J. Fish. Sci. Tech. 6(2), 81-87, 2003

JOURNAL OF
FISHERIES SCIENCE
AND TECHNOLOGY

Effects of Dietary Copper Exposure on Accumulation and Histopathological Change in Liver of Juvenile Rockfish, *Sebastes schlegeli*

Jae-Won Kim, Seong-Gil Kim*, Sang-Gyu Kim, Seoung-Yeup Song and Ju-Chan Kang

Department of Aquatic Life Medicine, Pukyong National University, Busan 608-737, Korea

Experiments were carried out to investigate the accumulation and the histopathological changes in liver of juvenile rockfish, *S. schlegeli*, after sub-chronic dietary Cu (0, 50, 125, 250 and 500 mg/kg) exposure for 60 days. Cu accumulation in liver was significantly increased with dietary exposure period and concentration for 60 days, and has a linear relation with dietary exposure days. After 60 days of Cu dietary exposure, the Cu concentration in the liver was 75.19 ± 12.05, 126.29 ± 22.11 and 360.44 ± 45.26 μg/g dry weight and was approximately 11-fold, 18-fold and 51-fold higher than in the control diet group at 125, 250 and 500 mg/kg Cu diet group. The accumulation factors were increased with the dietary exposure period in liver of rockfish. In the primary exposed stage, the effect of hepatic tissue in the rockfish exposed to dietary Cu observed enlargement of hepatocytes nuclei, activity of hepatic cells and the swelling of hepatic cells. While exposed time and concentration were increased, the distinct granulation, irregular shape and necrosis of hepatic cells were observed. It was observed that granule degeneration and necrosis showed a part of cells in hepatic tissue after 60 days at 500 mg/kg.

Key words: Copper, *Sebastes schlegeli*, Accumulation, Accumulation factor, Histopathology

Introduction

Generally, due to its central role in metabolism and its sensibility to metal, the liver of fish has been studied for accumulation and toxicological effect of metal in fish. In fish, the liver tends to concentrate metals and exhibits relatively high potential for bio-accumulation and detoxification (Roesijadi and Robinson, 1994).

As copper (Cu) is an essential metal for all organisms including fish, it plays an important role in organism metabolism, and its concentration is well regulated (Cousins, 1985). However, Cu is one of the most toxic metals to fish and affects various blood parameter (Christensen and Tucker, 1976), growth (Langston, 1990), enzyme activity (Roesijadi and Robinson, 1994), and reproduction (Horning and Nieheisel, 1979).

Although the sub-chronic toxic effects of metal on fishes are well documented, that is mostly in fishes exposed to waterborne metal, but few studies have been conducted on the effects of dietary metal (Handy, 1996). The realization that dietary uptake of metal is a major cause of long-term contamination in wild fish (Dallinger et al., 1987; Farag et al., 1995) has renewed interest in the nutritional and toxicological effects of metal in the food of fishes (Handy, 1996).

Fish accumulate Cu from polluted environment resulting in accumulation in their tissues. Cu accumulation between tissues varies depending on the source of uptake, food or waterborne (Sorensen, 1991). Bioaccumulation patterns of metals in fish tissues can be utilized as effective indicators of environmental metal contamination (Larsson et al., 1985). Moreover, histopathological approaches should be obligatory environmental for assessments, and may be used to formulate monitoring programs (Hinton and Laurén, 1990). The histopathological changes of waterborne Cu have been studied in fish (Baker, 1969; Segner

*Corresponding author: cosmas@bcline.com

and Braunbeck, 1990; Arellano et al., 1999), but relatively few investigations have defined the pattern of histopathological alteration in subchronic exposure to dietary Cu in important organ, especially in liver. Therefore, metal accumulation and histopathological change studies are important from the point of view of health protection and assessment of toxicological effects for different metallic contaminants.

The rockfish, *Sebastes schlegeli*, is an economically important food fish in Korea that is commonly cultured in marine based cages (Jung et al. 2001). Despite the importance of the rockfish in Korea, relatively little information is available in the effect of copper, particularly dietary exposure. The aims of present study were (1) to investigate the Cu accumulation and (2) to study the histopathological change in liver of juvenile rockfish, *S. schlegeli*, after sub-chronic dietary Cu exposure.

