the past decades, eutrophication in Chinese lakes has progressed rapidly, resulting in frequent outbreak of toxic cyanobacterial blooms in many large lakes such as Lake Chaohu and Lake Taihu where production of freshwater shrimps are an important industry. It is quite likely that oral consumption of these shrimps exposed to high MC levels could lead to chronic human intoxication. Therefore, it is urgently needed to clarify whether MCs are able to accumulate in these shrimps or not, and to evaluate quantitatively consumptive risk for humans.

The present research was conducted on two freshwater shrimps, *Palaemon modestus* and *Macrobrachium nipponensis*, with occasional sampling of the red swamp crayfish *P. clarkii*, in a large shallow, eutrophic subtropical freshwater lake (Lake Chaohu) where heavy cyanobacterial blooms occur in the warm seasons of every year. *P. modestus* and *M. nipponensis* are high in production and used directly for human consumption; and there was a 6-fold increase in shrimp harvest during 1979–2001. The purposes of this study are mainly to examine distributions and seasonal changes of microcystins-LR and -RR in various organs (stomach, hepatopancreas, gonad, muscle, egg and gill) of the shrimps and to evaluate the relative importance of different organs in the accumulation of MCs with comments on the potential risk to human health when these are consumed.

2. Materials and methods

Lake Chaohu, located in Anhui Province in the southeastern China, is among the five largest freshwater lakes in China. It is a subtropical lake with a surface area of 760 km^2, a mean depth of 3.06 m, and a mean retention time of 136 days. During the past decades, the lake has witnessed a steady increase in eutrophication, characteristic of a regular occurrence of cyanobacterial surface blooms (mainly composed of *Microcystis* spp. and *Anabaena* sp.) in the warm seasons of each year (Deng, 2004). In this lake, *P. modestus* and *M. nipponensis* are two important freshwater shrimps, and the red swamp crayfish *P. clarkii* are minor in the crustacean catch.

P. modestus has a natural range from Fujian Province of China in the south to Siberia of Russia in the north, and *M. nipponensis* is widely distributed in China with natural occurrence also in Vietnam and Siberia of Russia (Li et al., 2003). Generally, *M. nipponensis* prefers littoral habitats, dominating in macrophytic shallow lakes like Lake Honghu (Sun et al., 1999), while *P. modestus* prefers pelagic habitats, dominating in lakes like Lakes Chaohu and Taihu where there are more pelagic habitats (Shi, 1995).

The red swamp crayfish *P. clarkii* is one of the most widespread freshwater crayfish in the world. It is a native species from South America and used in many countries as a food resource (Perry and LaCaze, 1969; Vasconcelos et al., 2001). *P. clarkii* was transplanted into Japan in the early 20th century; from Japan, it was introduced into China in the late 1930s, and now is widely dispersed in natural waters of China (Guo and Zhu, 1997) perhaps due to its high migratory ability, resistance to environmental changes and high ability to tolerate low water quality (Johnson and