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# The Mexican Axolotl as a Pet and a Laboratory Animal

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## Basic taxonomy, life span and habitat preferences:

The axolotl is a urodele amphibian from the largest family of salamanders, the new world's Ambystomatidae or mole salamanders. The Mexican axolotl is known scientifically as *Ambystoma mexicanum*. The axolotl is originally from two large lakes, Lake Xochimilco and Lake Chalco, in the same central plateau where Mexico City is now located. One hundred years ago, these lakes and their connecting marshes, swamps, and lagoons covered over 200 square km (77 square mi). The lakes were filled by springs and meltwater from the nearby snow capped volcanoes.

Up until this century, the axolotl was also a common food source, taking the place of fish in the region. Today, the lakes have been sacrificed to provide farmland and space for Mexico City itself (Brandon, 1989). Lake Chalco is completely dry. Lake Xochimilco has shrunk to 314 hectares (personal communication, Virginia Graue). The reduction in size and the pollution of the axolotl's natural habitat has led to its being listed in Appendix II of the Endangered Species list.

European naturalists have been commenting on the axolotl as far back as the late 1500s (Smith, 1989). Unfortunately, it is now so rare in the wild that almost all scientific observations on behavior and preferences are based on laboratory animals. The axolotl has a long and honorable history as a lab animal. The ancestry of some individual animals can be traced all the way back to an original large group of axolotls imported from Mexico in 1864. Six of the specimens were given to M.A. Dumeril of the Jardin des Plantes of Paris. At least one more of the original group, and perhaps more, were subsequently added to collection (Smith, 1989; Newth, 1960).

Axolotls have enjoyed extensive use by scientists, particularly classical embryologists, since that time (Smith et al, 1971).

The name axolotl comes from Aztec mythology. The god of the dead, Xolotl, tried to evade banishment from earth by taking on a number of repulsive and unattractive forms. He was finally captured and killed while in the form of an axolotl and as such was used to feed the sun and moon. The name, which can be translated as water dog, is rendered ajolote in Spanish (Smith, 1989).

The term axolotl is often used to refer to any large branchiate salamander (one that has not undergone metamorphosis and still has its gills). Larval or neotenic tiger salamanders *(Ambystoma tigrinum)*, a closely related species, are also often called axolotls in the vernacular. In particular, many pet shops and pet books refer to larval or neotenic tiger salamanders as axolotls. Usually these animals differ significantly in appearance from the Mexican axolotl, being more olive in color and more stocky in build with shorter, stubbier toes, and they cannot be depended upon not to metamorphose. Mexican axolotls, on the other hand, ordinarily will not metamorphose unless they receive thyroxine, either by injection or by addition to the water or diet. They are inducible obligate neotenes, remaining in the water-dwelling, larval state for their entire life cycle unless artificially induced (see Martin et al, 1995, for definitions of the terms facultative, inducible obligate, and uninducible obligate).

The scientific name, *Ambystoma mexicanum,* was firmly agreed upon by the scientific community only in 1974. There are at least 48 other scientific names, especially Amblystoma, and variations in spelling that are used in pre-1974 literature (Brandon1989, Smith 1969).

There are at least eighteen other Mexican Ambystoma species, several of which closely resemble Ambystoma mexicanum. There is a subspecies of tiger salamander that is found in the same part of Mexico as the axolotl. Before metamorphosis, this subspecies of tiger salamander is virtually indistinguishable to the untrained eye (Brandon, 1989; Shaffer 1989; Smith 1989).

**Physical characteristics:**

The Mexican axolotl is a large, heavy-bodied tailed amphibian with relatively short legs, (Fig.1). The tail is normally as long as the head and body length combined. The dorsal tail fin extends from the middle of the back to the tip of the tail, and it is matched, caudal to the vent, by a vertical fin on the ventral side. Like other salamanders, axolotls have four toes (Figs. 2) on the front feet and five on the back (Figs. 4,5). The axolotl has a rounded, shovel-shaped head with a large mouth and small, low-set eyes. The body of the axolotl has several vertical costal grooves, which are especially prominent in the male (Figs 4,5). The most striking feature of the axolotl are its three-lobed, feathery gills, which extend over the neck and behind the head.

Axolotls commonly reach lengths of 25 cm or more, and adults may weigh anywhere from 150 to 350 grams. Most of their growth is reached during their first two years. Older animals and females are larger and heavier. In the laboratory, it is not unusual for axolotls to live ten years or more (Armstrong, et al, 1989). The oldest axolotl the first author has personally heard of from a reliable source is a female of 18 years (and counting). She has regularly produced a spawning each spring of her adult life (Pieter Nieuwkoop, personal communication).

The wild-type axolotl is typically a dark olive-green to blackish ground color with overlying yellow and black spots or speckles (Figs. 1,2). They may have pale gold or silver, iridescent patches. The eyes are black with reflective gold irises (Figs. 1,4). These colors are the result of combinations of three different pigment cell types, the melanophores (black), xanthophores (yellow), and iridophores (iridescent silvery and golden). Wide variation among wild-type coloration occurs depending on the distribution and relative abundance of the three pigment cell types.

