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Is prevention of water pollution and eutrophication the best option to ensure axolotl survival in its natural environment?

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One considerable advantage for axolotl (*Ambystoma mexicanum*) conservation planning could be the almost 150 years of captive propagation experience around the world (Griffiths et al. 2003). The real situation is that the scientific community does not have enough knowledge about the biological and ecological conditions of axolotls in its natural habitat (Graue 1998, Zambrano 2006). The tendency of axolotl population to diminish in Xochimilco Lake is drastic and the most probable causes (pollution, exotic species predation and irrational exploitation for human consumption) have not been quantified (Zambrano 2006) or certainly related (González 2006). Neither malformations nor disease symptoms or pathogens have been detected in natural populations of *A. mexicanum* (Graue 1998, Frías-Alvarez et al. 2008, Molina 2008). However, axolotl captive maintenance is affected by cosmopolite aquaculture pathogens that have developed high resistance to many antibiotics (Negrete & Romero 1999, Uribe 2002) or asymptomatic prevalence (Frías-Alvarez et al. 2008). In fact, it is during the younger stages of development in captive populations where a higher susceptibility to microbial infections and disease occur (Ensastigue 2003). Embryos and larval stages are the most critical stages of development in axolotls and in order to secure their survival into adults successfully, management programs should be geared towards promoting their survival in natural habitat (Zambrano et al. 2007).

Reintroduction plans for axolotls have been incipient in local and international institutions (Ensastigue 2003, Griffiths et al. 2003, Bride et al. 2008). I developed the present research with the objective to evaluate survival and growth possibilities for *A. mexicanum* embryos and hatchlings in their natural habitat. My hypothesis was that differential growth rate and survival occurred between treatments and I predicted that embryos and hatchlings had a higher growth rate on the treatment of Xochimilco Lake water but I did not do any prediction about survivorship.

I conducted this research between January and March 2003 in Centro de Investigaciones Biologicas y Acuicolas de Cuemanco axolotls colony, which is contiguous to Xochimilco Lake and holds a recent lineage of *A. mexicanum* captured from their endemic natural habitat in the Valley of Mexico (Griffiths et al. 2003). I located 150 eggs from a new clutch without hormonal inducing treatment and placed 50 in each three different water quality treated aquariums: the first consisted of water from the Cuemanco canal of Xochimilco Lake which I sifted to filter insect predators, the second aquarium was filled with Acriflavine (Biomaq) antibiotic (two drops diluted with one liter of water) and the third aquarium with potable water served as control. Each aquarium was filled with 20 l of water, constantly aired and maintained with similar conditions of temperature, a constant pH of 7.5 and natural photoperiod.

In each treatment, I counted weekly survival of embryos until they hatched out, and survival of hatchlings three weeks after hatch. After hatching, I introduced aquatic plants (*Ceratophyllum demersum*) into aquariums to prevent cannibalism between axolotls. One week later I fed hatchlings with *Daphnia* sp. produced locally (approximately 160 organisms in 100 ml of water every other day). Every week, I measured total length of five hatchlings and weighed 20 hatchlings with an electronic scale (0.1 g precision). Hatchlings for these measures were obtained at random from each treatment. Statistical analysis to corroborate independence from predicted and observed data in hatch and survivorship were carried out by G test in StatView 5.0.1. To test differences in growth of hatchlings between treatments Kruskal Wallis test were carried out in Software R 2.5.1.

In all three treatments, embryos started hatching out between weeks three and four. Canal water treatment had the higher embryo hatching rate with 100%, followed by antibiotic and potable water treatments with 88% and 78%, respectively. Hatching success did not differ between treatments if I expected success of 100% ($G^2 = 1.45$, $p = 0.48$) or even 50% ($G^2 = 0.99$, $p = 0.61$). Hatchlings and embryos survival rates were very similar in each treatment (Fig. 1). Canal water treatment had the higher number of survivors with 21 hatchlings alive at the end of the experiment, followed by antibiotic and potable water treatments with 16 and 9 hatchlings alive, respectively. Survival differed between treatments if expected survival rate of 50% (G^2= 6.31, p= 0.043), according to life table developed by Zambrano et al. (2004) for laboratory conditions.

