Opusc. Zool. Budapest, XXIX–XXX, 1997

On the protective role of maternal organism in amphibians

By
M. PUKY and N. OERTEL*

Abstract. The heavy metal accumulation characteristics of semi-aquatic amphibians (*Rana esculenta* complex, *Bombina bombina*) were studied to collect information on their possible use as bioindicators. Two different biotops were chosen for our investigations, an underseepage fed series of ponds at Göd, near the River Danube and the fish pond system of the Warmwater Fish Hatchery (TEHAG) at Százhalombatta, where a part of the water supply comes from the cooling water of the nearby power plant. Sample collection, storage, digestion and the atomic absorption analysis was made after Parker. A new example for the protective role of maternal organism has been found in an animal group, where it had not been proved before while comparing the heavy metal concentration of anuran ovaries and eggs, where significant differences were recognised. In most cases the heavy metal concentration of eggs was much lower, with the exception of Cu and Zn, which are known to have a coenzyme role. This way the adult organisms maximises the fitness of the future offspring. At the moment there are no data on how the metals are bound in the ovary and the eggs.

The pollution of receiving waters has dramatically increased in the last few decades and its character has also changed, containing more hazardous micropollutants including heavy metals. Because of their significance in environmental protection, the study of pollutants of anthropogenic origin (particularly heavy metals) and the use of organisms in biomonitoring have been focal points of scientific interest all over the world.

The decline of amphibians is a worldwide problem, which is in close correlation with increasing pollution (BLAUSTEIN and WAKE, 1990). Due to their considerable sensitivity to environmental changes (PAVEL and KUCERA, 1986) amphibians as bioindicator organisms were studied for 5 years. Since they are available in large numbers and in different water types, they also form an intermediate link in the aquatic food chain so they are good representatives of freshwater life. They also correspond to the late embryonic stage of higher vertebrates and undergo significant anatomical, histological and physiological changes during their development, making the formation of easily - recognisable deformities caused by environmental pollution possible (KHANGAROT et al, 1985). What is more, amphibians are not only important links between aquatic and terrestrial ecosystems but some species, e.g. the edible frog, *Rana esculenta* also serve as human food.

In spite of these facts there is only little information available on the heavy metal accumulation of amphibians. On the basis of LC1 values, some amphibian species generally exhibited equal or slightly greater sensitivity than that of being observed for the embryo-larval stages of the rainbow trout (BIRGE et al, 1980). TERHIVUO et al (1984) found

---

* *Dr. Miklós Puky* and *Dr. Nándor Oertel*, MTA Ökológiai és Botanikai Kutatóintézete, Magyar Dunakutató Állomás (Institute of Ecology and Botany of the Hungarian Academy of Sciences, Hungarian Danube Research Station), 2131 Göd, Jávorka S. u. 14, Hungary.

that the mercury concentration of common toad (*Bufo bufo*) kidneys originating from polluted areas was thirty times greater than at semi-natural control sites.

The present paper discusses the heavy metal (Ag, Cd, Cu, Fe, Hg, Zn) distribution in the eggs and ovaries of semi-aquatic, highly water-bound amphibian species (ZUG, 1978), *Rana esculenta* L. (Ranidae) and *Bombina bombina* L. (Discoglossidae).

Sites and method

Two different biotops were chosen to be investigated. A series of four ponds of about 1,800 m^2 at the Hungarian Danube Research Station 20 km north of Budapest, near the River Danube. It is alimented by an underseepage originating from gravel terraces situated above the Danube level. In former investigations it was established that its total salt content was higher than in the Danube, dominated by Ca^{2+} and HCO_3^- ions, the pH varied between 7.6 - 8.4 (DVIHALLY and KOZMA, 1966). Because of the water supply there is a small, but practically negligible current in the pond. The existence of non-point pollution sources (e.g. heavy metals) can not be excluded.

The other site is at Százhalombatta, 25 km south of Budapest, near the River Danube within the territory of a Warmwater Fish Hatchery (TEHAG). Samples were collected from 40 fish ponds of 100 - 300 m^2, individually, which can be considered as one network. The ponds get their water supply partly from the Danube, partly from the cooling water of a nearby power station. The latter also originates from the Danube, although it is warmer by 5 - 8° C. The chemical characteristics of the ponds and the Danube - including pollution - are nearly the same. There are smaller or greater currents in the ponds because the water supply is continuous. Fish farming sometimes causes extra heavy metal load by fertilising, feeding and disinfecting.