Material and Methods

Diet preparation

Diets were supplemented with 0 (control), 50, 125, 250 and 500 mg/kg diet, using $CuSO_4 \cdot 5H_2O$ (Aldrich, USA). Copper sulfate pentahydrate was dissolved in 1000 mL acidified water and mixed well with the other feed ingredients prior to pelleting. All ingredients were mixed and pelleted by a laboratory pellet machine without heating by a 2 mm diameter module (Baokyong Commercial Co., Pusan, Korea). The pellects were packed into small bags and stored at -20℃ until they were fed to the fish. Proximate analyses of the diets indicated a crude protein of 48.0 %, crude lipid 5.0 %, carbohydrate 4.0 %, ash 15%, calcium 1.0 % and phosphrous 2.7%.

Experimental fish and Treatment

The juvenile rockfish (*S. schlegeli*) were obtained from rockfish nursery in Koge island, Korea. The rockfish were acclimated in 1000 L aerated running seawater tanks for 1 month to the laboratory conditions (Table 1). Each tank received a flow of 7 L per min and was supplied with continuous aeration. Fish were fed Cu-free diet daily at a rate of 2 % body weight (as two 1% meals per day). After 1 month in acclimating tanks, fish were randomly transferred to 150 L of tank (flow=1.2 L/min), which were running water test with continuous aeration. Fish with mean length 11.83 ± 0.03 cm (mean ± S.E., n=600), body weight 26.02 ± 0.23 g were selected for the experiment of dietary Cu exposure. Each of the four experimental diets were fed to rockfish for 60 days.

Table 1. The chemical components of seawater used in the sub-chronic dietary copper exposure experiment. Values indicate mean ± S.E.

Parameter	Values
Temperature (℃)	18.0 ± 0.2
pH	8.1 ± 0.2
Salinity (‰)	32.7 ± 0.4
Ammonia (μg/L)	12.66 ± 1.25
Nitrite (μg/L)	1.37 ± 0.28
Nitrate (μg/L)	9.62 ± 1.01
Phosphate (μg/L)	5.05 ± 0.96
SS (mg/L)	5.62 ± 0.2
Dissolved oxygen (mg/L)	6.74 ± 0.84
COD (mg/L)	1.52 ± 0.08
Fe (μg/L)	5.02 ± 0.87
Cu (μg/L)	2.32 ± 0.12

Metal analysis

Fish were starved for 24 h prior to sampling to allow all feed to be excreted. The liver tissues were sampled every 10 days for analysis of metal concentration. Eight fish were removed each test concentration and the control. Tissue samples were dried at 65℃ and kept in a desiccators until digestion. Dry tissues were digested with 1:1 HNO_3 (Suprapur grade, Merck, Germany) and samples were fumed to near dryness on a hot plate at 120℃ for overnight. After digestion, the residue was dissolved in 20 mL of 0.2 N HNO_3 and kept in a refrigerator until analysis for Cu. Cu concentrations of livers were measured using a flame atomic absorption spectrophotometer (AAS, Perkin-Elmer 3300, USA). Cu concentration in the liver of rockfish was expressed as μg/g dry wt. Accumulation factor (AF) is often used to compare the body burden of an organism with the degree of contamination. The following definition is used here:

$$\text{Accumulation Factor (AF)} = \frac{[\text{Me}]_{\text{exp}} - [\text{Me}]_{\text{control}}}{[\text{Me}]_{\text{diet}}}$$

where $[Me]_{exp}$, $[Me]_{control}$, $[Me]_{diet}$ are the metal concentration in the experimental group, the control group and the diet group, respectively, in μg/g (Holwerda, 1991).

Histology and liver tissue processing

Ten fish were sampled from each tank every 10 days throughout the experiment. The liver were rapidly removed and fixed with Bousin's solution.

After dehydration in graded concentrations of ethanol, the liver tissues were embedded in paraffin blocks. Four to five micron sections were stained with Mayer hematoxylin and 0.5% eosin.

