Original article

Population structure of the African Clawed Frog (*Xenopus laevis*) in maize-growing areas with atrazine application versus non-maize-growing areas in South Africa

LOUIS H. DU PREEZ[1], KEITH R. SOLOMON[2], JIM A. CARR[3], JOHN P. GIESY[4], TIMOTHY S. GROSS[5], RONALD J. KENDALL[6], ERNEST E. SMITH[6], GLEN L. VAN DER KRAAK[7] AND CHÉ WELDON[1]

[1]*School of Environmental Sciences and Development, North-West University, Potchefstroom Campus, Private Bag X6001, Potchefstroom 2520, South Africa; drklhdp@puk.ac.za*
[2]*Centre for Toxicology, University of Guelph, Guelph, ON, N1G 2W1, Canada; ksolomon@tox.uogeulph.ca*
[3]*Department of Biological Sciences, Institute of Environmental and Human Health, Texas Tech University, Lubbock, TX 79416; james.carr@ttu.edu*
[4]*Zoology Department, National Food Safety and Toxicology Center and Institute of Environmental Toxicology, Michigan State University, East Lansing, MI, 48824, USA; jgiesy@aol.com*
[5]*Florida Caribbean Science Center-USGS-BRD, Gainesville, Florida, USA; Tim_S_Gross@usgs.gov*
[6]*The Institute of Environmental and Human Health and Department of Environmental Toxicology, Texas Tech University, Lubbock, Texas, USA; esmith@ttu.edu*
[7]*Department of Zoology, University of Guelph, Ontario NIG 2W1, Canada; gvanderk@uoguelph.ca*

Abstract.—The herbicide atrazine has been suggested to cause gonadal deformities in frogs and could possibly impact on reproduction. Since the early 1960s, atrazine has been used in large amounts in maize production areas of South Africa. These areas overlap with populations of the African Clawed Frog (*Xenopus laevis*) that has a wide distribution in southern Africa and is found in most water-bodies including those where atrazine residues are detected. The aim of this study was to compare various attributes of individual- and population-level responses of *X. laevis* from maize-growing and non-maize-growing areas. *Xenopus laevis* were studied in three reference and five maize-growing sites. Sex ratio, snout-vent length, body-mass and age profiles were found to be similar for populations in maize-growing and non-maize-growing areas. Our mark-recapture data indicated that all sites had robust populations. There were no significant relationships between exposure to atrazine and any of the parameters investigated in populations of *X. laevis*.

Key words.—*Xenopus laevis,* African Clawed Frog, population characterization, atrazine.

The central "high veld" region of South Africa is the major maize production area of the country. This region experiences dry winters and has somewhat irregular summer rains. Most *Xenopus laevis* are found in natural or constructed ponds on farms. Due to surface run-off and lateral movement through the predominantly sandy soils, these ponds usually contain residues of pesticides used in production of maize and other crops. Among these are triazine herbicides, such as atrazine, a broad leaf herbicide. Since the early 1960s, atrazine and related triazines have been used extensively in maize-growing areas. Although less atrazine is now being used and is being used in combination with other chemicals, it is the most widely used herbicide in the USA (and probably the world) and has been over the last four decades (Hopenhayen-Rich *et al.* 2002). Compared to other herbicides, atrazine still is a very cost-effective product.

Recent studies in mammals and reptiles suggest that atrazine may interfere with the endocrine regulation of reproduction (Crain *et al.* 1997; Cooper *et al.* 2000; Sanderson *et al.*