Tadpoles were caught by netting. Adult frogs and toads were collected by netting, fishing and by hand. Tadpoles were killed by 4% formalin solution, adults with ether. Species were identified in accordance with DELY (1967), developmental stages in accordance with ANGELIER and ANGELIER (1968), who differentiated ten stages (named as A, B, C, D, E, F, G, H, I) on the basis of outer morphological marks. Five to fifteen samples were collected from each locality at each sampling.

The same digestion method was used for tadpole and adult samples. After measuring the dry weights of the tissues and organs, digestion was made in teflon bombs with the mixture of 10 ml cc. HNO_3 and 5 ml cc. H_2O_2. Sample collection, storage, digestion and the atomic absorption analysis was made after PARKER (1972). Acid mixture without any samples provided control values. The atomic absorption analysis was made by a Varian Techtron AA-275 ABD type spectrophotometer: Fe and Zn were determined with flame atomisation; Ag, Cd and Cu with electrothermal atomisation using a Varian Carbon Rod Atomizer (CRA-90). The total Hg was digested at room temperature by OMANG's (1971) method. The determination was made by cold vapour technique with a Varian Model 65 type apparatus.

Results

The heavy metal distribution among *Bombina bombina* organs can be seen in Figure 1. In all cases the highest heavy metal concentration was found in the kidney (even if there is e.g. a larger Cu storage in liver due to the 1:9.27 weight ratio between the two organs).

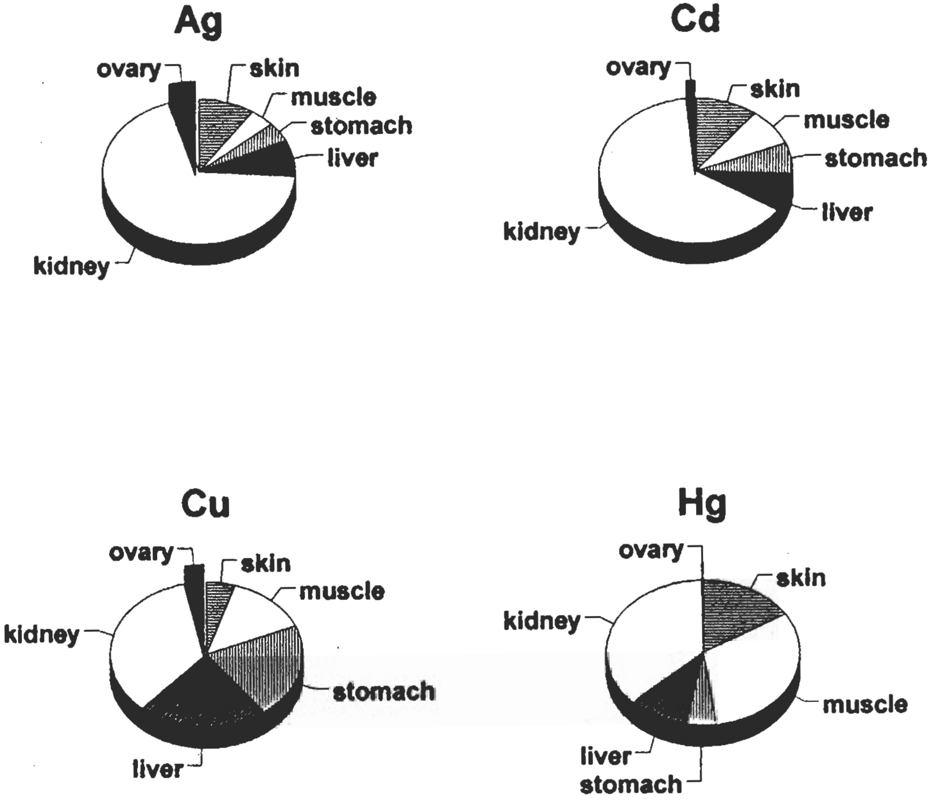

Fig. 1. Percentage distribution of heavy metals in *Bombina bombina*

Table 1. Mean heavy metal concentration of Rana esculenta c. ovary and eggs *(mgkg^{-1}, Hg in μgkg^{-1}).*

