Title: **Program for Environmental Enrichment**
Established:2018
Revision Date: October 2021

Program for Environmental Enrichment

Various terms are used to describe the welfare requirements of research animals, such as psychological wellbeing, environmental enrichment, behavioral needs, etc. The 1985 amendments to the Animal Welfare Act require facilities to provide exercise for dogs and programs to promote the psychological wellbeing of nonhuman primates. The Guide for the Care and Use of Laboratory Animals (2010) states that "The primary aim of environmental enrichment is to enhance animal well-being by providing animals with sensory and motor stimulation, through structures and resources that facilitate the expression of species-typical behaviors and promote psychological well-being through physical exercise, manipulative activities, and cognitive challenges according to species-specific characteristics".

The ARC is responsible for the Enrichment Program. The mission is to promote psychological well-being of all laboratory animals housed within the MSU research program through appropriate housing, enrichment, health care and activity, thereby allowing the animals to engage in species-typical behaviors. The goals of the program are to provide interaction and self-initiated behaviors by allowing a degree of control over their environment and to increase species-typical behaviors while decreasing pathological maladaptive behaviors.

I. **Methods of enrichment**
   a. include, but are not limited to:
      i. Social housing
      ii. Housing devices
      iii. Manipulative devices
      iv. Visual, Auditory, and Olfactory devices
      v. Food items

II. **Goals of Enrichment**
   a. To address the psychological wellbeing of all species housed in the facility.
   b. To provide interaction and self-initiated behaviors by allowing a degree of control over their environment.
   c. To increase species-typical behaviors while decreasing pathological maladaptive behaviors.

<u>Non-human Primates</u>

I. **Refer to the MSU Program for Psychological Well-Being of Non-human Primates for detailed information.**

II. **Manipulative Devices**

   a. A minimum of two toys are always provided. Attempts are made to rotate these toys to decrease boredom.

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i. Examples: brushes, paper towel rolls (peanut butter or honey), DNA Flexors, Dumbbells, PVC pipe, Kong Toys, balls, plastic milk jugs (with frozen water/fruit inside), hardwood logs, etc.
ii. Suspended toys include mirrors, plumbing products, stainless steel triangle, foraging devices such as puzzle feeders, etc.

b. Toys are sanitized and rotated biweekly.

III. **Audio Devices**

a. Devices such as radios or televisions are considered a sensory enrichment and are not necessarily a direct form of enrichment. These devices are part of our program, but not the main emphasis. The monkeys may view one movie/day during the week.

IV. **Food Items**

a. Cut up fruits and/or vegetables are given when research protocol allows, and usually with the PM feeding.
b. Supplemental food items are utilized as part of the enrichment program and usually provided with a foraging device or puzzle feeder.
    i. Examples include but are not limited to: air popped popcorn, frozen juice cubes, cut up vegetables, sunflower seeds, pumpkin seeds, trail mix, shredded wheat, etc.
c. All food enrichment items are documented in the daily log.
d. Juice is a reward item given by the research staff when the monkey is on study.

Rabbits

I. Housing Devices

a. Group Housing
    i. Rabbits are housed in groups on the floor, allowing no less than four square feet per rabbit (4-5.4 kg).
    ii. The maximum number of rabbits per room is 26, but the average is 10-15 per room.
    iii. Empty plastic barrels are provided as escape and lounging structures.
    iv. Empty bedding bags are also provided for escape and hiding.

b. Cage Housing (single or pair)
    i. When required for experimental protocols, health reasons or incompatibilities, rabbits are housed in single, stainless steel cages.
    ii. Cages provide four square feet per compartment and at least 16 inches high. When possible, the partitions separating the compartments are removed to allow pair housing or to allow for extra space for singly housed rabbits.
    iii. Cage racks for the rabbits in single cage housing situations are arranged so visual and auditory contact between rabbits are maintained.

II. Manipulative Devices

a. Toys
   i. At least one device is always available to cage housed rabbits and provided on a periodic schedule for floor housed rabbits.
   ii. Examples: Nylaballs, Jingle Balls, Nylabones, PVC Pipe, Kong Toys, wood chew toys, etc.
   iii. Shredded paper, straw or autoclaved alfalfa hay provides a soft nesting material or foraging device.
   iv. Stainless steel bowls provide noisy entertainment.

b. Toys are sanitized and rotated biweekly.