2000). In addition, several field surveys have linked amphibian deformities with pesticide use (Ouellet *et al.* 1997; Sower *et al.* 2000). Recent studies suggest that atrazine exposure during larval development may affect gonadal differentiation in *X. laevis*, although the threshold concentration for this response is unclear. Tavera-Mendoza *et al.* (2002a) reported that exposure of *X. laevis* tadpoles to atrazine (21 µg atrazine/L) for 48 hr reduced testicular volume and number of testicular nest cells. When *X. laevis* were exposed to 0.1 to 200 µg atrazine/L from hatching to metamorphosis, no effects of atrazine on larval growth, developmental rate, mortality, time to metamorphosis or size at metamorphosis, were observed in females or males. However, atrazine was reported to reduce the size of the male laryngeal muscle at concentrations >1 µg atrazine/L (Hayes *et al.* 2002). Atrazine exposures as small as 0.1 µg/L were also reported to induce gonadal abnormalities in males (Hayes *et al.* 2002). Carr *et al.* (2003), using a different strain of *X. laevis*, failed to demonstrate any effects on laryngeal muscle, but animals exposed to 25 µg/L atrazine were observed to have a small but significantly increased frequency of gonadal abnormalities. There are few data on pesticide residues in the tissues of field-collected amphibians. The effects of atrazine observed in laboratory tests with *X. laevis* have yet to be observed in native amphibian populations.

Despite the wide use of *X. laevis* as a laboratory model, little work has been done on the ecology of natural *X. laevis* populations. Literature on the life history of *Xenopus* is scattered and often in old and relatively obscure sources (Tinsley & Kobel 1996). There are no long-term, field-based ecological studies of population biology, generation times, and growth profiles of *X. laevis* in their native range in Africa (Tinsley & Kobel 1996).

We report the results of a study to assess the possible effects of exposure of *X. laevis* to atrazine and other pesticides used in maize pro-

duction in its natural environment in South Africa. The objective of this study was to determine if there are differences in the sex-ratios, age, and size classes of *X. laevis* inhabiting ponds in a maize-growing area (MGA) versus non-maize-growing area (NMGA) located in the Viljoenskroon and Potchefstroom regions of South Africa. Gross gonadal, testicular histology, and laryngeal responses in frogs from the same sites were characterised and are reported in a companion paper (Smith *et al.* 2005).

MATERIALS AND METHODS

This study was conducted in the Potchefstroom and Viljoenskroon areas in the Free State and North-West Provinces, South Africa (Fig. 1). Five ponds were selected in a MGA close to Viljoenskroon based on the presence of *X. laevis* and close proximity to maize production with previous use of atrazine, terbuthylazine and the presence of residues of atrazine (see analytical method below) in the ponds. No atrazine-free ponds could be found in the Viljoenskroon area. Three reference ponds were selected in the cattle farming NMGA 50 km to the north, near the city of Potchefstroom. Reference sites were selected based on the presence of *X. laevis*, lack of maize production and absence of measurable residues of atrazine and the structurally related triazine and terbuthylazine in the water.

Water samples for atrazine analyses were collected during late winter (August 2001). At each site four 1 l water samples were collected. To ensure that the water sample was representative of the total water-body, sub-samples, one from each quadrant of the water body, were combined and, from this, two 1-l sub-samples collected in labelled 1-l solvent rinsed (acetone and hexane) glass bottles. Water samples were transported in insulated containers containing ice and stored at 4 °C until analysed. Analyses were conducted at the chemical laboratories of the Microbiology Department of the North-

West University. For the liquid/liquid extraction, the method of Yokley and Cheung (2000) was used.

The study began during August 2001. Frogs were collected using 10 baited bucket traps per site. Traps were checked after 48 h and the frogs transferred to a labelled container and transported to the laboratory. Frogs were sexed, weighed, snout-vent length measured and two phalanges of the longest toe of the right hind foot removed and fixed in Bouin's fixative for histological examination through skeletochronology (Hemelaar & Van Gelder 1980). The interpretation of the skeletochronology proved to be difficult for some frogs as they had very faint or no visible growth lines in their phalanges. Specimens where the age could not be determined with certainty were excluded.