	Spring		Summer		Autumn	
	Göd	Százhalombatta	Göd	Százhalombatta	Göd	Százhalombatta
Ag						
ovary	1.08±0.79	50.3±7.1	30.0±18.1	94.3±4.8**	24.1±31.1	171±97***
eggs	0.59±0.35	0.00	7.26±4.99	47.1±8.2	3.52±3.17	81.6±37.8**
Cd						
ovary	13.5±1.1	77.0±38.2	114±47*	100±7	41.2±51.2	237±130***
eggs	8.78±0.71	0.11±0.08	18.8±6.4	50.8±12.3	8.99±0.09	69.6±62.9
Cu						
ovary	6.89±0.69	0.00	1.9±3.81	3.64±2.43	6.49±3.92	7.04±2.79
eggs	11.5±2.1	16.5±12.7	72.3±15.6***	2.07±3.93	14.3±2.1	5.95±2.86
Fe						
ovary	71.2±49.2	279±10	335±240	150±144	279±287	389±69
eggs	122±56	78.2±34.7	243±122	313±234	143±36	214±167
Hg						
ovary	17.2±15.3	88.9±23.4*	107±24**	33.5±7.3	3.8±1.2	41.3±122
eggs	3.68±3.29	35.2±11.3	4.27±1.41	13.5±6.2	0.00	11.3±4.9
Zn						
ovary	52.2±7.9	541±659	549±182	590±490	102±31	261±222
eggs	2736±446***	2229±1126***	2427±1691***	2736±550***	2084±1324***	1798±105**

Göd = Semi-natural ponds at Göd, Százhalombatta = fish ponds at Százhalombatta * ** *** significant difference at p = 5%, 1%, 0.1% level

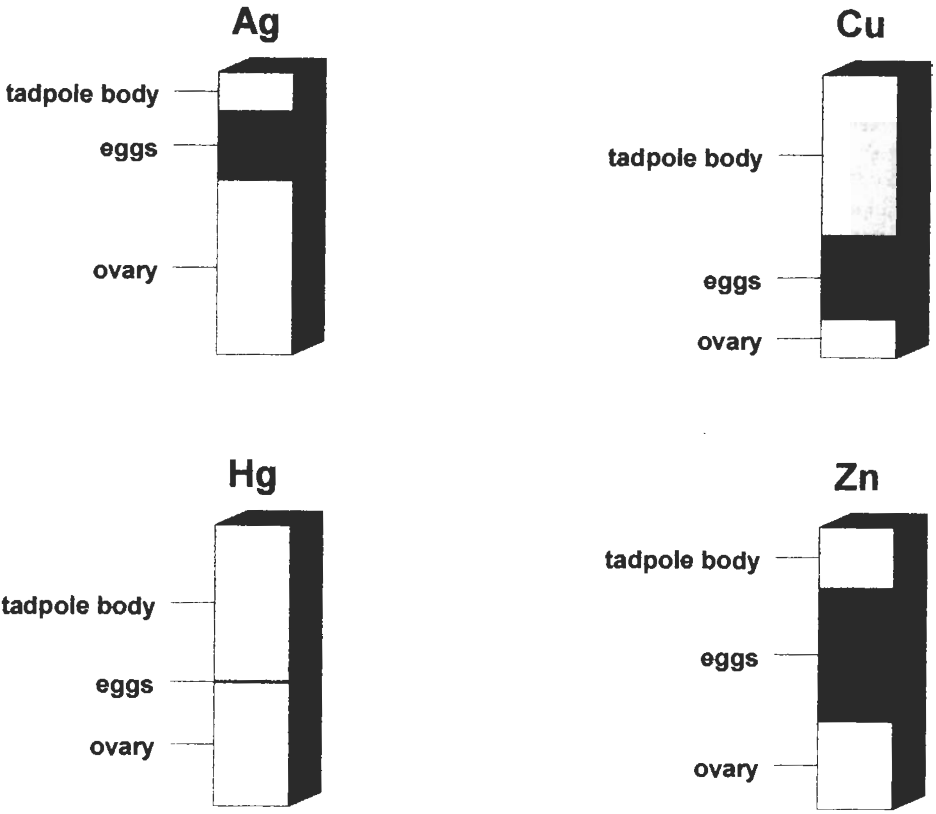

Fig. 2. Heavy metal concentration changes during the development of *Bombina bombina* (ovary, eggs, stage C tadpole) at Százhalombatta.

