Astronautls

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Sometime in the 1990's, a pair of axolotls may boldly go where no axolotl has ever gone before. Their mission: to mate and spawn fertile eggs that will develop in the zero-gravity environment of the Space Shuttle in earth orbit.

The groundwork for this scenario is being developed now in a collaborative effort between Star Enterprises, a private company, and several investigators at Indiana University. The project is one of the more far-out uses of axolotls in biological research, and we offer here a brief description of the work as it stands, and where we believe the interesting questions are to be found.

The project arose from the convergence of three separate lines of research. First was NASA's long-held interest in how space travel might affect human and other forms of life, as well as how biological experiments in space may tell us about the role of gravity in life on earth. Second was the developmental biologist's interest in the possible role of gravity in specifying pattern formation during early amphibian development. Third was the reproductive biologist's interest in mating behavior and how the structure of living spaces might influence that behavior. All of these interests could be met, and experiments could be done, if animals could be sent into space, but that required designing suitable animal habitats that would not only keep the animals alive, but also permit them to mate and bear offspring, and permit the young to grow and develop.

Star Enterprises, Inc., was begun to fill the need for this kind of animal care facility in space. One of the projects of Star Enterprises is an Aquatic Animal Breeding Facility (AABF, NASA loves acronyms). The AABF is designed to (1) maintain a variety of aquatic organisms in zero gravity for missions of 14 to 28 days; (2) provide a suitable environment for mating behavior of these animals, including space for mating dances, biocompatible substrata, food, and light cycle and temperature control; (3) provide for the growth of young animals; (4) permit videorecording of animal growth and behavior; and (5) fit into the Middeck Locker of the Space Shuttle, where it will run without the attention of the astronauts.

The axolotl is one of three test animals for the AABF (the other two are zebrafish, *Brachydanio rerio*, and the Japanese newt *Cynops pyrrogaster*). We chose the axolotl for four reasons. First, much is known about its early development. Any abnormalities that appear during space flight can be compared with the rich background of developmental studies on earth. Second, it is relatively hardy, easy to raise, and easily available given our proximity to the Indiana University Axolotl Colony. Third, and much to our relief, it can survive completely submerged, indefinitely. This last feature is essential because the AABF must be filled completely with water. (In zero gravity there is no "up," and an air space would break up into bubbles distributed throughout the water. This would hamper visiblity, interfere with pumps and purification, probably confuse the animals, and generally be a nuisance). Although axolotls have lungs and gulp air, they do not need to gulp air to survive. In fact, in our AABF axolotls have survived for 19 days submerged without an air space, with no ill effects. Finally, we chose the axolotl because it will eat a prepared diet (salmon chow) that can be easily stored for days or weeks aboard a spacecraft.

Our work with the axolotls requires us to consider three aspects of the animal's biology: physiology, reproductive biology, and developmental biology. Under physiology, our main concerns are with the axolotl's requirements for gas exchange, primarily its oxygen metabolism, and its requirements for removing ammonia. Most of what we know about the axolotl's oxygen metabolism comes from work done by Dr. Henry Prange and his graduate student, Chuck Zwemer, of the department of Medical Sciences, Indiana University. They have found that axolotls can tolerate quite low dissolved oxygen levels before they change their rate of oxygen consumption. Moreover, animals raised for several months under hypoxia of 50% normal saturation grow only slightly slower than controls, suggesting that 50% saturation is not particularly stressful. Apparently, the axolotl's ability to tolerate low oxygen levels means that its gills and skin are efficient at extracting enough oxygen for the animal's needs even when oxygen is not abundant. This is probably why they can survive in the AABF without using their lungs, and we have used these data to establish a design criterium that oxygen levels in the AABF not go below 85% saturation.

The most toxic byproduct of the axolotl's metabolism is ammonia. We are currently determining the levels of ammonia axolotls can tolerate for long periods. Our preliminary results suggest that axolotls, both larve and adults, survive ammonia concentrations of 10^{-4} M, 10 times higher than a level that would kill most aquarium fish. Measured ammonia levels in the AABF do not exceed 10^{-4} M, even after two weeks with two adult animals in the tank. However, although we know that axolotls can survive elevated ammonia concentrations, they may be stressed subclinically by lower levels. We therefore are designing a biological filter, in principle similar to the gravel filter found in most home aquaria, to keep ammonia levels low.

Our work on reproductive biology borrows heavily from the studies of axolotl mating behavior being done by Heather Eisthen, a graduate student in the Department of Psychology, Indiana University (see Eisthen in this issue of the Axolotl Newsletter). Our goal is practical, however. We want to provide an environment that will encourage and support breeding behavior in zero gravity. Our primary concerns are (1) whether males will deposit spermatophores in zero gravity, or do they require gravity to attach spermatophores to horizontal surfaces and (2) whether females will be able to respond to the male's courtship behavior and pick up spermatophores without a gravity cue. After that point, we believe that physiology will supersede and that spawning will ensue, regardless of the presence or absence of gravity.

We know from casual observation that males will deposit spermatophores on cylindrical objects such as air supply tubing. Similarly, females will deposit eggs on branches of natural and plastic plants, and prefer to do so rather than to shed them directly into the water. It seems likely that they would behave the same way in zero gravity, but this behavior has never been studied carefully. In particular, we do not know whether females will follow males and find the spermatophores deposited on such three-dimensional surfaces. In the laboratory, matings have always proceeded on horizontal surfaces of rocks. We are currently designing different shapes of substrata and presenting these to males and females to determine their preferences. If they will, for example, reliably deposit spermatophores on a vertical tube at normal gravity, and the female can pick them up, they will probably be able to do the same thing at zero gravity.

Our final interest is in developmental biology. The physiological effects of space travel on mature organisms are relatively well documented, particularly for humans. Loss of muscle mass, fluid imbalances, and demineralization of bone are all short-term effects, but ones that are reversible upon return to normal gravity. There may be more serious effects, for example on the structure of long bones, if an animal develops under zero gravity. Or, the vestibular system that allows animals to orient to gravity on earth may develop abnormally in zero gravity. Gravity may effect embryogenesis quite early, since studies in frog embryos have shown that the establishment of the dorsal/ventral axis is sensitive to orientation to gravity during a window of time immediately after fertilization but before the first cell division.

For these reasons, we are designing the AABF not only for maintaining adults, but also for nurturing and studying offspring. The AABF will be equipped with a video camera to record both the behavior of adults and the development of larvae. The design challenge for us is to provide a mechanism for sequestering hatched larvae from cannibalistic adults.

The mission of Star Enterprises is to provide not just a holding tank that will keep animals alive, but as much as possible a small environment that provides for all of the axolotl's physical and behavioral needs, except for gravity. In a sense, we are asking the axolotl to design its own habitat. The challenge for us is learn to ask questions in ways that the axolotls can answer. If we are successful, then soon there may be a small colony of astronautls ready to earn their wings.