

Histopathological findings in Gonads of *Xenopus laevis* from Central Chile

Hallazgos histopatológicos en Gónadas de *Xenopus laevis* de Chile central

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RESUMEN

A fin de realizar un estudio histológico gonadal, se capturaron ejemplares adultos de *Xenopus laevis* en cuatro sitios de la región central de Chile. Los resultados evidenciaron ausencia de alteraciones gonadales en hembras, en cambio, los machos presentaron anomalías histológicas testiculares características de disrupción endocrina, posiblemente generadas por contaminación ambiental.

PALABRAS CLAVES: Disruptores endocrinos, anfibios, anomalías histológicas, ovocitos testiculares.

ABSTRACT

In order to perform a gonadal histological study, adult specimens of *Xenopus laevis* at four sites in the central region of Chile were captured. The results showed no alterations in gonadal female instead the males showed testicular histological abnormalities, features of endocrine disruption, possibly generated by environmental pollution.

KEYWORDS: Endocrine disruptors, amphibians, histological abnormalities, testicular oocytes.

The decline of amphibian populations has been recognized as a worldwide phenomenon. In this regard, Hayes *et al.* (2010) suggest that the main causes for this decline are due to environmental contaminants, atmospheric changes, habitat modification, invasive species and pathogens. Environmental pollutants are potential primary factors in the decline of these animals (Blaustein *et al.* 2003), since amphibians are susceptible to exposure to xenobiotics in the water such as endocrine disrupting compounds (EDCs) which can enter through their highly permeable skin (Hayes *et al.* 2006), generating endocrine disruption that may affect the fitness of amphibians. The objective of the present study was to determine the presence of histological abnormalities in gonadal specimens of *Xenopus laevis* of central Chile, which could be attributed to environmental contamination by endocrine disruptors.

80 adult specimens of *X. laevis* were caught between October

and November of 2011 (20 per site: 10 females and 10 males) from the following locations: (1) Pitama irrigation reservoir, located in Casablanca District, Valparaíso Region (33°14'S, 71°28'W), an area of agricultural use, with a history of water contamination by material runoff from a waste deposit site (Boettiger 2011), (2) La Cigüeña irrigation reservoir, located in the Municipality of Cartagena, Valparaíso Region (33° 29' S, 71° 34' W) located in an area of agricultural use, (3) Batuco wetland in the field of Puente Negro, Municipality of Lampa, Metropolitan Region (33° 16' S, 70° 48' W), with visible signs of contamination in the form of illegal dumping of solid waste and residues from a water treatment facility (Isler 2013), and (4) watersheds of Carampangue sector, District of Talagante, RM (33° 41' S, 70° 54' W) a site considered clean without signs of contamination. Frogs were captured manually using a scoop and baited funnel traps (Lobos & Measey 2002). Specimens were euthanized intraperitoneally with 1 mL of 2% lidocaine (Núñez *et al.*

2003), as recommended euthanasia for *X. laevis* (Reed 2005). Gonadal tissues were removed by dissection from males and females and the samples preserved in 3.7 % formalin. The gonads were subjected to routine inclusion techniques through an automatic tissue processor (Shandon Citadel 2000), sectioned with a 5 µm sample cut with a microtome (Leitz) and stained with hematoxylin-eosin (HE) variant Lillie Mayer (1965), periodic acid-Schiff (PAS) and Mallory (Lynch *et al.* 1972). The histological plates were evaluated using a light microscope (Leica DM 1000), under a blind study scheme, using an attached digital camera (MOTICAM 2300), and the software Motic Images Plus 2.0 for Windows (Motic China Group CO., LTD.). Descriptions of Hecker *et al.* (2006) and Wolf *et al.* (2010) were considered to differentiate between normal and abnormal conditions on gonadal histopathology, comparing it with those described by Wiechman & Wirsig-Wiechmann (2003).

The histological examination showed that the oviducts of the 40 females had normal characteristics, according to previous studies established by Hecker *et al.* (2006). In contrast, histological abnormalities were observed in male testes (Table 1): four specimens coming from the reservoir Pitama (S1) had only increased intertubular wall thickness (IWT), seven specimens from La Cigüeña reservoir (S2) presented the same findings (IWT, tubular lumen dilation (TLD) with decreased germ cells, atrophy of the seminiferous tubules (AST) (Figure 1C) and others. In addition, all the specimens from the Batuco Wetland in Puente Negro (S3) presented the same histological abnormalities and two oocytes were observed in testes of one of these animals (Figure 1D). In Talagante (S4) only two specimens had histological abnormalities (one of this with TLD, the other with AST and both with IWT).