Color variants also result from mutations which affect the presence, distribution, and abundance of the different pigment cell types. There are four common variants, each controlled by a recessive gene: white (d), melanoid (m), albino (a), and axanthic (ax). An axolotl is considered to have the wild-type phenotype if it expresses none of these mutations.

The white mutation (Figs. 5,6) may be the best known and is certainly the first-described pigment variant. A white axolotl was included among the first axolotls received in Paris, and all white axolotls today are descended from that animal (Smith, 1989). The white mutant is often mistakenly called an albino because the animal is white with pink gills, but it also has black eyes and is really not an albino at all. It carries a defect in the dermis which prevents the migration of normal pigment cells from the neural crest over the rest of the animal. Very young white larvae have black and yellow pigmentation on the head and along the top of the spine, but even that pigmentation disappears as the animals mature. White axolotls often acquire spotty black pigmentation as they age, especially on the gills (which may appear bluish), legs, and tails.

Melanoid axolotls (Figs. 7, 8) have more abundant melanophores than the wild-type animal and few xanthophores. Iridophores are absent. Axolotls homozygous for this trait are dark gray to velvety black. They also have solid black eyes without the reflective yellow ring normally produced by iridophores.

The albino gene was introduced into the genome of the Mexican axolotl from a tiger salamander (Humphrey, 1967). Albino axolotls (Figs. 2,3) completely lack melanin, but xanthophores and iridophores are not affected by the mutation. Therefore the albino Mexican axolotl is a deep, shiny gold color with golden eyes and gills, (Figs. 5,6).

The axanthic animal lacks both xanthophores and iridophores and is uniformly gray in appearance. Their eyes are solid black. Adult axanthic axolotls cannot be distinguished unequivocally from adult melanoid animals. The two phenotypes must be differentiated, therefore, when the animals are very young. Both melanophores and xanthophores can be discerned in hatchling melanoid axolotls examined under a dissecting scope; axanthic hatchlings have melanophores only.

All four pigment genes segregate independently, so many combinations are possible. For example, axolotls that are homozygous white and albino (Figs. 2,3) are white to translucent in appearance with pale pink eyes and pink-tinged skin (Figs. 5,6). A combination of albino and axanthic yields a young axolotl that is virtually colorless, but that becomes pale yellow over time as it acquires riboflavin from its diet (Bukowski et al., 1990).

Occasionally other color variants have been described. For instance, Scott (1981) describes a white and black spotted axolotl, the harlequin, which may be a variation on the white mutant. The gold starburst pattern (Frost, 1989) axolotls display the typical wild-type coloration at first, but several months after hatching develop golden yellow starbursts, 0.5 to 1.0 cm in diameter, that are particularly prominent on their legs. They often have golden gill fronds mixed among the black (Figs. 3,4). The size and brilliance of the starbursts decreases with age. The starbursts are probably caused by especially abundant iridophores in an otherwise wild-type axolotl.

Axolotls can change their color to a small degree, depending on the lighting conditions and the color of the surface they are on (Pietsch et al, 1991). This slight color change is especially noticeable in larvae and juveniles, who use it to good advantage for camouflage. It is normal to place young axolotls in a new tank and be able to spot them easily at first, only to have them vanish into the background by the next day. As the axolotls age, they become darker and tend more towards a deep green to charcoal in color.

Numerous other mutations have been described in axolotls. Most notable are the genes eyeless (e), cardiac nonfunction (c), and short toes (s). Armstrong (1985) has provided an overview of axolotl mutants.

**Housing Adults, Water Quality and Temperature:**

Axolotls can be successfully housed in a variety of ways. Many laboratories house them individually in glass or plastic containers. Flow-through tanks, tanks that are periodically flushed, and filtered aquaria have also been used. The critical elements of any system are water quality and temperature. Other considerations are lighting and available bottom space (since axolotls spend the majority of their time resting on the bottom). So long as these parameters are within appropriate limits, any method chosen will do.

Square 24" x 24" x 10" tanks constructed of glass bonded by aquarium silicone (standard silicone can contain arsenic) are used with great success in the Manitoba colony. One tank may house as many as 8 to 10 juvenile axolotls or up to four large adults. Each tank has a standard aquarium undergravel filtration system (Spotte, 1979). Four centimeters of well washed #3 poultry grit (individual stones approximately 0.5 - 1.0 cm) instead of the often much finer standard pet shop gravel reduces costs and prevents ingestion. Smaller pebbles or gravel may be ingested by axolotls, especially if they are underfed. Although they regurgitate the gravel eventually, it's easier to avoid the problem. Each tank should have an independent air supply and filtration system in order to avoid the transmission of disease from one tank to another. If a power filter is used, be careful not to circulate the water too fast. Continuous rapidly circulating water is stressful to axolotls.