At the end of the experiment I did not register increment in the hatchlings length from antibiotic water treatment; however, in potable and canal water treatments, hatchlings length increased until three and six millimeters, respectively (Figure 2A). Growth in length differed between treatments at the end of the experiment (X^2= 6.46, p= 0.039).

On account of the little number of survivors in the potable and antibiotic treatments, it was not possible to weigh hatchlings after weeks four and five, respectively. However, data suggests that antibiotic treatment induced diminution of weight between weeks four and five, while canal water induced an increase of 0.5 g in weight in hatchlings three weeks after hatching (Figure 2B).

Surprisingly, *A. mexicanum* embryos and hatchlings showed higher survival and growth rates in canal water treatment despite its content of high levels of nutrients (Zambrano et al. 2009), heavy metals (González 2006) or organophosphorus pesticides (Robles-Mendoza et al. 2009) deposited for several years in Xochimilco Lake. Apparently, embryos and juveniles have efficient physiological mechanisms for detoxification and cellular protection against adverse effects of pollutants (González 2006, Mendoza 2009, Robles-Mendoza et al. 2009). Similar resilient response has been observed in the Xochimilco endemic fish, *Girardinichthys viviparous* (López-López et al. 2006). However, in all treatments high mortality was observed around day 40 of development, which seems to be the critical time for survival in *A. mexicanum* (Legorreta 2002, Ensastigue 2003, Zambrano et al. 2004).

Hatching rate of 100% in canal water treatment is similar to that obtained by Legorreta (2002) with the same organism densities. However, final hatchlings survival rate of 42% at canal water treatment was higher than previously obtained by Legorreta (2002) who found 30% survival rate. Legorreta (2002) did not report volume of water employed neither air condition in the aquariums, which could facilitate nutrients oxidation. Furthermore, the eggs he employed were obtained by hormonal induced lay. All of these factors differing between our methods could have affected survival results.

I observed fungus growth on embryos in potable and canal water treatments in week three. Defoliation of aquatic plants could have accelerated eutrophication in aquariums from potable and canal water treatments. Also, it could owe to lack of water change in aquariums. Weekly water changes in canal water treatment prevented fungus growth on hatchlings. Phytoplankton in canal water treatment could have given off natural antimicrobial that inhibited pathogens (Hernández-Cruz et al. 1994). Although, antibiotic water treatment prevented successfully fungus or bacteria diseases in embryos and hatchlings, this treatment had evidently lower survival and growth rates.

Furthermore microalgae from Xochimilco Lake could have provided oxygen interchange between egg membrane which optimized embryos development in canal water

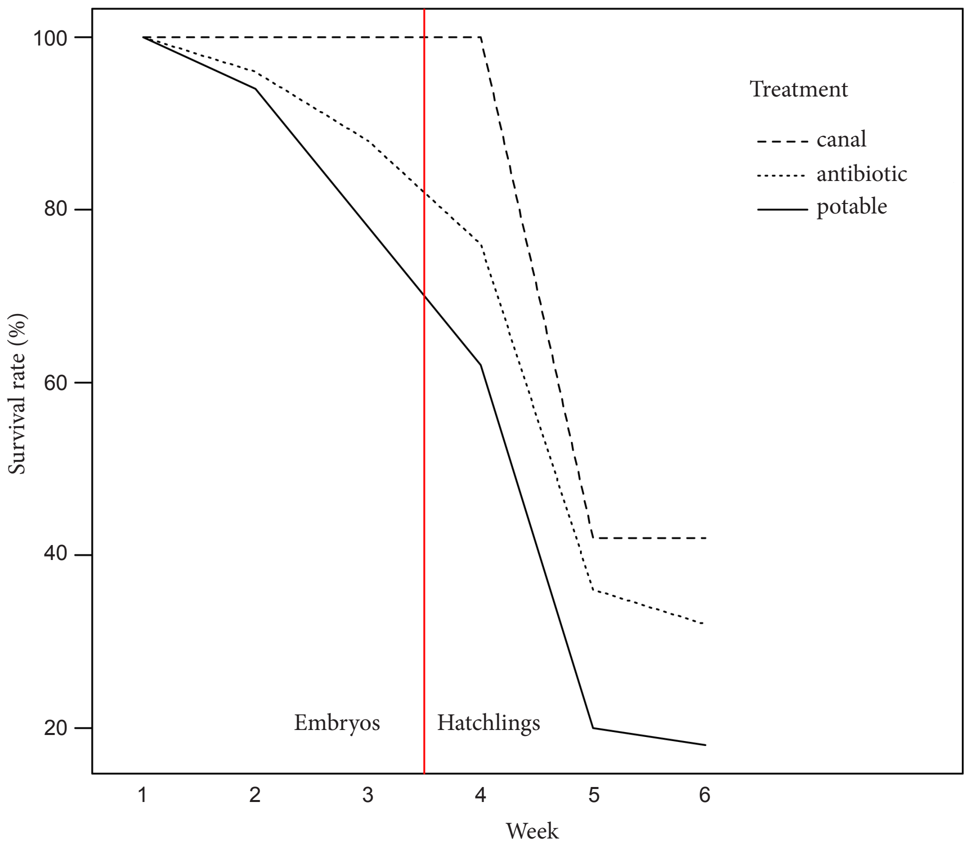

Figure 1. Survival rate of embryos and hatchlings in three treatments of water (see text).

