CADMIUM TOXICITY TO RUDD (SCARDINIUS ERYTHROPHTHALMUS (L.)) LARVAE AFTER SHORT-TERM EXPOSURE

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ABSTRACT. Rudd, *Scardinius erythrophthalmus* (L.), larvae aged four days post-hatch and at the very beginning of swimbladder inflation were exposed to cadmium (0.0, 0.1, 0.2, and 0.3 mg dm⁻³) at 22.0°C (\pm 0.5°C) for 24 h. Following exposure, the larvae were reared in pure water in a recirculating system at 25.0°C (\pm 0.5°C) for the subsequent nine days. Cadmium resulted in reduced larval growth and survival, retarded swimbladder inflation, and the delayed onset of exogenous feeding on live Artemia nauplii. All these effects were dependent on concentration. The differences between the effects of cadmium at the lowest and the highest concentrations were significant (P \leq 0.05). The results demonstrated the highly toxic effect of short-term exposure to 0.1-0.3 mg Cd dm⁻³ in rudd larvae.

Key words: RUDD (SCARDINIUS ERYTHROPHTHALMUS), LARVAE, CADMIUM, GROWTH, SURVIVAL, SWIMBLADDER INFLATION, EXOGENOUS FEEDING

INTRODUCTION

Water pollution resulting from various human activities has led to the considerable increase of heavy metal levels in many water bodies. In trace concentrations, these metals are normal constituents of natural waters and some of them, including copper, zinc, and manganese, are essential for fish. Cadmium is one of the heavy metals that does not play any known biochemical role in living organisms, and it also has a particularly high capacity for bioaccumulation (Svobodová et al. 1993). The cadmium present in surface waters may be either dissolved or insoluble. The dissolved forms, including the simple Cd²⁺ ion and various inorganic and organic complex ions, may be poisonous to fish (Svobodová et al. 1993). It is widely known that long-term exposure to even very low concentrations of cadmium may produce specific effects in fish, especially on their growth and survival

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(Benoit et al. 1976, Peterson et al. 1983). However, there are few studies on cadmium toxicity to fish after short-term exposure (Morsy and Protasowicki 1990, Woo et al. 1993, Melgar et al. 1997, Witeska et al. 2006).

The susceptibility of fish to heavy metal toxicity decreases with age; however, results obtained by various authors indicate that it can increase during such critical periods in larval ontogeny as swimbladder inflation, digestive tract opening, gill development, or the beginning of exogenous feeding (review in Jezierska and Witeska 2001). It is suggested that even the short-term presence of cadmium in the water during the swimbladder inflation period could affect fish larvae considerably.

In the present study, the effect of cadmium on larval rudd, *Scardinius erythrophthalmus* (L.), was examined. This cyprinid fish species, widely distributed in Europe, inhabits the littoral zone of lakes and slowly-flowing rivers (Załachowski 2000). According to Lelek (1987), this fish is sensitive to all types of pollutants, especially those of industrial origin. However, except for papers describing their content in adult specimens of this species (Svobodová et al. 1975, Perkowska and Protasowicki 2000), little is known about the effects of heavy metals on *S. erythrophthalmus*.

The aim of the present study was to evaluate the effect of high levels of ionic cadmium, which simulated a sudden, short-term discharge of this metal into the aquatic environment, on *S. erythrophthalmus* larvae. The effects of cadmium on their growth, survival, and ontogenetic rate (time of swimbladder inflation and commencement of exogenous feeding) were assessed.

MATERIAL AND METHODS

FISH AND EXPERIMENTAL PROCEDURES

Yolk sac *S. erythrophthalmus* larvae at an age of four days (*i.e.*, 96 h post-hatch) were used in a ten-day experiment under laboratory conditions. The experiment was comprised of two consecutive phases: cadmium exposure (D1) and larval rearing (D2-D10). The larvae were obtained from a commercial hatchery and were the pooled progeny of several females and males. They were stocked into eight 10 dm³ glass aquaria filled with experimental cadmium solutions at 22.0°C (\pm 0.5°C). The initial stocking density was 200 larvae aquarium⁻¹. The initial mean total length and mean wet body weight of the fish were 5.64 \pm 0.22 mm and 0.98 \pm 0.08 mg, respectively.