Axolotls housed in nonfiltered systems don't need continuously circulating water if the water is replaced regularly. They are extraordinarily resourceful when it comes to gas exchange. Axolotls often lie still on the bottom, merely flicking their gills periodically. Moving the fronds back and forth quickly stirs up the surrounding water and disperses any carbon dioxide that may have accumulated about the gills and mixes in fresh, oxygenated water. Axolotls often swim to the surface and gulp air as well (Fig. 5) because, although the neotenic axolotl never relinquishes its gills, it still undergoes "cryptic" metamophosis and develops lungs (Tompkins, 1978).

Water quality is critical for maintaining the health of the animals. Whatever the system for housing, pH, hardness, and levels of ammonia must be kept within acceptable limits. Chlorine and heavy metals must be eliminated before the water is used. Axolotls do well in water with pH between 6.5 and 7.5. Higher, more alkaline values are acceptable if ammonia levels are kept very low. In a filtered tank, pH may gradually decrease and should be monitored regularly. Hard water is best. Distilled or deionized water should be avoided. In many laboratories, axolotls are kept in a dilute saline system in order to discourage parasites, fungus, and bacteria (Armstrong et al, 1989). If ammonia and chlorine are present in the water source, they must be removed with a commercial chlorine, ammonia, and chloramine remover before the water is used.

Thereafter ammonia derived from the metabolic wastes can be controlled by frequent water turnover (in bowls or in flow-through or flush tanks) or by the maintenance of a biological filter in a filtered tank. A biofilter consists of specialized bacteria that convert ammonia to nitrites and nitrites to nitrates. Of the three, nitrates are the least toxic to animal life. Even so, nitrate levels should be monitored regularly to avoid buildup to toxic levels. Regular partial water changes of a filtered tank are recommended to keep pH and nitrates within acceptable limits.

Axolotls thrive in water maintained between 16 and 20 C. They do not tolerate heat well, so temperatures that exceed 22 C should be avoided. and they should be kept out of direct sunlight. Axolotls tolerate cold temperatures below 16 (and above freezing) well, although at low temperatures they become torpid and eat little.

Usually axolotls can be housed in groups without problems. Groups can be same sex or mixed sex. Axolotls often associate together within a tank and do not seem to establish territories. Bigger axolotls, however, especially fast growing juveniles, regard almost anything smaller than they are as potential food. Since the juveniles have widely varying growth rates, they must be closely monitored and large animals must be separated from smaller ones. Otherwise the smaller animals will lose limbs, and more, to larger tankmates. Sometimes an axolotl will not thrive in a group tank. An animal that appears to be eating, but is not growing well should be moved into a tank with slightly smaller companions, or it can be put by itself in a bowl. The occasional aggressive axolotl can be moved into a tank with slightly larger animals or housed individually.

Identification of individual animals within a group tank can be difficult. For this reason, animals were housed in individual bowls in the Indiana colony for many years. Sometimes individuals can be distinguished by housing contrasting pigment phenotypes together. A small number of animals can be identified by individual tail shapes and unique coloration.

Tattoos, tail notches, toe clipping, and freeze etching with liquid nitrogen are not desirable or reliable methods of identification because they stress the animals, and axolotls regenerate too well. Electronic identification using transponders and a reader are currently in use at the Indiana colony. The transponders are implanted in the muscle of the upper back to either side of the spine. The needle should be inserted at a shallow angle and pushed in all the way so that the transponder will be properly seated. The site of injection should be cleaned with a disinfectant such as mecurochrome before and after placement of the transponder, and the needle and transponder should be sterile. Transponders properly inserted remain in place and seldom cause problems for the animal.

**Feeding Adults and Juveniles:**

Axolotls will eat a wide variety of food. Snails, insects, earthworms, small, whole aquarium-raised fish, or chopped pieces of fish, canned tuna, and chicken and pork organs are all consumed with relish. Axolotls do not tolerate any form of processed meats such as bologna or hot dogs. Any live food, particularly snails, should not come from a wild source, to prevent parasite infestations. Similarly, fresh water minnows, sold for bait, carry heavy parasite loads and should be avoided.

For many years juvenile and adult laboratory axolotls were fed beef liver or heart almost exclusively with no identifiable problems. These diets based on single organs are not well balanced, however. Liver in particular is likely to be too high in vitamin A. Furthermore, hand feeding is usually necessary, which is very labor intensive. The Indiana colony now uses commercially available salmon pellets (from Rangen, Inc.) exclusively to feed juvenile and adult axolotls. These soft, sinking pellets are readily accepted by the animals, nutritionally balanced, and promote rapid growth. Others have reported using trout pellets (Balsai et al, 1994). Juvenile axolotls are fed 5 pellets (4-5 mm in diameter) daily. Adults are fed 5 pellets 3-5 days per week. In the Manitoba colony, each axolotl is hand fed pieces of beef heart with tweezers three to four times per week supplemented with other meats and live food meals two to three times a month.

