

CFR : 3.81

**Photographer: K. FRANK**

**Date and Time: 09-AUG-17 1500**

**Inspection No: 2016082568698097**

**Description:**

Examples of toys that have diminished integrity in the Environmental enhancement plan.

**Certificate: 93-R-0066**

**Legal Name:**

**SALK INSTITUTE FOR  
BIOLOGICAL STUDIES**

## Animal Resources Department

---

### **Environmental Enrichment Program**

#### **Purpose:**

Environmental enrichment (EE) is a critical component of animal care, health, and well-being for all species. The goal of the Animal Resources Department enrichment program is to improve the quality of life of animals under our care by stimulating physical activity and natural behaviors, and preventing or reducing stereotypical behaviors.

#### **Benefits:**

Environmental enrichment provides important benefits not only to the animal but also to research by reducing stress, research variability, and adverse behavioral patterns. Environmental enrichment helps meet specific behavioral needs such as the need for shelter/hiding or foraging and offers an environment in which opportunities for exploration and social interaction are encouraged.

#### **Regulatory requirements:**

The Animal Welfare Act and the *Guide for the Care and Use of Laboratory Animals* both include requirements for environmental enrichment of research animals. Salk's Public Health Service Assurance and accreditation by the Association for Assessment and Accreditation of Laboratory Animal Care International (AAALAC) also mandate compliance with these standards, unless justification for withholding is approved by the IACUC.

### **Species Specific Activity and Enrichment**

- Non-Human Primates
  - Macaques
  - Marmosets
- Rabbits
- Guinea Pigs
- Ferrets
- Rodents (Mice and Rats)
- Frogs / Aquatics

### **Monitoring and Assessment:**

1. Daily physical checks by Animal Care Technicians
2. Routine rounds by Veterinary Services
3. Behavioral monitoring by Animal Care Technicians and Veterinary Services for atypical behavior patterns and changes in the proportion of normal behavior. Variations in previously established behavior patterns may warrant early intervention with enhanced enrichment or changes in social housing (Both Husbandry Supervisor and Veterinary Services will determine the appropriate treatment).
4. All significant observations must be recorded in room maintenance logs, on enrichment sheets, food and water consumption logs, or in the clinical record.

## **Non-Human Primates (NHP - Macaque)**

### **Normal Behavior:**

- Vocalization: sounds produced primarily from the throat and mouth such as cries, grunts, moans, hiccups, and whistles
- Yawning: wide open mouth display
- Aggression/Threats: full face display, opening the mouth and bearing teeth; shaking the cage violently
- Submit/Displaying: the animal will present a body part (usually hind quarter and/or back)
- Sitting: an animal that is showing contentment
- Standing: an animal showing curiosity (the animal is usually alert to its surroundings)
- Sleeping: relaxed and comfortable
- Foraging: fulfilling a natural instinct/behavior/need
- Manipulation: operate with hands (i.e. toys, cage parts/section, self (masturbation))
- Self-grooming: when an animal is grooming oneself
- Scratching: when an animal is rubbing or scraping an area of its body

### **Aberrant Behavior:**

- Circling: walking in a circle pattern within the cage
- Pacing: repetitive movement, traversing the same pathway at least twice
- Rocking: movement side to side or up and down
- Jumping/Hopping: when an animal is springing up and down and/or skipping
- Head toss: so-called “weavers” swing their heads rhythmically, at the same time pawing alternatively with their feet
- Auto-mutilation: when individuals turn a normal grooming behavior into an excessive behavior; this behavior may be expressed as exaggerated licking, gnawing and scratching at parts of the body and may cause ulceration.
- Abnormal aggressiveness: explosion of aggressive behavior that can be directed to a conspecific, including humans. The thwarted drive then suddenly finds an outlet in an attack upon the first available “adversary”.
- Apathy: separation from a mate or companion to which an animal is strongly attached can evoke a state of inactivity comparable to depression.
- Overeating: constant eating due to boredom
- Refusal of food: food can be refused after stressful situations like capture, manipulation or after a change of quarters
- Aberrant appetite (pica): the tendency of animals to eat unusual (non food-type) objects that could affect their health (i.e. toys, feces, bedding, etc.)
- Manipulation of self: when an animal is biting a part of its body “self-biting”; when an animal is removing body hair “self-plucking” pulling it out with hands, feet or mouth/teeth; when an animal is grabbing itself “self-grasping” using their hands/feet to grab or hold a body part (usually done because of stress)

