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DISTURBANCES OF EARLY DEVELOPMENT OF FISH CAUSED BY HEAVY METALS (A REVIEW)

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Abstract: Waterborne metals adversely affect development and viability of early developmental stages of fish, larvae being the most susceptible to intoxication. In the present study, the effects of Cd and Cu intoxication during embryonic and larval development were evaluated, and discussed at broad literature background.

The effects of metals on developing eggs involved malformation of embryos Structural and functional disturbances during embryonic development resulted in an increase in mortality of embryos before and during hatching, and reduced number of hatched larvae. Metal exposure of embryos resulted in high share of deformed newly hatched larvae. The larvae obtained from metal-exposed eggs showed poor quality, and reduced viability.

Exposure of larvae to waterborne metals reduced their survival and development rate. Cd and Cu intoxication inhibited yolk sac resorption, and swim bladder inflation. Metal strongly impaired feeding activity of fish, reducing their ability to perceive, search and capture prey. The exposure to heavy metals resulted in decreased growth of fish which is one of the most sensitive responses to Cu and Cd intoxication.

Long metal exposure increased the frequency of body malformations in fish, mainly vertebral curvatures, that were related to metal-induced inhibition of bone calcification.

Metal intoxication increases mortality of fish offspring, and weakens their condition, resistance and viability.

Key word: heavy metals, fish, early development
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Introduction

In the polluted water, waterborne metals may induce disturbances in structure and function of fish organism, which results in higher mortality, and inhibition of development of young stages. The effects of metals on various fish organs, tissues, and metabolic processes were discussed in details by Jezierska, Witeska (2001). The cited data indicate that fish mortality is caused mainly by disturbances in the functions of the gills, liver, kidneys, blood circulation, and digestive tract, neurotransmission disorders, muscular dysfunction, immunosuppression, and other detrimental changes caused by metals.

Young stages of fish, especially the larvae, are the most sensitive to environmental factors, including intoxication with waterborne metals. Most data cited by Jezierska, Witeska (2001) indicate that susceptibility of fish to metal toxicity decreases with age. It is obvious that even under optimum environmental conditions early stages of fish show considerable mortality, often occurring during so called critical developmental periods, e.g. fertilization, hatching, gill development, digestive tract opening, or at the beginning of exogenous feeding, and during swim bladder inflation. Most individuals showing any structural and functional disturbances that impair their locomotor and feeding activity die.

Susceptibility to intoxication may be measured in acute toxicity tests, and expressed as LC_{50} value, or as survival in long-term exposures. Time is a very important factor affecting metal accumulation in fish tissues. High metal concentrations in the

organism may cause pathological changes, observable after certain time of exposure. According to Jones (1964), we may determine: 1. minimum exposure time necessary to initiate toxic action, 2. time necessary to produce typical response, 3. minimum time to cause irreversible changes, 4. time necessary to kill fish. Thus, long- term exposures to heavy metals during laboratory culture may affect larval development.

The investigations of embryos, larvae and juveniles of *Cyprinus carpio* carried out in the Department of Animal Physiology of the University of Podlasie concern toxic effects of copper and cadmium on young stages of fish.

Materials and Methods

The studies were done on common carp embryos and larvae reared under laboratory conditions. Development was observed and registered using the microscope and computer image analysis system MultiScan. The eggs obtained from artificially induced spawning were fertilized using "dry method". Dechlorinated tap water (temp.20-22ºC, DO saturation about 90%, hardness 210 mg $CaCO_3 \times dm^{-3}$, pH 7.2-7.8) was used for embryonic development and larval culture. Metal solutions were prepared using $CdCl_2 x2½H_2O$, and $CuSO_4 x5H_2O$. The embryos were exposed to 0.1-0.2 $mg \times dm^{-3}$ of Cu or 0.1-0.2 $mg \times dm^{-3}$ of Cd, from the beginning of development until hatching.

The embryos were carefully placed on slides divided with a pen into four sections, three eggs in each, using a small natural bristle brush. Each egg was numbered, beginning from the float side of slide, clockwise, in order to identify individually each embryo. When the eggs well stuck to the slide surface, the slides supplied with styrofoam floats were put into 12 l aquaria (five slides in each).

The observations of development of each embryo from experimental groups, were made every 2 hours using the binocular connected with the camera, and computer image analysis system MultiScan. The deformations of embryos and larvae were recorded in photographs.

Common carp larvae were obtained from the own laboratory hatchery. Only correctly developed fish were selected for the experiment. One day old larvae were divided into 3 groups: Control, Cu, and Cd. The fish were exposed to 0.2 $mg \times dm^{-3}$ of Cu or 0.2 $mg \times dm^{-3}$ of Cd. Each group consisted of 300 fish stocked into 180 dm^3 tanks. Water was changed every 3 days to maintain nominal metal concentrations. The fish were fed brine shrimp nauplii *ad libitum*, three times a day, and from the 25 day after hatching, additionally with dry feed.

Development of 25 or 50 larvae from each group was observed and registered daily. Photographs were taken from the1 to 30 day after hatching. Photographs of older larvae were taken every 10 days (40, 50, 60, 70, and 80 days after hatching). Skeletal calcification was evaluated using selective staining of cartilage and bone tissues. Every 10 days three fish were randomly sampled from each experimental group, anesthetized in 2-phenoxyethanol, and preserved in 3% formaldehyde solution. The fish were then trypsin-digested to make bones visible, and stained with Alcian blue (cartilage), and Alizarin red (calcified bone), according to Taylor (1967), in order to make a detailed analysis of bone calcification.

The photographs were used for measurements of length (*longitudo caudalis*), and body perimeter areas of larvae. Body perimeter area was used instead of body mass in order to avoid fish mortality related to manipulation and blotting.

From all experimental groups, normal larvae, without any visible malformations were chosen for further observations, and prey capture tests. Prey capture activity of the larvae at different age was measured as a number of *Artemia salina nauplii* consumed within 5 minutes (for 10 single larvae, each provided 10 - series A, or 20 – series B *nauplii*). Before the test the larvae were starved for 24 h.