**Sexing:**

The age at which axolotls reach sexual maturity is dependent on various factors such as the amount of food available, the temperature at which they are raised, and the degree of crowding that they experience. Under optimal conditions, they can reach sexually maturity by twelve months. Although one year old animals will spawn, it is usually best to wait to breed them until they are 14 to 18 months old and have a little more size. They can be easily and frequently bred up to six years of age. After this age, spawning rates begin dropping.

Up until the axolotl reaches sexual maturity, it is impossible to tell females and males apart. A male animal can rarely be distinguished from sexually immature or female animals at about 10 cm length by early development of the subcaudal glands, but it is best to wait until the animals are at least 15 to 20 cm in length. Thereafter, it is easy to sex axolotls. The male axolotl has glands which form a large cloacal bulge immediately behind the hind legs (Figs 3,4). Viewed from the top, the male's costal grooves are much deeper, and the animal is considerably thinner than the broad female. Females, due development of the oviducts and egg production, are more rotund (Figs 7,8). The female lacks the subcaudal glands. Before spawning, if she is egg heavy, her costal grooves will have virtually disappeared. Immediately after spawning, she will be noticeably thinner, but still broader than the male, with less obvious grooves.

**Obtaining Natural Spawnings:**

Getting a natural spawning in healthy, well-cared-for animals is not difficult. During the past decade, the Indiana colony has relied exclusively upon natural matings to obtain nearly 3000 spawnings. Place healthy male and female axolotls together in any suitably sized container. The water should at least cover the animals. The bottom of the container must be textured or contain rocks to which the male can attach his spermatophores, small cones of clear jelly with a white, sperm-containing cap. Males deposit more spermatophores more frequently if they are placed with a female only when a spawning is desired, and we have found in the Indiana colony that males housed individually give spermatophores more often than those housed in groups. It is usually possible to tell within a few minutes if the spawning will be successful, because the male will immediately start the courtship behavior (Eisthen, 1989; Duellman and Trueb, 1986) by waving his tail in front of the female and nudging her. The female will follow close behind the male as he executes his courtship dance and pick up with her cloaca spermatophores that the male has deposited in front of her. Two or more excited courting males may result in larger spawnings. White males flush to a bright pinkish red during courtship.

Leave the animals together overnight. It is possible to get a spawning a few hours after the animals have been ignoring each other, but this is unusual. If a spawning is imperative, it is best to set up three or four breeding sets at one time. While it is possible to get spawnings at all times of the year providing the animals are on an unvarying light cycle, midwinter to early summer is the most successful time and midsummer to late fall the least.

Twenty-four hours after placing the animals together, check the tank for spermatophores. If spermatophores are present the female can be moved into a separate tank with plastic plants and large rocks for the female to lay on. (Long glass rods of 2 cm diameter can also be used and are easier to remove the eggs from.) The female will always lay the eggs widely dispersed on these plants and rocks, never in large clumps. In the Indiana colony, she is placed by herself in a glass bowl, which is covered with a towel.

Usually the female will begin to lay her eggs 12 to 20 hours after mating. Rarely, she will wait several days to begin. Once started, she sheds the eggs over a period of one to two days, usually in groups separated by intervals of inactivity. The female will normally lay anywhere from 200 to 800 eggs, although smaller spawnings will occur in younger females and much larger spawnings have been recorded. The eggs are about 2 mm in diameter (although those from young females will be somewhat smaller) and are encapsulated by a clear jelly coat. The eggs are initially sticky and difficult to remove without damaging them, but within a short while can easily be handled with forceps or wide-mouth pipet. After about six hours, the jelly coating will have swelled up with water tripling the diameter, and the stickiness is considerably reduced. Eggs can then be easily and safely scrapped off surfaces with a razor blade. Eggs laid in the bottom of a bowl can be collected with a wide-mouth pipet. It is best to collect the eggs during the intervals when the female is not laying to avoid disturbing and upsetting her.

To achieve year-round natural spawnings the lights should kept on a timer to provide an unvarying light cycle. If the axolotls are allowed to experience a normally changing light cycle, they will usually require artificial induction to spawn at times other than late winter to spring. Changes in daylength, rather than any specific amount of light seems to be critical for spawning success. If the light period is shortened, spawning behavior will be greatly reduced. On the other hand, it is possible to encourage spawning by adjusting the lighting conditions to increase the daylength by 15 minute increments over a period of a week or two. If the light-dark periods are constant (12-14 hours of light is suitable), seasonal behavior will be minimized (Løvtrop, 1981).

Females should be given the opportunity to spawn at least twice a year, but it is best to wait at least three months after a spawn before the same female is used again. Males can be used more frequently, but they produce more spermatophores if at least a week is allowed to lapse between matings.