### **Enrichment:**

Psychological enrichment is an essential element of any program to ensure the well-being of NHPs. They are very complex animals and providing them with environmental enrichment helps to minimize the development of stereotypical and/or abnormal behaviors. The physical environment of NHP should therefore allow the animal to display normal behaviors such as walking, climbing, jumping, and running. It should also promote important behavior patterns such as foraging, physical contact and social interactions between animals and caretakers. The efficiency of an EE program is also dependent on novelty. This can be achieved by providing NHP with a variety of destructible and reusable objects to manipulate. These objects as well as their spatial arrangements should be rotated regularly. The involvement of the animal care staff is another important component of a successful EE program. Available enrichment devices for use may include:

REUSABLE ITEMS	DESTRUCTIBLE MATERIALS	FORAGING FOOD ITEMS	FIXED FEATURES
Fire hose	Paper plates	Fresh fruits and vegetables	Multiple level perches
2" Plastic Chains	Paper bags	Sunflower seeds	Barred human access door
55 gallon drums	Paper towel rolls	Oatmeal and rice	Panoramic windows
20 gallon pool	Plastic water/juice bottles	Granola	
Puzzle feeder balls	Phone books	Chicken scratch	
Prima-Hedrons	Shredded paper	Monkey mix	
Hammocks	Cardboard boxes	Popcorn	
Swings	Sani-Chip® bedding		
Mirrors	Egg cartons	Cereals	
Plastic pallets		Dried bean mix	

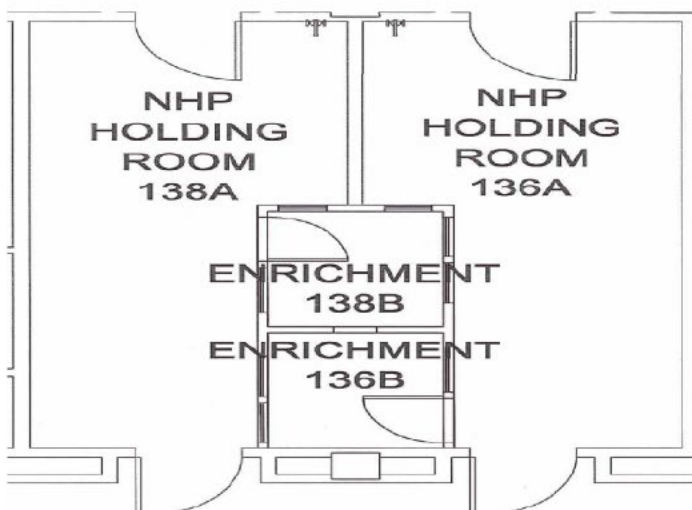
### **Environmental Enrichment (EE) Cubicles:**

EE cubicles provide a physical environment which encourages and enables expression of beneficial behaviors including interaction with personnel, foraging, and manipulation of enrichment devices. The NHP suites contain four (4) cubicles (36 ft<sup>2</sup> X 8 ft in height) constructed of a stainless steel with panoramic 1/2" laminated glass windows, methyl methacrylate (MMA) flooring, and a central drain. Cubicles are paired between two (2) holding rooms to provide visual contact with the adjacent holding room and were designed side-by-side with removable dividers to allow doubling floor space as needed. They are equipped with multiple fixed perching levels and smooth eyebolts on the walls and ceiling to attach enrichment devices.

Curtains are installed for situations causing stress to the animals within the holding rooms and/or the enrichment units.