The larvae were exposed to cadmium for 24 h. The nominal concentrations of this metal were 0.0 mg dm⁻³ (control group), 0.1, 0.2, and 0.3 mg dm⁻³ (each in duplicate). The solutions were made using pure cadmium chloride (CdCl₂ × 2 H₂O, P.P.H. Polskie Odczynniki Chemiczne, Gliwice, Poland). During cadmium exposure, gentle aeration was applied directly in the aquaria with airstones.

To terminate cadmium exposure, approximately 90% of the experimental solutions were siphoned out. Simultaneously, pure water originating from the recirculation system began to be supplied to the aquaria at a constant rate of 11 dm³ h⁻¹. Within the next 24 h (D2), the water temperature in the aquaria was gradually increased to the target level of 25.0°C (\pm 0.5°C). Throughout the second phase of the experiment (D2-D10), the dissolved oxygen content in the aquaria was maintained at about 90% of air saturation. The total ammonia and nitrite concentrations were below 0.2 and 0.02 mg dm⁻³, respectively, and pH was 7.8-8.3.

Larval exogenous feeding was begun 21 h after the start of cadmium exposure. The fish were given freshly hatched, live Artemia nauplii (EG grade, INVE Aquaculture B.V., Belgium) in a quantity considered to be *ad libitum*. After the completion of cadmium exposure (*i.e.*, from D2 onwards), the larvae were fed nauplii *ad libitum* four times a day at 08:00, 11:00, 14:00, and 17:00.

The aquaria were artificially illuminated for 12 h day⁻¹ (08:00-20:00). Fluorescent tubes provided a light intensity of 700 lx at the water surface. Dead fish were removed and the bottoms of the aquaria were cleaned every morning.

MEASUREMENTS AND DATA ANALYSIS

The total length (TL, ± 0.001 mm) and wet body weight (BW, ± 0.01 mg) of individual fish were measured on the first (D1) and last (D10) days of the experiment. On D10, the length of the posterior chamber of the swimbladder was also measured using a stereoscopic microscope (to the nearest 0.001 mm at a magnification of 100 x). The initial fish sample consisted of 25 larvae. All final samples comprised 25 individuals per aquarium (*i.e.*, 50 per treatment group). At the termination of the experiment, all fish in each aquarium were counted in order to calculate the final survival rates.

At the onset of the experiment, the percentage of larvae with partially inflated swimbladders was determined (n = 50). From D1 to D10, the percentages of larvae with inflated swimbladders and those with Artemia nauplii present in their gut were

assessed daily. A group of 25 fish was selected from each aquarium, lightly anaesthetized with CO₂, and then observed under a stereoscopic microscope at a magnification of 100 x. After observations, they were returned live to their aquaria.

Duncan's multiple range test was used to compare the significance of the differences in larval TL, BW, and the length of the posterior chamber of the swimbladder among experimental groups. Survival percentages, percentages of larvae with inflated swimbladders and of those with Artemia nauplii present in their gut were normalized using angular transformation (Sokal and Rohlf 1969). The level of significance was set at $P \le 0.05$.

RESULTS

GROWTH

The maximum mean larval final size of 9.02 mm TL and 9.56 mg BW was recorded for the control group, but no significant ($P \le 0.05$) differences in the final TL values were found between this group and those exposed to cadmium at 0.1 and 0.2 mg dm $^{-3}$ (Table 1). Larvae growth was significantly slowest at the highest cadmium concentration of 0.3 mg dm⁻³ at which they reached 8.09 mm TL and 6.87 mg BW.