III. Visual and auditory devices

a. Attempts are made at all times to group/floor house rabbits. This allows direct visual, tactile, auditory, and olfactory contact.
b. Cage racks are arranged so that visual, auditory, and olfactory contact with other rabbits in the room is maximized.

IV. Food Items

a. Food enrichment is provided on a rotating basis for both the floor and cage housed rabbits.
b. Bunny Blocks
   i. Periodically, Bunny Blocks are hung to provide additional stimulation.
c. Straw and hay
   i. Autoclaved barley straw and alfalfa hay is provided as enrichment for the floor and cage-housed rabbits to facilitate foraging.
d. Fruits/Vegetables
   i. At a minimum of once a week the rabbits are provided with various vegetables or fruits.

<u>Rodents</u>

I. Housing Devices

a. Group Housing
   i. Most rodents at the ARC are housed in groups that are dictated by the experimental protocol, current use of the animals as well as age/bodyweights of animals.
   ii. Rodents used for breeding are either monogamous pairs, trios or harem breeding.
   iii. Animals held for stock are housed in numbers according to age, sex, and strain, not to exceed the recommendations of the *Guide for the Care and Use of Laboratory Animals*.
b. Single Cage Housing

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i. Individually housing rodents is avoided whenever possible, except when required by experimental protocols, health concerns or incompatibilities with other animals.

c. Lounging, hiding or escape structures.
   i. These devices are used whenever possible.
   ii. Mouse houses are used for mice, polycarbonate tunnels are used for rats
   iii. These devices are sanitized or disposed of as needed.

II. Manipulative Devices

a. Some type of manipulative device is maintained in all rodent cages, unless the experimental protocol prohibits this practice.
b. Examples include but are not limited to:
   i. EnvrioDri
   ii. Nestlets
   iii. Mouse Igloos
   iv. Wood blocks for chewing/gnawing
   v. Pulp paper bio tunnels are provided for rats.

III. Visual, auditory, and olfactory devices

a. Attempts are always made to group house rodents to allow them visual, auditory, olfactory, and tactile exposure to conspecifics.

IV. Food Items

a. Rodent treats may be provided on an intermittent basis for foraging.

Guinea Pigs

I. Housing

a. Guinea pigs are provided with at least 60 square inches of floor space if the weigh under 350 grams or greater than 101 square inches if over 350 grams, and all have a cage height of 7 inches.
b. Group housing
   i. Pair-housing is preferred for guinea pigs.
   ii. Triplicate-housing is permitted if an odd number of animals exists with daily monitoring for any incompatibilities between animals as well as cage soil level.
c. Single Cage Housing
   i. Individual housing of guinea pigs is avoided whenever possible, except when required by experimental protocols, health concerns or incompatibilities with other animals.

II. Manipulative Devices

a. Enrichment includes poly-carbonate huts/shelters inside the cage.
b. Small wooden blocks are given for chewing and gnawing.

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III. Visual, auditory, and olfactory devices

a. Guinea Pigs are housed in open style cages with grid tops, this allows greater auditory, and olfactory exposure to conspecifics. Pair/group housed animals have visual and tactile exposure to conspecifics.

IV. Food items

a. Guinea pigs receive cut up fruit and/or vegetables 3x per week (typically Monday, Wednesday, and Friday). Caution should be used to ensure that the amount of treats is limited to ensure that the animals eat their nutritionally balanced diet.

Aquatic Species

I. Housing

a. Xenopus laevis: adults should be housed at 1 per 2 liters of water, juveniles (froglets) at 1 per liter of water. Xenopus tropicalis: 1 adult per liter of water, 1 juvenile (froglet) per 0.25 liters of water. Zebrafish: 5 fish per 1 liter of water on flow through system, 1-2 fish per liter of water in static tanks.

b. Aquatics Species are group housed as standard. Isolation may be required for health or study/scientific reasons.

