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Review

Effect of heavy metals on fish larvae deformities: A review

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ABSTRACT

Heavy metals have been associated with many fish deformities in natural populations and in laboratory produced specimens as well. Deformities in general have devastating effects on fish populations since they affect the survival, the growth rates, the welfare and their external image. Although the embryonic stage in respect to heavy metal exposure has been extensively studied, there is not much information available as to what happens in fish larvae and adults. In the present article, we present the available information on the effect of heavy metals on fish larvae deformities. We also address the need for more research towards the effects of metals on the subsequent life stages in order to assess the long-term consequences of heavy metal poisoning on fish organisms and possibly correlate these consequences with the environmental contamination (use as biomarkers).

1. Introduction

Heavy metals have been associated with many fish deformities in natural populations as well as in laboratory produced specimens (Bengtsson and Larsson, 1986; Cheng et al., 2000; Jezierska et al., 2009a, 2009b). The presence of pollutants and especially heavy metals in the aquatic environments of fish (seas, rivers, lagoons) leads to severe adverse effects on the organisms and has been a subject of concern for many decades (Bengtsson et al., 1979; Muramoto, 1981). Early in the 1970s, many attempts were made to identify the effects of heavy metals on organisms such as the effect of cadmium on vertebral deformities of *Cyprinus carpio* (Muramoto, 1981) and *Pimephales promelas* (Pickering and Gast, 1972) or the effect of zinc on the vertebral column of *Phoxinus phoxinus* (Bengtsson, 1974).

In general, metals can be categorized as biologically essential and nonessential. The nonessential metals (e.g., aluminum (Al), cadmium (Cd), mercury (Hg), tin (Sn) and lead (Pb)) have no proven biological function (also called xenobiotics or foreign elements), and their toxicity rises with increasing concentration. Essential metals (e.g., copper (Cu), zinc (Zn), chromium (Cr), nickel (Ni), cobalt (Co), molybdenum (Mo) and iron (Fe)) on the other hand, have a known biological role, and toxicity occurs either at metabolic deficiencies or at high concentrations. The deficiency of an essential metal can therefore cause an adverse health effect, whereas it's high concentration can also result in negative impacts which are equivalent to or worse than those caused by non-essential metals (Kennedy, 2011). The most commonly found heavy metals in fish organisms are cadmium, lead, mercury, zinc, copper, nickel, cobalt, molybdenum, chromium and tin. Amongst them, the most frequently studied, with respect to fish deformities, include cadmium, copper, lead, zinc, mercury and chromium.

The most bioavailable form of metals that results in toxicity is believed to be the dissolved ionic form. Significant metal toxicity in fish can also derive from the organic forms of several metals including tin and mercury. Multiple physiological systems are affected by metals (commonly the gills) and toxicity depends on metal form and speciation, bioavailability, toxicokinetics (absorption, distribution, biotransformation, and excretion), and toxicodynamics (interactions with ligands) (Kennedy, 2011). Heavy metals accumulate in the tissues of aquatic animals and become toxic when concentrations reach certain toxicity thresholds, values which vary considerably among metals, metal species, taxonomic species and organism life stages. Fish absorb metals mainly through the gills and the digestive track, and to a lesser extent, through the skin (Kennedy, 2011). For more information on heavy metals, environment and fish interactions see Kennedy (2011).

On the other hand, fish deformities have devastating effects on fish populations since they affect their survival, growth rate, welfare and external morphology (Boglione et al., 2013; Divanach et al., 1996). Some of the most common deformities can be located in the vertebral column (Koumoundouros et al., 2002; Sfakianakis et al., 2006), the swimbladder (Chatain, 1994; Divanach et al., 1996), the cephalic region (Georgakopoulou et al., 2007), the fins

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(Favaloro and Mazzola, 2003; Koumoundouros et al., 2001, 1997; Sfakianakis et al., 2003) and the lateral line (Sfakianakis et al., 2013a). The most frequent of them are the ones in the vertebral column and especially lordosis (V-shaped dorsal–ventral curvature), kyphosis (Λ-shaped dorsal–ventral curvature) and scoliosis (lateral curvature). Fish deformities (especially skeletal ones) are quite important since they interfere with the organism's ability to interact with the environment. A distinctive example is the impairment of the swimming ability (Sfakianakis et al., 2011) which is the most crucial characteristic in accomplishing life important actions such as prey hunting, predator avoidance, traveling etc.

Heavy metals may adversely affect various metabolic processes in developing fish (embryos in particular), resulting in developmental retardation, morphological and functional deformities, or death of the most sensitive individuals. Additionally, heavy metals activate energy-consuming detoxification processes; thus, in intoxicated fish less energy can be used for growth. Most of the studies of heavy metals on developing fish (embryos or larvae) report high incidences of mortality, hatching delay, altered body shape, and body anomalies (Jezierska et al., 2009a, 2009b).

Fish embryonic and larval stages are generally considered to be the most sensitive in terms of toxicity during the entire fish life cycle (Osman et al., 2007; Zhang et al., 2012). On the other hand, adult exposures are not entirely risk-free; metal exposure of spawners may result in contamination of eggs and sperm, and reduce fish fertility and embryonic development. In many cases it is proven that exposure of adults to heavy metals leads to the latter being deposited in the testes and ovaries (Jezierska et al., 2009a). Generally, prenatal or early postnatal life is very vulnerable and sensitive to any type of xenobiotics (Ankley and Johnson, 2004) and exposure during these critical periods may cause profound effects during the entire lifetime of a fish (Birnbaum, 1994).