TABLE 1

to different cadmium concentrations					
		Cadmium concentration (mg dm ⁻³)			
Parameter	Ν	0.0	0.1	0.2	0.3
Initial total length (mm)	25	5.64 ± 0.22			
Final total length (mm)	50	9.02 ± 0.39^{a}	8.88 ± 0.40^{a}	8.76 ± 0.57^{a}	8.09 ± 0.82^{b}
Initial body weight (mg)	25	0.98 ± 0.08			
Final body weight (mg)	50			8.22 ± 2.00^{b}	
Final survival rate (%)	400	97.7 ^a	86.7 ^b	76.2 ^c	62.7 ^d

Initial and final characteristic of *S* erythronhthalmus larvae exposed

N – number of measurements; Length and weight data are presented as mean \pm SD. Data in rows with different superscripts are significantly different at $P \leq 0.05$.

SURVIVAL

Throughout cadmium exposure, only in the control group did fish losses not occur. At the end of D1, larval survival rates calculated based on the number of dead specimens found were then 100.0, 98.5, 95.0, and 90.3%, respectively for the consecutively increasing

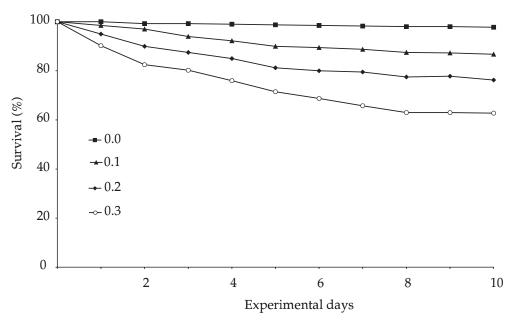


Fig. 1. Daily survival rates of *S. erythrophthalmus* larvae exposed to cadmium at concentrations of 0.0, 0.1, 0.2, and 0.3 mg dm⁻³.

cadmium solutions (Fig. 1). All these values differed significantly, as did the final survival rates, from the maximum of 97.7% recorded for the control group and the minimum of 62.7% for the group exposed to the highest cadmium concentration (Table 1). Most of the dead larvae did not have inflated swimbladders and/or any food in their alimentary tracts.

LARVAL BEHAVIOR AND SWIMBLADDER INFLATION

At the beginning of the experiment, the posterior chamber (PC) of the swimbladder was partially inflated in 10% of the larvae (Fig. 2). Throughout D1, the fish in the control group exhibited active swimming behavior and attempted to inflate their PCs. At the same time, most of the larvae in the remaining groups stayed motionless on the aquaria bottom and only a few of them tried to swim to the water surface. At the very end of cadmium exposure (D1), 100% of the larvae in the control group had partially inflated PCs, whereas for concentrations of 0.1, 0.2, and 0.3 mg Cd dm⁻³, the respective values were 86, 78, and 74% (Fig. 2). All these values differed significantly except the last two. From D4, when the share of larvae with inflated PCs increased to 98-100% in all experimental groups, all differences became insignificant.

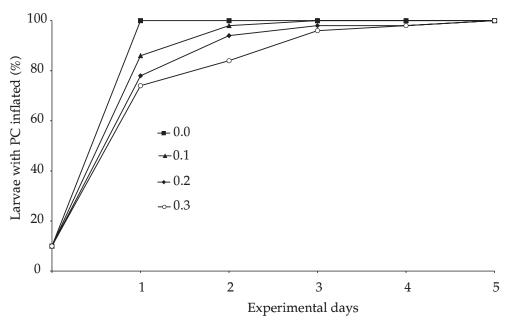


Fig. 2. Daily percentages of *S. erythrophthalmus* larvae with an inflated swimbladder posterior chamber (PC) after exposure to cadmium at concentrations of 0.0, 0.1, 0.2, and 0.3 mg dm⁻³.