Statistics

Data are expressed as means±standard error (SE). Statistics were performed with SPSS, using one-way analysis of variance (ANOVA) followed by Duncan's multiple comparisons test of mean values if significant differences were found ($P<0.05$).

Results

Copper accumulation

Cu accumulation in liver of *S. schlegeli*, as a function of dietary exposure time and exposure concentration are shown in Fig. 1. Cu accumulation in liver was significantly increased with dietary exposure period and concentration for 60 days ($P<0.05$). During first 10 days, Cu concentration increased sharply reaching a value 75.19 ± 3.21 μg/g (10-fold increase compare to control) at 500 mg/kg Cu diet group. Cu accumulation was significantly increased after first 10 days at 125. 250 and 500 mg/kg Cu diet group. Finally, after 60 days of Cu dietary exposure, the Cu concentration in the liver was 75.19 ± 12.05, 126.29 ± 22.11 and 360.44 ± 45.26 μg/g dry weight and was approximately 11-fold, 18-fold and 51-fold higher than in the control diet group at 125, 250 and 500 mg/kg Cu diet group, respectively. On the other hand, Cu accumulation in liver did not vary significant at 50 mg/kg Cu dietary exposure during the first 10 days. After 60 days of exposure, Cu concentration values were 22.80 ± 0.285 μg/g. For fish exposed to 50, 125, 250 and 500 mg/kg of dietary Cu, the following linear relations were obtained:

Cu (50 mg/kg) = 5.51 days + 7.06
(r^2=0.9306, $P<0.01$)
Cu (125 mg/kg) = 2.10 days + 13.89
(r^2=0.9826, $P<0.01$)
Cu (250 mg/kg) = 1.09 days + 6.32
(r^2=0.9455, $P<0.01$)
Cu (500 mg/kg) = 0.24 days + 6.26
(r^2=0.9822, $P<0.01$)

The accumulation factors are presented for liver at 50, 125, 250 and 500 mg/kg Cu dietary exposure in Fig. 2. The accumulation factors were increased with the dietary exposure period in liver. Although the accumulation factor in liver increased with dietary exposure concentration, there was no significance.

Histopathological change

Liver of the control individuals did not show any histopathological changes by light microscopy (Fig. 3A). In Cu dietary exposed to fish at 50 mg/kg (10 days), the tissue showed hydropic swelling of hepatocytes with the pyknotic nuclei in some cells and at 250 mg/kg (30 days), observed in many cells (Fig. 3B, C). After 40 days of Cu dietary exposure, liver changes were characterized by irregular shape

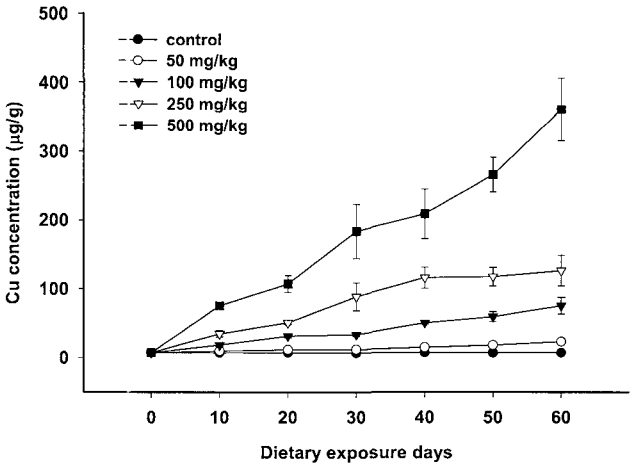

Fig. 1. Daily variation of copper concentration in the liver of juvenile rockfish, *Sebastes schlegeli* exposed to dietary copper for 60 days. Vertical bars denote a standard error of the mean.