Immediately before release, we used a cryogenic brand (cooled to -180 °C in liquid nitrogen; Daugherty 1976) to mark the abdomen of frogs with a numeral corresponding to the site. With each capturing event, the number was marked at a different angle from the lateral in order to indicate when an individual frog was collected. Population estimates were calculated according to the Jolly-Seber Stochastic estimator of population size (Donnelley & Guyer 1994).
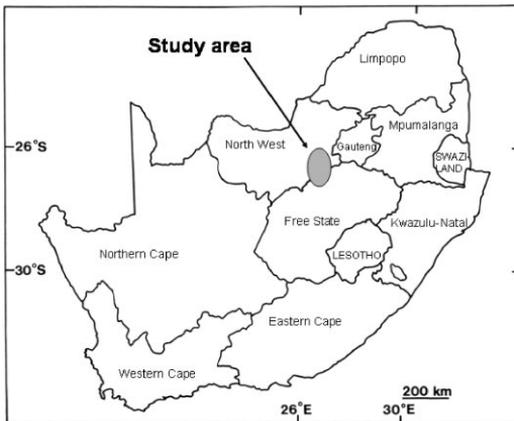

Figure 1. Map of South Africa showing the location of the study site.

Statistical analyses.—Because there are real differences between individual sites when comparing them for sex ratio and body mass, it is invalid to pool all the animals into the two groups (exposed and reference) for a χ^2 test of homogeneity. However, a simple two sample *t*-test using the male percentages at each site can be used to compare the two groups of sites. Arcsine or similar transformations were not necessary as the percentages were between 30-70% (Snedecor & Cochran 1980).

The difference in age distributions between the reference and exposed sites was tested by first pooling the animals from all sites in each group. Then the reference and exposed sites are compared by means of a χ^2 homogeneity test statistic. Unfortunately, the conventional *P*-value produced by this χ^2 test is invalid because there are real differences between sites in both the exposed and reference groups. Rather, a permutation or randomisation test (Efron & Tibshirani 1993; Manly 1997) distribution was used to calculate the *P*-value for this test: All possible ways that the 8 sites can be partitioned into two groups of three ("reference") and five ("exposed") were constructed and the pooled χ^2 homogeneity statistic computed for each. The permutation test *P*-value is simply that fraction of combinations for which the χ^2 statistic equals or exceeds the statistic actually obtained. Other methods exist for comparing reference and exposed sites with respect to relevant aspects of the age structure. For example, the mean age for each site can be calculated from individual age values (i.e., not the age classes) and used in a t-test.

RESULTS

Physical and Chemical Properties of the Sites.—Reference site R3 is a small heavily vegetated pond with clear water of medium depth. Ponds R1 and R6 are large, earth-walled dams with muddy water. Vegetation in ponds R1 and R6 was limited to the periphery and mainly consisted of stands of *Juncus excertus*

Table 1. Physical and chemical properties of reference sites (R) in non-maize-growing regions and exposed sites (E) in maize-growing regions in the Viljoenskroon region of South Africa.

Site no.	Surface area (m²)	Conductivity (µS/cm)	pH	Dissolved oxygen (mg/L)	Secci depth (mm)	Atrazine (µg/L)
R1	20 500	2.61	8.3	3.39	115	0
R3	2 000	4.77	7.3	3.39	320	0
R6	14 860	8.6	8.2	3.14	65	0
E1	7 406	186.0	10.1	6.01	Light penetrates to bottom	1.23
E3	46 076	144.0	9.1	3.96	Light penetrates to bottom	0.32
E4	2 400	116.7	8.2	4.51	Light penetrates to bottom	0.12
E6	68 722	57.5	8.4	3.88	2070	0.68
E8	2 400	54.0	10.2	4.35	Light penetrates to bottom	0.84

and *Paspalum* sp. Pond R3 was shallow and had dense stands of especially *J. excertus* throughout the pond. The exposed sites in the Viljoenskroon area varied from small to large (Table 1). All sites are heavily vegetated around the periphery with species such as *J. excertus*, *Persicaria salicifolium* and *Potamogeton pusillus*. In most sites floating *Ceratophylum demersum* was present. All exposed sites had clear water with light penetrating to the bottom except site E6 which had a Secci depth of 2070 mm.