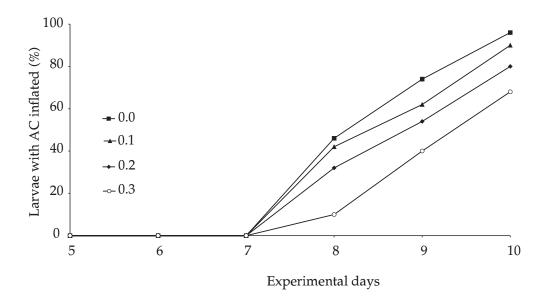


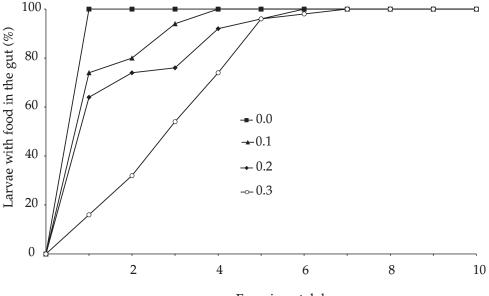
Fig. 3. Daily percentages of *S. erythrophthalmus* larvae with an inflated swimbladder anterior chamber (AC) after exposure to cadmium at concentrations of 0.0, 0.1, 0.2, and 0.3 mg dm⁻³.

The difference in mean final length of PCs between the control group (1.07 mm) and that exposed to 0.1 mg Cd dm⁻³ (1.04 mm) was not significant, likewise the difference between the latter and the group exposed to 0.2 mg Cd dm⁻³ (1.02 mm). Significantly, the lowest value (0.93 mm) was found for the group exposed to the highest cadmium concentration.

The larvae started to inflate the anterior chambers (AC) of their swimbladders on D7 in all experimental groups (Fig. 3). At the end of the experiment, the percentages of fish with inflated ACs were 96, 90, 80, and 68%, for cadmium concentrations of 0.0, 0.1, 0.2, and 0.3 mg dm⁻³, respectively. These values differed significantly except those found for the groups exposed to 0.0 and 0.1 mg Cd dm⁻³ and 0.1 and 0.2 mg Cd dm⁻³.

EXOGENOUS FEEDING

At the end of D1, 100% of the fish in the control group had Artemia nauplii present in their gut, whereas for the groups at 0.1, 0.2, and 0.3 mg Cd dm⁻³, the respective values were 74, 64, and 16% (Fig. 4). All these figures differed significantly. The differences among all experimental groups was insignificant from D6, when the share of individuals that fed successfully on nauplii reached 98-100%.



Experimental days

Fig. 4. Daily percentages of *S. erythrophthalmus* larvae with food present in the gut after exposure to cadmium at concentrations of 0.0, 0.1, 0.2, and 0.3 mg dm⁻³.

DISCUSSION

Due to discharges of heavy metals into the aquatic environment, fish may be subject to high concentrations of these toxicants for shorter or longer periods. The effects of high concentrations of heavy metals on adult fish organs, tissues, and blood parameters are well documented (review in Jezierska and Witeska 2001). Little is known, however, about the effects of these metals on fish larvae. Some data indicate that fish are more sensitive to various toxicants, including heavy metals, in the larval period of ontogeny than in earlier or later periods of their lives (*e.g.*, Hwang et al. 1995, Yin et al. 1997). It is also known that the susceptibility of larvae to metal toxicity may vary substantially during development (Rombough and Garside 1982, Wright et al. 1985, Słomińska 1998, Williams and Holdway 2000). In more developmentally advanced larvae, heavy metal uptake is much higher than in newly hatched individuals since these toxicants enter the fish mainly through functioning gills (Pärt and Svanberg 1981, Pedersen et al. 1998). In contrast with older larvae, newly hatched specimens, with their poorly-developed gills and closed mouths, appear to be more tolerant of heavy metals (Middaugh and Dean 1977, Kuroshima et al. 1993).

Cadmium is considered to be one of the most toxic heavy metals to fish (Jones 1964, Hellawell 1989). According to these authors, only mercury (Hg) and copper (Cu) are more dangerous. The cadmium concentrations of 0.1-0.3 mg dm⁻³ applied in the present study are regarded to be high for larval cyprinids such as common carp, *Cyprinus carpio* L. (Sarnowski 2003, 2004). The concentration of 0.57 mg Cd dm⁻³ was found to be lethal (96-h LC₅₀) for the larvae of this species (Sarnowski 2005).