TABLE 1. Testicular histological abnormalities observed in adult male specimens of *Xenopus laevis*, considering the capture site.

TABLA 1. Anormalidades histológicas testiculares observadas en ejemplares machos adultos de *Xenopus laevis*, considerando el sitio de captura.

SITE	TESTES ABNORMALITIES							PRESENCE OF HISTOLOGICAL ABNORMALITIES	ABSCENSE OF HISTOLOGICAL ABNORMALITIES	TOTAL
	IWT ^a	TO ^b	TLD ^c	AST ^d	DSL ^e	NSL ^f	DNS ^g			
Reservoir Pitama	4/10	-	-	-	-	-	-	4	6	10
Reservoir La Cigüeña	7/7	-	7/7	7/7	7/7	7/7	7/7	7	3	10
Batuco Puente Negro	10/10	1/10	10/10	10/10	-	-	-	10	0	10
Talagante	2/2	-	1/2	1/2	-	-	-	2	8	10
Total								23	17	40

^aIWT = Increased intertubular wall thickness

^bTO = Testicular oocytes

^cTLD = Tubular lumen dilation and reduced germ cells

^dAST = Atrophy of the seminiferous tubules

^eDSL = Degeneration of spermatogenic line

^fNSL = Necrosis of spermatogenic line

^gDNS = Decreased number of sperm

There was significant association between the presence/absence of testicular histological abnormalities linked to the capture site ($X^2 = 15.04$, $n = 40$, $P < 0.002$), whereas all males captured in S3 (a site with visible signs of contamination) showed histological gonadal abnormalities, in contrast with S4 where the smallest number of abnormalities were found (a site considered clean without records of contamination). Considering the total number of abnormalities there were no significant differences in the contribution of histological abnormalities ($X^2 = 6.39$, $n = 23$, $P > 0.090$).

Xenopus laevis has proved to be a relevant model to study

the estrogenic activity in aquatic animals. A variety of morphologic changes have been reported to occur in the gonads of *X. laevis* in response to estrogenic substance exposure (Hecker *et al.* 2006, Wolf *et al.* 2010). Its morphology and normal histology are well known, with existing standardized protocols for detecting pathologies (Wiechman & Wirsig-Wiechmann, 2003). In this regard, the males captured in the S1-S3 sites (reservoirs and wetland agricultural contaminated areas), had a higher incidence of associated histopathological endocrine disruption (Hayes *et al.* 2003, Wolf *et al.* 2010). Moreover, the presence of testicular oocytes (TO) in one of the males of S3 represents a

finding that has been described as a direct result of exposure of specimens of *X. laevis* to EDCs 17 β -estradiol (Wolf *et al.* 2010) and the atrazine herbicide (Hayes *et al.* 2006). Moreover, problems in the management of solid waste and water pollution residues have been described in the town of Batuco (Cox 2007). Also a sewage treatment plant (STP) is located in this area that discharges their effluent to the wetland and has presented operational problems (Isler 2013). Such discharges could be generating estrogenic effects, considering studies performed in rivers that receive STP discharges identified as alkylphenols (Jobling *et al.* 1996) and 17 α -ethinylestradiol, one of the active ingredients of most hormonal contraceptives (Sumpter & Jobling 2013). However, is possible to expect synergy effects generated by complex mixtures of chemicals, considering that during the capture of specimens in S3, solid residues, debris, waste water discharges and foam were observed. The findings of

this study motivate to develop a research line involving/ combining systematic observations of wild specimens of this and other amphibian species (e.g. *Calyptocephalella gayi*), chemical analyses of the aquatic environment inhabited by these animals, the use of biomarkers for the detection of estrogenic compounds and manipulative experiments in the laboratory. In relation of the emission of EDC to the aquatic environment the European Union restricted the non-ionic surfactants based on nonylphenol (NP) and placed ethinyl estradiol on a draft list of priority pollutants. In 2012 the U.S. Environmental Protection Agency has recommended alternatives to NP-based surfactants. Finally water companies in United Kingdom and the United States have engaged with scientist to better understand the problem and find solutions to it (Sumpter & Jobling, 2013). Unfortunately, in Chile the regulators haven't considered the EDC in the policy for protection of aquatic environments.