Conductivity of reference sites was generally low and varied for exposed sites from 54 µS/m (site E8) to 186 µS/m (site E1). The pH was slightly alkaline for all sites. (Table 1).

Atrazine Concentrations.—Sites R1, R3 and R6 had no detectable concentrations of atrazine when sampled in August 2001 and were selected as reference sites in the NMGA. Atrazine concentrations measured at exposed sites at the same time varied from 0.12 µg/L in site E4 to 1.23 µg/L in site E1 (Table 1). Sites E1, E3, E4, E6 and E8 were selected as exposed sites in the MGA.

Sex Ratio.—For all the sites, except sites R3 and E1, more females than males were collected (Fig. 2). However, only in sites E4, E6, and R1 did the sex ratios deviate significantly from a 1:1 ratio (χ^2 goodness-of-fit tests, $P < 0.05$). The percentage of males collected ranged from 34% for sites E4 and E6 to 52% for site R3.

This variation in sex-ratio is unlikely to be due entirely to sampling error. A χ^2 test of homogeneity indicated that the true proportion of female and male frogs was not the same for all sites ($P = 0.03$). This does not, however, provide evidence of a systematic sex-ratio difference between the set of reference sites and the set of exposed sites. This hypothesis needs to be explicitly tested. The mean percentage of males for the reference and exposed sites were 45% and 42%, respectively. The *t*-test indicates that this difference between the two mean percentages is far from being significant ($P = 0.67$). Thus, while there were certainly indications of real site-to-site variation in the sex ratio, there is no evidence that the ratios differed between exposed and reference sites.

Body Mass.—Mean body-mass of male frogs ranged from 17 g to 29 g in reference sites and between 14.5 g and 21 g in exposed sites. In the case of female frogs, mean body-mass ranged from 35 g to 45 g at reference sites and between 21 g and 34 g at the exposed sites (Fig. 3). The mass data appear skewed toward larger values and the within-site variability of frog mass differed between sites. These characteristics make the conventional analysis of variance methods less useful. A logarithmic transformation of the mass data reduced the heterogeneity of variance although there was still some residual heterogeneity. Therefore, a nonparametric Kruskal-Wallis test was used to test if the median weights were the same for all sites. Using the two-sample *t*-test on these data, the result-

ing mean weights of female frogs at reference sites (39 g) and exposed sites (28 g) were found to be significantly different ($P = 0.02$). The observed mean mass of males over the five exposed sites (18 g) was less than the mean of the three reference sites (22 g). But in this case, the observed difference was not statistically significant ($P = 0.24$).

Snout-Vent Length.—Average snout-vent length (SVL) of male frogs varied from 53 mm to 63 mm in reference sites and from 50 mm to 58 mm in exposed sites. Females were from 66 mm to 72 mm in reference sites and from 57 mm to 69 mm in exposed sites (Fig. 4). In our data, SVL was highly correlated with mass. In fact, the relationship between log (mass) and log (SVL) is practically linear with highly significant correlations of 0.965 ($P < 0.0001$) for males, 0.971 ($P < 0.0001$) for females, and 0.973 ($P < 0.0001$) for both sexes combined. The null hypothesis that the average snout-vent length was identical across sites was again rejected for both females ($P < 0.0001$) and males ($P < 0.0001$) using a Kruskal-Wallis test. The more relevant comparison, that between reference and exposed groups, was based on the mean SVL calculated for each site. For females, the mean snout-vent lengths were 68 mm and 62 mm for reference and exposed groups, respectively. Although these means differed in a similar manner to mass (above), mean SVLs were not significant ($P = 0.07$). Similarly, for males, the mean SVL was 57 mm

for reference sites and 53 mm for exposed sites but these were not significantly different ($P = 0.25$).