Many studies reveal that growth is the most sensitive indicator of heavy metals intoxication in fish (*e.g.*, Rombough and Garside 1982, Woltering 1984). The effect of these metals on growth is often concentration related, and cadmium and copper are known to be the most powerful growth inhibitors (review in Jezierska and Witeska 2001). The results obtained in the present study which indicate that the more sensitive measure of metal intoxication is fish body weight rather than length concur well with the data of other authors (Shukla and Pandey 1988). *S. erythrophthalmus* larvae exposed to cadmium concentrations of 0.1 and 0.2 mg dm⁻³ were of the same final total length but of significantly lower final body weight in comparison with the control group.

There are several possible explanations for fish growth inhibition under the influence of heavy metals: lower food intake (Holdway 1992), disturbances in its utilization or metabolic disruptions (Kuzmina et al. 2002), or delays of growth hormone expression (Jones et al. 2001). As suggested by Marr et al. (1996), decreased energy conversion for growth may be related to heavy metal detoxification, and this process has high energetic costs, especially as concerns metallothionein (MT) synthesis (Floriańczyk 1999). The main features of MT are its ability to bind heavy metals and its involvement in the normal homeostasis of copper and zinc metabolism (Olsson et al. 1989). However, some authors proved that cadmium can easily displace these metals and can be bound to MT (Olsson and Haux 1986, Dallinger et al. 1997).

According to Woltering (1984), survival is the second most sensitive fish response to heavy metal intoxication. Significantly reduced *S. erythrophthalmus* final survival rates were observed in the present experiment, and this phenomenon was evidently dependent on cadmium concentration. Most of the dead larvae were individuals exhibiting abnormalities in swimbladder inflation. It should be stressed that larval *S. erythrophthalmus* losses occurred not only during cadmium exposure, but also during the nine-day post-exposure period of observations. Delayed larval mortalities, induced by heavy metals, are also known from other studies. For instance, Holdway (1992) observed them in purple-spotted gudgeon, *Mogurnda mogurnda* (Rich.), and chequered rainbow fish, *Melanotaenia splendida inornata* (Cast.), that were exposed to water-borne uranium.

In the present study, the final larval survival rates ranged from 86.7% in the group exposed to 0.1 mg Cd dm⁻³ to 62.7% obtained at the highest cadmium concentration. These data indicate that *S. erythrophthalmus* is a species with a similar tolerance to cadmium as tench, *Tinca tinca* (L.), another cyprinid fish. When exposed during swimbladder inflation to the same cadmium concentrations under similar experimental conditions, the larvae of the latter attained final survival rates of 88.0 and 66.2%, respectively (Sikorska and Wolnicki 2006). However, both these cyprinids seem to be distinctly less tolerant to cadmium than *C. carpio*. The larvae of this species, exposed continuously at 22.0°C to 0.2 mg Cd dm⁻³ for 40 days, survived at a rate of 65% (Sarnowski 2005).

Swimbladder inflation with air is essential for active larval swimming; therefore, any failure in this process may have serious implications including reduced feeding activity and increased mortality (Korwin-Kossakowski 1988). Heavy metals are known to inhibit swimbladder inflation (Słomińska 1998, Słomińska and Jezierska 2000, Sarnowski 2004). The latter author observed either retardation in swimbladder inflation or a significant reduction in its size in cadmium- or copper-exposed larval *C. carpio*. In the present experiment, cadmium affected negatively larval swimming activity, including their ability to swim up to the water surface. This resulted in a considerable, concentration-dependent delay of swimbladder inflation and the reduction of the length of its posterior chamber. Such pronounced effects indicate that the process of swimbladder inflation is particularly sensitive to heavy metals intoxication.