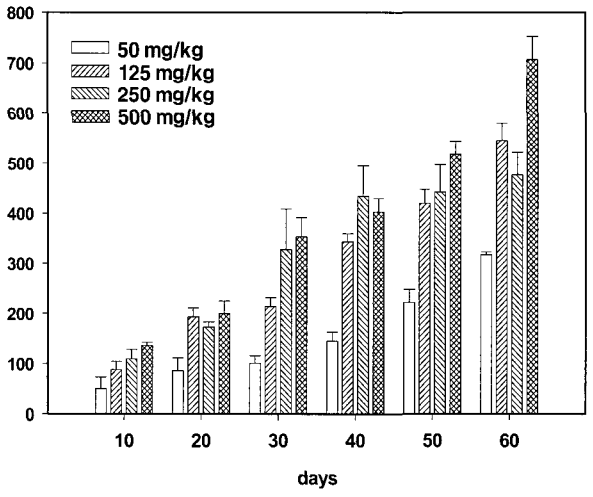

Fig. 2. Accumulation factor (AF) over time in the liver of juvenile rockfish, *Sebastes schlegeli* (mean ± S.E.), exposed to 50, 125, 250 and 500 mg/kg Cu diet, respectively.

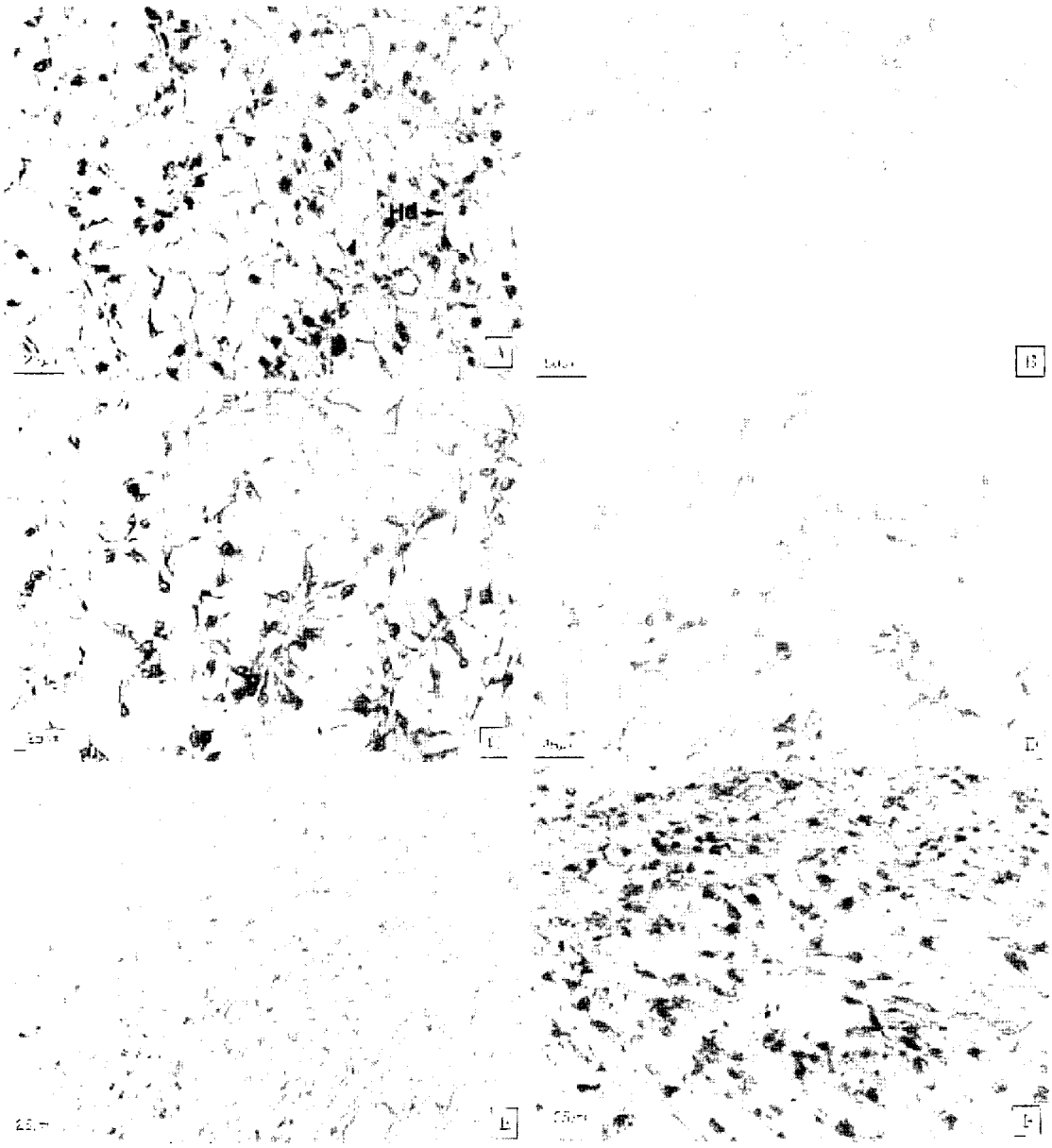

Fig. 3. Histopathological changes of liver of the rockfish, *Sebastes schlegeli* exposed to dietary Cu. A, Control. The hepatic cord and hepatic cell; B, Cu 50 mg/kg (10 days). The liver tissue showed enlargement of hepatocytes nuclei and the swelling of hepatic cells; C, The magnification of figure B; D, Cu 250 mg/kg (40 days). The liver tissue showed cellular disarray; E, Cu 500 mg/kg (50 days). The liver tissue showed some the granulation and necrosis of hepatic cells; F, Cu 500 mg/kg (60 days). The liver tissue showed distinct the granulation and necrosis of hepatic cells. Abbreviations: Hc, hepatic cell; Hd; hepatic cord; N, necrosis.

of hepatocytes at 250 mg/kg (Fig. 3D). At 250 mg/kg (60 days) and 500 mg/kg (50 days), there were the granulation and necrosis of some hepatic cells (Fig. 3E). After 60 days of exposure, the liver of the rockfish occurred a distinct granulation and necrosis at the 500 mg/kg Cu diet group (Fig. 3F).

Discussion

Metal accumulation in tissues of fish is dependent on exposure dose and time as well as other factors such as temperature, age of fish, interaction with other metals, water chemistry and metabolic activity of the fish (Pagenkopf, 1983; Goyer, 1991; Heath,

1995). Cu accumulation in liver of the rockfish increased with dietary exposure periods and concentration. Similar patterns of Cu accumulation were also shown in other studies with aquatic animals (Miller et al., 1993; Berntssen et al., 1999; Wong et al., 1999; Kamunde et al., 2002).