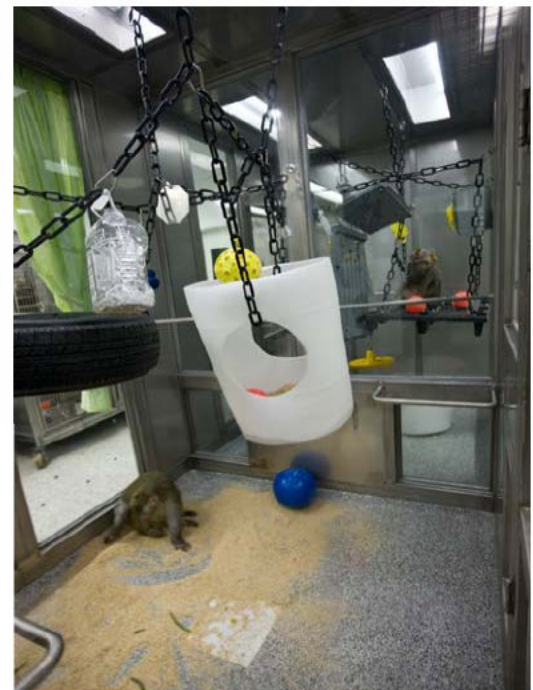


**Figure:** Panoramic view of EE cubicles: units were designed to facilitate operations and animal monitoring while providing as much visual contact across rooms to stimulate social interactions among members of the colony.



**Figure:** Floor plan portion of EE cubicles within CRAF.

**Figure:** Floor space allows for a foraging activity, multiple levels and perches create a three dimensional environment.



## **Animal and Human Interaction:**

Positive interaction between an animal and care technician can be done in a safe manner to increase relationships and to provide an ideal research model for training conditions. Technician time provides the non-human primates with daily positive human contact. This time includes husbandry routines with consistent staffing, technicians providing treats, in addition to positive verbal and nonverbal communication.

## **NHP Pair Housing:**

Pair housing will be encouraged when the process does not conflict with research objectives. Every effort will be made to maintain imported NHPs arriving as pairs. Veterinary Services must always be on alert when pair housing is in action for trauma issues that may arise. The following are general considerations for establishing pairs:

**Non contact familiarization period:** Allows visual, olfactory and auditory communication in order to allow dominance- subordinate relationships should be developed before pair housing. Monkeys are housed next to each other with clear partition between them.

**Tactile interaction:** Monkeys are permitted contact with one another via wire partition.

**Pair housing:** Partition removed to pair monkeys (assessing facial expressions, postures and physical distance between the animals).

**Note:** Mild aggression may be tolerated to allow monkeys to establish dominance. Partitions will be available to separate in case of excessive aggression. Pairs are deemed incompatible if one animal continually tries to escape or if aggression doesn't subside within a week. See NHP Pair Housing Interaction Levels table:

Level 1	Aggressive behavior/threats with no physical contact
Level 2	Aggressive behavior/threats with one non-injurious physical contact
Level 3	Aggressive behavior/threats with multiple non-injurious physical contacts that cease upon intervention
Level 4	Aggressive behavior/threats with injurious physical contact(s) that cease upon intervention
Level 5	Aggressive behavior/threats with injurious physical contact(s) that does not cease upon intervention

\*For the purpose of this process, injurious is defined as a laceration or wound that may or may not require veterinary intervention.

The individual executing the pairing will intervene when the pair demonstrates behaviors from Levels 3, 4 and 5.



**Compatible pair formed:** Length of time together is increased gradually, but they are separated for feeding and sleeping if needed.

**Note:** Using the natural social structure of macaques will assist in forming compatible pairs or groups of monkeys. Examples:

- bachelor troops/groups, often males can be pair-housed successfully
- pairing of a young animal with an older animal, so that the dominance hierarchy is clear and fighting is minimized.

### **Safety:**

The EE cubicle animal doors are designed to be an exact fit to our transport cage with fitting guillotine style doors to avoid animal escapes. Before NHPs are introduced into the EE cubicles of any NHP, all obvious safety issues are evaluated such as: clips to use for enrichment items, object's sturdiness and safety, central drain inaccessibility to animals, layout of chains, and inaccessibility to screws or caulking that could be ingested by animals. During the training period, 3/4" round neon colored stickers are placed on the transparent windows to signal the presence of the glass to the animals, thus avoiding accidental collisions.