Results and Discussion

1. The influence of metals on number and viability of fish larvae

Our observation of embryonic development indicate that the effects of metals on developing eggs include body malformations of embryos). Most of them resulted from disturbances of cleavage and gastrulation, and included changes in craniofacial, cardiovascular, and vertebral deformities. The examples of observed malformations are shown in Figure 1. Some deformed embryos died before hatching, while others hatched as deformed larvae (Ługowska 2005). In consequence, hatchability in metal-exposed group was reduced. She also observed also normal embryos sometimes hatched as deformed larvae (Figure 2) (Ługowska 2005). Thus, metal exposure of embryos results in a high contribution of deformed hatch. Vertebral curvatures, tail shortening, head enlargement, edema and deformation of heart and yolk sac are the most typical malformations (Jezierska et al. 2000). Most of the deformed larvae died in first days after hatching but some of them recovered (Ługowska 2007). Our results indicate that even in unpolluted water survival of normal larvae exposed during embryonic development to heavy metals was lower comparing to those that developed under optimum conditions (Ługowska, Witeska 2004).

Metals induce disturbances during embryonic development which may result in increased mortality of embryos before and during hatching process, thus in a considerably reduced hatching success. An increase in frequency of body deformations in larvae exposed during embryonic development to heavy metals was observed also by many other authors (Heisinger, Green 1975, Holcombe et al. 1976, Weis, Weis 1977, Klein-MacPhee et al. 1984, Munkittrick, Dixon 1989, Stouthart et al. 1994, Sarnowski, Jezierska 1999, Stasiūnaitė 1999) who observed a wide range of malformations. Their data, however, indicate that spinal curvatures are most commonly observed (also under optimum conditions), and deformations are not metal-specific.

It seems that adverse conditions during embryonic development may reduce survival of newly hatched larvae not only immediately but also within several days post hatching. The delayed effects of embryonic exposure were described also by Brent, Herricks (1998), and Samson et al. (2001).

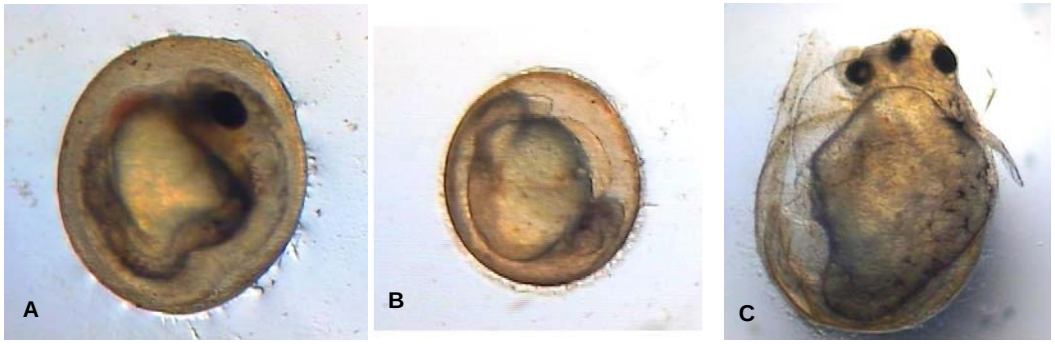

Figure 1. Examples of carp embryonic malformations: A. vertebral curvature, B. lack of tail, C. cranial malformation.

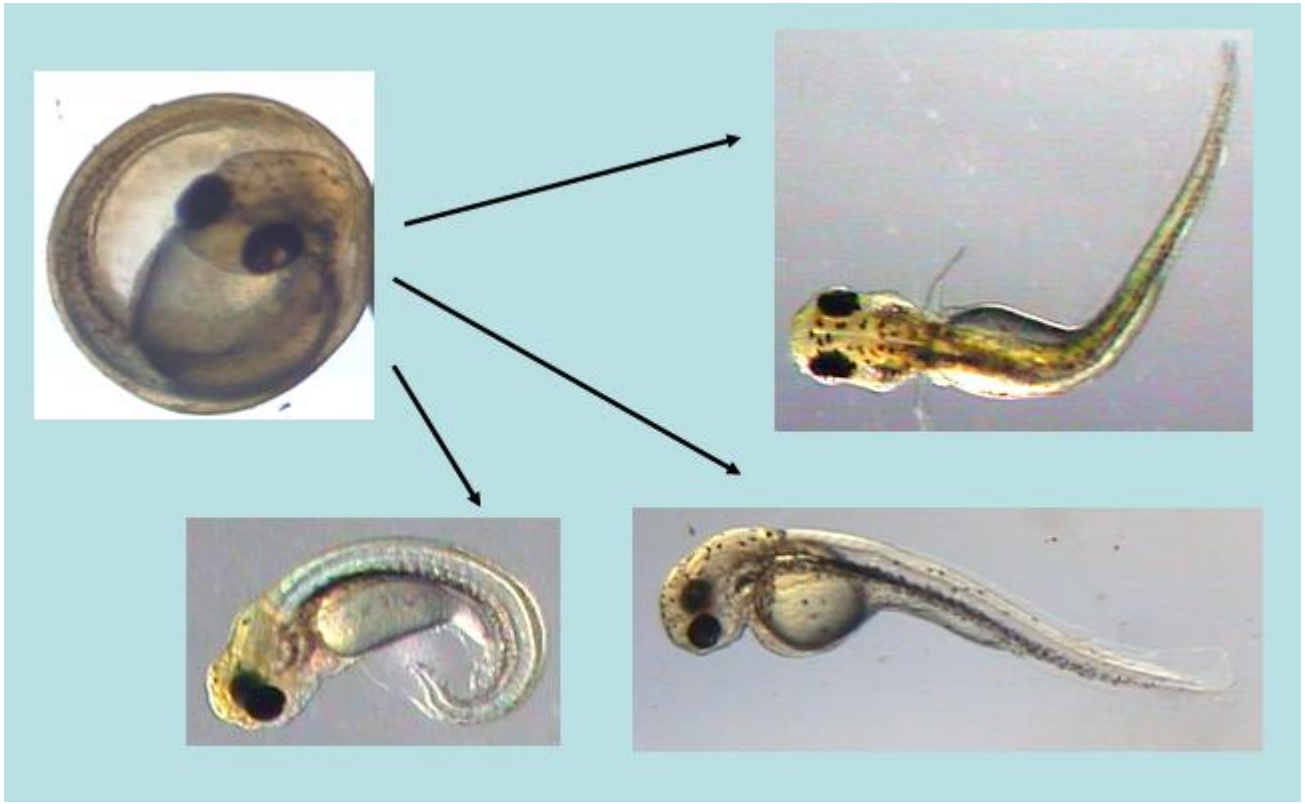

Figure 2. Normal embryo may hatch as deformed larva.

Table1. Survival of common carp larvae under different laboratory conditions.

