Bull. Environ. Contam. Toxicol. (1994) 53:25–31
© 1994 Springer-Verlag New York Inc.

Environmental Contamination and Toxicology

Natural Levels of Abnormalities in the Trilling Frog (*Neobactrachus centralis*) at the Olympic Dam Mine

J. L. Read,[1] M. J. Tyler[2]

[1]Environmental Department Olympic Dam Operations, P.O. Box 150, Roxby Downs, South Australia 5725, Australia
[2]Department of Zoology, University of Adelaide, GPO Box 498, Adelaide, South Australia 5001, Australia

Received: 20 April 1993/Accepted: 1 November 1993

Frogs are more susceptible than most vertebrates to environmental contaminants. Unlike amniotes, the frog egg is not protected by a semi-impervious shell, and hence is readily exposed to pollutants. In addition, tadpoles develop in wetlands to which many noxious substances drain from the surrounding landscape. Coupled with this high exposure rate, frogs are also very sensitive to trace elements, some pesticides, heavy metals especially when coupled with exposure to low pH (Tyler 1989; Power *et al.* 1989; Browne and Dumont 1979) and ionizing radiation (Tyler 1989; Nishimura 1967). Frogs commonly exhibit discernible deformities following exposure to teratogenic contaminants, and therefore are valuable indicators of the existence of noxious substances in the environment (Tyler 1989; Emery and McShane 1980; Henle 1981; Hazelwood 1970). Tadpoles and frogs have also been used extensively in radiobiological surveys (Ahmad 1976; Giannetti and Zubrzycki 1990; Blinov 1962).

Australian deserts support a surprisingly high concentration of frogs considering the harshness of the environment (Morton *et al.* 1993; Read 1992). The abundance and ease of sampling of frogs, along with their sensitivity to environmental contaminants, makes them ideal organisms for environmental monitoring in the Australian arid zone. The study of abnormalities in frogs has therefore become an integral part of the Environmental Management Programme of the Olympic Dam Operations (ODO) copper-uranium-gold-silver mine in northern South Australia (Fig. 1).

The Trilling Frog (*Neobatrachus centralis*) is the only frog species which has been recorded at Olympic Dam (Read 1992). These frogs emerge *en masse* to breed following major rain events which occur sporadically in central Australia and hence sampling occurs opportunistically. *N. centralis* spend the majority of their life underground and usually only emerge following at least 5 mm of rain (Read 1992). Although the ecology of this species is poorly understood, it is known that adults are nocturnal, feed predominantly on termites and other insects, and are seldom recorded large distances from suitable moist burrowing sites (pers obs). It is therefore likely that these frogs, are relatively sedentary, thus enhancing thier value as indicator organisms. A pilot survey in 1989 documented frog deformity levels

Correspondence to: J. L. Read

comparable to those found at undisturbed sites in Australia and in other countries (Read and Tyler 1990). Here we document a larger study conducted in February and March 1992 when heavy rains provided another opportunity to survey the frog population.

MATERIALS AND METHODS

A total of 1537 frogs were collected by several different techniques around Olympic Dam during February and March 1992: 178 frogs were trapped in pitfall traps at the Pitgrid site (Fig. 1), 524 frogs were collected by hand from water bodies at the Mine, Administration Claypan, EV 308, SBS, RB4 and RB10 (Fig. 1) and 835 were collected from roads in the district. The road collected samples were separated into Roxby Downs roads, south of Olympic Dam Village, and Olympic Dam roads, north of the village. Collecting frogs as they hopped across roads at night proved to be the most effective method of sampling large numbers of frogs. Throughout the collecting period, frogs from each collection region were either retained in separate tanks for several days or collected from widely separated sites, to prevent sampling the same individuals twice. Frogs were not toe-clipped for identification because of the problems of burrowing frogs injuring their feet (Tyler *et al.* 1985) which could mimic toe clipping, and also to avoid confusion with the most common deformity; ectrodactyly.

All captured frogs were thoroughly investigated for gross external abnormalities. Normal frogs were ultimately released to their region of capture and deformed frogs were euthanised. Chi-squared tests were performed to test for significant differences in abnormality rates between samples.

Samples of 18 to 36 tadpoles were collected for radionuclide analysis from ponds at the mine, metallurgical plant and control site EV 308. The tadpoles were placed in fresh water and provided with fish food flakes for several days to clean detritus from their digestive tracts which may have contaminated the samples (Read and Tyler 1990; Baudo 1976). The tadpoles were sacrificed in chloroform and dissolved in nitric and perchloric acids. Radiochemical separation and analysis methods as specified by the Australian Nuclear Science and Technology Organisation (Brown and Ring 1988) were used. Polonium and lead are separated from a hydrochloric acid solution using a 1% solution of di-ethylammonium diethyldithiocarbamate in chloroform. Uranium and thorium are separted from an aliquat 336 solution using oxalic/perchloric acids solution and hydrochloric acid solution respectively. Concentrations of the radionuclides U 238, Th 230, Ra 226, Pb 210 and Po 210 from the prepared tadpoles and water samples from the same sites, were determined in the ODO low level radiation counting laboratory. U 238, Th 230 and Po 210 levels were determined by alpha spectorscopy on thin samples electro-deposited on stainless steel disks. Pb 210 was determined by gross beta counting of the Bi 210 daughter nuclide in equilibrium with the parent Pb 210. A radon de-emanation method was used to determine Ra 226 levels (Davey 1993).