For those who find natural spawnings insufficiently reliable or who require simultaneously fertilized eggs, induced spawning and artificial insemination procedures are readily available (Armstrong et al, 1989). Ovulation in the female is easily induced by hormonal injections, usually HCG (human chorionic gonadotropin). Lower doses are used if the goal is merely to increase the percentage of successful matings when the female and male are placed together. Larger doses force ovulation and are used if artificial insemination is to be attempted. No method is as yet available for inducing spermatophore production in the male. Spermatophores for artificial insemination can be obtained from a male stimulated with the same or a different female, or from the macerated vas deferens of a sacrificed male.

**Raising Young Axolotls:**

The internally fertilised eggs take about two weeks to develop and hatch although the rates are highly temperature dependent (Bordzilovskaya et al. 1989). Place the embryos in a shallow bowl; avoid overcrowding. Discard any excess jelly, and do not allow embryos to clump. Embryos in the interior of a clump may not receive enough oxygen. It is best to sort the embryos when they have reached blastula stage (about one-day- old at room temperature) to remove infertile or nonviable eggs. During development keep the water topped up and stir occasionally if the embryos begin to clump together. Remove any dead embryos. As the embryos develop, the jelly capsule will gradually expand to accommodate the growing embryo.

The young axolotls usually are ready to eat soon after hatching. Begin feeding them when yolk is no longer visible in their bellies. Feed them live, freshly hatched brine shrimp daily. The animals that eat successfully can be easily spotted because they are still translucent. Their bellies will distend and the orange shrimp will be visible inside. If they are not fed right away, they may swallow air and get air bubbles in their stomach. When they begin to eat, however, they will be able to expel the air.

There are several ways to raise the larvae. If only a few individuals are to be raised from each spawning, the pond tank method is easy and efficient. A pond tank is a well-aged 40-gallon mini-ecosystem without an undergravel filter and with minimal or no aeration. Only water and an overhead 60 watt incandescent light bulb or sunlight need be provided. The pond tank should contain water plants and many small organisms, in particular an abundance of Daphnia. Organisms for starting pond tanks can be obtained from any university's introductory biology teaching unit. They can also be purchased from any biological supply company's living plant and animal demonstration kits. The organisms are simply tossed into the aquarium, where they soon establish a self-sustaining population (Reynolds, 1969).

Starter organisms for a pond tank should be obtained only from other well-established pond tanks or directly from a supply company to avoid predators (particularly dragon fly and water beetle larvae) and parasites. If you must start from a wild source, use an eyedropper to select individual Daphnia from a shallow white tray. Snails that are several generations from the wild are usually safe since the majority of their most harmful parasites require a large animal vector.

A partial list of organisms found in the Manitoba colony pond tanks include Spirogyra, Chlamydomonas, Amoeba, Planaria, Vorticella, Rotifera, Paramecium, Euglena, Cyclops, various cyanobacteria, assorted free living nematodes, and at least three species of Daphnia (including Daphnia magnum), rams horn and common pond snails, water ferns and duck weed.

The axolotl larvae are left to fend for themselves in the pond tank. They are removed when they have grown big enough to be easily seen, have all four legs, and the Daphnia population begins to drop visibly. This normally takes three to six weeks.

In the Manitoba colony, after the axolotls have been removed from the pond tank they are trained to take raw beef heart daily. At first the raw beef is dropped from tweezers and allowed to fall in front of them. They snatch at it as if it were live food. Over two weeks they are gradually trained to take the beef heart directly off the tweezers or from the bottom. During this training period, their diet should be supplemented with live Daphnia and/or brine shrimp after each attempted beef heart feeding. A couple of baby guppies in the same container will regularly disappear at a rate of 2 or 3 a week and can be replaced as necessary.

Alternatively the young axolotls can be raised to this stage in shallow bowls. Feed the larvae newly hatched brine shrimp, Daphnia, red worms, or similar fare. Tubifex is best avoided. Very young axolotls prefer live food and are usually reluctant to eat frozen brine shrimp. Feed enough shrimp so that all of the young larvae have orange bellies afterward, but few shrimp are left uneaten in the bowl. This method is labor intensive because the water must be changed daily, but large numbers of larvae can be raised this way.

When the young axolotls have all four legs or at about four centimeters length, they can be weaned off brine shrimp to pellets (2-3 mm in diameter) or other food as described above. Their diet should be supplemented with live brine shrimp during the transition period because some animals will take longer than others to adjust to a new food source. At first only one or two pellets per animal is enough, but as they grow the number of pellets should be increased. By the time they are 10 cm long they can be switched to larger pellets (4-5 mm in diameter).

To promote rapid growth, larval and juvenile axolotls in the Indiana colony are fed pellets daily. When they are about 20 cm long (usually about one year of age), reduced to three to five days a week. In the Manitoba colony, feedings begin twice daily, but are juveniles are fed with decreasing frequency and in increasing amounts until they are about six months old. Eventually, when they are about 10-15 cm long, they are fed three or four pieces of beef heart three times a week.

