

# The Axolotl Newsletter

Issue number 27

Fall, 1998

The *Axolotl Newsletter* is prepared at the Indiana University Axolotl Colony, Bloomington, Indiana 47405. The Newsletter is distributed free of charge and is supported, in part, by funds from the National Science Foundation.

## I.U. Axolotl Colony

Director: George M. Malacinski  
Assistant Director: Susan T. Duhon  
Curator: Sandra J. Borland  
Editor, *Axolotl Newsletter*: Susan T. Duhon

### Communicating with the Axolotl Colony

Telephone: (812) 855-8260

Fax: (812) 855-6705

Email:

Susan Duhon:

[duhon@indiana.edu](mailto:duhon@indiana.edu)

Sandra Borland:

[sjborlan@indiana.edu](mailto:sjborlan@indiana.edu)

World Wide Web:

<http://www.indiana.edu/~axolotl>

Mailing address:

IU Axolotl Colony

Jordan Hall 407

Bloomington, IN 47405

Back issues 1-26 of the Newsletter are available. Single issues cost \$1.50. Unless individual numbers are requested, issues 1-9 and 11-14 are sent reduced in size and bound together. The set containing issues 1-9 costs \$10, and the set containing issues 10-14 costs \$5. Contents are listed on our website. Indices for numbers 1-15 are in issue 15 and for numbers 16-20 in issue 20. Issue 25 contains indices for issues 21-25. Beginning with issue 26, the Newsletter is available free online at the Axolotl Colony website.

Copyright 1998, The Trustees of Indiana University

## Contents

Note from the Director George Malacinski .....	2
Frontiers of the Biology of Amphibia Symposium notice .....	2
Axolotl Colony Price List for 1998-1999 .....	3
Breeding the Axolotl in its Native Habitat <i>V. Graue, J. Sánchez Robles, G. Castro, O. Cuamatzi, Y. Márquez, and M. Vázquez</i> .....	4
Xochimilco Today <i>S.T. Duhon</i> .....	7
Axolotls in the Second Grade Chapter Two <i>V. Coppedge</i> .....	11

### The Axolotl Newsletter is going electronic!

This is the last issue of the Axolotl Newsletter that will be distributed on paper. Beginning in 1999, the Newsletter will be on line. We will continue to accept contributions: research reviews or notes, technical comments, announcements, and axolotl lore of all kinds for the online Newsletter.

Visit the Axolotl Newsletter on the  
Web!

<http://www.indiana.edu/~axolotl/newsletter/newsltr.html>

## Note From the Director

The Colony continues its supply functions, which of course represent our main activity. In addition, however, in order to expand the usefulness of the axolotl as a model system, we are attempting to make transgenic animals. We are trying several methods, with no notable successes to date. Included in our endeavors are procedures which are similar to those which have been successful with *Xenopus*. For example, we have been able to swell sperm, but unable to drive cleavage with those injected sperm. We are also injecting a LTR-GFP construct (viral long-terminal repeat sequences flanking the green fluorescent protein marker) in an attempt to produce "tran-

sient" transgenic animals. In addition, we soon will begin injecting a foreign-gene construct directly into the artificially inseminated eggs, near the animal pole (where the zygote pronucleus resides).

Dr. Roy Tassava of Ohio State University is collaborating with us and kindly providing gene constructs for injection. We expect to expand that collaboration in the future, and we are optimistic that one or another method will eventually work. In the meantime, we are always anxious to entertain suggestions for alternative strategies. We are also willing to inject potential marker-gene/viral constructs or other genetically engineered samples readers might like to provide.

George Malacinski  
December 16, 1998

### Frontiers of the Biology of Amphibia

An international symposium entitled "The Frontiers of the Biology of Amphibia" will be held March 22 - March 24, 1999, in Hiroshima, Japan. The purpose of the symposium is to encourage international cooperation among amphibian biologists.

The program will include oral presentations by the invited speakers and poster presentations, for which all who wish may submit an abstract. The official language of the symposium is English. An optional tour of the Laboratory for Amphibian Biology at Hiroshima University is also planned. The deadline for submitting an abstract is November 30, 1998. The registration deadline is January 31, 1999.