The commencement of external feeding is recognized as the most vulnerable period in the early ontogeny of fish, as this is when they exhibit considerable sensitivity to many factors, including toxic substances (review in Kamler 1992). Various data indicate that larvae are more susceptible to metal intoxication at the beginning of exogenous feeding than yolk sac larvae or juveniles (Wright et al. 1985, Sarnowski 2003). In the present study, it was found at the end of D1 that 74% of larvae exposed to 0.3 mg Cd dm⁻³ already had inflated PCs, but only 16% of them had food present in the gut. These results suggest that cadmium intoxication is the main reason for the delay in exogenous feeding and not the lack of an inflated swimbladder PC.

In conclusion, the yolk sac of *S. erythrophthalmus* larvae proved to be highly sensitive to cadmium intoxication at a concentration of 0.1-0.3 mg dm⁻³ when exposed to this heavy metal at the beginning of swimbladder inflation over a period of only 24 h.

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STRESZCZENIE

TOKSYCZNOŚĆ KADMU DLA LARW WZDRĘGI (*SCARDINIUS ERYTHROPHTHALMUS* (L.)) PO KRÓTKOTRWAŁEJ EKSPOZYCJI

U larw wzdręgi badano krótkotrwały wpływ kadmu w stężeniach 0,0 (grupa kontrolna), 0,1, 0,2 i 0,3 mg dm⁻³ na: wzrost ryb, przeżywalność, napełnianie pęcherza pławnego i pobieranie pokarmu (naupliusy solowca). Doświadczenie składało się z 24-godzinnej ekspozycji larw na kadm w 22,0°C i ich podchowu przez 9 dni w czystej wodzie o temperaturze 25,0°C.

Końcowe długości całkowite ryb eksponowanych na kadm w stężeniach 0,0-0,2 mg dm⁻³ nie różniły się istotnie (P \leq 0,05; tab. 1). Najmniejszą długość (8,09 mm) miały ryby z grupy o stężeniu 0,3 mg Cd dm⁻³. Ostatniego dnia istotnie najcięższe były ryby z grupy kontrolnej (9,56 mg), a najlżejsze z grupy o najwyższym stężeniu kadmu (6,87 mg). Końcowa przeżywalność larw (rys. 1) w kolejno rosnących stężeniach kadmu wyniosła odpowiednio 97,7, 86,7, 76,2 i 62,7% (różnice istotne).

Na zakończenie ekspozycji na kadm, częściowe napełnienie tylnej komory (PC) pęcherza pławnego stwierdzono u 100% larw w grupie kontrolnej (rys. 2). W kolejnych rosnących stężeniach udział ryb z napełnioną PC był istotnie mniejszy – odpowiednio 86, 78 i 74%. Od czwartego dnia do końca doświadczenia udział osobników z napełnioną PC wynosił 98-100% (różnice międzygrupowe nieistotne). Napełnianie przedniej komory (AC) pęcherza pławnego zaczęło się siódmego dnia (rys. 3). Dziesiątego dnia napełnioną AC miało 96% larw z grupy kontrolnej. W stężeniach 0,2 i 0,3 mg Cd dm⁻³ udział ryb z napełnioną AC był wtedy istotnie niższy (odpowiednio 80 i 68%).

Na zakończenie pierwszego dnia 100% larw z grupy kontrolnej miało pokarm w jelicie (rys. 4). W stężeniach 0,1, 0,2 i 0,3 mg Cd dm⁻³ pokarm był obecny odpowiednio u 74, 64 i 16% ryb (wszystkie różnice istotne). Od szóstego dnia udział żerujących osobników osiągnął we wszystkich grupach 98-100% (różnice nieistotne).

Uzyskane wyniki dowodzą istnienia silnej, zależnej od stężenia, toksyczności kadmu dla larw wzdręgi. Krótkotrwałe zanieczyszczenia wód powierzchniowych tym metalem – w zakresie stężeń 0,1-0,3 mg dm⁻³ – mogą być dużym zagrożeniem dla tego gatunku w larwalnym okresie ontogenezy.