Young larvae are very vulnerable to disease, but difficult to treat. Therefore, the most effective strategy is to feed them generously, pay strict attention to water quality, guard against overcrowding, and grow them up out of the vulnerable stage as quickly as possible.

**Handling and Shipping:**

Axolotls are sensitive to skin damage and should therefore be handled with care. Fine mesh nets that do not disrupt the natural protective slime layer on their surface should be used. Alternatively, the water of older juveniles and adults housed in bowls can be changed by carefully pouring it (with the axolotl) into a plastic colander. With a bit of practise, they can also be easily captured and held in bare hands. The best way to do this is to cup one hand in front of the axolotl's face, to stop forward motion, and use the other hand to loosely enclose the animal's body. Using this method, the axolotl is caged in the hands, rather than squeezed, which could result in bruising or other injury.

Axolotls, especially embryos and larvae, ship easily and well. The Indiana colony ships all ages double-bagged in heat-sealed plastic bags. Pack them in insulated (Styrofoam) containers. Juvenile and adults should be bagged singly and have ice (or artificial ice packs) placed in the container with them. It is important to send them by overnight courier or express.

Alternatively axolotls can be shipped by placing them in insulated containers in ice water. Large, four-litre insulated drink jugs with wide mouths work well. This method is also appropriate for transporting axolotls by automobile.

If possible, do not feed axolotls for a day or two before shipping so that they do not regurgitate their food in transit and foul their water.

Cooling the animals during travel slows their metabolism and reduces stress. it also makes more oxygen available to them. Care must be taken not to overcool early embryos, however. It is best not to cool embryos before first cleavage below room temperature. Embryos between first cleavage and blastula stage can be cooled to about 10 C. Post-blastula embryos will tolerate temperatures down to 1-2 C (Ginsburg et al, 1987). Embryos can also be shipped in ice water providing that they are past the stage of neural tube closure.

**Anesthesia:**

For surgery and other laboratory procedures, axolotls can also be left out of the water for up to two hours if they are anesthetised and left wrapped in moist cloths. The least amount of disruption to the slime layer occurs when the wrapping material is a 40% cotton, 60% polyester blend (unpublished data). Paper towelling is not recommended as a wrap for animals left out of the water as it draws more of the protective slime off. The more the slime layer is disrupted, the greater the skin risk of skin damage and infection.

Vanable 1985, found 0.02% benzocaine gives superior anesthesia to the more commonly used 0.35% MS222. It is certainly cheaper. Benzocaine, must first be dissolved in absolute ethanol (see appendix). Properly diluted (0.1 %) and pH adjusted to 7, MS222 is routinely and successfully used in many laboratories.

**Maintaining Normal Health:**

Axolotls normally walk in slow motion on the bottom or remain motionless for extended periods of time except for the occasional flap of their gills. Axolotls sometimes swim up to take gulps of air from the surface. A healthy axolotl often seems curious and alert. It snaps eagerly at its food and appears to stalk it like a cat. Axolotls normally move in slow motion, but the movements should be fluid and graceful. A startled animal may thrash and swim away. An axolotl's pigments are normally clear and bright. White animals have a pinkish cast, and white and albino animals usually have bright red gills because they are filled with oxygenated blood. The pigment in the gills of dark-colored animals, however, usually obscures this red color.

**Signs of Illness:**

Poor gill development in an axolotl is often an indication of chronic stress or illness. On the other hand, an axolotl with particularly luxuriant gills may harbor parasites or may be compensating for some other problem. Ill axolotls commonly behave sluggishly, fail to eat, and have ragged gill filaments. Dull, grayish- or yellow-tinged skin (except, of course, in a golden albino) may be an early sign of poor health. More severely ill animals may be strongly jaundiced or anemic (lacking all red color in the gills), or they may suffer from edema or ascites. Small open sores on the skin and subcutaneous hemorrhage are common symptoms. An animal which floats as though it has gas which it cannot expel is probably ill. Sometimes sick animals bleed from the gills or bruise easily, resulting in red spots along the tailfin, especially noticeable in white axolotls. If an axolotl seems to move stiffly and does not react to be touched, it is probably a sickly animal.

**Dealing with health problems:**

Axolotls are usually vigorous and easy to maintain. When their health does fail, it almost always can be attributed to one or more of three causes. The first and most important is environmental. The others are bacterial infection and parasitic infestation.

Poor water quality and other inappropriate conditions of husbandry can be a direct cause of distress and death (for instance, toxic levels of ammonia, nitrites, or nitrates), and, as well, can be a source of chronic stress to axolotls, leading to secondary infection by opportunistic pathogens and the inability to cope with parasitic infections. Whenever a problem with axolotl health is suspected, one should first determine that water quality has been consistently maintained. In particular, check ammonia level, especially if the animals are kept in a non-filtered system. In a filtered system, both ammonia and nitrate levels should be checked. (Cloudy, bulging eyes can be a sign of prolonged exposure to nitrates.) In either case, make sure pH is within an acceptable range.

