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THE NATURE AND INCIDENCE OF POST-AXIAL, SKELETAL ABNORMALITIES IN THE FROG *NEOBATRACHUS CENTRALIS* PARKER AT OLYMPIC DAM, SOUTH AUSTRALIA

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Summary

READ, J. L. & TYLER, M. J. (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.* 114(4), 213-217. 30 November, 1990.

Samples of 315 specimens of the frog *Neobatrachus centralis* from four sites at Olympic Dam, South Australia, included 12 specimens exhibiting skeletal abnormalities of the limbs. Examination revealed a predominance amongst the abnormal specimens of partial or complete ectrodactyly, most commonly involving terminal components of the fourth toe. The overall incidence of abnormalities is comparable to those occurring at undisturbed sites in other countries.

Radionuclide levels in tadpoles from the sampled sites were very low or not detectable, and were not associated with the incidence or nature of the abnormalities there.

KEY WORDS: Skeleton, abnormalities, *Neobatrachus centralis*, radionuclides.

Introduction

Olympic Dam Operations (O.D.O.) manages a large copper-gold-uranium-silver mine at Olympic Dam, approximately 500 km north of Adelaide. Mining commenced in 1984 and the metallurgical plant there started production in August 1988.

Airborne, aquatic and biotic environments are monitored stringently for both conventional (e.g. $S0_2$, SO_3 and total particulates), and radioactive emissions by Olympic Dam Operations, in accordance with the South Australian Government approved Environmental Management Program (Roxby Management Services 1986), and the Waste Management Program, Olympic Dam Project (O.D.P. 1987). The plant and animal communities in all habitats in the Olympic Dam region are monitored regularly to determine the possible effects, if any, of the mining and processing operations on species diversity, abundance and condition. There have been no measurable effects on the environment, outside the immediate vicinity of the metallurgical plant, that can be attributed to the mining or processing operations (O.D.P. unpubl.).

In addition to the general monitoring program, more detailed studies are conducted on certain indicator organisms, such as frogs, to enhance the sensitivity of the monitoring program. Frogs are very sensitive to radiation (Emery & McShane 1980) and have proved to be useful indicators of radioactive emissions (Nishimura 1967; Tyler 1989) and trace elements (Browne & Dumont 1979). Frogs are also the most common vertebrate animals associated with claypans: regions of natural heavy metal and radionuclide accumulation. A photograph of one of the claypans at Roxby is presented by Tyler (1989, plate 3).

Following rains in the semi-arid Olympic Dam region, the frog *Neobatrachus centralis* is exceptionally common adjacent to claypans and flooded swales (O.D.O. unpubl.). The ease of capture and identification of physical abnormalities in live specimens makes it an ideal subject as a potential indicator of environmental emissions at Olympic Dam.

Here we document an initial survey of frog deformity levels at control sites where there are negligible emission levels, and at sites in close proximity to the metallurgical plant where emission levels, while remaining very low, are detectable (O.D.O. 1989[1]).

Materials and Methods

On 12.iv.1989, approximately 80 tadpoles were collected from two water bodies within 1 km of the Olympic Dam mine and metallurgical plant (Claypan, ENW5), and from a pond 16 km south of the mine (EV 308) (Fig. 1). The EV 308 site is near a continuous radionuclide and airborne emission monitoring site. It has not detected any emissions from the mine, and hence is a valid control site.

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[1] Olympic Dam Operations (1989) Environmental Radiation Monitoring Annual Report. (Report to the S.A. Govt) Unpubl.