RESULTS AND DISCUSSION

The incidence of gross external abnormalities found in frogs collected within the ODO mining lease (Administration Claypan, Olympic Dam roads, RB10, RB4 and Mine Pond) ranged from 1.1 to 3.1%. Frogs collected between 2

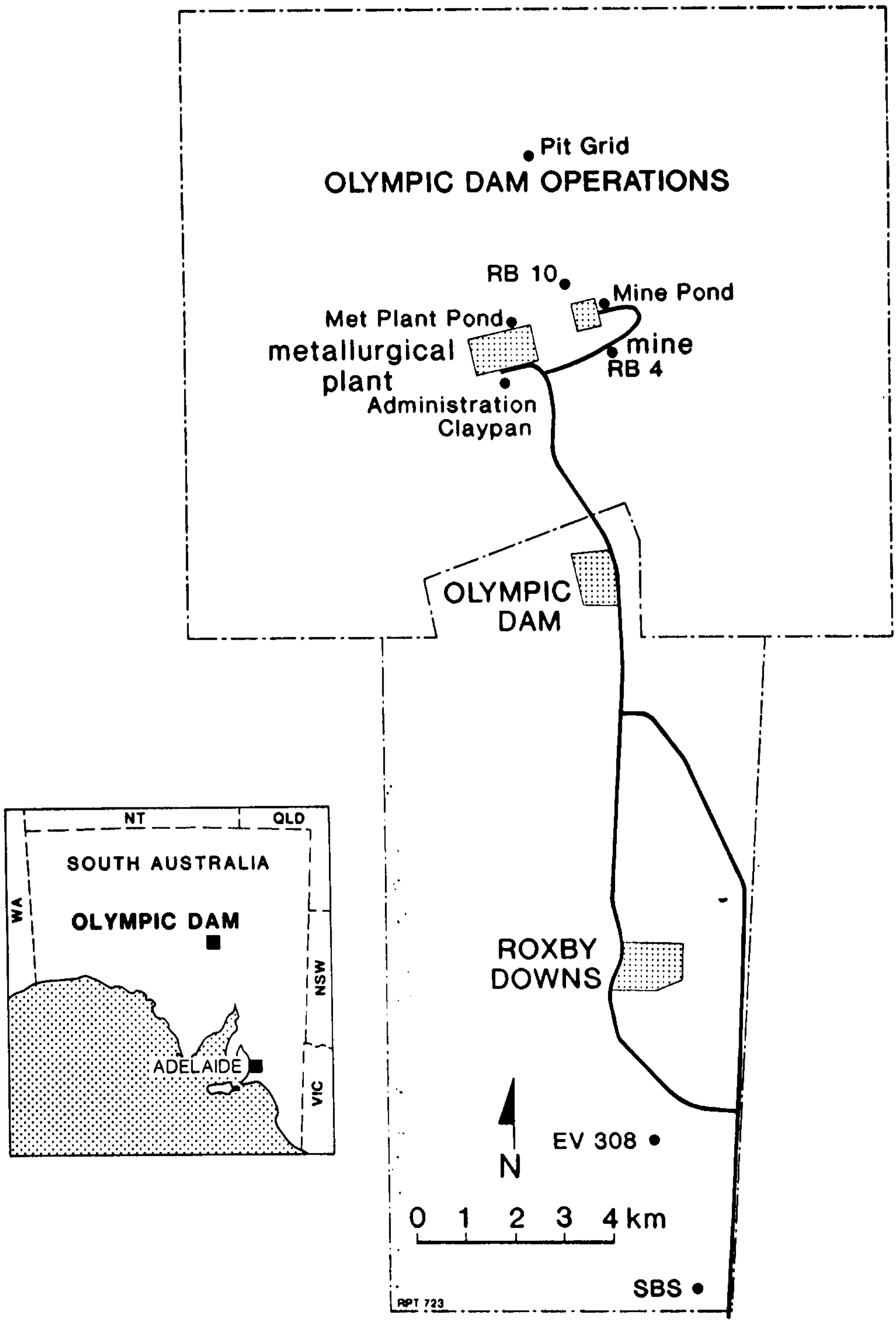

Figure 1. Location of Olympic Dam and Frog Monitoring Sites

Table 1. Details of abnormalities detected

Location	Sample Size	Incidence %	Frog Ref.	Abnormality
SBS	25	0		
EV 308	122	1.6	1	Dilation TI-III right foot
			2	Partial ectrodactyly T IV left foot
Roxby Roads	198	0.5	1	Partial ectrodactyly T IV right foot
Pitgrid	178	2.2	1	Partial ectodactyly T IV left foot
			2	Gross ectrodactyly of left foot: digits II-IV replaced by 2 stumps
			3	Ectrodactyly F I right hand, syndactyly right hand
			4	Partial ectrodactyly T IV left foot
Olympic Dam Roads	609	1.1	1	Gross truncation in bones of right hand
			2	Partial ectrodactyly T IV left foot
			3	Gross fusion of elements of right foot
			4	Partial ectrodactyly T IV right foot
			5	Partial syndactyly of base of F III with F IV of left hand
			6	Gross abnormality of left foot
			7	Partial ectrodactyly F II left hand
Administration Claypan	32	3.1	1	Partial ectrodactyly T IV left foot
RB 10	193	3.1	1	Partial ectrodactyly T IV right foot
			2	Partial ectrodactyly T IV left foot
			3	Partial ectrodactyly F II right hand
			4	Bilateral partial ectrodactyly F II both hands
			5	Partial ectrodactyly F III right hand
			6	Partial ectrodactyly FI and FII right hand
RB 4	83	2.4	1	Partial ectrodactyly T IV right foot
			2	Partial ectrodactyly T IV left foot
Mine Pond	97	2.1	1	Partial ectrodactyly of right foot: all digits truncated distal to T II
			2	Gross abnormality right foot: only T IV normal
TOTAL	1537	1.6	25	

and 10 km from the mine (Pitgrid and Roxby roads) had a 0.5 to 2.2% incidence of abnormalities. Frogs from the other locations which were at least 10 km from the ODO mining sites possessed gross abnormalities within the range of 0 to 1.6%. Most abnormalities detected (56%) involved partial ectrodactyly, or loss of distal tarsal bones, of a single digit (Table 1).

Radionuclide concentrations in the tadpoles (wet weight) and water bodies from three sites are presented in Table 2. All samples yielded very low or undetectable levels of radionuclides and there was no consistent trend between the traces detected in the water and tadpoles from the same sites.