Population Estimates.—The population of *X. laevis* at site R3 was approximately 360, while sites R1 and R6 had respective populations of 713 and 950 individuals. Site E8 had a small population of approximately 216 frogs. Populations at sites E4 and E1 were estimated as 354 and 370 frogs, respectively, while sites E6 and E3 had estimated numbers of 909 and 1 218 frogs respectively. Although the population sizes for the 8 sites were diverse, the mean population sizes were comparable for the reference sites (674) and the exposed sites (613). These two means are not significantly different (*t*-test, $P = 0.84$).

Age Structure.—Only 12% of frogs examined were older than three years of age. The oldest frog recorded from a reference site was a six-year old frog from site R1, while the oldest frog from an exposed site was an eight-year old frog from site E1 (Fig. 5). The mean ages differed significantly among the eight sites (Kruskal-Wallis test, $P < 0.0001$). By combining all ages of 4 years or more into a single category age class (in order to eliminate sparse categories) the relative abundance of the four age classes (1, 2, 3, and 4+ years) can also be compared using categorical data methods. A χ^2 test of homogeneity indicates highly significant dif-
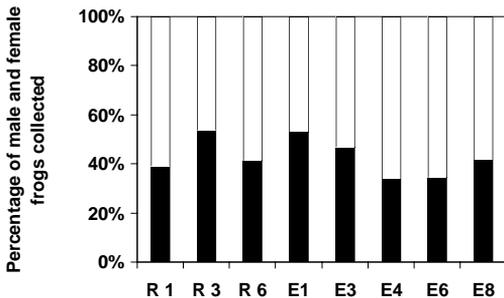

Figure 2. Histogram of the number of male (dark bar) and female *Xenopus laevis* as percentage collected from reference (R) non-maize-growing sites and exposed (E) maize-growing sites from South Africa.
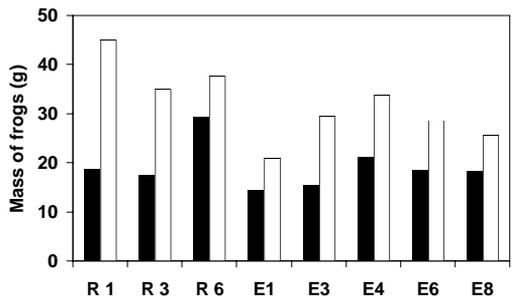

Figure 3. Histogram of the average mass of male (dark bar) and female *Xenopus laevis* collected from reference (R) non-maize-growing sites and exposed (E) maize-growing sites from South Africa.

ferences in age-structure among the eight populations ($P < 0.0001$).

A t-test based on the percentage of frogs in age-class 1 found that the means of the reference sites, 49%, and that of exposed sites, 38%, were not significantly different ($P = 0.51$). This same procedure found a similar lack of significance for the 2-year ($P = 0.82$), the 3-year ($P = 0.56$), and the 4+-year ($P = 0.55$) age classes. More efficiently, all the age-class data can be used together to test the null hypothesis that the distribution of frogs in the four age classes is the same for exposed and reference sites. For reference and exposed groups, mean ages of 1.9 years and 2.1 years, respectively were determined, and a non-significant P-value of 0.46. Consequently, while there are age differences among sites, there is no evidence of a systematic difference in age structure between exposed and reference sites.

DISCUSSION

In terms of morphometry, sex ratios, and age class, there were no significant differences between the populations of African Clawed Frogs in the exposed and the reference sites. The observed difference in female mass was not surprising since the mass of females is dependent on the state of ovarian development, which might be expected to vary with north-south location and differences in seasonality. The trend in female mass appeared to parallel the atrazine concentrations measured in the sites prior to sampling. With the exception of Tavera-Mendoza *et al.* (2002b), reports in the literature have not suggested that female frogs respond to atrazine exposures. Sufficient measures of exposures prior to sampling of frogs were not available to adequately characterise exposures during the development of these females. This apparent trend may be an artefact of sampling in sites where exposures were subsequently shown to be highly variable (Hecker *et al.* 2004). The use of atrazine and other pesticides in maize production does not appear to have adverse effects on the population structure of *X. laevis* under field exposure conditions.