The Animal Resources Department always takes in consideration the safety of the animals in our care, by inspecting, testing, and documenting all enrichment items utilized in the enrichment program.



**Figure:** ARD personnel verifies physical integrity of enrichment objects to ensure that they are free of sharp edges, defects, cracks, or other evidence of diminished integrity.

## **Non-Human Primates (NHP - Marmoset)**

### **Normal Behavior:**

- Calm locomotion
- Allogrooming and self grooming
- Exploration of environment
- Playing: playful chasing, wrestling, biting, batting, rolling. Solitary play common.
- Scent markings: rubs sterna (tummy) or anogenital area over surfaces is common but a negative behavior is done excessively
- Allomark: lifts tail and rubs genital area on other marmosets
- Nuzzling: face/body/anal, can include licking, sniffing and hugging
- Vocal calls: whirr/trill – contact call between animals and chirp – like bird call pleasant to human ears are associated with positive behavior
- Leaping and vertical flight
- Head cock stare: inquisitive/studying behavior
- Leg-stand: marmoset stands tall when a new noise or motion is noticed
- Hang by feet and vertical cling
- Flop: marmoset lays down on belly often hanging feet and hands off limbs/ledges
- Affiliative behavior with other marmosets: huddling, food sharing

### **Aberrant Behavior:**

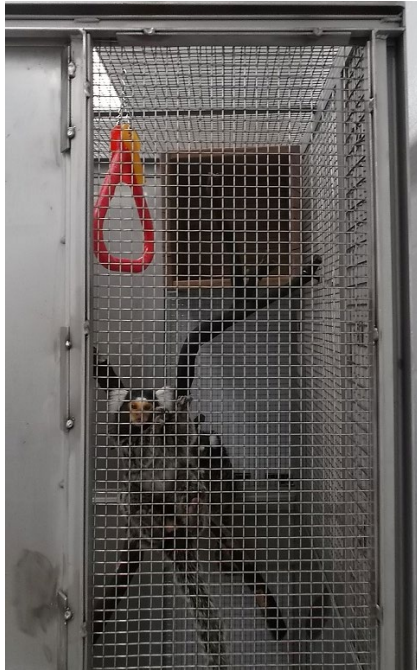
- Anogenital tail present
- Vocal calls: marmosets use a wide variety of vocalizations based on level of anxiety: squeal/scream, see/seep, chatter (angry, cackle), loud shrill, Ek, cough, Tsik, Rapid fire mobbing call, alarm calling (lek, tsik, seeps and mobbing call)
- Hair fluffing/bristling up, nervy locomotion, mobbing behavior are signs of distress
- Stereotypical behavior: repeated circling, weaving
- Self-injurious: excessive self scratching, picking at hair and biting
- Withdrawal gesture: backward movement with arms outstretched
- Cringe: more violent than withdrawal gesture
- Hair standing on end and swaying with alarm calls are signs of agitation
- Other negative behaviors: excessive scent markings, scratching, gouging, agitated locomotion except during play, chasing, cuffing, snap biting and stealing food

### **Enrichment:**

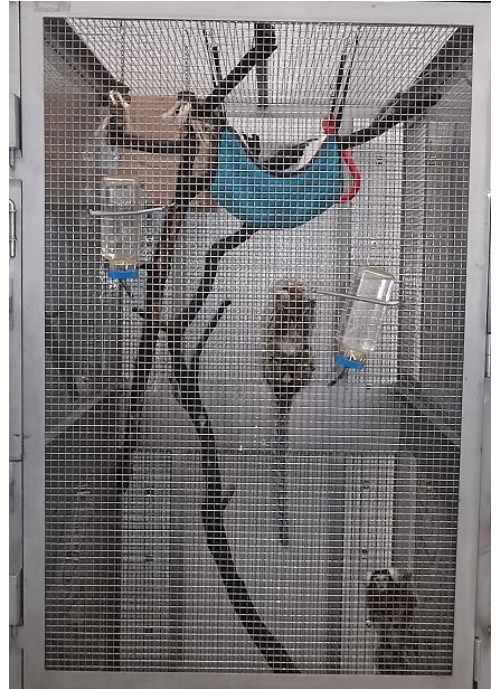
- Marmosets spend a significant amount of their day foraging for food. Multiple feeding times coupled with edible enrichment given throughout the day will help facilitate this behavior
- These small agile monkeys feel most safe high in the trees away from many predators. Adding branches and hammocks will allow them to demonstrate many normal behaviors. A place to hide such as a nesting box is crucial as well
- Marmosets are naturally curious animals who will study and investigate new things placed in their cage and the room itself. Varying the types of enrichment and items used is a good way to enrich this behavior. Watch closely for items that cause negative behaviors
- Enrichment items ideas: foraging foods wrapped in paper/box/bottle, frozen juice blocks with fruit in