Experiment			Survival (%)			References
No	Exposure	n	3 days after hatching	12 days after hatching	30 days after hatching	
1	Control Cu 0.1 mg/l	100	100 96	100 85	90 20	Jezierska, Słomińska 1997
2	Control Cd 0.2 mg/l Cu 0.2 mg/l	100	100 80 80	100 78 60		Sarnowski 2003
3	Control Cd 0.2 mg/l Cu 0.2 mg/l	50	100 92 95	98 76 64	95 68 56	Sarnowski 2005
4	Control Cd 0.2 mg/l Cu 0.2 mg/l	50	100 91 80	97 83 64	95 73 51	Sarnowski 2005
5	Control Cu 0.1 mg/l Cu 0.2 mg/l	20	100 90 80	100 75 65		Sarnowski un-published

Table1 shows the data obtained in various experiments in which common carp larvae hatched from the eggs incubated in unpolluted water, and then exposed to heavy metals after hatching. The results indicate that survival in exposed groups decreased, especially during fist period after hatching. At the end of the long-term metal exposures, the numbers of fish in metal exposed groups were significantly lower comparing to the control. Mortality was concentration-related, and higher in copper-exposed groups comparing to the cadmium-treated ones.

Some data indicate that the older the larvae, the more tolerant they are to metal toxicity (e.g. Holdway 1992, Jezierska, Witeska 2001). Larval susceptibility to metal toxicity may change during the development (Rombough, Garside 1982, Wright et al.1985, Woodward et al.1989). Roch, McCarter (1984) studied growth and survival of *Oncorhynchus tshawytscha* exposed to zinc, copper and cadmium. Their results showed a slight increase in tolerance in 8 weeks post hatching. Also metallothionein level increased with the age of larvae.

Some data, however, show high tolerance of the larvae, or at least certain larval stages. Meisner and Quan Hum (1987) suggest that zinc is equally toxic to juvenile and subadult *Oncorhynchus mykiss*. According to Middaugh, Dean (1977), 1-day old *Fundulus heteroclitus* larvae were less susceptible to cadmium than 7-days old ones. Similar results were obtained for cadmium-exposed *Pagrus major* – 1-day old fish were more tolerant than older larvae (Kuroshima et al. 1993). The authors explained that newly hatched larvae had closed mouth and their gills were not developed yet. In older fish with functioning gills metal uptake rate is higher, and gill epithelium is susceptible to metal-induced damage. It is also suggested that a higher rate of metal accumulation results in high concentration of cadmium in fish before the onset of metallothionein synthesis which results in a severe damage of tissues. Also Wright et al. (1985) observed that 7-days old *Morone saxatilis* larvae were more susceptible to cadmium than 1-day old ones, and concluded that yolk sac larvae were more tolerant than the post yolk sac ones. Ingersoll et al. (1990 a) noted that swim up fry of *Salvelinus fontinalis* were more sensitive to aluminum than yolk sac fry. According to Munkittrick, Dixon (1989), higher metal tolerance of yolk sac larvae is related to metal deposition in the sac, and after yolk resorption, fish susceptibility increases. It seems, however, that the way of feeding itself is the most important. The results obtained by various authors who determined LC_{50} values indicate higher susceptibility to metal intoxication at the beginning of exogenous feeding (Hazel, Meith 1970, Wright et al. 1985, Jezierska, Słomińska 1997, Słomińska 1998, Sarnowski, Jezierska 1999). It is a critical period when all the structural and functional developmental disturbances affecting feeding efficiency may express. The fish showing such anomalies die probably due to feeding difficulties. The results of long-term exposures of the larvae revealed that mortality considerably increased at that stage (Słomińska 1998). Munkittrick, Dixon (1988 b) distinguished the following critical developmental stages at which the fish are most sensitive to metals: first swimming, full development of gill circulation and gall bladder, mouth and pectoral fin movements, inflation of swim bladder, beginning of exogenous feeding, and complete yolk absorption.

2. Metal-induced development retardation

The mortality of larvae may be related to the delay of development. In our experiments we measured time of onset of various larval stages (according to Vasnetsov et al. 1957) of *Cyprinus carpio* exposed to cadmium and copper (Table 2). We observed extended duration of each stage, beginning from C1 (start of exclusively exogenous feeding), which resulted in retarded development.

Table 2. Development rate of common carp larvae under different laboratory conditions

Experiment			Developmental stages (% ind.)			References
No	Exposure	n	10 days after hatching	20 days after hatching	30 days after hatching	
1	Control Cu 0.1 mg/l	10	C_2>50 C_1>50	D_1>50 C_2>50	E>50 C_2-33, D_1-66	Słomińska, Jezierska 2000
2	Control Cu 0.1 mg/l Cu 0.2 mg/l	10	C_2>50 C_1>50 C_1>50	D_2>50 D_1>50 C_2>50	F>50 E>50 D_2>50	Słomińska, Jezierska 2000
3	Control Cu 0.2 mg/l	10	C_1>50 C_1>50	D_1>50 C_2>50	E>50 C_2>50	Słomińska, Jezierska 2000
4	Control Cu 0.2 mg/l Cd 0.2 mg/l	50	C_1-100 B-75, C_1-25 B-33, C_1-67	D_1-66, D_2-34 C_2-88, D_1-12 C_2-71, D_1-29	D_1-12,D_2-73,E-15 C_2-30,D_1-36,D_2-34 C_2-26,D_1-30,D_2-44	Sarnowski 2005
5	Control Cu 0.2 mg/l Cd 0.2 mg/l	50	C_1-100 B-86, C_1-14 B-12, C_1-88	C_2-5,D_1-70,D_2-25 C_2-100 C_2-79, D_1-21	D_1-21,D_2-60,E-19 C_2-22,D_1-35,D_2-43 C_2-27,D_1-26,D_2-47	Sarnowski 2005
6	Control Cu 0.2 mg/l Cd 0.2 mg/l	25	C_1-100 B-60, C_1-40 B-76, C_1-24	C_2-4,D_1-80,D_2-16 C_2-96, D_1-4 C_2-77, D_1-23	D_1-25,D_2-66,E-9 C_2-29,D_1-40,D_2-31 C_2-16,D_1-35,D_2-49	Sarnowski 2005

According to Vasnetsov et al. (1957)
B – inflation of posterior chamber of swimbladder, mixed endo-exogenous feeding
C_1 – exogenous feeding, yolk sac resorbed
C_2 – fin fold transformation into a heterocercal caudal fin
D_1 – inflation of anterior chamber of swimbladder (smaller than a posterior one)
D_2 – both chambers of swimbladder show similar size
E – bone rays in all fins, two intenstine loops

Table 3. The effect of metals on yolk sac resorption of common carp larvae.