The mean numbers of adult male and female frogs in each age class were not significantly different between the reference and exposed sites. Populations of *X. laevis* from both exposed and reference sites appear to do well in the study area and, given the sizes of the sites, were judged to be robust. *Xenopus laevis* have been reported to migrate between ponds, particularly when ponds dry up (Wager 1986). No migration of marked frogs between any of the sites was recorded. The rainfall for 2001 was higher than average and none of the sites dried up over the winter period. The possibility of migration cannot be excluded, but it is highly unlikely as the farmers reported that none of
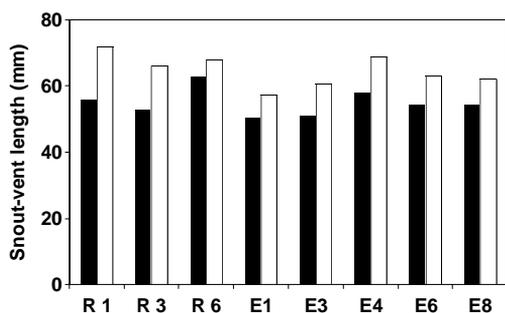

Figure 4. Histogram of the average snout-vent-length of male (dark bar) and female *Xenopus laevis* collected from reference (R) non-maize-growing sites and exposed (E) maize-growing sites from South Africa.
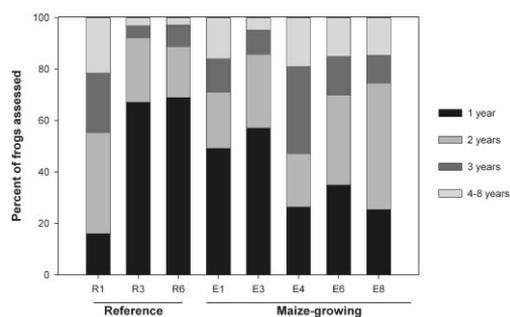

Figure 5. Histogram of the age profile of *Xenopus laevis* collected from reference (R) non-maize-growing sites and exposed (E) maize-growing sites from South Africa.

the sites studied had dried up for a number of years prior to the study.

One would expect that if atrazine had a negative effect on *X. laevis*, it would be reflected in the population dynamics of the species. Detection of atrazine in the exposed sites confirmed exposure during the previous maize-production season. However historic concentrations in these sites, and therefore the exposure history of the resident populations are not known. In the case of the reference sites, significant historical exposure is unlikely since the reference area is almost exclusively a cattle-farming area, due to topography and poor soil conditions.

The naturally occurring *X. laevis* populations in MGA might have been selected for tolerance or resistance to certain chemicals, including natural and anthropogenic endocrine disrupting chemicals. Atrazine is the most widely used herbicide in the catchment area of the exposed sites, and has likely been used in this area since 1956. This might be the case and has been suggested by Hayes *et al.* (2003) to explain the presence of robust populations of native frogs in areas of the USA where atrazine is used and is present in amphibian habitats. Resistance to atrazine has been observed in plants but this occurs at the site of action in the chloroplast, a process that is specific to plants (De Prado *et al.* 2000). For selection of resistance to have occurred, effects on survival and/or reproduction must be sufficient to create a selection pressure. As atrazine is not highly toxic to frogs in acute or chronic exposures (Howe *et al.* 1998; Allran & Karasov 2000, 2001; Diana *et al.* 2000) and does not appear to affect reproductive development to a significant extent (Carr *et al.* 2003), it appears unlikely that selection for resistance has occurred. However, if resistance has occurred, it does not appear to have affected *X. laevis* at the population level.

The size and SVL of frogs captured during the present study can be regarded as average for *X. laevis*. Under favourable conditions, females may reach a length of 130 mm (Channing 2001). In captivity *X. laevis* will survive for long periods, the greatest recorded age is 30 years and four months (Channing 2001). In the present study, the age of the oldest frog captured was eight years. Furthermore, 88% of the frogs captured were younger than three years. This could indicate high levels of predation in its natural environment, and although *X. laevis* does have a chemical defence against predators, they are frequently taken by egrets, cormorants, herons, fish eagles, terrapins, water snakes, clawless otters, and largemouth bass (Channing 2001). The key to this frog's success is probably due to the fact that it is hardy and opportunistic, and can be found in a wide variety of water-bodies from a roadside pool to large dams and rivers (Du Preez 1996).