Generally, uptake of metal in aquatic organisms can occur by two major routes. These involve gill, in the case of dissolved forms, and the digestive organs, in the case of metals associated with ingested material such as food or sediment (Leland and Kuwabara, 1985). Moreover, dietary exposure to Cu is considerably less toxic than waterborne exposure (Handy, 1996). In this study, Cu accumulation in the liver of rockfish was approximately 51-fold, 18-fold and 11-fold higher than in the control diet group at 500, 250 and 125 mg/kg Cu diet group, respectively. Miller et al. (1993) reported that in rainbow trout elevated Cu concentrations in the water of more than 11 times increase from normal levels already induced an accumulation of liver Cu. These authors concluded that diet appeared to be dominant source of Cu in liver compared to waterborne Cu. Kamunde et al. (2002) found that the Cu level in the liver of rainbow trout, *Oncorhynchus mykiss*, was 33-fold higher than control at 282 mg/kg dietary exposure and they suggested that the role of the liver is central in fish Cu metabolism. The same results were observed that rainbow trout (Lanno et al., 1985; Julshamn et al., 1988), Atlantic salmon (Lorentzen et al., 1998) and channel catfish (Gatlin and Winson, 1986). In fish, Cu exposure resulted in increased Cu accumulation of liver, because it plays a major role in detoxification and excretion of metals through the induction of metal-binding proteins such as metallothioneins (MTs; Roesijadi, 1992). Therefore, it can be concluded that liver of rockfish is a more important storage organ than other organs and Cu accumulation clearly reflected the level of dietary exposure.