There was no significant difference ($P > 0.5$) between the abnormality levels at the different sample sites or between sites close to and distant from the

Table 2. Levels of radionuclides in frogs and water at three sites near Olympic Dam

Sample Type	Location	Sample Size	Uranium 238 Bq/kg +/-	Thorium 230 Bq/kg +/-	Radium 226 Bq/kg +/-	Lead 210 Bq/kg+/-	Polonium 210 Bq/kg +/-
Tadpole	Mine Pond	9.27g	1.6+/-0.8	8 +/-4*	6 +/- 2	N.D.	7 +/- 2
Tadpole	Met Plant Pond	6.05g	2 +/- 1	5 +/-3*	N.D.	N.D.	9 +/- 2
Tadpole	EV 308	4.25g	N.D.	N.D.	N.D.	N.D.	16+/- 3
Water	Mine Pond	0.5L	1.31+/-0.09	1 +/-0.3*	1.48+/-0.06	3 +/-0.1	1.8+/-0.1
Water	Met Plant Pond	0.5L	0.17+/-0.03	0.02+/-0.01*	0.07+/-0.01	N.D.	0.019+/-0.007
Water	EV 308	0.5L	N.D.	N.D.*	N.D.	N.D.	0.020+/-0.007

ND = No statistical difference from background (only counting errors considered).

* Low confidence due to radio chemical losses during analysis

Counting error at 95% confidence interval (+/- 2) mine (P > 0.5). Abnormalities are a natural occurrence invertebrate populations (Tyler 1989). Genetically linked abnormalities are the building blocks from which natural selection drives the speciation process. However, the degree and incidence of deformities may be increased following exposure to various environmental insults. Significant increases in deformity rates may therefore indicate that the environment has become more hostile.

In none of the samples did the incidence of abnormalities exceed that recorded from supposedly undisturbed localities in six countries: 0-3.1% (Tyler 1989). Therefore the sites were pooled to give a meaningful sample size. The overall incidence of abnormalities in *N. centralis* at Olympic Dam was 1.6%, which was less than that detected in a pilot study at the same localities in 1989 (Read and Tyler 1990). In the pilot study abnormality rates in the sites close to the mine averaged 1.7% compared with 9% at a control site (Read and Tyler 1990). The disparity between sites was attributed to natural variation and low sample sizes, thus necessitating the pooling of samples wherever appropriate. Because the frog deformity level at Olympic Dam apparently reflects the background levels, it may be assumed that the mining activity at this site has not resulted in an elevated incidence of gross skeletal abnormalities in adults.