The Hg concentration of muscle was significantly high. In general, the heavy metal concentration of ovary can be considered as relatively moderate.

There is a close relationship between the heavy metal concentration of *Rana esculenta* ovary and eggs (Table 1). Their concentrations often changed in the same direction between the seasons, which indicates a close physiological relation. With the exception of copper at Göd and zinc at both sites, the metal concentration was generally higher in the ovary. In mammals, high concentration of zinc was detected to lower the toxicity of cadmium (SENDELBACH et al, 1989). Besides, maternal metallothioneins are known to change the heavy metal distribution completely between the maternal organism and the offspring and ovary can act as a barrier (BRUS et al, 1995).

The hypothesis was checked again in 1987. Similarly to 1984, the relative concentrations of four heavy metals in the eggs remained low in 1987 though the concentration of some metals (Cd, Cu) increased several times in the water.

Besides eggs and ovary, the heavy metal concentration of tadpoles was also monitored. Figure 2 demonstrates how development affected the heavy metal concentration of *Bombina bombina*. This species had a more diverse metal distribution than *Rana esculenta c.*, which only had higher zinc concentration in the eggs than in the tadpole body of an early developmental stage (C). GRILLITSCH and CHOVANEC (1995) draw similar conclusions while investigating the Cd, Cu Pb and Zn concentrations of *Rana ridibunda*, *R. dalmatina* and *Bufo bufo*. They also found higher Zn and lower Cd and Cu concentrations in the eggs. *Bombina bombina* eggs seem to have a significantly low mercury concentration. There was a gradual decrease in silver concentration, just the opposite of what was measured with copper. The role of zinc in the embryonic phase is emphasised further by the presented results. The actual heavy metal concentration of the tadpoles are greatly influenced by the larval ecotype (WARINGER-LÖSCHENKOHL, 1988).

Discussion

Every parent tries to maximise its offspring's fitness, even through ways, which can be harmful for itself by lowering its own chances of survival if the survival chances of the offspring are raised to a greater extent (STILTING, 1992). During the investigation of the heavy metal accumulation in amphibians a phenomenon in agreement with this theory has been recognised. Having no hair or feathers heavy metals burden e.g. the kidney, liver, gonad and skin (BAUDO, 1976; SUZUKI & TANAKA, 1983; SUZUKI et al, 1983; TERHIVUO et al, 1984). After the eggs hatch, heavy metals could affect the skeletal system, growth and development, changes in the pigmentation and behaviour. These effects are probably caused by the inhibition of collagen synthesis, general membrane dysfunction and the osmotic regulatory system (BIRGE et al, 1983; CHANG et al, 1973; KHANGAROT et al, 1985).

There was a close relationship between the heavy metal concentration of *Rana esculenta* ovary and eggs. Their concentrations changed often in the same direction between the seasons which refers to a close physiological connection, with the exception of copper at Göd and zinc, ovary concentration can generally be said higher. The fire-bellied toad, *Bombina bombina* exhibited the same characteristics, Cu, Zn (and Fe) concentrations were higher in the eggs while others (i.e. Hg) were lower. The reason of the high Zn (and Cu) content in the eggs might be the higher quantity of enzymes with Zn (and Cu) coenzyme in the embryonic life. This is supported by the fact that all of the metals except Zn had a higher concentration in larval stage C than they had in eggs.

We followed the larval development and could conclude that with the exception of Cu and Zn in *Rana esculenta*, Ag and Zn in *Bombina bombina*, the heavy metal concentration of the tadpoles of the next developmental stage were higher than those of the eggs. Maternal organism may have a protective effect against heavy metals and its discontinuance can result in the increase of the concentration after hatching.