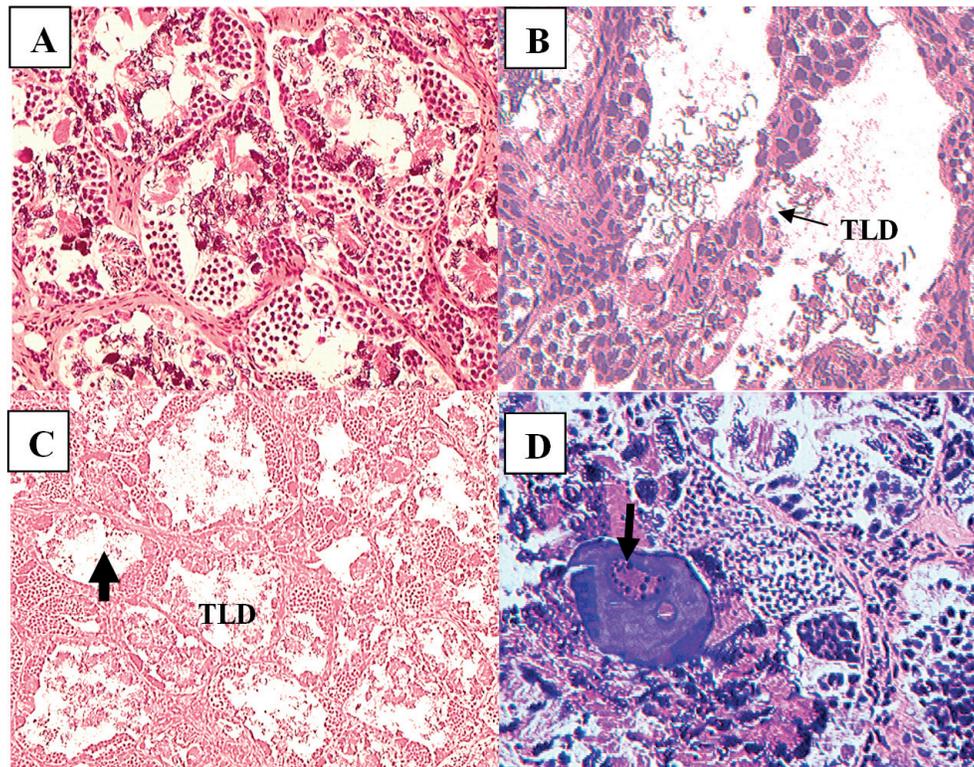


FIGURE 1. Histological sections of testes of *X. laevis* captured in Talagante (A) and the Batuco wetland in the sector of Puente Negro (B, C and D). Hematoxylin eosin staining (HE). No alterations are observed in the seminiferous tubules and interstitial tissue. 200x (A). There is dilation of the seminiferous tubules (TLD) and area of degeneration and necrosis of spermatogenic line (Arrow). 200x (B). Testicular atrophy characterized by decreased number of sperms, seminiferous tubule dilation (TLD), degeneration and necrosis of spermatogonia (arrow). 40x (C). Presence of oocyte (arrow) between seminiferous tubules. 40x (D).

FIGURA 1. Cortes histológicas de testículos de especímenes de *X. laevis* capturados en Talagante (A) y en el humedal de Batuco en el sector de Puente Negro (B, C y D). Tinción hematoxilina eosina (HE). No se observan alteraciones en los túbulos seminíferos y el tejido intersticial. 200x (A). Se observa dilatación de lumen de túbulos seminíferos (TLD) y área de degeneración y necrosis de la línea espermatogénica (Flecha). 200x (B). Atrófia testicular caracterizada por disminución del número de espermios, dilatación del lumen de los túbulos seminíferos (TLD), degeneración y necrosis de espermatogonias (Flecha). 40x (C). Presencia de ovocito (Flecha) entre conductos seminíferos. 40x (D).