Experiment			Yolk sac resorption (days after hatching)		References
No	Exposure	n	50%	100%	
1	Control Cd 0.2 mg/l Cu 0.2 mg/l	25	3 3 5	6 10 12	Sarnowski 2003
2	Control Cd 0.2 mg/l Cu 0.2 mg/l	50	2.5 2.5 3	6 11 14	Sarnowski 2005
3	Control Cd 0.2 mg/l Cu 0.2 mg/l	50	3 3 4	6 10 12	Sarnowski 2005
4	Control Cd 0.2 mg/l Cu 0.2 mg/l	25	3 3 4	7 12 13	Sarnowski 2005
5	Control Cu 0.1 mg/l Cu 0.2 mg/l	20	3 4 4	6 9 11	Sarnowski unpub-lished

Considerable retardation occurred also at the D1 stage (beginning of anterior swim bladder inflation). Słomińska (1998) observed that copper-exposed fish reached the fry stage after 40 days, while the control group already after 32 days.

Metal-induced inhibition of development was reported also by Holdway (1992), and Stouthart et al. (1996). The mechanism of inhibition is not quite clear. According to Stouthart et al. (1996), it may result from underdevelopment of upper jaw, which prevents air ingestion. The authors also suggest that copper affects gas exchange, which may impair floating ability.

The most distinct developmental retardation involves a delay of yolk sac resorption and swim bladder inflation. During first days after hatching, development and growth is related to utilization of yolk, and yolk sac diameter is an indicator of yolk resorption rate. In our study yolk sac perimeter area is an indicator of yolk resorption rate. Our results (Table 3) indicate that inhibition of development is related to the metal-induced inhibition of yolk sac utilization. In the control groups fish utilized over 50% of yolk during first three days post hatching, and the yolk sac was completely resorbed within 6-7 days, while in the metal exposed groups 50% resorption occurred in 4-5 days, and the complete yolk sac utilization, and shift to exclusively exogenous feeding were considerably delayed (at 0.2 mg dm^{-3} of Cu took place between the 11 and 14 day post hatching).

The important role of yolk sac resorption rate, and the effect of various environmental factors on this process was stressed by Kamler (2002). Correct yolk sac utilization is essential for further fish development, and metal exposure may disturb this process. McKim, Benoit (1971) observed slower growth and yolk resorption by *Salvelinus fontinalis* larvae. According to Peterson et al. (1983), 2 μg dm^{-3} of cadmium reduced growth and yolk-conversion efficiency in *Salmo salar*. The authors supposed that cadmium might interfere with yolk utilization by inhibition of calcium uptake. Calcium is probably important in mobilization and transport of yolk proteins. Also Hwang et al. (1995) reported that *Oreochromis mossambicus* larvae treated with 0.2 mg dm^{-3} of cadmium for 4 days were shorter and had larger yolk sacs, comparing to the control. Wu et al. (2003) reported that yolk absorption rate of *Oreochromis mossambicus* larvae was significantly suppressed when they were exposed to copper (0.03-0.4 mg Cu/l).

Yolk sac edema in embryonic and larval fish, following metal exposure, has commonly been identified by changes in the shape of the yolk sac and space between the yolk and yolk sac itself (Cheng et al. 2000). However, Johnson et al. (2007) did not observe such abnormalities or deformations in any of the individuals analysed after Cu exposure, which, therefore, suggests that the increased yolk sac area, with increasing copper concentration, was not caused by edema, but a reduction in yolk utilisation. The decreased length and yolk sac absorption in those fish exposed to copper support the idea of delayed development.

Another structural disturbance caused by metal exposure is inhibition of swim bladder inflation. Our results indicate that common carp larvae from all experimental groups started to inflate their first (posterior) chamber of swim bladder (PCS) in 3 days after hatching but heavy metals considerably inhibited this process, and especially growth of second – anterior chamber of swim bladder (ACS). High individual variability in the effect of metals on the rate of swim bladder development was observed. The data shown in Table 3 indicate that in the control group, on the 12-14 day post hatching, the mean size of PCS was 46-52 mm and some fish already started to inflate the ACS, on the 20-21 day all fish already showed ACS inflated (56-58 mm). Metal-exposed fish started to inflate the ACS later and some of them showed only PCS until the end of the experiment (30 days after hatching). At this time, the total size of swim bladder (two chambered) of fish from control groups was signicantly larger than of the one chambered swim bladder of metal-exposed ones. Słomińska (1998) observed that some copper-treated fish did not develop the ACS after 40 days.

The data obtained by other authors indicate that various environmental factors and toxicants my adversely affect fish swim bladder

inflation (Korwin-Kossakowski 1988, Martin-Robichaud, Peterson 1998, Ostaszewska et al. 1999, Uotami et al. 2000). Inhibitory effect of mealas on swim blader inflation was observed by Holdway (1992), and Stouthart et al. (1996). These authors, however, did not report at which stage inflation was inhibited.

The inhibition of swim bladder inflation may result in a reduction of locomotor activity of the larvae, and the difficulties in swimming. Metals may also affect metabolic activity in fish and inhibit gas passage to each swim bladder chamber. According to Stouthart et al. (1996), copper reduces air uptake ability of fish, and disturbs secretion and absorption of gas that fills the bladder. Similar hypothesis to explain lack of swim bladder inflation by *Salmo salar* larvae was developed by Poppe et al.(1997).These authors observed the changes in the pneumatic duct used for bladder inflation. The factors inhibiting swim bladder inflation may act directly or indirectly on epithelial cells of the bladder itself, and of the air duct. That would explain also inhibitory effect of heavy metals. According to various authors cited by Jezierska and Witeska (2001) heavy metals cause damage to epithelia in various fish organs: gills, body surface, intestine, or kidney tubules. Typical changes include swelling and proliferation of epithelial cells, followed by necrosis, and sloughing. It is always preceded by heavy mucus secretion. Therefore, it seems that similar changes may be expected in swim bladder and pneumatic duct wall epithelia. Probably, in metal-exposed larvae excessive amounts of mucus may congest the duct. Congestion may also result from histological changes in epithelium, such as hypertrophy. Ostaszewska et al. (1999) observed that hypertrophic secretory epithelium reduced the lumen of swim bladder itself, and of the pneumatic duct in pikeperch (*Stizostedion lucioperca* L.) larvae. Pneumatic duct congestion disturbs or precludes inflation of the ACS.

Table 4. The effect of metals on swimbladder inflation (mean values).