#### Invited speakers as of 18 August 1998

Enrique Amaya—Transgenesis  
Jeremy Brockes—Regeneration  
Sylvia Evans—Embryonic morphogenesis  
David Hillis—Molecular phylogeny and evolution  
Kristen Kroll—Transgenesis  
Michael Schmid—Cytogenetics  
Yun-Bo Shi—Metamorphosis  
Hubert Vaudry—Cellular and molecular neuroendocrinology  
Cris Wright—Embryonic morphogenesis  
Mitsugu Maeno—Mesoderm induction  
Masafumi Matsui—Phylogenetic systematics  
Noriyuki Sakata—Molecular mechanism of cell-cycle regulation  
Masanori Taira—Embryogenesis  
Kazuhito Takeshima—Embryogenesis and metamorphosis  
Naoto Ueno—Embryonic morphogenesis  
Masakane Yamashita—Molecular mechanism of oocyte maturation

#### For more information contact

M. Sumida  
Secretariat  
The Frontiers of the Biology of Amphibia  
Laboratory for Amphibian Biology  
Faculty of Science  
Hiroshima University  
Higashihiroshima 739-8526  
JAPAN

Telephone: 81-824-24-7482  
Fax: 81-824-24-0739  
Email: msumida@ipc.hiroshima-u.ac.jp

You may also wish to contact the chairman of the organizing committee,  
Katsutoshi Yoshizato.  
kyoshiz@ipc.hiroshima-u.ac.jp



**Indiana University Axolotl Colony**  
**Price List**  
*current Fall 1998*

**Embryos**

	One-time or occasional ( <b>&lt;1500 embryos annually</b> )	Multiple ( <b>&gt;1500 embryos annually, frequent shipments</b> )
normals, wildtype, and albinos (a/a)	\$30 per 100 embryos or fraction thereof, billed monthly	\$30 per 100 embryos or fraction thereof, billed quarterly
white (d/d), melanoid (m/m), axanthic (ax/ax)	\$20 per 50 embryos or fraction thereof, billed monthly	\$20 per 50 embryos or fraction thereof, billed quarterly
eyeless (+/e X +/e), cardiac (+/c X +/c), short toes (+/s X +/s), and other specific gene crosses	\$20 per 50 embryos or fraction thereof, billed monthly	\$20 per 50 embryos or fraction thereof, billed quarterly

Darkly pigmented embryos are sorted at blastula.  
 Albino embryos are NOT sorted, viability varies.

**Larvae and Juveniles**

Phenotype	Size Range**	Cost per Animal (\$)***
normals and wildtype	hatchlings	0.35
	2-3 cm larvae	0.75
	3-5 cm larvae	1.50
	5-8 cm juveniles	7.50
	8-15 cm juveniles/sub-adults	12.00
white (d/d), albino (a/a), melanoid (m/m), axanthic (ax/ax)	hatchlings	0.50
	2-3 cm larvae	1.00
	3-5 cm larvae	2.00
	5-8 cm juveniles	10.00
	8-15 cm juveniles/sub-adults	15.00

\*\*All sizes may not be available. Call or e-mail to find out current availability. In some cases when space allows, animals can be raised to fill orders placed in advance.

\*\*\* Billed monthly.

**Adults**

Type	Phenotype	Price per Animal (\$)***	
		Female	Male
Healthy, Non-breeding	all phenotypes	15	15
Breeders (ask about availability)	normal, wildtype	25	20
	white, albino, melanoid, axanthic	30	25

\*\*\*Billed monthly

## Breeding the Axolotl in its Native Habitat

V. Graue, J. Sánchez Robles, G. Castro, O. Cuamatzi, Y. Márquez, and M. Vázquez

**Centro de Investigaciones Biológicas y Acuícolas de Cuemanco (CIBAC)  
Universidad Autónoma Metropolitana (UAM), Xochimilco, México, D. F.**

Since 1995 our university has operated a government-owned center for the ecological restoration of the Xochimilco aquatic fauna. At that time our existing colony of axolotls, some 20 animals, which had been at UAM since 1992, was transported to this center, known as CIBAC, which is located right beside Cuemanco, one of Xochimilco's widest remaining channels.