LITERATURE CITED

ALLRAN, J.W. & W.H. KARASOV. 2000. Effects of atrazine and nitrate on northern Leopard Frog (*Rana pipiens*) larvae exposed in the laboratory from posthatch through metamorphosis. Environ. Toxicol. Chem. 19: 2850-2855.

ALLRAN, J.W. & W.H. KARASOV. 2001. Effects of atrazine on embryos, larvae, and adults of anuran amphibians. Environ. Toxicol. Chem. 20: 769-775.

CARR, J.A., A. GENTLES, E.E. SMITH, W.L. GOLEMAN, L.J. URQUIDI, K. THUETT, R.J. KENDALL, J.P. GIESY, T.S. GROSS, K.R. SOLOMON, & G.J. VAN DER

KRAAK. 2003. Response of larval *Xenopus laevis* to atrazine: Assessment of growth, metamorphosis, and gonadal and laryngeal morphology. Environ. Toxicol. Chem. 22: 396-405.

CHANNING, A.C. 2001. Amphibians of Central and Southern Africa. Protea House, Pretoria. 470pp.

COOPER, R.L., T.E. STOKER, L. TYREY, J.M. GOLDMAN & W.K. MCELROY. 2000. Atrazine disrupts the hypothalamic control of pituitary-ovarian function. Toxicol. Sci. 53: 297-307.

CRAIN, D.A., L.J. GUILETTE, A.A. ROONEY & D.B. PICKFORD. 1997. Alterations in steroidogenesis in alligators (*Alligator mississippiensis*) exposed naturally and experimentally to environmental contaminants. Environ. Health Perspect. 105 :528-533.

DAUGHERTY, C.H. 1976. Freeze branding as technique for marking amphibians. Copeia 1976: 836-838.

DE PRADO, R., N. LOPEZ-MARTINEZ & J. GONZALEZ-GUTIERREZ. 2000. Identification of two mechanisms of atrazine resistance in *Setaria faberi* and *Setari viridis* biotypes. Pestic. Biochem. Physiol. 67: 114 -124.

DIANA, S.G., W.J. RESETARITS JR, D.J. SCHAEFFER, K.B. BECKMAN & V.R. BEASLEY. 2000. Effects of atrazine on amphibian growth and survival in artificial aquatic communities. Environ. Toxicol. Chem. 19: 2961-2967.

DONNELLEY, M. & C. GUYER. 1994. Estimating population size. Pp. 183-205. *In* W.R. Heyer, W.R. Donnelet, W. McDiarmid, L.C. Hayek and M.S. Foster (eds.), Measuring and Monitoring Biological Diversity: Standard Methods for Amphibians, Smithsonian Institution Press, Washington.

DU PREEZ, L.H. 1996. Field Guide to the Frogs and Toads of the Free State. University of the Free State, 92pp.

EFRON, B. & R. J. TIBSHIRANI. 1993. An Introduction to the Bootstrap. Chapman and Hall, London.

HAYES, T.B., A. COLLINS, M. MENDOZA, N. MORIEGA, A.A. STUART & A. VONK. 2002. Hermaphroditic, demasculinized frogs after exposure to the herbicide atrazine at low ecologically relevant doses. Proc. Nat. Acad. Sci. U.S.A. 99: 5476-5480.