Experiment			Beginning of ACS inflation by first fish in group		Beginning of ACS inflation by last fish in group		End of experiment		References
No	Exposure	n	Days after hatching	Size of PCS (mm^2)	Days after hatching	Size of PCS (mm^2)	Days after hatching	Size of ACS+PCS	
1	Control Cd 0.2 mg/l Cu 0.2 mg/l	50	12 18 21	0.48 0.43 0.46	21 - -	0.58	30	1.42 1.06 0.91	Sarnowski 2004
2	Control Cd 0.2 mg/l Cu 0.2 mg/l	50	13 17 20	0.46 0.36 0.42	21 - -	0.56	30	1.33 1.11 0.88	Sarnowski 2004
3	Control Cu 0.1mg/l Cu 0.2 mg/l	20	14 16 16	0.52 0.44 0.42	20 - -	0.56	30	1.51 1.17 1.16	Sarnowski unpublished

ACS – anterior chamber of swimblader
PCS – posterior chamber of swimbladder

Therefore, inflation is delayed, and among metal-exposed 19 days old larvae, some individuals showed reduced size of ACS, while the others completely failed to inflate it. At the same time, all the fish from the control group showed correctly developed two-chambered swim bladder.

The uninflated, or incorrectly developed swim bladder disturbs arrangement of fish internal organs, which often results in vertebral malformations. Disturbances in swim bladder inflation adversely affect fish locomotor activity, and their feeding abilities.

The results of our experiment indicate that metals may affect feeding efficiency by the fish. We observed that cadmium and copper-exposed larvae showed very poor food consumption. The analysis of photographs revealed poor filling of digestive tract with Artemia *nauplii* (Słomińska 1998, Sarnowski 2003, Sarnowski 2005). In Figure 3 five days old larvae are shown: from the control with digestive tract filled with Artemia *nauplii*, and from Cu-exposed group with the gut only slightly filled.

In our experiments the effects of heavy metal exposures on Artemia *nauplii* capture and ingestion by common carp larvae were measured. We observed differences in feeding behavior of the control and metal-exposed larvae. The larvae of the control groups actively searched, detected, and pursued prey, efficiently captured and ingested them, while the metal-exposed ones were less active, and probably did not perceive *Artemia* passing in their vicinity.

The data in Table 5 (Jezierska et al. 2006) show that feeding efficiency increased with the age of larvae. It was the most pronounced in the control groups. Larval exposures to heavy metals considerably impaired feeding activity of fish, copper being the most toxic. The suppressive effect of larval exposure initially increased with time, but after some time adaptation was observed, especially in cadmium-exposed fish.

Metal-induced reduction of feeding activity was observed by various authors (Menezes, Qasim 1984, Lowe-Jinde, Niimi 1984, Cleveland et al. 1986, Woodward et al. 1989, Ghosh, Chakrabarti 1990, Handy 1993, Woo et al. 1993, Wilson et al. 1994, Weis, Weis 1995, De Boeck et al.1997, McGeer et al. 2000).

The reduction of feeding activity may be related not only to the appetite loss but also to the locomotor activity and perception disturbances which cause the difficulties in prey capture. Many authors cited by Jezierska, Witeska (2001) reported that waterborne metals may affect the behavior and reduce metabolic rate and fish activity. They may injure fish receptors, and reduce ability of prey search and capture.

Table 5. The effect of metal exposure on live food capture by common carp larvae (Jezierska et al. 2006).

Exposure	In test		Days after hatching			
	n fish	n prey	5	9	13	21
			% of captured and ingested Artemia nauplii			
Control	10	10	66	94	100	100
Cd 0.2 mg/l			43	92	90	98
Cu 0.2 mg/l			37	26	40	80
Control	10	20	46	58	58	86
Cd 0.2 mg/l			25	42	41	72
Cu 0.2 mg/l			18	18	17	51

Most of the available data concern the effects of copper on lateral line, olfactory receptors, eyes (Cancalon 1982, Rehnberg, Schreck 1986, Saucier et al. 1991, Bjerselius et al. 1993, Saucier, Astic 1995).
Such changes might have impaired the ability of prey perception and identification by the fish. Appetite loss and reduced growth rate in copper-exposed *Oncorhynchus kisutch* was reported by Buckley et al. (1982) but the authors suggested also metal-induced inhibition of metabolic processes and food utilization. They explained recovery of feeding activity and growth rate after 16 weeks of exposure with acclimation to waterborne metal.

3. The effect of metals on growth

The exposure to heavy metals under laboratory conditions resulted in decreased growth of common carp larvae. Table 6 shows the data concerning length, perimeter area and weight of fish. Metal exposed fish were smaller comparing to the control group. Our data indicate the ranking of sensitivity of various endpoints to metal toxicity: length < perimeter area < weight.
Fish growth rate, particularly at the early developmental stages, is highly variable and extremely sensitive to environmental changes including pollution with heavy metals. Also other authors reported growth reduction in metal-exposed fish (Peterson et al. 1983, Woltering 1984, Norberg-King 1989, Nguyen, Jensen 2002, Hansen et al. 2002, Kim et al. 2004). Among metals, cadmium and copper are probably the most powerful growth inhibitors. Rombough, Garside (1982) reported that growth rate was the most sensitive indicator of cadmium toxicity to *Salmo salar* alevins. Fish length was related to cadmium concentration and exposure time. Kaviraj, Ghosal (1997) observed that growth of *Cyprinus carpio* in respect of lengh and weight was reduced by all levels (1.0. 2.5 and 5.0 mgxdm^{-3}) of cadmium. Dose-dependent growth reduction over the range of Cu treatments was reported by Hansen et al. (2002) who suggested that it was related to metal accumulation in exposed fish. Other data also demonstrated a clear relationship between metal residues, and growth reduction in exposed fish (Peterson et al.1983, Collvin, 1984, Marr et al. 1996, De Boek et al. 1997, Farkas et al. 2002, Thongra-ar et al. 2003).

Table 6. The effect of metals on growth of common carp larvae.