To this original stock we added axolotls

*tarium,*" a deeper hole (45 centimeters deep) lined with plastic and filled with water from Cuemanco's channel through its connection to our pumping system. It has a drainage outlet that returns water back to the channel, establishing in this way a water flow.

Later, a second axolotarium was constructed at the back of the center, right beside the water. It was covered by a cage with a mesh to prevent birds and water snakes from getting in since it was farthest from our view where we work. Also, the cover gives nice shade for work outside.

In order not to lose track of the animal's registration number (our identification system is based on individual characteristics), floating cages were devised, so that each pair could be given a number. The floating cages are light cubical structures made of PVC and nylon mesh that allows the water to flow through.

Near the end of the 1997-1998 spawning



**Our uncovered axolotarium has a bridge for reaching the cages in the middle of the pond and for children's visits. The yellow balloon with big eyes is our effective scarecrow.**

taken from the lake for an evaluation of the wild population. Breeding was undertaken, since repopulation is an ultimate goal of the program.

Dr. George Malacinski visited us in the spring of 1995, and in January, 1997, Susan Duhon and Sandi Borland stayed for two weeks working with us at CIBAC. From these visits very useful ideas and suggestions on the handling of adult breeding and raising of larvae resulted.

We had noticed better results in breeding with pairs kept in a hole we had dug outside and filled with channel water than in aquaria. This led to the construction of our "axolo-



season, we decided to evaluate larval survivorship in the covered axolotarium as compared to the uncovered one, since we had already noticed high predation of eggs in the floating cages in the latter axolotarium by voracious insect larvae.

Twelve pairs of sexually mature axolotls were chosen, and each pair was put in a floating cage with a stone for spermatophore deposition and casuarine tree branches for egg deposition. Six pairs were put in the covered and six in the uncovered axolotarium between May 22 and May 29, 1998.

We obtained five spawnings in the covered axolotarium and only three in the other one, perhaps because we were already at the end of the spawning season.

The adults were removed after they spawned, and the eggs were counted in each spawning. Temperature was 21°C, pH 9.4, and dissolved oxygen 12.9 mg/l (parameters measured at noon) in both axolotariums.

To keep to a minimum the entrance of predators or their eggs with the canal water, a filter was placed over the faucet of the covered axolotarium.

Survivors were counted after one month (June 29). In the uncovered axolotarium, with no filtration in the water, there were no survivors. Before the eggs hatched, we had observed coricids and chironomid larvae, which entered from the air or with the unfiltered water and flourished in the spawnings. Also some frogs, which had escaped from the ranarium, were found inside the cages. Temperatures were at a record high in May, so populations of insects were very high.

Survivorship in the covered axolotarium was better, ranging from 4.9% to 32.2%. We attribute the wide variation to some cages having holes in the mesh at the end of the experiment because of handling. Some larvae escaped through these holes and thus could not be counted. We also noticed genetic differences in the spawnings themselves, since one male produced spawnings much stronger than others.

It was not possible to apply any statistical analysis because the mortality of all the larvae in the unprotected axolotarium prevented statistical comparison, but we plan to repeat the experiment next spawning season after improving the design of our system. One of



The construction of our covered axolotarium



the problems we have to solve is that, when we handle the floating cages, some larvae get underneath the tubes and are crushed when we put the cage back in the water. Also, separating the larvae that grow faster from their smaller siblings to prevent cannibalism is more difficult in this system than in aquaria.

The advantages we have found to this form of rearing are reduced costs and labor in the feeding of larvae, since in the floating cages the axolotl larvae are taking advantage of the rotifers, copepods, cladocerans, and insect

## **Axolotl Newsletter Number 27**

larvae of the natural waters of their habitat. Water flux in these systems frees us from spending time cleaning containers, and also we presume the larvae will have to handle less stress when they are put in the lake from this seminatural condition than they would if transferred from aquaria into the lake. In this way we hope we can optimize breeding for repopulation purposes.

Our preliminary results show success with the protection factor although this will have to be corroborated with future studies.