HAYES, T.B., K. HASTON, M. TSUI, A. HOANG, C. HAEFFELE & A. VONK. 2003. Atrazine-induced hermaphroditism at 0.1 ppb in American Leopard Frogs (*Rana pipiens*): Laboratory and field evidence. Environ. Health Perspect. 111: 568-575

HECKER, M., J.P. GIESY, P.D. JONES, A.M. JOOSTE, J.A. CARR, K.R. SOLOMON, E.E. SMITH, G. VAN DER KRAAK, R.J. KENDALL & L.H. DU PREEZ. (2004). Plasma sex steroid concentrations and gonadal aromatase activities in African Clawed Frogs (*Xenopus laevis*) from the corn-growing region of South Africa. Environ. Toxicol. Chem. 23: 1996-2007.

HEMELAAR, A.S.M. & J.J. VAN GELDER. 1980. Annual growth rings in phalanges of *Bufo bufo* (Anura, Amphibia) from the Netherlands and their use for age determination. Neth. J. Zool. 30: 129-135.

HOPENHAYN-RICH, C., M.L. STUMP & S.R. BROWNING. 2002. Regional assessment of atrazine exposure and incidence of breast and ovarian cancers in Kentucky. Arch. Environ. Contam. Toxic. 42: 127-136.

HOWE, G.E., R. GILLIS & R.C. MOWBRAY. 1998. Effects of chemical synergy and larval stage on the toxicity of atrazine and alachlor to amphibian larvae. Environ. Toxicol. Chem. 17: 519-525.

MANLEY, B. F. J. 1997. Randomization, Bootstrap and Monte Carlo Methods in Biology. 2nd ed. Chapman and Hall, London.

OUELLET, M., J. BONIN, J. RODRIGUE, J.L. DESGRANGES & S. LAIR. 1997. Hindlimb deformities (ecromelia, ectrodactyly) in free-living anurans from agricultural habitats. J. Wildl. Dis. 33: 95-104.

SANDERSON, J.T., W. SEINEN, J.P. GIESY & M. VAN DEN BERG. 2000. 2-Chloro-s-triazine herbicides induce aromatase (CYP19) activity in H295R human adrenocortical carcinoma cells: a novel mechanism for estrogenicity? Toxicol. Sci. 54: 121-127.

SMITH, E.E., L.H. DU PREEZ, A. GENTLES, K.R. SOLOMON, B. TANDLER, J.A. CARR, G.J. VAN DER KRAAK, R.J. KENDALL, J.P. GIESY. & T. GROSS. 2005. Stereological assessment of testicular cell types in *Xenopus laevis* (Anura Pipidae) inhabiting agricultural and non-agricultural areas of South Africa. Afr. J. Herpetol. 54: 69-76.

SNEDECOR, G.W. & W.G. COCHRAN. 1980. Statistical Methods. 7th ed. Iowa State University Press, Ames, Iowa, U.S.A.

SOWER, S.A., K.L. REED & K.J. BABBITT. 2000. Limb malformations and abnormal sex hormone concentrations in frogs. Environ. Health Perspect. 108: 1085-1090.

TAVERA-MENDOZA, L., S. RUBY, P. BROUSSEAU, M. FOURNIER, D. CYR & D. MARCOGLIESE. 2002a. Response of the amphibian tadpole (*Xenopus laevis*) to atrazine during sexual differentiation of the testis. Environ. Toxicol. Chem. 21: 527-531.

TAVERA-MENDOZA, L., S. RUBY, P. BROUSSEAU, M. FOURNIER, D. CYR, & D. MARCOGLIESE. 2002b. Response of the amphibian tadpole *Xenopus laevis* to atrazine during sexual differentiation of the ovary. Environ. Toxicol. Chem. 21: 1264-1267.

TINSLEY, R.C. & H.R. KOBEL. 1996. Preface. Biology of *Xenopus*. Oxford Science Publications and The Zoological Society of London. 440pp.

WAGER, V.A. 1986. Frogs of South Africa: Their Fascinating Life Stories. Delta Books, Craighall, 182pp.

YOKLEY, R.A. & M.W. CHEUNG. 2000. Analytical method for the determination of atrazine and its dealkylated chlorotriazine metabolites in water using gas chromatography/mass selective detection. J. Agric. Food Chem. 48: 4500-4507.