No	Experiment		Days of experiment	Mean size of fish on the end of experiment as % of control fish			References
	Exposure	n		Length mm	Perimeter area [mm^2]	Weight [g]	
1	Cu 0.1 mg/l	100	41	75	-	40	Jezierska, Słomińska 1997
2	Cu 0.2 mg/l	100	40	70	-	-	Słomińska, Jezierska 2000
3	Cd 0.2 mg/l Cu 0.2 mg/l	50	30	86 57	67 45	53 27	Sarnowski 2005
4	Cd 0.2 mg/l Cu 0.2 mg/l	50	30	80 68	73 47	56 25	Sarnowski 2005

The effect of metals on fish growth rate becomes clearly visible during long-term exposures. Jezierska, Witeska (2001) presented the results of various studies indicating reduced rate of body length or mass increase in long-term metal treatments, body mass being more sensitive to metal intoxication than the length. According to Shukla, Pandey (1988), the growth rate of *Opiocephalus punctatus* was reduced by 11% and 16% for length and mass, respectively, under the stress of cadmium. Some authors reported that metal exposure reduced body mass increase, while body length was the same as in the control. Vosylienė, Petrauskienė (1995) studied growth of *Oncorhynchus mykiss* at various copper concentrations (0.025-0.2 mg dm^{-3}). No changes in body length increase occurred but the increase in body mass was reduced after 2 and 3 months of exposure to 0.1 and 0.2 mg dm^{-3}.

Many data indicate that fish growth is related to the food intake, nutrient utilization rate, and energy conversion. All these processes may be disturbed by toxic action of metals. Growth rate reduction is, according to some authors, related to a decrease of food intake (Waiwood, Beamish 1978, Lanno et al. 1985). According to Woo et al. (1993), *Oreochromis aureus* at 0.5-20 mg dm^{-3} of cadmium were anorectic and lost weight. Marr et al. (1996) explained reduced growth of copper-exposed *Oncorhynchus mykiss* with a decrease of feeding activity. Holdway (1992) observed a decline of body growth rate in two species of tropical fish treated with uranium. They also supposed that the fish lost appetite.

Fish growth rate may be also adversely affected by metal-induced metabolic disturbances, such as disorders in water-electrolyte balance (Woodward et al. 1989, Wood et al. 1990a, b, Sayer et al. 1991a, b, Woodward et al. 1991, Hwang et al. 1995, Stouthart et al. 1995). Some data indicate that metal exposure may induce iono-regulatory disturbance and increase in metabolic cost McGeer et al. (2000).

Metals may also affect the levels of nucleic acids, RNA/DNA ratio, and protein synthesis. Relationship between growth rate and the level of nucleic acids is stressed by many authors (Bulow 1970, Haines 1973, Barron, Adelman 1984, Cleveland et al. 1986). The RNA/DNA ratio is an indicator of growth rate, and metals do affect the level of nucleic acids, thus - the rate of protein synthesis. Any disturbance of protein synthesis would always affect fish growth.

Metals may also reduce growth rate of fish affecting hormonal regulation, e.g. cadmium delays growth hormone expression during fish development (Jones et al. 2001). Lower levels of T_4 and T_3 in fish from water polluted with various toxicants including cadmium and copper were reported by Hontela et al.1996, and Levesque et al. 2003.

Decrease of energy conversion for growth may be related to activation of metal detoxification (Dixon, Sprague 1981, Marr et al. 1996).

Therefore a reduction in growth is a sensitive indicator of reduction in an organism fitness which may result in reduced survival of an individual or population (Hansen et al. 2002).

4. Skeletal deformation of young stages of fish

In our experiments we observed body malformations of some common carp individuals, mainly vertebral curvatures observed in fish treated with copper, lead and cadmium (Jezierska, Słomińska 1997, Słomińska 1998, Słomińska, Jezierska 2000, Sarnowski 2005).

In our studies we observed lordosis in carps exposed to copper and showing underdeveloped swim bladder (Figure 4). These two disturbances often coexist. These data are similar to those obtained by other authors. Andrades et al. (1996) reported that juvenile lordotic *Sparus aurata* L. displayed uninflated swim bladders but all lordotic adults possessed an inflated functional swim bladder. Divanach et al.(1997) observed that lordosis in *Dicentrarchus labrax* was related

to the lack of swim bladder or its delayed inflation. Deformed spinal column of these fish looks as if it fitted deformed swim bladder. Fish with uninflated bladder show reduced growth rate and survival, and often suffer from severe vertebral malformations Marty et al (1995). The malformations and disturbances of swim bladder functions result in altered behavior, reduced locomotor and feeding activity, and in consequence – in reduced growth rate and viability of fish.

The vertebral curvatures are sometimes observed also in fish with correctly developed swim bladder (Figure 5).

We also observed inhibition of skeletal ossification. Figure 6 shows two fish at the same age and of similar size: from the control group, and the copper-exposed one. Fish from the control group shows completely calcified vertebral column, and parts of fin rays and skull, while in the copper-exposed one entire skeleton is not calcified Sarnowski (2005).

Many literature data cited by Jezierska, Witeska (2001) indicate that metal intoxication reduces gill calcium uptake, and bone calcium accumulation which results in the changes in bone properties – they become flexible and easy bend. Metals may also induce disturbances of neuro-muscular transmission which often results in muscular contractures that may lead to skeletal deformities, especially if it is flexible (Figure 7).

Figure 3. The effect of copper on digestive tract filling in 5 days old carp larva: A. control, B. Cu exposure.

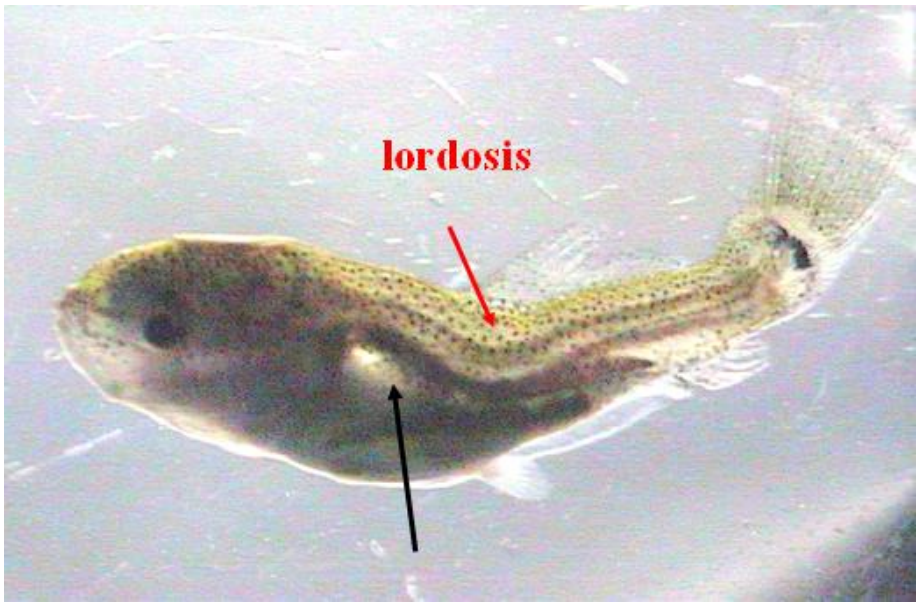

Figure 4. Coexistence of lordosis and underdeveloped swim bladder.