them, frozen bananas, whole pieces of fruit for them to break into, dry foraging items spread out through bedding or enrichment device, television and radio



**Figure:** Nesting boxes are placed high in cage facing away from the front to afford the marmosets a sense of safety



**Figure:** Tree branches, hanging toys and hammocks are installed for climbing and nesting



**Figure:** Marmosets spend the majority of their time high in the trees. Placing tree branches and toys/devices near the top of the cage is preferred.

## **Ferret (*Mustela putorius*)**

### **Normal Behavior:**

- Ferrets tend to sleep 14 to 18 hours a day and have poor eyesight (this is important when enriching their environment).
- Ferrets are naturally inquisitive and can escape if the caging equipment is not appropriate for housing.
- Ferrets have a natural instinct for tunneling and their inherent curiosity frequently places them in potentially dangerous situations.
- Domestic ferrets use many different types of behaviors, including body posturing, animations, vocalizations, and scent markings.
- These behaviors may differ somewhat from ferret to ferret.

**Note:** When handling ferrets one must remember that it is natural for them to be nippy.

The domestic ferret is best understood by observation and recognition of its behavior patterns and interactions as it plays and communicates with both humans and animals within its home environment. Changes in routine are often an indication of a problem, including a normally active ferret suddenly becoming quiet or vice-versa; any sudden increase or decrease in daily food and water intake; routine behaviors performed out of context or order; sudden increase or decrease in the speed at which routine behaviors are performed (such as urination, defecation, grooming, food, or water intake); sudden changes in personality or attitude toward other ferrets or toward other animals or people.

### **Enrichment:**

Ferrets are complex and curious animals; the enrichment program consists of structural enrichment such as: hammocks, nesting boxes, litter boxes for excretions and burrowing. Similar to rabbits and guinea pigs the ferrets are offered a verity of enrichment devices. Jill's are pair housed; however; there are cases were Jill's are single housed such as pregnancy, age, and/or size. Technician time can provide ferrets with a positive human contact, this will increase easy of handling and restraint when necessary.



**Figures:** Enrichment items utilized for sleeping and daily activity.

## **Rabbit (*Oryctolagus cuniculus*)**

### **Normal Behavior:**

- Rabbits are active, curious animals that spend large amounts of time exploring objects in their environment.
- Rabbits normally sit on all four feet, with their body weight evenly distributed and the head held level with the top of the back.
- Rabbits move around the cage by hopping and occasionally stand on their hind legs.
- Rabbits are more active at twilight than during the day.
- Rabbits feed and drink water mostly in the late afternoon and early evening and sleep during the day.
- Air-boxing: fast forward flicking of forelimbs whilst rabbit sits upright on haunches. Usually precedes body grooming.
- Body-rolling: the rabbit throws itself onto the bottom of cage in a sideways roll, may lie immobile in outstretched position on side with eyes half closed for some seconds after roll (often performed as a dust-bath).
- Chin-marking: rubbing the chin over an object or conspecific, releasing secretion from the chin gland.
- Coprophagy: a rabbit eats soft, mucus-covered, grape-like clustered pellets directly from anus.
- Crouching: a submissive behavior, animal freezes and presses head and shoulders against bottom of cage with ears flattened.
- Gnawing helps to maintain tooth sharpness and oral health (rabbit's teeth grow continuously).