The calculated accumulation factor has two major purposes: first, to measure how much Cu is accumulated with respect to exposure concentration; second, to find the finite limit in the ability of fish to accumulate metals (Sorensen, 1991). The accumulation factor of rockfish increased with exposure period. Similar patterns of accumulation factor were also shown in carp (Cinier et al., 1999) and eel (Yang and Chen, 1999). Thus, Cu accumulation in rockfish strongly influenced by dietary exposure periods.

The liver of vertebrate not only acts as a storage organ of metal, but is also the primary site for detoxification mechanisms (Brown et al., 1984; Olsson et al., 1989) and is known to be target organs for Cu (Brungs et al., 1973; Buckley et al., 1982). The histological changes observed in various studies on livers exposed to pollutants include increased vacuoles in the cytoplasm, enlarged lysosomes, changes in nuclear shapes, focal necrosis (death of cells in a localized area), ischemia (blockage of capillary circulation), hepatocellular shrinkage, regression of hepatocytic microvilli at the bile canaliculi, fatty degeneration, and loss of glycogen (Heath, 1995). Studies concerning the histopathological effects of copper toxicity in teleost fish have been limited to investigations involving waterborne copper. In *Fundulus heteroclitus* treated with copper (800 μg/L), Ortiz et al. (1999) observed a disorganization of hepatic structure, focal necrosis, and accumulation of erythrocytes. However, in the milkfish, *Chanos chanos* (Segner and Braunbeck, 1990) and Senegales sole, *Solea senegalensis* (Arellano et al., 1999), the Cu concentration of 100 μg/L in the water did not lead to pathological hepatic damage. In addition, it has been reported that the consumption of diets containing elevated levels of dietary copper results in a dramatic increase in the copper content of the liver of channel catfish (Murai et al., 1981) and rainbow trout (Lanno et al., 1987).

In this study, as increasing dietary Cu of all exposure group for 60 days we have found that liver tissue showed more distinct hydropic swelling of hepatocytes with the pyknotic nuclei, and irregular shape, the granulation and necrosis of hepatic cells. Therefore, our result indicates that the dietary Cu could be responsible for the changes observed in the liver of rockfish.

In conclusion, this study observed highly Cu accumulation in the liver at all dietary Cu exposure group and dietary Cu exposure results in histopathological change. For the rockfish, the maximum allowable Cu concentration in feed supplies seems to be 50 mg/kg. The European Union (EU) has set maximum permitted concentrations for Cu in fish feed are currently 35 mg/kg dry weight (Lundebye, 1999). However, this EU guideline is based primarily on research done on poultry, and data on fish are scarce. Therefore, further research is necessary to set concentration of feed guideline, to protect fish health and human safety.

References

Arellano J.M., V. Storch and C. Sarasquete. 1999. Histological changes and copper accumulation in liver and gills of the senegales sole, *Solea senegalensis*. Ecotoxicol. Environ. Safe., 44, 62-72.

Baker, J.T.P. 1969. Histological and electron microscopical observations on copper poisoning in the winter flounder (*Pseudopleuronectes americanus*). J. Fish. Res. Bd. Can., 26, 2785-2793.

Berntssen, M.H.G., K. Hylland, S.E. Wendelaar Bonga and A. Maage, 1999. Toxic levels of dietary copper in Atlantic salmon (*Salmo salar* L.) parr. Aquat. Toxicol., 46, 87-99.

Brown, D.A., S.M. Bay, J.F. Alfafara, G.P. Hershelman and K.D. Rosenthal. 1984. Detoxification/toxification of cadmium in scorpionfish (*Scorpaena guttata*): acute exposure. Aquat. Toxicol., 5, 93-107.

Brungs, A., E.N. Leonard and J.M. McKim. 1973. Acute and long-term accumulation of copper by the brown bullhead *Ictalurus nebulosus*. J. Fish. Res. Bd. Can., 30, 583-586.

Buckley, J.T., M. Roch, J.A. McCarter, C.A., Rendell and A.T. Matheson. 1982. Chronic exposure of coho salmon to sublethal concentrations of copper. I. Effect on growth, on accumulation and distribution of copper, and on copper tolerance. Comp. Biochem. Physiol., 72C, 15-19.