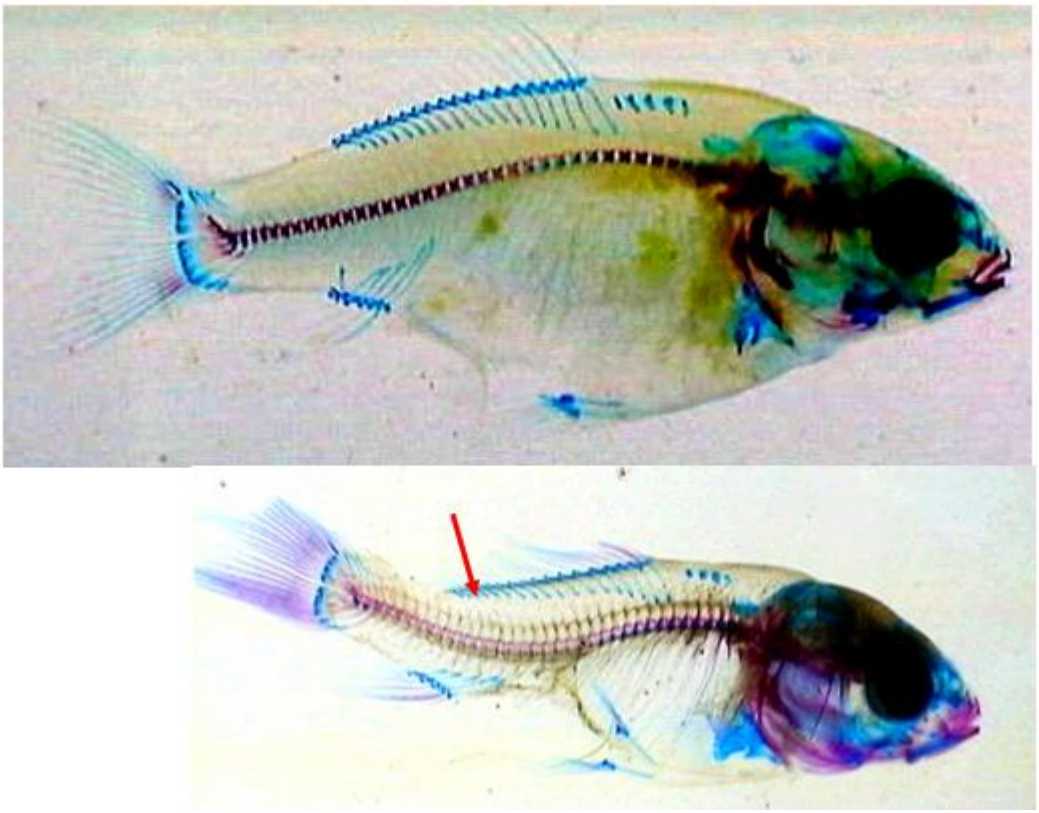

Figure 5. Vertebral malformations of 40 days old carp: A. Pb exposure, B. Cd exposure.

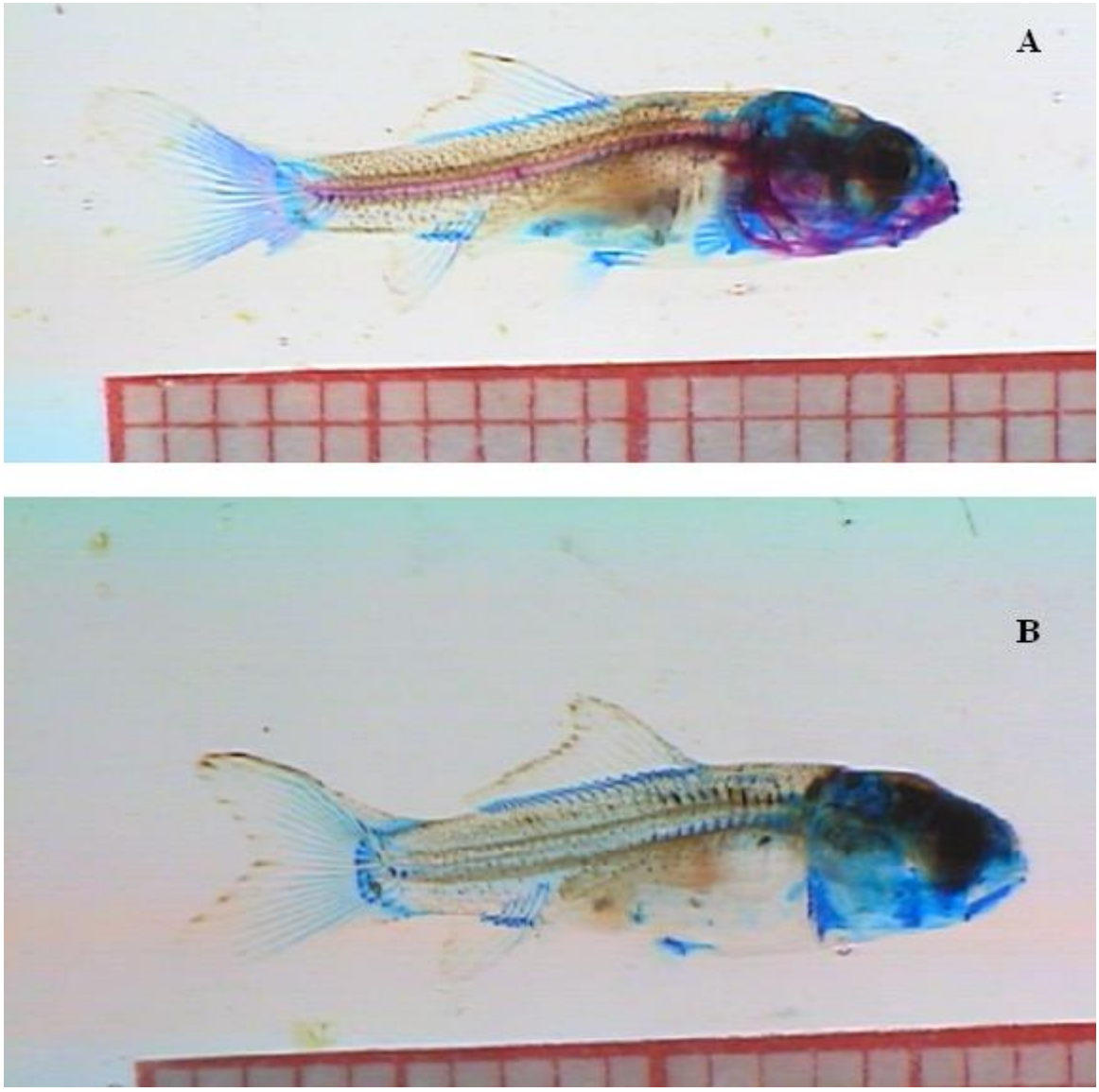

Figure 6. The effect of copper on bone calcification in 60 days old carp: A. control, B. Cu exposure.