**The  
finished  
axolotarium**



**The floating cages  
showing the faucet  
covered by a filter**

## Xochimilco Today

**Susan T. Duhon**  
**Indiana University Axolotl Colony**  
**Jordan Hall 407**  
**Bloomington, IN 47405**

Sandi and I had the opportunity and privilege in January 1997 to travel to Mexico and see for ourselves the last remaining natural habitat of the axolotl first hand. We were hosted in Mexico by Virginia Graue of UAM, Director of CIBAC (Centro de Investigaciones Biológicas y Acuícolas de Cuernavaca— a research center for the study of the aquatic life of Xochimilco), and a specialist on the axolotl in Xochimilco.

Virginia is directing an evaluation of the axolotl population in its natural habitat today. Another important goal is to raise and breed axolotls at CIBAC with the aim of boosting the natural population with offspring from this breeding project. Sandi and I were there in large part to help establish the breeding project. While there, I also gave a talk at UAM on the IU Axolotl Colony and axolotls in research.

Xochimilco today is an irregular network of wide and narrow canals on the southern edge of Mexico City. Some of the canals wind among the streets and houses of the village of Xochimilco, now part of the capital city. Much of the landscape is still rural, however, and the canals are part of modern-day chinampas, which are cultivated, especially for flowers, or used for pasture.

CIBAC is located adjacent to the Canal de Cuernavaca, one of the major canals of Xochimilco, and opposite the Pista Olímpica, a long pool for rowing and other water sports built for the 1968 Olympics. The center was constructed for the study of the native aquatic fauna (especially axolotls, frogs, and native carp) of Xochimilco as part of a larger program of ecological restoration for Xochimilco.

A small, white, stucco-coated building with several small rooms provides space for an office and for hatching embryos and raising small larvae and juvenile axolotls. Adults are housed in a large open building, the *módulo*, near the canal. Although the side walls of this building reach to the eaves, the end walls rise only about half way. A large worktable stands in the center. Concrete shelves line the walls

## Views of CIBAC and Xochimilco

CIBAC: The *estanques*, or in-ground tanks, are in the foreground. The *módulo* is in the rear on the left, and the *ranario* is on the right. The *piletas* are out of sight to the right.



The *piletas*. The *estanques* are to the right, out of the picture.





The interior of the *módulo*

Virginia Graue (left) with the author



A view down one of the canals. Sandi Borland is videotaping.



Salvadore Soto casting the *atarraya*

and support the large plastic basins that house the axolotls.\* CIBAC also has eight large in-ground tanks (used mainly for carp) and six above ground tanks (*piletas*) out-of-doors. The *piletas* can be filled with water directly from the canal and are used both for axolotls and to hold the *Daphnia* and Corixid beetles that are kept to feed the axolotls. Also near the canal and adjacent to the *módulo* is a *ranario* or frog house, a large polygonal structure for tadpoles and adults of the species *Rana montezumae*.

The axolotls that form the initial breeding colony at CIBAC were caught in the canals as part of a baseline study of the natural population of axolotls in the canals. This study included a program of sampling carried out over the course of a year. A report containing the data collected during this program is currently being prepared. While Sandi and I were in Mexico, we were taken out onto the canals in the *trajinera* belonging to CIBAC so that we could see more of Xochimilco and see how the sampling had been done. A *trajinera* is an open, flat-bottomed wooden boat with a rounded canopy to provide protection from the sun and rain. Typically, they are painted bright colors, given romantic names, and poled slowly about the canals, carrying tourists. CIBAC's *trajinera* is propelled by an out-board motor with poles as a backup. On this occasion, a smaller craft, a sort of skiff called a *canoas* was tied to the side of the *trajinera*. The *canoas* belonged to Salvador Soto, a local fisherman and farmer, who helps with the sampling. We made a circuit to several of the

Xochimilco at dusk. The volcano Ixtaccíhuatl is on the left and Popocatepetl is on the right.



sampling sites. At each site, Salvador poled his *canoas* away from the *trajinera* in order to cast the *atarraya* (a fishing net, six meters in diameter, that can be cast and drawn up by one person). At each sampling location, the net was cast three times. Meanwhile those still in the *trajinera* took measurements of water temperature and pH and noted pertinent observations. At the several sampling

New housing has now been developed. See the article by Virginia Graue in this issue

locations that we visited, the net yielded a few carp most of the time, but only two axolotls altogether.