II. Environmental Enrichment

a. Xenopus laevis and tropicalis receive a polycarbonate cubic tunnel in each tank. Zebrafish are provided with aquarium plants and are fed artemia 1x per day as live prey.

Conclusions

All enrichment devices are approved by the Attending Veterinarian and the Primary Investigator. Any animal that shows signs of illness is evaluated by the veterinarian and exempted from the enrichment program as needed. Since enrichment is a continually evolving practice, the items stated in this document are considered minimums. New and novel ideas may be tried at any time. If deleterious behaviors are noted in any animal housed at the ARC, the veterinarian is contacted. A health assessment is performed. Additional efforts are made to improve individual animal psychological well-being and decrease or eliminate deleterious behavior.

Approval Dates
Attending Veterinarian: 19th April 2021
MSU IACUC: 21st April 2021

SOP Number: ARC SOP 20.4
ARC SOP Title: Non-Human Primate Daily Husbandry & Health Checks
Established: June 2009
Revision Date: October 2021

Non-Human Primate Daily Husbandry & Health Checks

1. Purpose and Scope

This Standard Operating Procedure (SOP) details the procedures for performing Non-Human Primate (NHP) daily husbandry and health checks in the ARC.

2. References

2.1. Regulations

The contents of this document will conform to the most current editions of all institutional, local, state and federal regulations, policies and guidelines for working with animals used in scientific research projects.

2.2. SOPs and Plans

NHP Psychological Well-Being Plan
SOP Entering and Exiting the NHP ABSL2 Containment Area

3. Roles & Responsibilities

Trained ARC Staff are responsible for performing NHP husbandry and health checks as described in this SOP.

4. Special Requirements

4.1. Equipment and Supplies

Adequate amounts of food, bedding, and environmental enrichment. Appropriate disinfectant (e.g., Rescue), foamer, trashcans lined with biohazard waste autoclave bags.

Safety Requirements

Occupational Health Assessment and clearance must be obtained prior to work with NHPs.
Staff must be trained on the risks and mitigations for potential Herpes B Virus exposure.
Personal protective equipment (PPE) required for performing husbandry tasks as described in the applicable SOP.
Personnel performing procedures must do so in a manner that reduces the risk of personal injury from NHPs.

5. Applicable Locations

ABSL2 East Containment NHP housing rooms - ARC

6. Space Requirements

6.1. When required by experimental protocol, health reasons or incompatibilities with other animals, rhesus monkeys are singly housed in stainless steel, one-over-one slide floor cages. Whenever possible and reasonable, animals should be given additional floor space.

6.2. The cages provide 6.2 sq. ft. of internal floor space and 82 inches of height. At a minimum, USDA category Group 4 monkeys weighing between 22 and 33 lbs. (10-15 kg must be provided with 6.0 sq ft of floor space and 32 inches of cage height. Thus the caging configuration provided exceeds the USDA minimal standard. Animals weighing > 15 kg must be given 8 sq. ft. of floor space and 36 inches of cage height. Therefore, animals weighing greater than 15kg must be given an extra one-over-one cubicle.

7. Procedures and Instructions

Follow the steps required to enter the ABSL2 housing area (see relevant SOP).

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Document completion of the following procedures on the NHP Monthly Room Log. Some procedures require additional documentation as described below.

Note: Prior to entering an NHP room; look through the door window to ensure no loose animal can be seen.