Heavy metals also act as endocrine disrupters in fish, e.g., cadmium was reported to reduce thyroid hormone levels (Hontela et al., 1996), inhibit estrogen receptors and disrupt growth hormone expression (Guével et al., 2000), while lead inhibits thyroid hormone synthesis by affecting iodine metabolism (Chaurasia et al., 1996). Prooxidative properties of metal ions may result in oxidative stress in fish and oxidative damage to the cell membranes. Cadmium, copper mercury and lead also exert a genotoxic effect on fish (Çavaş, 2008; Cavas et al., 2005).

Over the past decades, it has been suggested by scientists and public organizations that fish deformities can be used as biomarkers of environmental exposures (Au, 2004). Morphological deformity assessment is believed to be one of the most straightforward methods to study the effects of contamination on fish due to the ease of recognition and examination when compared with other types of biomarkers (Sun et al., 2009). In many cases, fish deformities are easy to distinguish – even macroscopically – and thus offer a tremendous advantage over other methods especially for scientists working on the field away from the laboratory equipment.

Toxicants such as heavy metals in water have posed a serious risk to aquatic organisms and public safety. These contaminants are frequently incorporated into food chain via water, microorganisms, plants, fish, and then enter human bodies through drinking water and fishery foods. Many recent studies exhibit the accumulation of various metals on animals' livers and kidneys especially in closed seas such as the Mediterranean and address the need for close scene monitoring (Storelli et al., 2005).

The embryonic stage has been extensively studied (reviewed by Jezierska et al. (2009a)) probably due to fact that specimen manipulation and handling is easier and faster. However, there is still quite a big gap of knowledge as to what happens in fish larvae and adults. In the present review we tried to summon the – limited – relatively recent literature on the matter and address the need for more research, targeted in life stages after the embryonic cycle. In order to do so, we focused on the studies that test the effect of metal on a larvae (or adult), but due to the surprisingly small number of experiments, we also included studies which, although the exposure to metals was carried out on the embryonic stage, the assessment of their effects was performed on live hatched larva.

2. Heavy metal exposure in laboratory fish populations

2.1. The effect of cadmium (Cd)

Cadmium is a naturally occurring non-essential trace element and its' tendency to bioaccumulate in living organisms often in hazardous levels, raises environmental concern (Kalman et al., 2010; Liao et al., 2011). The severity of cadmium toxicity in aquatic organisms as well as the fact that human activities such as disposal of industrial wastes and mining, are the primary routes of its release in the environment, have rendered it a priority pollutant research wise (Kalman et al., 2010; Maunder et al., 2011). Cadmium accumulates in aquatic organisms via dietary or aqueous exposure (Liao et al., 2011). The toxicity of Cd to aquatic species depends on speciation, with Cd^{2+} being the mainly absorbed species, following primarily the gill and intestine uptake pathways and secondarily the branchial one (McGeer et al., 2011). The main mechanism of toxicity is the antagonistic interaction between the uptake of Ca^{2+} and Cd^{2+}, which disrupts Ca^{2+} absorption (McGeer et al., 2011) leading to acute hypocalcaemia and growth reduction, problematic reproduction, as well as impairments in development and behavior (Dang and Wang, 2009; Maunder et al., 2011; McGeer et al., 2011). A concentrated list of cadmium induced deformities in fish is presented in Table 1.

Witeska et al. (1995) exposed Common carp (*C. carpio*) eggs at different concentrations of cadmium (0.001–0.05 ppm) until hatching. They reported head deformities and spinal curvatures which ranged from 0% to 47% in the different populations. Interestingly, the higher concentration of cadmium (0.05 ppm) presented one of the lower deformities incidence (5%). The authors also used Common carp larvae younger than 10 days, between 10 and 20 days old and older than 20 days for 96-h acute toxicity tests (0–0.017 ppm) but did not observe any deformities. The results of this study indicate the protective role of the egg shell, as the newly hatched larvae proved to be more susceptible than the eggs. Moreover, the results show that susceptibility decreases with age since the 96-h LC_{50} for fish under 10 days, between 10 and 20 days and over 20 days was 0.002 ppm, 0.005 ppm, 0.007 ppm of cadmium respectively.

Williams and Holdway (2000) studied the effects 2 h pulse-exposure of cadmium on early life stages of Australian crimson spotted rainbow fish (*Melanotaenia fluviatilis*). The range of the concentrations used was 0.033, 0.1, 0.33, 1, and 3.3 mg/L. The age of the embryos was 3, 46 and 92 h (post fertilization). Cadmium affected hatching, larval survival and spinal deformities (without specifying which ones). Higher cadmium concentration and smaller embryo age resulted in more adverse effects. Deformed specimens (spinal deformities) reached up to 27% in the populations. The authors also tested the effect of zinc on the species larvae without however checking for deformities.

Nguyen and Janssen (2002) studied the African catfish (*Clarias gariepinus*) with the effect of metal starting after fertilization and lasting for 5 days. Concentrations used varied from 0.05 to 5 mg/L ($CdCl_2 \cdot 2.5H_2O$). The main deformity they reported was reduction of body pigmentation which reached percentages of up to 100% at the highest concentration and was significantly higher from the control (0 mg/L) at concentrations above 0.5 mg/L.