Sandi and I spent approximately two weeks in Mexico. It was a great opportunity to meet with our Mexican colleagues and to see the

natural habitat of the axolotl. We also came away with a greater understanding of the pressures that are exerted on the native population of axolotls by the urbanization of the region.

## **Axolotls in the Second Grade Chapter Two\***

**Virginia Coppedge  
Spencer Elementary School  
Spencer, Indiana**

In February, 1997, two of our adult black axolotls mated and produced 164 live embryos. Of those, 120 were adopted by children from Spencer Elementary. Of those babies, we estimate there are some 70-75 still alive. The deaths of the others have been duly recorded along with the stories of their demises, most of which have been attributed to cats or jumping out of the tanks. Very few have been reported as dying of a disease or noticeable physical condition. Many have spent some time in somebody's refrigerator, but then recuperated. Large numbers of kids reported that their axolotl did not eat much at all over the summer this year. Many owners floated bags of ice in their tanks during the summer, which the animals seemed to like to hide under.

At present, we have five adult animals at Spencer Elementary including the parents of the 164 babies. At least seven faculty members have some of the babies, who have grown amazingly well. Fred Walden has one black

that is at least 12 to 14 inches long. It grins all the time. The kids say it is because it knows it is the biggest one. We have been hand-feeding one baby who was returned to us this August that we felt sure we would lose. It had been put in a fish tank since its adoption, and, when we got it back, it had little or no gills, malformed front legs, and a big chunk taken out of its tail. Most likely the goldfish in the tank were responsible for this atrocity (let alone the humans). It measured less than three inches at 19 months of age. The children and I and even the custodian set up an every-three-hours feeding schedule over 18 hours a day, and it has slowly begun to recuperate. The tail has not regenerated, but the front legs and gills look much better. The torso is more rounded, and it eats 12 to 15 small pellets a day. Its name is Peewee, but I do think it is gaining ground. The children and the custodian consider this a huge life-saving venture!

Since we have two adult pairs in my classroom at this time, Mr. Walden and I plan to mate both pairs around Christmas, but we will probably keep only enough eggs for all the kids in our two classes.

We have shared axolotls with at least five other schools, who keep in touch regularly. It continues to be an exciting adv

---

\* The first "chapter" appeared in Axolotl Newsletter number 24, pages 4-5. A related article by Fred Walden followed on page 6.

## Instructions for Contributors

Beginning with Issue 28, the Axolotl Newsletter will be available only online. Contributions will along the lines suggested below will continue to be accepted.

Contributions pertaining to axolotls or other urodeles or more generally to amphibians are welcome. All of the following categories are encouraged:

- short reviews
- research notes
- technical comments
- material requests or material available
- announcements
- inquiries for information
- colony descriptions or directories
- disease control notes

Authors are encouraged to submit line drawings, diagrams, or photographs to accompany the written contribution.

The Axolotl Newsletter is an informal, non-peer reviewed, publication. Contributions should be written in a style appropriate to both the nature of the material and the character of the Newsletter. The contribution need not be based on new research, but may be a distillation of previously published work.

Manuscripts should be submitted electronically, either on disk or as an email attachment. You may be asked for a printout of the manuscript in some cases.

There is no predetermined length, but please inquire before submitting a manuscript longer than 15 typewritten pages. Manuscripts are not cut to fit space.

Copy editing is light to medium. For most manuscripts, only typographical and spelling errors and grammatical mistakes will be corrected. More extensive editing is occasionally necessary, but in these cases the author will be consulted.

Manuscripts should be submitted on a 3 1/2" disk if a word processor is used. Both PC and Macintosh disks are accepted. Please send a hardcopy of the manuscript along with the disk.

Any computer-produced graphics should be sent as separate files (in PICT, TIFF, or other standard format) on the disk. Please indicate the type of file by an appropriate extension or other means.

Address inquiries and contributions to:

Susan T. Duhon  
Department of Biology  
Indiana University  
Bloomington, IN 47405  
USA

email: [duhon@indiana.edu](mailto:duhon@indiana.edu)