7.1. Environmental Monitoring, Sanitation, and Security

7.1.1. Record room temperature and humidity. Report readings outside the acceptable range of 68-79°F/ 30-70% to ARC Supervisor.
7.1.2. Using a scraper and dustpan remove solid waste from grid floor and soiled bedding from pans and dispose in a trashcan lined with both a biohazard waste autoclave bag and standard trash bag. Do not manipulate cages unless trained and proficient to do so, or under supervision of someone who is proficient.
7.1.3. Refill pans with clean bedding as needed.
7.1.4. Uneaten biscuits should be counted and removed from cages where possible. The standard method is to allow biscuits to drop through into pans by manipulating grids/flooring.
7.1.5. If biscuits are on perches or other areas of the cage, close off the animal from the cage securing a divider between the cage and the animal, use an instrument to dislodge the biscuit so that it falls into the pan. The animal can then be allowed back into the cage.
7.1.6. If it is not safe to remove a biscuit from the cage, do not do so, make a note in the feed log so the biscuit is not counted more than once.
7.1.7. Hose down heavily soiled areas of the caging. Remove pans if necessary, to prevent accumulation of water in the bedding. Avoid wetting the animals, moving them to other areas of the cage if necessary.
7.1.8. Foam the floor with appropriate disinfectant, allow at least 3 minutes of contact time, then rinse with water and use the squeegee to remove as much excess water from the floor as possible.
7.1.9. Avoid spraying the cages with water and clean drain covers that become clogged during the process.
7.1.10. Animal security must be checked following all cage manipulations. Check that locks are engaged and positioned correctly. Confirm all locks again at the afternoon check.
7.1.11. Ensure that all vertical dividers in the Carter type caging that are to remain closed to prevent animal contact or escape have safety clips in place. These are dividers that are normally never used in cage manipulation.
7.1.12. Animal room lights are on timers but the those in the surrounding areas are not. Each day prior to leaving confirm that all hallway, and prep room lights are off.

7.2. Feeding and Watering

7.2.1. Note any special feeding requirements for specific animals such as DNF (Do Not Feed) or biscuit soaking etc.
7.2.2. Remove any uneaten biscuits in NHP cages during AM checks, following the steps outlined above. Count, but no not remove, biscuits before PM feeding.
7.2.3. Separate pair housed animals prior to feeding if required.
7.2.4. Communicate with laboratory staff on timing of regrouping, as necessary.
7.2.5. Each NHP is fed a pre-determined number of approved NHP specific diet biscuits twice a day.
7.2.6. On weekends/holidays the AM and PM biscuits are all provided in one serving. Fresh items such as fruit/vegetables are provided at the PM check.

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7.2.7. Record the number of biscuits provided on the NHP Weekly Food Log. At the next scheduled feeding count the number of biscuits left (not consumed) to calculate the amount eaten and update the log.
7.2.8. Refill water bottles via the animal or prep room sink for non-water restricted animals each morning and check/refill as needed in the afternoon. If bottle is soiled in any way, rinse when filling. If heavily soiled, replace with new clean bottle.
7.2.9. Water bottles will have a red tag placed to indicate that animal is on fluid restriction.
7.2.10. Secure water bottles in holders when replacing and ensure animals cannot access the security devices.
7.2.11. Water restricted animals are provided measured water rations by the lab or ARC when directed to do so. Rations are documented on the Water Delivery Confirmation Log. Contact the appropriate lab staff on any occasion when it is unclear as to whether water restricted animals have received their ration.
7.2.12. Treats may be placed in food hoppers or offered by hand. When offering a treat by hand, the treat much extend beyond the operators' hands/fingers such that it can be grasped by the animal without operator contact.

7.3. Enrichment

7.3.1. Enrichment is provided in accordance with the MSU NHP psychological wellbeing plan.
7.3.2. Check that all animals have appropriate enrichment items.
7.3.3. Turn on audio visual devices in the room to provide sensory enrichment. These devices must be turned off prior to leaving the facility each day.
7.3.4. Provide standard enrichment by adding browse to feeder boards and/or puzzle feeders each morning.
7.3.5. Additional food items such as fruits and vegetables may be provided in the afternoon when allowed by the research protocol.
7.3.6. Document all activities using the NHP Enrichment & Behavioral Management Log.

7.4. Health Checks and Treatments

7.4.1. NHPs receive twice daily health checks, an AM and a separate PM check. Checks are typically carried out alongside husbandry duties.
7.4.2. Visually assess each animal for activity levels, ease of movement, posture, hair coat and body condition. Check the animal's caging environment especially the waste pan for signs of any abnormal fluid/discharge/blood.
7.4.3. Report medical concerns (e.g., injury, disease, or abnormal behavior) immediately to the Attending Veterinarian or vet on call as appropriate.
7.4.4. Document abnormal observations, timings and actions taken in the animal's individual medical record.
7.4.5. Some animals may receive medical treatments. Document all treatment administration in the appropriate treatment sheet/medical record.