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REFERENCES

- BLAUSTEIN, A.R., ROMANSIC, J.M., KIESECKER, J.M. & HATCH, A.C. 2003. Ultraviolet radiation, toxic chemicals and amphibian population declines. *Diversity and Distributions* 9: 123-140.
- BOETTIGER, C. 2011. Embalse Pitama: Jurisprudencia de daño ambiental. *Actualidad Jurídica Universidad del Desarrollo* 24: 405-423.
- COX, C. 2007. Metodología de diseño de una red de monitoreo de recursos hídricos para humedales: Aplicación en la Laguna de Batuco. Memoria de Título. Departamento de Ingeniería Civil, Universidad de Chile, Santiago, Chile.
- HAYES, T., HASTON, K., TSUI, M., HOANG, A., HAEFFELE, C. & VONK, A. 2003. Atrazine-Induced Hermaphroditism at 0.1 ppb in American Leopard Frogs (*Rana pipiens*): Laboratory and Field Evidence. *Environmental Health Perspectives* 111: 568-575.
- HAYES, T., CASE, P., CHUI, S., CHUNG, D., HAEFFELE, C., HASTON, K., LEE, M., MAI, V.P., MARJUA, Y., PARKER, J. & TSUI, M. 2006. Pesticide mixtures, Endocrine disruption, and amphibian declines: Are we underestimating the impact? *Environmental Health Perspectives* 114: 40-50.
- HAYES, T.B., FALSO, P., GALLIPEAU, S. & STICE M. 2010. The cause of global amphibian declines: a developmental endocrinologist's perspective. *The Journal of Experimental Biology* 213: 921-933.
- HECKER, M., MURPHY, M.B., COADY, K.K., VILLENEUVE, D.L., JONES, P.D., CARR, J.A., SOLOMON, K.R., SMITH, E.E., VAN DER KRAAK, G., GROSS, T., DU PREEZ, L., KENDALL, R.J. & GIESY, J.P. 2006. Terminology of gonadal anomalies in fish and amphibians resulting from chemical exposures. *Reviews of Environmental Contamination and Toxicology* 187: 103-131.
- ISLER, B. 2013. Calidad microbiológica de los recursos hídricos del sector de la laguna de Batuco en la Comuna de Lampa, Región Metropolitana. Memoria de título. Escuela de Medicina Veterinaria, Universidad Mayor, Santiago, Chile.
- JOBLING, S., SHEAHAN, D., OSBORNE, J.A., MATTHIESSEN P. & SUMPTER, J.P. 1996. Inhibition of testicular growth in rainbow trout (*Oncorhynchus mykiss*) exposed to estrogenic alkylphenolic chemicals. *Environmental Toxicology and Chemistry* 15: 194-202.
- LOBOS, G., & MEASEY, G.J. 2002. Invasive populations of *Xenopus laevis* (daudin) in Chile. *Herpetological Journal* 12: 163-168.
- LYNCH, M.J., RAPHAEL, S.S., MELLOR, L.D., SPARE P.D. & INWOOD, M.J. 1972. Tinción de los cortes. En: *Métodos de Laboratorio*: pp. 1145-1154. 2ª edición. Nueva Editorial Interamericana SA de CV México DF, México.
- NÚÑEZ, H., NAVARRO, J., GARÍN, C., PINCHEIRA-DONOSO D. & MERIGGIO, V. 2003. “*Phrynosaura manueli* y *Phrynosaura torresi*, nuevas especies de lagartijas para el Norte de Chile (Squamata: Sauria)”. *Boletín del Museo Nacional de Historia Natural, Chile* 52: 67-88.
- REED, B.T. 2005. Guidance on the housing and care of the African clawed frog, *Xenopus laevis*. Research Animals Department – The Royal Society for the Prevention of Cruelty to Animals RSPCA. UK.
- SUMPTER, J.P. & JOBLING, S. 2013. The occurrence, causes, and consequences of estrogens in the aquatic environment. *Environmental Toxicology and Chemistry* 32: 249-251.
- WIECHMAN, A. & WIRSIG-WIECHMANN, C.R. 2003. *Color Atlas of Xenopus laevis Histology*. Kluwer Academic Publishers. Boston. London. 133 pp.
- WOLF, J.C., LUTZ, I., KLOAS, W., SPRINGER, T.A., HOLDEN, L.R., KRUEGER, H.O. & HOSMER, A.J. 2010. Effects of 17 β -estradiol exposure on *Xenopus laevis* gonadal histopathology. *Environmental Toxicology and Chemistry* 29: 1091-1105.

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