REFERENCES

1. ANGELIER, E. & ANGELIER, M. L. (1968): Observations sur la development embryonnaire et larvaire de Rana temporaria L. (Batrachien, Anoure). – Ann. Limnol., 4: 113-131.

2. BLAUSTEIN, A. R. & WAKE, D. B. (1990): Declining amphibian populations. A global phenomenon? – Trends Ecol. Evolut., 5: 203-204.

3. BAUDO, R. (1976): Heavy metals concentrations (Cr, Cu, Mn and Pb) in tadpoles and adults of Rana esculenta L. – Mem. Ist. Ital. Idrobiol., 33: 325-344.

4. BIRGE, W. J., BLACK, J. A., WESTERMAN, A. G. & RAMEY, B. A. (1983): Fish and amphibian embryos. - A Model system for evaluating teratogenicity. – Fundam. Applied Toxicology, 3: 237-242.

5. BRUS, R., KOSTRZEWA, R. M., FELINSKA, W., PLECH, A., SZKILNIK, R. & FRYDRYCH, J. (1995): Ethanol inhibits cadmium accumulation in brains of offspring of pregnant rats that consume cadmium. – Toxicol. Lett. 76(1): 57-62.

6. CHANG, L. W., REUHL, K. R. & DUDLEY, A. W. (1973): Effects of methylmercury chloride on Rana pipiens tadpoles. – Environm. Res., 8: 82-91.

7. DELY, O. Gy. (1967): Kétéltűek. – Fauna Hungariae, 20:1-80.

8. DVIHALLY, ZS. T. & KOZMA, V. E. (1966): Beiträg zur Kenntnis eines kleinen Zuflusses der Donau bei Alsógöd (Ungarn). – Opusc. Zool. Budapest, 6: 109-119.

9. GRILLITSCH, B. & CHOVANEC, A. (1995): Heavy metals and pesticides in anuran spawn and tadpoles, water and sediment. – Toxicol. Environm. Chemistry, 50: 131-155.

10. KHANGAROT, B. S., SEHGAL, A. & BHASIN, M. K. (1985): "Man and Biosphere". - Studies on the Sikkim Himalayas. Part 5. Acute toxicity of selected heavy metals on the tadpoles of Rana hexadactyla. – Acta Hydrochim. Hydrobiol., 13: 259-263.

11. OMANG, S. H. (1971): Determination of mercury in natural waters and effluents by flameless atomic absorption spectrophotometry. – Analyt. Chim. Acta, 53:415.

12. PARKER, C. R. (1972): Water analysis by atomic absorption spectroscopy. – Varian Techtron Pty. Ltd., Springvale, 78 pp.

13. PAVEL, J. & KUCERA, M. (1986): Cumulation of heavy metals in frog (Rana esculenta). – Ekol. (CSSR), 5: 431-440.

14. SENDELBACH, L. E., BRACKEN, W. M. & KLAASSEN, C. D. (1989): Comparisons of the toxicity of $CdCl_2$ and Cd-metallothionein in isolated rat hepatocytes. – Toxicology, 55(1-2): 83-91.

15. STILTING, P. D. (1992): Ecology. – Prentice-Hall, Inc. London, pp. 597.

16. SUZUKI, K. T. & TANAKA, Y. (1983): Induction of metallothionein and effect on essential metals in cadmium-loaded frog Xenopus laevis. – Comp. Biochem. Physiol. C., 74: 311-317.

17. SUZUKI,K.T., TANAKA Y. & KAWAMURA, R. (1983): Properties of metallothionein induced by zinc, copper and cadmium in the frog, Xenopus laevis. – Comp. Biochem. Physiol. C., 75: 3-37.

18. TERHIVUO, J., LODENIUS, M., NUORTEVA, P. & TULISALO, E. (1984): Mercury content of common frogs (Rana temporaria L.) collected in southern Finland. – Ann. Zool. Fennici, 21: 41-44.

19. WARINGER-LÖSCHENKOHL, A. (1988): An experimental study of microhabitat selection and microhabitat shifts in European tadpoles. – Amphibia-Reptilia, 9: 219-236.

20. ZUG, G. R. (1978): Anuran locomotion - structure and function. 2. Jumping performance of semiaquatic, terrestrial and arboreal frogs. – Smiths. Contrib. Zool., 276: 1-31.