**Figure:** Rabbit actively playing with a Jingle Ball.

### **Aberrant Behavior:**

- Rabbits are sensitive to noise and may react violently to loud or sudden sounds
- Rhythmic biting of water valve and/or pulling out stoppers
- Biting, chewing or licking bars and food hoppers
- Pawing or digging in the corner of the cage, pawing the walls or the food hopper
- Rapid circling, sometimes including kicking the walls
- Head swaying or weaving, repeatedly sliding the nose between the bars
- Pushing hoppers and drinkers with the head
- Somersaulting



### **Enrichment:**

Suitable enrichment for rabbits includes roughage such as: hay blocks, loose Timothy hay, fresh fruits and vegetables. Enrichment devices are rotated as scheduled to provide the rabbits with activity time in their cage environment. Devices utilized with the rabbits include but not limited to the following: plastic chains, plastic curtain rod rings, Jingle balls, plastic Dumbbells, plastic balls, and Nyla bones.

### **Enrichment Pens:**

Group-housed or pair-housed rabbits represent a stimulating form of enrichment. The quality of life of group/pair-housed rabbits is significantly improved in comparison with singly housed animals, even for those individuals regarded as subordinate. Group/pair-housed rabbits need to be able to initiate or withdraw from social and visual contact at will; this is facilitated by providing plenty of space and structures such as refuges and partitions.



**Figure:** Pair-housed rabbits in enrichment pen.

Technician time can provide rabbits with positive human contact, increasing ease of handling and restraint.

## **Guinea Pig (*Cavia porcellus*)**

### **Normal Behavior:**

- Guinea pigs often vocalize and at a much louder volume than most rodents. When in pain or distress, they emit a loud squeal.
- Guinea pigs often play with their food and water, resulting in wastage.
- Guinea pigs have relatively short legs and stocky bodies; as a result, they cannot climb or jump.
- The teeth of guinea pigs constantly grow which means that chewing items are essential for their dental health.

### **Aberrant Behavior:**

- Barbering is common in guinea pigs housed together. The dominant animal will chew the hair of subordinates leaving bare patches that can be confused with a skin problem.
- Guinea pig may appear bored, listless, won't wheel or talk, pop, run around, etc. Guinea pigs interact with each other with their acute sense of smell and hearing as well as speech and touch. To remove the ability for a social animal to interact with another can dramatically impact the health and overall emotional state of the animal

### **Enrichment:**

Guinea pigs are provided similar enrichment devices as the rabbits. Guinea pigs are provided shelters such as tubes and huts in addition to enrichment devices. Roughage such as hay blocks, loose Timothy hay, fresh fruits and vegetables are also given on a scheduled basis. Guinea pigs received required vitamin C through their regular pellet diet and also supplemented through fresh fruits and vegetables. Sows are group housed to take care of their social needs. Technician time can provide guinea pigs with positive human contact, increasing ease of handling and restraint.



**Figure:** Guinea pigs utilizing a hut to hide.

## **Rodents (*Mus musculus*, mouse and *Rattus norvegicus*, rat)**

### **Normal Behavior:**

- Mice and rats are normally more active at night.
- Normal posture is with all four feet on the cage floor, eyes and ears alert.
- Mice groom themselves almost constantly, which helps them maintain a smooth, glossy hair coat.
- Mice have a high metabolic rate and are constantly active except when sleeping.

### **Aberrant Behavior:**

- Lack of grooming behavior, a dull hair coat, decreased activity and hunched posture are early signs of stress or disease.
- Barbering, fighting, circling, pacing
- Porphyrin is a red discharge appearing from either the nose or eyes. The substance is usually not blood. The discharge is produced by a special gland behind the rat's eyes called the Hardarian gland. Overproduction of this discharge can be caused by stress or illness.
- Bruxism, or teeth grinding, may be observed in a stressed animals.

Rodents attempt to divide their living space into separate areas for feeding, resting, and excretion. The cage design provides them this opportunity: the wire bar feeder provides the area above the cage floor for feeding and paper based shredding material provides nesting and resting areas. In some cases rodents are provided huts for breeding and resting purposes. Both mice and rats are grouped housed for socializing opportunities; however, there are cases where single housing is required depending on research needs.