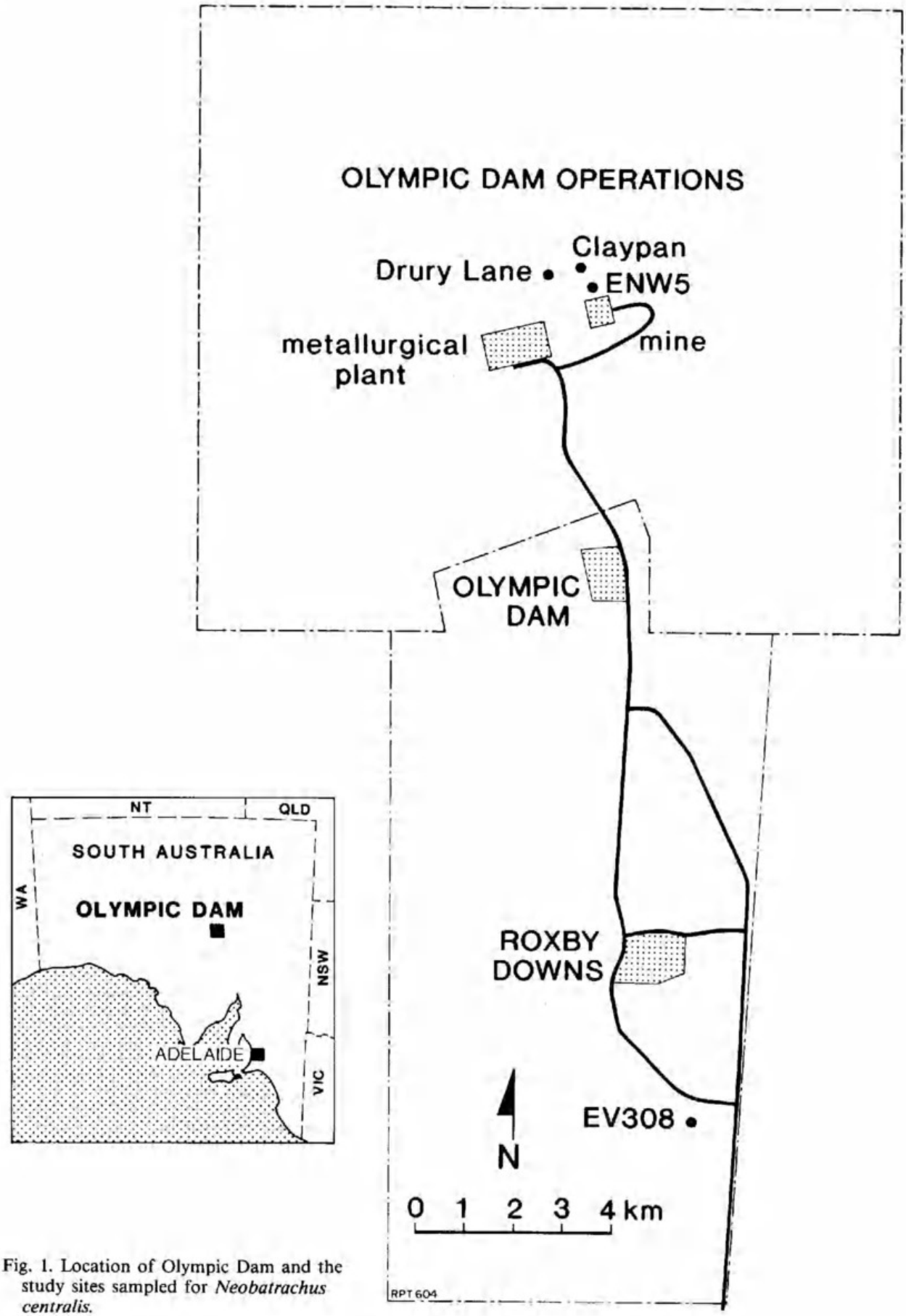

Fig. 1. Location of Olympic Dam and the study sites sampled for *Neobatrachus centralis*.

A sample of the tadpoles from each site (approximately three weeks old at the time of capture) were weighed and measured, and their stage of development recorded (Table 1). The tadpoles were housed in clean glass aquaria in the Environmental Laboratory at the Olympic Dam Village. Mud and water from each study site was used in the respective aquaria and, because individuals were in the non-feeding stage near metamorphic climax, food was not provided. Care was taken to ensure that the tadpoles were not subjected to any contaminants in the laboratory. In addition, 70 recently metamorphosed frogs were collected from a roadside near the mine (Drury Lane Site).

Following metamorphosis (i.e. complete resorption of the tail: Gosner (1960), stage 46) external features were examined under a Wild M5 dissecting microscope. Individuals with physical abnormalities were killed by exposure to 3% chloral hydrate solution and cleared and stained with Alizarin Red following the method of Davis & Gore (1947).

Five to ten metamorphosing frogs were sampled subsequently from the three water bodies and placed in a clean beaker with distilled water for several days to permit total evacuation of the gut. Earlier trials showed that freshly caught frogs and tadpoles contained considerable quantities of soil and waste matter which contributed significantly to levels of heavy metals and other contaminants. The evacuated frogs were sacrificed in ether and dissolved in Nitric and Perchloric acids. Levels of radionuclides (Po210, Pb210, Ra226, U238, Th230) were determined in the low level radiation counting laboratory of the O.D.O. Environmental Department.

Results

The average sizes, wet weights and developmental stages of the tadpoles captured at the three sites are presented in Table 1. Considerable variation existed between the sites: specimens from EV 308 (control) and Claypan being considerably heavier than those from ENW5, while individuals from EV 308 and ENW5 were more advanced than specimens from Claypan.

Of the 315 frogs examined, 12 individuals exhibited externally detectable abnormalities of the hindlegs. Further skeletal abnormalities of other elements were revealed in one when the series had been cleared and stained. The nature of the abnormalities observed in the series is documented in Table 2.

Radionuclide levels in the frogs from all three sites are presented in Table 3. These results indicate that radionuclide levels at the control site, EV 308, were slightly higher than at the sites close to the metallurgical plant.