High temperatures, especially temperatures above 25 C, or a constant, too rapid circulation of water, especially if a power filtration system is used, often are a source of chronic stress. Other environmental sources of stress to consider are possible overcrowding, too frequent disturbance, or rough handling. If any problems are discovered, it is essential to correct them immediately. Also be sure to maintain cleanliness standards in the facility.

In the laboratory, surgical procedures or other experimental manipulations are common stressors. Particular attention should be paid to the conditions under which experimental animals are kept. During the immediate recovery period, it is often helpful to keep the axolotls cold (between 5 and 15 C). After healing is underway they can be returned to their usual housing.

For a few days after breeding, the animals involved, particularly females, should be closely watched for signs of disease because the stress of spawning increases their susceptibility to disease.

Axolotls, especially those chronically stressed, often become infected with bacterial pathogens such as Aeromonads and Pseudomonads. Other bacterial pathogens, mostly gram negative, have been reported (see Duhon, 1989). Typically all of these organisms are opportunistic. That is, they are continuously present in the animals' living environment. Most axolotls will successfully resist infection unless acutely or chronically stressed. That is why the first consideration when a problem is suspected is to investigate the conditions in which the animals have been kept. Nevertheless, particularly in a large facility, some sources of stress (e.g., experimental procedures, spawning) are inevitable. If bacterial infection is suspected, the affected animals can be treated with antibiotics. It is usual to choose a broad- spectrum antibiotic, since often the specific cause of illness cannot be identified. Furthermore several pathogens may be involved.

In the Indiana Colony we have found that gentamicin, amikacin, and enrofloxacin are well tolerated by the axolotls. These are best given by injection. An appropriate dose is approximately 5 mg/kg body weight. Prepare a dilute solution with physiologic saline, so that this dose is delivered in a volume between .05 and 1.5 ml. Each animal should receive three to five doses of gentamicin or amikacin, spaced 48 hours apart. Enrofloxacin should be administered daily for seven to ten days. The injections are given intraperitoneally, in the flank, just anterior to the hind limb and approximately midway along the dorsal ventral axis. The needle should be directed roughly parallel to the animal's body.

In order to administer the injection, grasp the animal firmly with one hand (the left hand for those that are right handed). The forefinger and middle finger of the hand should be to either side of the head, in front of the forelimbs. If the animal thrashes its tail, brace it against the rim of the container. Wait till the animal has stopped resisting before giving the injection. It is possible that these antibiotics can be placed in the water instead of injected, especially with smaller animals (larvae and juveniles), but the concentration needed for an effective dose has not been established. Tetracycline and related antibiotics are poorly tolerated by axolotls and should be avoided if at all possible.

Sick animals are also often benefited by being kept cold (between 5 and 15 C). It is possible to keep a sick animal refrigerated for several weeks with only an occasional water change while it recovers.

Parasitic, especially protozoan, infections can be internal or external. Axolotls may acquire parasites, such as Hexamina, Opalina, Trichodina, or Costia, from their diet (e.g., bait minnows) or from being housed with other animals carrying parasites. Parasites should be suspected if the axolotls go off their feed and become scrawny.

Those suffering from internal parasites will have a red and inflamed gastrointestinal tract. Severe infestations of skin parasites may cause the axolotl's skin to redden and become inflamed. It also becomes sticky and comes off on the hand when the animal is handled. Parasites can also be easily seen under the microscope by examining skin scrapings from affected animals.

Internal parasites can be treated effectively with metronidazole, which can be mixed with their food (500 mg/100 g food for three or four feedings). Skin parasites can be treated with formalin (25-50 ppm up to eight hours or 100 ppm for 1-2 hours). Infected animals will shed enormous amounts of slime and skin cells following this treatment and must be kept in a different tank with frequent water changes. Keep them in 50% modified Holtfreter's solution, a saline solution, (Asashima et al, 1989) for one week after fromalin treatment. Uninfected animals will not shed or will shed very little.

If the external parasites are to be completely eliminated, all animals must be treated simultaneously, and all bowls and other possibly contaminated surfaces must be disinfected. Axolotls suffering from skin parasites can also be treated by placing them in a high salt bath, which has the advantage of being very well tolerated by the animals. Animals stressed by poor water quality or other husbandry problems suffer more from parasites. Skin parasites are seldom a problem (even if they are present) in a healthy, well-maintained colony.

Fungal infections ordinarily occur only in animals which have been weakened from other causes, such as prolonged illness. No effective treatment is known. Large wart-like growths, possibly of viral origin, can occur and spread within a tank. The animals do not appear to show any distress or discomfort and will eat, swim and spawn normally with the growths. Because axolotls are susceptible to infection following skin breaks, removal of these growths is not recommended.

With proper care and feeding the axolotl will live a long and healthy life in either the laboratory or the pet keeper's aquarium. The key to success with axolotls is to keep them healthy by providing clean water, proper food, and the correct temperatures. Once these are available, the axolotl requires a minimum of effort and thrives in captivity.