Mice are typically housed on 1/4 inch corn cob with the addition of nesting material (ex: Bed-r'Nest or Nestlets). They may also be provided with red huts or perches. This promotes animal activity and satisfies natural instincts to nest and burrow.

Rats do not response to nesting opportunities as much as mice. They however are very active gnawers, and will quickly work through a nyla-bone. They are provided fresh fruits and vegetables and a destructible enrichment device (i.e. paper balls or paper tubes) with a foraging item, as well as, red, polycarbonate hiding tubes.



**Figure:** Examples of mouse enrichment: nesting material and a mouse hut.





**Figure:** Examples of rat enrichment: disposable cardboard and fresh fruit. Rats do not typically build nests.



**Figure:** Rat enrichment: polycarbonate hiding tubes



**Figure:** Mouse perch provides enrichment as well as protection in the case of accidental cage flooding.

**Figure:** Barbering in mice, resulting from a dominant mouse over grooming cage mates. Note: the unaffected mouse is normally the culprit and the dominant animal (arrow).



**Figure:** Porphyrin staining in rats is an indicator of stress. It is usually seen around the eye and nares and can be mistaken for blood.



**Frog (*Xenopus laevis*, African clawed frog and *Xenopus tropicalis*, tropical frog)**

**Normal behavior:**

- Frogs are relatively non-specific predators that hunt on the surface and forages on the bottom for a wide taxonomic range of prey.
- Both species of frogs will continue to eat even when their stomachs are full (monitoring diet is important).
- It is also important to keep them in a secure tank and keep the water a few inches from the top of the tank, they are good jumpers.
- The frogs also enjoy a place to hide, in the wild they would hide in the murky water.

**Aberrant Behavior:**

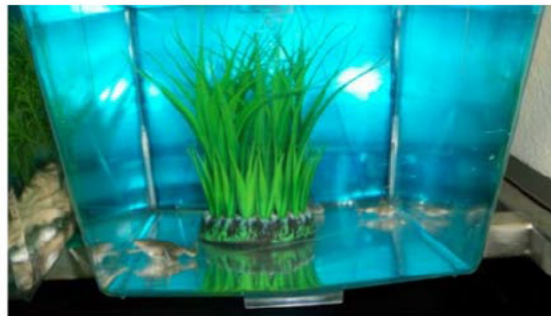
- Excessive stress may lead to the production of excesses mucin (cloudiness of the water), repeated attempts to escape, and hyper startle response to movement, noise, vibration.
- Often with stress, superficial ulcerations develop from running into objects within the cage.

**Enrichment:**

Both male and female frogs are socially housed. When applicable, the frogs are offered floating plastic balls, plastic aquatic plants, floating lily pads and PVC Pipes to hide in.



**Figure:** When frogs are housed in small clear recirculation tanks gravel may be used as enrichment. Plastic aquatic plants are used to simulate a natural-looking habitat.



**Figure:** When frogs are housed in large opaque tanks: floating lily pads can be used for hiding.



## Axolotl *Ambystoma mexicanum*

### Normal behavior:

- Axolotls are neotenic - meaning that they attain sexual maturity but remain permanent larvae. They do not metamorphose into the adult stage.
- Axolotls are quite cold tolerant, but usually thrive in water kept at room temperature (72 to 76 F) as well.
- Axolotls are voracious salamanders that consume all manner of smaller pond-dwelling creatures, including smaller examples of their own kind. They find their food by sight, by scent and, perhaps, by touch.

### Aberrant Behavior:

- Axolotls spend a lot of time near the water's surface when their gills aren't able to take in enough oxygen from the water. This lack of oxygen can be due to gill shrinkage, and it can also be due to inadequate aeration or water contamination.
- Loss of appetite for more than 3 days, usually a sign something is wrong. Check the following: water temperature, water contamination, and signs of illness.

### Enrichment:

Axolotls are given plastic natural looking plants for hiding. Technicians train axolotls to come towards the surface for feeding.

**Figure:** Hand feeding axolotls creates a bonding moment between technician and animal.



---

	Signature	Title	Date
Approved:	(b) (6), (b) (7)(C)	Sr. Director, ARD	10/17/11
	(b) (6), (b) (7)(C)		

---