Note: Animal security should always be double checked before leaving the NHP housing room if cage manipulations have taken place.

8. Forms

8.1. NHP Monthly Room Log

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8.2. NHP Weekly Food Log
8.3. Water Delivery Confirmation Log
8.4. NHP Enrichment & Behavioral Management Log

9. Records Management

Records are kept on file at the ARC for a minimum of 3 years.

Approval and Dates
Attending Veterinarian: [redacted] May 2021
MSU IACUC:

SOP Number: **21.5**
ARC SOP Title:
Daily Husbandry, Health, Life Support System Checks for *Xenopus laevis*
Established: 2011
Revision Date: February 2021

Daily Husbandry, Health, and Life Support System Checks for *Xenopus laevis*

1. Purpose and Scope

This SOP details the procedures for the daily, weekly, and monthly husbandry tasks for *Xenopus laevis*.

2. References

2.1. Regulations

The contents of this document will conform to the most current editions of all institutional, local, state, and federal regulations, policies, and guidelines for working with animals used in scientific research projects.

3. Roles & Responsibilities

ARC Staff are responsible for carrying out the procedures in this SOP.

4. Special Requirements

4.1 Equipment and Supplies

In room thermometer, flashlight, pest traps, system screen, squeegee, salt, bicarbonate, measuring cup, reverse osmosis (RO) water

4.2 Safety Requirements

Gloves must be worn to handle frogs. Other personal protective equipment (PPE) as appropriate.

5. Applicable Locations

[redacted]

6. Procedures and Instructions

6.1. Daily Husbandry

6.1.1.Parameters

6.1.1.1. Room Temperature: 63-66°F
6.1.1.2. Humidity: 30-70%
6.1.1.3. Water Temperature: 63-66°F
6.1.1.4. pH: 7-8
6.1.1.5. Conductivity: 800-1200 µS
6.1.1.6. Housing: Adults should be housed at 1 per 2 liters of water, juveniles (froglets) at 1 per liter of water.
6.1.1.7. Feeding: Tuesday and Friday, females receive 1 gram of food per frog and males receive 0.5 grams of food per frog.

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Attending Veterinarian: [redacted] April 2021
MSU IACUC: 19th May 2021

6.1.2. Record information in the animal holding room logs binder (white binder) on the room log. For the following steps refer to parameters in 6.1.1. If readings are not within parameters, report to operations manager.

6.1.2.1. Record the room temperature and humidity.

6.1.2.2. Record the water temperature, pH, and conductivity, which can be found on the P*ro-face* system screen located on the side of the tank rack.

6.1.2.3. Check all animals for general health. Check all animals in every tank; use a flashlight with a red filter if necessary to complete check.

6.1.2.4. Ensure enrichment is provided; use a flashlight when tanks are obscured by darkness.

6.1.2.5. If an animal health case arises, record the case in the animal health and husbandry records log (blue binder) then notify operations manager and the attending veterinarian (AV).

6.1.2.6. Use siphon to remove any excess debris from tanks by inserting hard plastic tube end of the siphon into the tank, lowering the pump end, and priming the pump by squeezing the bulb repeatedly until water flows freely.

6.1.2.7. Check pest traps. If a pest is found in the trap, report to operations manager and replace with a new pest trap.

6.1.2.8. Replace pre-filter by cutting a filter to fit in the holder.

6.1.2.9. If floor is excessively wet, use squeegee to push water into drains.

6.1.2.10. Check the salt and/or bicarb tanks.

6.1.2.11. If they are low, refill with RO water and measure out to appropriate ratio of 30 g per liter of salt for low conductivity, and 30 g per liter of sodium bicarbonate for low pH.

6.2. Weekly Husbandry

6.2.1. Parameters

6.2.1.1. Water Temperature: 63-66°

6.2.1.2. pH: 7-8

6.2.1.3. Conductivity: 800-1200 µS

6.2.1.4. NH3: <0.5 mg/L

6.2.1.5. NO3: <50 mg/L

6.2.1.6. NO2: <0.5 mg/L

6.2.1.7. Chlorine: 0 mg/L

6.2.2. