PROGRESS REPORT

*Ex situ* management of five extant species of *Atelopus* in Ecuador: assisted reproduction essays for *A. balios, A. elegans, A. nanay, A. spumarius,* and *A. sp.*

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EXECUTIVE SUMMARY

We began the managing and ex situ breeding of assurance colonies of five extant species of harlequin frogs Atelopus in Ecuador. Given the threats this genus faces, ex situ management is one of the proactive solutions to save these extant species from extinction, and assisted reproduction is a technological tool to accelerate breeding of species, which have shown to be difficult to breed. Between June 2011 and June 2012 we further equipped the ex situ facilities of the program Arca de los Sapos of Centro Jambatu; we did 8 field trips and collected founders of each of 4 species (A. sp., A. elegans, A. spumarius, and A. balios), and acquired captive born (donation of Amaru Zoo, Cuenca, Ecuador) tadpoles of A. nanay. Our field collecting efforts and lab-produced frogs increased the founder colonies, although current numbers are still insufficient to maintain genetically viable populations. We performed four essays of reproduction (two of them assisted with hormone treatments with HGC) and produced first descendants of three species (Atelopus elegans, A. spumarius and A. sp., spumarius-pulcher complex). We recorded for the first time details of an oviposition event of Atelopus sp. Also, we raised and documented (or are documenting) the ontogenic color and morphological changes of four species: A. balios, A. elegans, A. nanay, and A. sp., based on wild caught and lab born tadpoles. Keeping healthy individuals and successful breeding of these five species of Atelopus remains challenging. Assisted reproduction essays and rearing of tadpoles were generally successful, although management errors produced mortalities. Maintenance of wild caught adults and lab-reared juveniles remain difficult and produced mortalities, some of which are yet of undetermined causes. A web page providing details of management of each of the species is being developed and scientific publications are being prepared.
RESULTS

Field collections

We conducted eight field trips in the lowlands of eastern and western Ecuador between June 2011 and June 2012 to look for parental individuals and or tadpoles of four species included in this project (*Atelopus balios, A. elegans, A. spumarius, A. sp. (spumarius-pulcher complex)*). For *A. nanay*, we obtained a backup colony of tadpoles (captive born) donated by the Zoológico Amaru (of Cuenca, Provincia del Azuay). Localities, and number of males, females and juveniles (founders) kept before this project started and the ones currently kept at Centro Jambatu are summarized and indicated in Table 1.

Reproduction essays and feeding

Reproduction essays were performed in breeding tanks of glass 60cm long x 35cm wide x 30cm high. We used stream enclosures mostly as described by Poole (2006) with an open system of filtered water. We used four irrigation periods every day, each of 5 minutes length. Irrigation was done with a micro spraying system.

Terraria contained large stones, live plants for perching, moving water (generated by a water pump), flat stones arranged so as to form shelters and caves in contact with water so that the female can lay their eggs. Physical, chemical, and environmental variables were controlled in each of the reproduction essays.

Tadpoles were fed two times a day with SAR type III (Super Food for Tadpoles) powder. SAR is a mixture of fiber, protein, carbohydrates and minerals developed specifically to feed the tadpoles of harlequin frogs. The powder was extra fine so the tadpoles can feed it when it sinks to the bottom of the aquarium. A much more efficient feeding mechanism was by spreading humid SAR on sterile stones, drying it, an placing it at the base of the aquarium.
Frogs in early juvenile stages were fed with small springtails (*Folsomia candida*). Later, the diet included fruit flies (*Drosophila melanogaster*), crickets (*Gryllus* sp., *assimilis* complex) and newly hatched weevils (Curculionidae). Adults were fed with small crickets (between 1 and 5 days of hatching), fruit flies, and weevils.

Both youth and adults were fed three times a week. The insects were dusted with vitamin supplements (Repashi) once a week before being placed in the terrarium.

Follows a brief summary for each of the species studied:

(1) *Atelopus balios*
Initially we kept five males collected along the river Patul on the borderline of the provinces of Azuay and Cañar, which were collected on March 20, 2011.
June 7, 2011. An adult female (extremely thin) and two males were collected in a water channel at the Patul river. The female apparently had already laid eggs before being collected. Tadpoles were collected.

June 7, 2011. An amplexant couple was found and collected, nonetheless the female died during transport to the laboratory. Tadpoles were collected.

June 2012. A male was collected.

June 2012 to June 2012. Several males died. Some tadpoles died, others were raised to the juvenile stage. Their ontogenic development was documented.

30 June 2012. Currently we keep two males, one female, and a juvenile. To date no trials have been initiated because the only female kept at CJ is currently gaining weight and developing oocites.

(2) Atelopus elegans
Initially we kept a colony of four adults (three males and one female) collected in Durango, Province of Esmeraldas.

May 18, 201. Two adult males were collected in Durango river on the riverbank vegetation

September 1, 2011. A female was found walking during day collected from a stream close to the Durango river.

April 15, 2012. 20 females were collected and kept in isolation for observation.

30 June 2012. We currently maintain 17 females and three males.

Reproduction essay 1
January 31, 2012. Two males and one female were placed in a breeding tank.
February 1, 2012. An amplexus is recorded, the non-amplectant male returns to his terrarium.
February 5, 2012. The male was found dead under a rock. The female returns to its home cage.

Reproduction essay 2
May 9, 2012. Two males and two females were placed in the same breeding tank and they amplexed. Each individual was weighted before placed in the breeding cage.
May 23, 2012. Both pairs continue with the amplexus. One female was stimulated with HGC (Human Chorionic Gonadotropin) in a dose of 0.05 ml. The dose used was standardized after several trials since 2010 and is 10 IU per gram weight of the individual, based on reports from Kouba \textit{et al.} (2009) and Browne \textit{et al.} (2006). The hormone was administered with an insulin syringe (3ml) in the lateral region of the belly at level under the skin. While in amplexus the injection was done in a period of less than 20 seconds to avoid stressing the couple. The amplexant pair was returned to the breeding tank.

May 24, 2012. A clutch of eggs was observed under a stone.

May 28, 2012. Adults were returned to their terrarium.

June 1, 2012. Embryos were recorded.

June 4, 2012. Embryos continue their development. We began to document their ontogenic development.

(3) \textit{Atelopus nanay}

Initially we kept a male collected on 2011 in the surroundings of Patul in Azuay Province.

October 2011. 30 tadpoles were donated by the Amaru Zoo. Their ontogenic development was documented.

30 June 2012. An adult male adult and three surviving juveniles are kept.

(4) \textit{Atelopus spumarius}

Initially we kept a male collected on 2011 in the banks of the river Pucayacu in Pastaza Province.

August 27, 2011. We collected an amplexant pair in the riverbank climbing on a leaf at about 80cm from the ground. Another female was found near the couple and collected.

March 18, 2012. A female was collected on a fern at about 30 cm from the ground, at about 300 m from the margin of the Pucayacu river. Three males were collected at the same forest. They were on undisturbed primary forest on top of a hill.

\textit{Reproduction essay}
November 10, 2011. We placed a gravid female in a breeding tank.

November 14, 2011. Two males were placed in the breeding cage.

November 18, 2011. A pair is seen in amplexus. The solitary male is moved to its terrarium.

January 3, 2012. The female is stimulated with HGC at a dose of 0.03 ml.

January 4, 2012. A small clutch of eggs is recorded during the morning. It was placed among the roots of a plant. The couple is still in amplexus, the female apparently did not release all the eggs. In the afternoon (17: h00) the male releases the female and the two frogs return to their maintenance terraria.

January 9, 2012. Embryos are recorded.

January 12, 2012. Most embryos are dead. A possible cause was a drop in water temperature recorded on the night of 10 to 11 January 2012.

January 24, 2012. We removed the plants and stones in the aquarium to check for surviving tadpoles. There are three tadpoles, which were transferred to a new aquarium.

March 6, 2012. Two small tadpoles survive.

May 1, 2012. We found the two tadpoles dead.

**Atelopus sp. (spumarius-pulcher complex)**

Initially we kept five adults (one female and four males) collected in 2010 in southeastern Ecuador.

October 1, 2011. A field trip was done to San Carlos City of Limón, Provincia Morona Santiago. Individuals were collected on the banks of the river and the forest at 200 and 300 m from the riverbank. We collected two adult males and two females.

30 June, 2012. Currently we keep 30 individuals (four females, seven males and 19 juveniles).

**Reproduction essay I**

October 10, 2011. An amplexant pair recently collected is placed in the breeding tank.
October 11, 2011. The couple is seen immersed in water at 08:40h; it started spawning at 09:20h. Laying of about 500 eggs ended at 16:55 pm. The event was nearly totally recorded by means of lab notes, video recordings and photographs. Details of this event will be described in a scientific paper in preparation.

October 19, 2011. Photographs of embryos were taken.
October 23, 2011. Tadpoles begin to hatch from the egg capsule. They remain close to each other for several days.

November 2, 2011. We placed tadpoles into several groups at different aquariums.
November 16, 2011. We moved another group of tadpoles to various aquariums.
December 5, 2011. We moved another group of tadpoles to various aquariums.
January 1, 2012. First tadpoles with four legs (Stage 42 of Gosner) are recorded.
January 6, 2012. First early metamorphs (juveniles) are recorded and transferred to terrariums.
June 1, 2012. 19 juveniles are maintained. Many of the juveniles died for unknown reasons. Individuals visually in good condition were dying. All dead individuals were fixed and preserved in 75% ethanol.
LITERATURE CITED


BUDGET EXPENSES

The budget for this project was used to search for specimens in the field, to equip the labs and place them in captivity, to start assisted reproduction, and to develop webpages. Matching funds were provided by Saint Louis Zoo. In yellow we indicate new items (not initially budgeted) that were paid with AARK project. Items initially budgeted were bought with funds from other projects. Details below:

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<th>Item details</th>
<th>Budgeted</th>
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<td>10 Water pumps $50 each</td>
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<td>3 Ebottoms $200 each</td>
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<td>Materials</td>
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<td>Several lab materials (spumaflex, louver, silicone, plastic containers, nets, gloves, primers for molecular analyses of chytrid and Atelopus DNA analyses, etc)</td>
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<td>1 Video camera</td>
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<td>1 Ph meter</td>
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<td>5 Thermohygrometers $40 each</td>
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<td><strong>Atelopus balios</strong></td>
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