Bunton, T.E. and J.M. Frazier. 1989. Hepatocellular ultrastructure in white perch (*Morone americana*) with abnormal hepatic copper storage. Mar. Environ. Res., 28, 375-382.

Christensen, G.M. and J.H. Tucker. 1976. Effects of selected water toxicants on the in-vitro activity of fish carbonic anhydrase. Chem. Biol. Interactions, 13, 181-192.

Cinier, C.C., M. Petit-Ramel, R. Faure, D. Garin and Y. Bouvet. 1999. Kinetics of cadmium accumulation and elimination in carp *Cyprinus carpio* tissues. Comp. Biochem. Physiol., 122C, 345-352.

Cousins, R.J. 1985. Absorption, transport and hepatic metabolism of copper and zinc: Special reference to metallothionein and ceruloplasmin. Physiol. Rev., 65, 238-309.

Dallinger, R., F. Prosi, H. Segner and H. Back. 1987. Contaminate food and uptake of heavy metals by fish: a review and a proposal for further research. Oecologia, 73, 91-98.

Eisler, R. and G. Gardner. 1973. Acute toxicology to an estuarine teleost of mixtures of cadmium, copper and zinc salts. J. Fish. Biol., 5, 131-142.

Farag A, M. Stansbury, C. Hogstrund, E. MacConnell and H.L. Bergman. 1995. The physiological impairment of free-ranging brown trout exposed to metals in the Clark Fork River, Montana. Can. J. Fish. Aquat. Sci., 52, 2038-2050.

Gatlin, D.M. and R.P. Wilson. 1986. Dietary copper requirement of fingerling channel catfish. Aquaculture, 54, 277-285.

Goyer, R.A. 1991. Toxic Effects of Metal in Casarett and Doulls Toxicology; Basic Science of Poisons. 4th ed., Pergamon Press, Oxford, 1033 pp.

Handy, R.D. 1996. Dietary exposure to toxic metals in fish. In: Toxicology of Aquatic Pollution, Taylor E.W. ed., Cambridge University Press, New York, pp. 29-60.

Heath, A.G. 1995. Water Pollution and Fish Physiology. CRC Press, Boca Raton, Florida, 359 pp.

Hinton, D.E. and D.J. Laurén. 1990. Integrative histopathological approaches to detecting effect of environmental stressors on fishes. Am. Fish. Soc. Symp., 8, 51-66.

Holwerda, D.A. 1991. Cadmium kinetics in freshwater clams. V. Cadmium-copper interaction in metal accumulation by *Anodonta cyngneaand* characterization of metal binding protein. Arch. Environ. Contam. Toxicol., 21, 432-437.

Horning, W.B. and T.W. Nieheisel. 1979. Chronic effect of copper in the bluntnose minnow, *Pimephales notatus* (Rafinesque). Arch. Environ. Contam. Toxicol., 8, 545-552.

Julshamn, K, K.J. Andersen, O. Ringdal and J. Brenna. 1988. Effect of dietary copper on the hepatic concentration and subcellular distribution of copper and zinc in the rainbow trout (*Salmo gairdneri*). Aquaculture, 73, 143-155.

Jung, S.H., J.W. Kim, I.G. Jeon and Y.H. Lee. 2001. Formaldehyde residues in formalin-treated olive flounder (*Paralichthys olivaceus*), black rockfish (*Sebastes schlegeli*), and seawater. Aquaculture, 194, 253-262.

Kamunde, C., M. Grosell, D. Higgs and C.M. Wood. 2002. Copper metabolism in actively growing rainbow trout (*Oncorhynchus mykiss*): interactions between dietary and waterborne copper uptake. J. Exp. Biol., 205, 279-290.

Langston, W.J. 1990. Toxic effects of metals and the incidence of metal pollution in marine ecosystem. In: Heavy Metals in the Marine Environment, Furness R.W. and P.S. Rainbow. eds. CRC Press, Boca Raton, Florida, pp. 143-182.