**Recommended Reading in addition to the Reference List:**

Armstrong, J. B. & G. M. Malacinski (eds.). (1989) Developmental Biology of the Axolotl. (pp. 228-235). New York: Oxford University Press. (This reference includes many excellent articles on practical axolotl care as well as their natural history, pigmentation mutants, and common diseases and treatments. See the Reference list below for a partial summary of available articles.)

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**Appendices:**

1) Modified Holtfreter's solution for axolotls (from Armstrong & Malacinski, 1989) is made with NaCl, 3.46 g, KCl 0.05 g, $CaCl_2$ 0.1 g, $NaHCO_3$ 0.2 g, plus 1 liter of distilled water, pH adjusted to 7.4. Use 50% Holtfreters for adults and 25% for larvae. (100% for surgical procedures)

2) Vanable's benzocaine solution can be prepared by the following method (Vanable, 1985). 0.2 grams of benzocaine is first dissolved in 10 mL of absolute ethanol.This is added to 1 litre of a stock solution such as Holtfreter's listed above. If the animal is not completely anesthetised after about ten minutes the amount of benzocaine can be inceased to 0.3 grams for larger animals or the length of time of immersion increased until the animal does not execute the righting reflex after being placed on its back. Recovery is variable but takes an average of 45 to 60 minutes in anesthesia free water and at least two hours if the animal is wrapped in moist cloths.

3) The Indiana University Axolotl Colony publishes a regular newsletter as well as offering resources for scientists interested in working with axolotls. (See the reference list for many specific articles.) For reprints or more information write to SD.

**Figures:**

Figure 1: Shown is an eight centimeter four month old wild type jeuvenile axolotl. The Mexican axolotl is a large, heavy-bodied tailed amphibian with relatively short legs. The dorsal tail fin extends from the middle of the back to the tip of the tail, and it is matched, caudal to the vent, by a vertical fin on the ventral side. (Photo by John D. Latham, Winnipeg)

Figure 2: Another view of the same animal as in Fig 1. Like other salamanders, axolotls have four toes on the front feet and five on the back. The front toes of this animal are clearly visible. The axolotl has a rounded, shovel-shaped head with a large mouth and small, low-set eyes. The wild-type axolotl is typically a dark olive-green to blackish ground color with overlying yellow and black spots or speckles. They may have pale gold or silver, iridescent patches. The eyes are black with reflective gold irises. These colors are the result of combinations of three different pigment cell types, the melanophores (black), xanthophores (yellow), and iridophores (iridescent silvery and golden). Wide variation among wild-type coloration occurs depending on the distribution and relative abundance of the three pigment cell types. The most striking feature of the axolotl are its three-lobed, feathery gills, which extend over the neck and behind the head. (Photo by John D. Latham, Winnipeg)

Figure 3: An unusual variation of the wild type animal is the starburst pattern shown on this two year adult male axolotl who is gulping air as healthy axolotls occasionally do. The tail is normally as long as the head and body length combined as shown in this picture. The male's large cloacal bulge is clearly visible. Note the normal gold coloration in the eye. (Photo by John D. Latham, Winnipeg)

Figure 4: Top view of the same male as in Fig. 3. Note the relatively narrow body, deep costal ridges, and the bulge in the cloacal area behind the back legs. This photo also shows the five toes on the hind feet. (Photo by John D. Latham, Winnipeg)

Figure 5: White versus albino mutatation in the axolotl: These sibling animals are six months old and about ten centimeters in length. The animal on the right with is a white mutant showing typical lack of pigmentation except for some scattered speckling on the head and in the black eyes. This animal is not an albino. It carries a defect in the dermis which prevents the migration of normal pigment cells from

the neural crest down over the rest of the animal. The middle animal is a true albino. Albino axolotls completely lack melanin, but xanthophores and iridophores are not affected by the mutation. Therefore the albino is a deep, shiny gold color with golden eyes and gills. Both the white and albino mutations are inherited as a simple recessive. The left animal has both mutations appearing to be a white animal without the pigmented eyes and lacking the freckling. (Photo by John D. Latham, Winnipeg)

Figure 6: Top view of the animals in Fig. 5. It is impossible to sex animals of this age. (Photo by John D. Latham, Winnipeg)

Figure 7: A large five year old female axolotl displaying the melanoid trait. Melanoid axolotls have more abundant melanophores than the wild-type animal and few xanthophores. Iridophores are absent. Axolotls homozygous for this trait are dark gray to velvety black. They also have solid black eyes without the reflective yellow ring normally produced by iridophores. Adult axanthic animals are indistinguishable from melanoid. (Photo by John D. Latham, Winnipeg)

Figure 8: Top view of the same animal. Even though this female spawned recently, she still shows the relatively broader and rounder body and the wider costal ridges. Females ready to spawn are even more obviously broad and rounded. The yellow mark on the head is a light reflection. (Photo by John D. Latham, Winnipeg)

**Byline:**

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