The dominant abnormality at Olympic Dam in 1992, was partial ectrodactyly, predominantly of the fourth toe. This supports the findings of the 1989 survey (Read and Tyler 1990) which suggested this may be a common mutant rather than being an environmentally induced deformity. Breeding studies will be required to validate this supposition.

Radionuclide levels in both tadpole and water samples are provided to contribute to world-wide knowledge of baseline levels. There are no known studies of radionuclide levels in frogs or unfiltered water samples. Water samples were not filtered due to the high affinity of radionuclides to colloidal clay particles. In addition, some radionuclides behave as colloids in near neutral (Kolthoff and Elving 1966) solutions and hence are readily filtered out of solution (Olympic Dam Operations 1990). Natural variations in radionuclide levels in soil (United Nations Scientific Committee on the Effects of Atomic Radiation 1977) probably account for the variation recorded between sites at Olympic Dam. Although there are no known benchmarks with which to compare the levels of radionuclides in tadpoles, the low levels recorded support the finding of the frog abnormality study. *N. centralis* at Olympic Dam does not appear to be accumulating or being influenced by the very low levels of radionuclides present there.

Acknowledgments. This research programme was supported by the Olympic Dam Operations Joint Venturers, Western Mining Corporation and British Petroleum. Radionuclide data were analysed by Pat Garland and John Davey of the ODO Environmental Department. Thanks go to Tamra Chapman, Mick Evans, Shane Badman and Marilyn Holden for assistance with collecting the samples. Sheri Bacon typed the manuscript.

REFERENCES

Ahmad M (1976) The effects of ionizing radiations on prometamorphic anuran tadpoles. Karachi Univ J Sci 4 (rev Suppl 1) 26-37

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

Blinov VA (1962) Sensitivity of amphibian embryos to x-rays at various stages of development. Prob Radiobiol 1: 133-153

Brown SA, Ring RJ (1988) Analytical methods for the determination of Lead 210 and Polonium 210 in feed and product materials from the Olympic Dam Operations. Report to Western Mining Corporation - Olympic Dam Operations, Australian Nuclear Science and Technology Organisation Report C102

Browne CL, Dumont JN (1979) Toxicity of selenium to developing *Xenopus laevis* embryos. J. Toxicol Environ Hlth 5: 699-709

Davey JF (1993) Report on low level radiation counting facilities at Olympic Dam Operations. Radiation Protection in Australia (in press)

Emery RM, McShane MC (1980) Nuclear waste ponds and streams on the Hanford site: an ecological search for radiation effects. Health Phys 38: 787-809

Giannetti M, Zubrzycki Z (1990) *Xenopus laevis* South African clawed toad - a potential indicator for radioactive contamination in ecological studies? In: W Hanke (ed) Biology and physiology and amphibians. Fischer, Stuttgart, pp 279-285

Hazelwood E (1970) Frog pond contaminated. Brit J Herpetol 4: 177-185

Henle K (1981) A unique case of malformations in a natural population of the Green Toad (*Bufo viridis*) and its meaning for environmental politics. Brit Herp Soc Bull 4: 48-49

Kolthoff IM, Elving PJ (1966) Analytical Chemistry of the Elements. In: Treatise on analytical chemistry Part II, Volume 6. Interscience Publishers, New York

Morton SR, Masters P, Hobbs TJ (1993) Estimates of abundance of burrowing frogs in spinifex grasslands of the Tanami Desert, Northern Territory. The Beagle: 10: 67-70

Nishimura K (1967) Abnormal formation of visual organs of amphibian larvae induced by radioactive rainwater. Mie Med J 16: 263-267

Olympic Dam Operations (1990) Environmental Radiation Annual Report June 1989-May 1990. Olympic Dam, South Australia

Power T, Clark KL, Harfenist A, Peakall DB (1989) A review and evaluation of the amphibian toxicological literature. Technical Report No 61, Canadian Wildlife Service, Headquarters

Read JL (1992) Influence of habitats, climate, grazing and mining on terrestrial vertebrates at Olympic Dam, South Australia Rangel J 14: 143-56

Read JL, Tyler MJ (1990) The nature and incidence of post-axial, skeletal abnormalities in the frog *Neobatrachus centralis* Parker at Olympic Dam, South Australia. Trans R Soc S Aust 144: 213-217

Tyler MJ (1989) Australian Frogs. Viking O'Neill, Melbourne

Tyler MJ, Davies M, Walker KF (1985) Abrasion injuries in burrowing frogs (Amphibia: Anura) from the Northern Territory, Australia. Zool Anz 214: 54-60

United Nations Scientific Committee on the Effects of Atomic Radiation (1977) Sources and effects of ionising radiation. United Nations, New York