Lanno, R.P., B. Hicks and J.W. Hilton. 1987. Histological observations on intrahepatocytic copper-containing granules in rainbow trout reared on diets containing elevated levels of copper. Aquat. Toxicol., 10, 251-263.

Lanno, R.P., S.J. Slinger and J.W. Hilton. 1985. Maximum tolerable and toxicity levels of dietary copper in rainbow trout (*Salmo gairdneri* Richardson). Aquaculture, 49, 257-268.

Larssson, A., C. Haux and M. Sjöbeck. 1985. Fish

physiology and metal pollution: Result and experiences from laboratory and field studies. Ecotoxicol. Environ. Safe., 9, 250-281.

Leland, H.V. and J.S. Kuwabara. 1985. Trace metals. In: Fundamentals of Aquatic Toxicology, Rand G.M. and S.R. Petrocelli. eds., Hemisphere Publishing Corporation, New York, pp. 374-415.

Lorentzen, M, A. Maage and K. Julshamn. 1998. Supplementing copper to a fish meal based diet fed to Atlantic salmor parr affects liver copper and selenium concentration. Aquacul. Nutr., 4, 67-72.

Lundebye, A.K., M.H.G. Berntssen, S.E. Wendelaar Bonga and A Maage. 1999. Biochemical and physiological responses in Atlantic salmon (*Salmo salar*) following dietary exposure to copper and cadmium. Mar. Pollut. Bull., 39, 137-144.

Miller, P.A.. R.P. Lanno, M.E. McMaster and D.G. Dixon. 1993. Relative contributions of dietary and waterborne copper to tissue copper burdens and waterborne-copper tolerance in rainbow trout (*Oncorhynchus mykiss*). Can. J. Fish. Aquat. Sci., 50, 1683-1689.

Murai, T., J.W. Andrews and R.G. Smith. 1981. Effects of dietary copper on channel catfish. Aquaculture, 22, 353-357.

Olsson, P.E., A. Larsson, S. Maage, C. Haux, K. Bonham, M. Zafarullah and L. Gedamu. 1989. Induction of metallothionein synthesis in rainbow trout, *Salmo gairdneri*, during long-term exposure to water borne cadmium. Fish Physiol. Biochem., 6, 221-229.

Ortiz, J.B., M.L. Gonzáles de Canales and C. Sarasquete. 1999. Quantification and histopathological alterations produced by sublethal copper concentrations in *Fundulus heteroclitus*. Cien. Mar., 25, 119-143.

Pagenkopf, G.K. 1983. Gill surface interaction model for trace-metal toxicity to fishes: role of complexation, pH and water hardness. Environ. Sci. Technol., 17, 342-347.

Roesijadi, C. and W. Robinson. 1994. Metal regulation in aquatic animals: Mechanisms of uptake, accumulation and release. In: Aquatic Toxicology, Molecular, Biochemical and Cellular Perspectives, Malins, D.C. and G.K. Ostrander. eds., CRC Press, Boca Raton, Florida, pp. 387-420.

Roesijadi, G. 1992. Metallothioneins in metal regulation and toxicity in aquatic animals. Aquat. Toxicol., 22, 81-114.

Segner, H. and T. Braunbeck. 1990. Qualitative and quantitative assessment of the response of milkfish, *Chanos chanos*, fry to low-level copper exposure. In: Pathology in Marine Science, Perkins, F. and T. Cheng. eds., Proc. of PAMAQ III, Gloucester Point, Virginia, pp. 347-368.

Sorensen, E.M. 1991. Cadmium. In: Metal Poisoning in Fish. CRC Press, Boca Raton, Florida, pp. 175-234.

Wong, P.P.K., L.M. Chu and C.K. Wong. 1999. Study of toxicity and bioaccumulation of copper in the silver sea bream *Sparus sarba*. Environ. Int., 25, 417-422.

Yang, H.N. and Chen, H.C. 1996. Uptake and elimination of cadmium by Japanese eel, *Anguilla japonica*, at various temperatures. Bull. Environ. Contam. Toxicol., 56, 670-676.

(Received January 2002, Accepted June 2003)