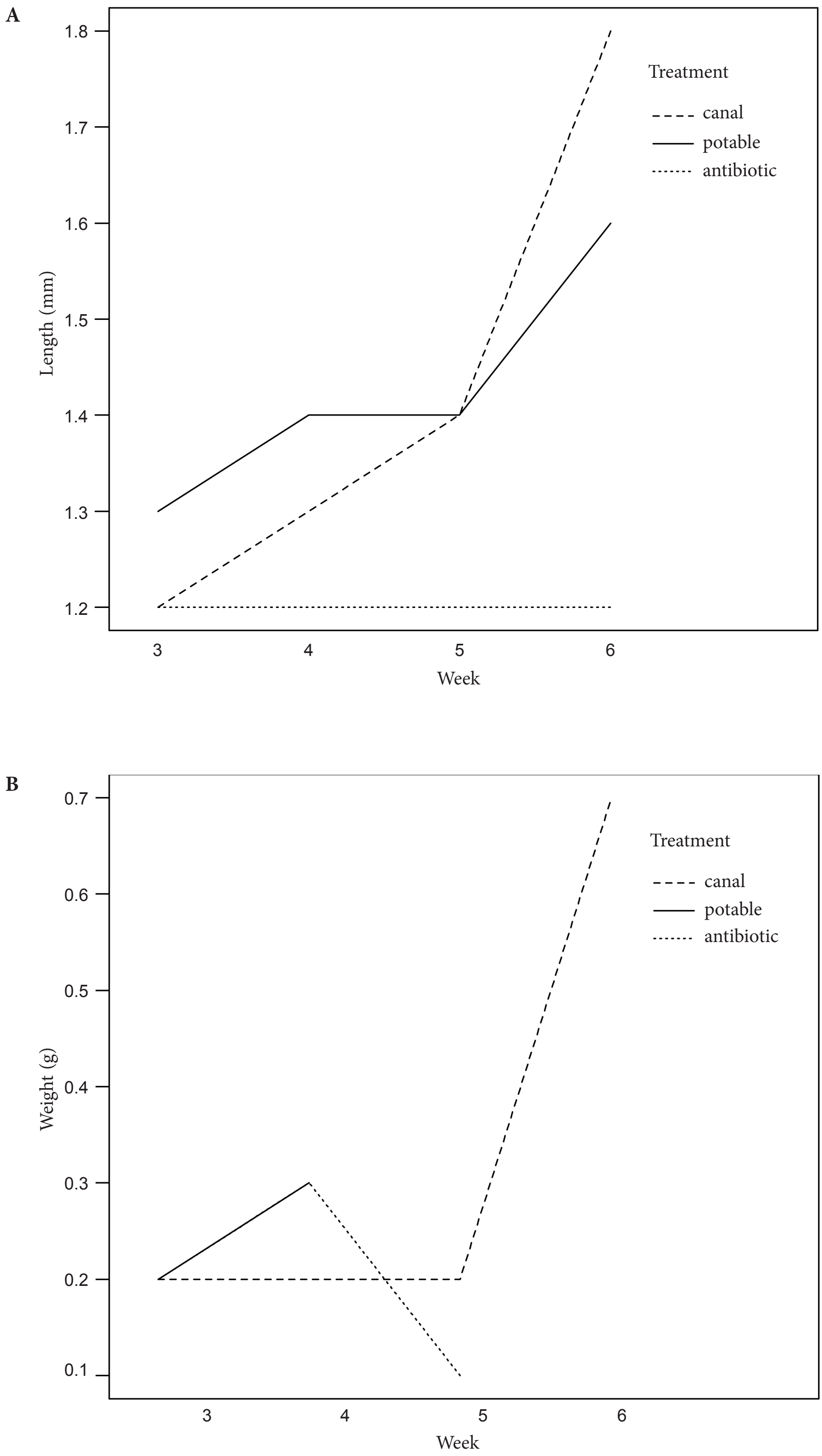

Figure 2. Changes in length (A) and weight (B) of hatchlings in three treatments of water.

treatment (Pinder & Friet 1994) and maybe provided nutrition to hatchlings (Seale 1980). However, Legorreta (2002) reported that after 10 weeks, axolotl hatchlings performed better in potable water treatment than canal water treatment and showed increase in both length (mean 4.08 vs. 2.95 mm, respectively) and weight (mean 0.97 vs. 0.69 g, respectively). Those results suggest that the time or age at which the embryos and hatchlings of axolotls are exposed to canal water (which could be polluted with organic and inorganic chemicals) could have affected axolotl's health and general condition (Robles-Mendoza et al. 2009).

González (2006) suggested after *in situ* experiments that bioaccumulation of heavy metals could be diminishing body conditions of axolotls without apparently having serious consequences on their health. On the other hand, laboratory experiments that exposed *A. mexicanum* to organophoshorus pesticides and nitrogen components have demonstrated malformations and early mortality among embryos and hatchlings (Mendoza 2009, Robles-Mendoza et al. 2009). Although these studies have found serious damage from pollutants, those are considerably in lesser degree for axolotls than for other amphibians. Although pollution resistance does not exempt the importance of restoration or improvement of water quality in Xochimilco Lake, the present data supports that the drastic diminution in population could be the effects of exotic species interfering food chain and illegal capture (Graue 1998), instead of high pollution in water as primary cause.

Amphibian conservation programs have procured pristine and innocuous conditions for maintenance of populations for future reintroduction planning (Odum & Zippel 2008). This way of conservationism has strongly criticized natural water conditions, which imply interaction with biotic and abiotic factors. Natural interactions between axolotls and their natural aquatic environment require more physiological research prior to reintroduction process. High resilient behavior of endemic species from Xochimilco also requires special attention of scientific community before extinction wipes out the species. Unfortunately, most of Mexican researches about natural populations of *Ambystoma* are kept secretly in libraries. This is also one exhort to make the information available for scientific discussion.

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