Conclusion

In Figure 8 a scheme of the effect of metals on development and growth of fish is summarized.

Metal intoxication increase fish offspring mortality, and weaken their condition, resistance and viability.

Therefore, sensitivity of early stages of fish to metal intoxication may cause a decrease of population, even in case of incidental pollution.

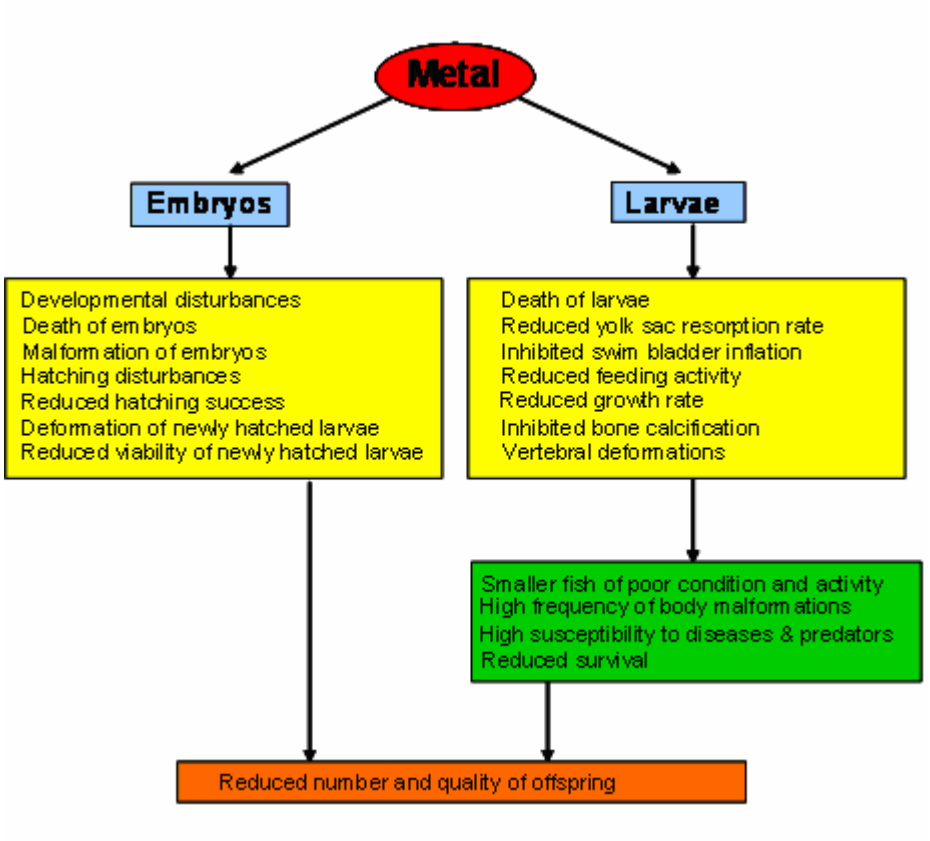

Figure 7. Toxic effects of metals on fish resulting in vertebral deformities.

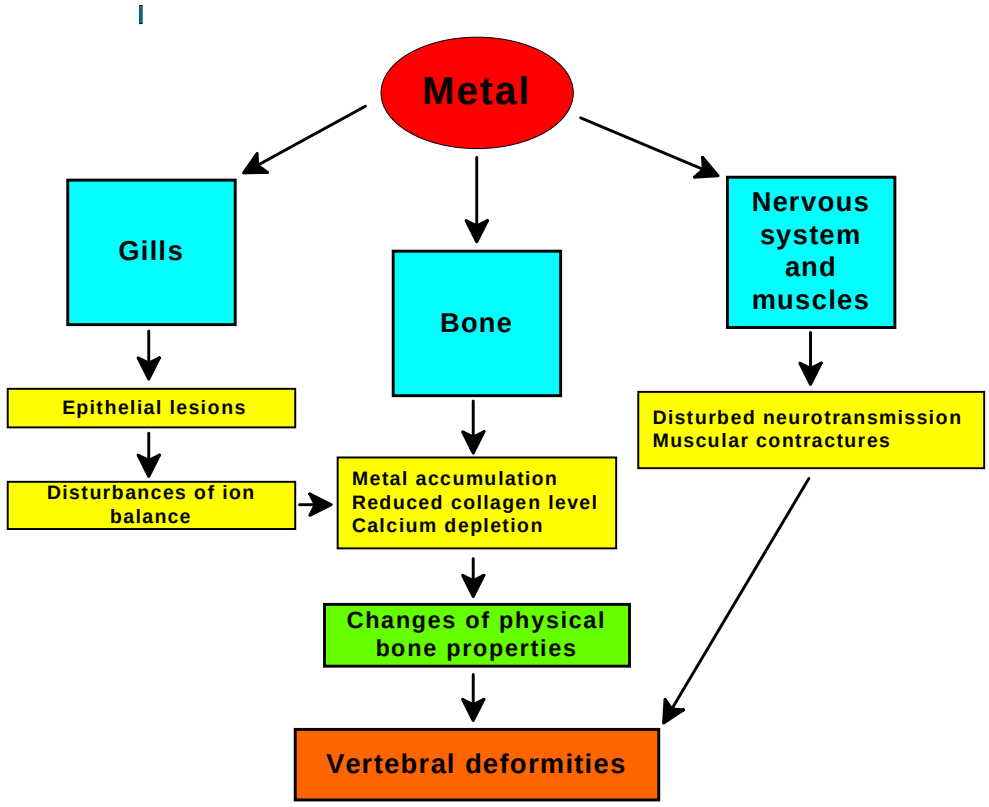

Figure 8. Scheme the effect of metals on development and growth of fish is summarized.

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