Discussion

In all populations of vertebrate animals there are skeletal abnormalities, whose nature and incidence may be influenced by exposure to a variety of environmental insults. It is therefore important to establish baseline data when any environmental perturbation may have an impact upon that incidence.

Tyler (1989) reported that skeletal abnormalities in the limbs of vertebrates were up to 3%: this being the naturally occurring abnormalities in any population. In the case of frogs, from supposedly undisturbed localities in six countries, limb abnormalities ranged from 0 to 3.09%, but with only one sample exceeding 2%. The impact of trauma, being physical injury within the life of individuals, needs to be distinguished. It was recorded in 2742 specimens (representing seven species) from Jabiru, N.T., and was estimated at 0.19 to 0.99%.

The incidence of abnormalities at Roxby Downs (Table 2) is equivalent to 3.8%, which is close to that reported by Tyler (1989) from undisturbed areas elsewhere. There is no evidence of trauma in our

TABLE 1. *Mean wet weights and measurements of tadpoles from three study sites at Olympic Dam.*

Site	Sample size	Weight (g)	Body length (mm)	Tail length (mm)	% with hind legs stage 24 (Gosner 1960)	% that had reached stage 42 (Gosner 1960)
EV 308	16	2.6	25	29	100	60
ENW5	28	1.3	20	21	68	32
CLAYPAN	22	2.2	24	27	77	0

TABLE 2. *Details of abnormalities detected.*

Site	Sample Size	Incidence	Frog Ref.	Abnormality
ENW5	85	3.5%	1.	Brachymely of R leg. Urostyle inclined dextrally. Sacrum misshapen. Ectrodactyly of F3 of R hand.
			2.	Bilateral ectrodactyly T4 mainly involving antepenultimate phalanx which is discoid.
			3.	Ectrodactyly T4 R leg; one phalanx lost.
CLAYPAN	73	1.4%	1.	L foot with abnormal metatarsals 3-5: 3 abbreviated; 4 with distal excrescences; 5 with proximal and distal excrescences.
EV 308	89	9.0%	1.	R foot with ectrodactyly of T4; proximal phalanx dilated distally.
			2.	L foot lacking digits 1-2.
			3.	Brachymely of R leg involving tibia, tarsi and all digital elements.
			4.	L foot with ectrodactyly of T4: lacking 3 terminal phalanges.
			5.	L foot with ectrodactyly of T4: 2 terminal phalanges missing, antepenultimate phalanx broadened.
			6.	R foot as for 5.
			7.	L foot as for 5.
			8.	Brachydactyly of all digits of R foot; T4 only one lacking terminal phalanges.
DRURY LANE	71	0%		

TABLE 3. *Levels of radionuclides detected in metamorphosing frogs from three study sites at Olympic Dam.*

Site	Weight sampled (g)	Uranium — 238 Bq/g	Thorium — 230 Bq/g	Radium — 226 Spec Act/Bq/g	Lead — 210 Spec Act/Bq/g	Polonium — 210 Bq/g
EV 308 (control)	6.46	0.0003 +/- 0.0002	0.004 +/- 0.002	.004 +/- .002	0.011 +/- 0.006	0.053 +/- 0.006
ENW5	11.65	N.D.	N.D.	.0007 +/- .0005	0.001 +/- 0.002	0.005 +/- 0.001
CLAYPAN = ENW5 (n)	11.23	N.D.	0.001 +/- 0.0004	.0019 +/-.0009	0.006 +/- 0.004	0.132 +/- 0.011
STANDARD	—					

N.D. = Not detected
Deviation quoted is counting statistic

samples. Low abnormality rates at sites near the mine (0 to 3.5%) contrasted with higher rates from the control site (EV 308). Conspicuous amongst the abnormalities at Roxby Downs is the predominance of partial ectrodactyly, principally of the fourth toe. It is expressed more commonly by a unilateral reduction of the normal phalangeal formula. Although ectrodactyly is one of the most common forms of skeletal abnormality in Australian frogs, the similarity of the digital target in this sample is noteworthy. Breeding experiments will be required to determine if this is a common mutant.

Variations in weight and size of the tadpoles from the different sites is probably related to food availability, water temperature and turbidity and genetic factors rather than pollutant levels in their ponds. Richards (1962) and Sokol (1984) have also demonstrated that growth suppressing substances released by tadpoles results in slower growth rates in densely crowded tadpoles compared with tadpoles reared at lower densities.

Slightly higher levels of radionuclides at the control site compared with the mine sites is not an effect of the project but a natural phenomenon. Due to the natural variation in soil radionuclide concentrations (United Nations Scientific Committee on the Effects of Ionising Radiation 1977) geographical variation in radionuclide levels can be expected.

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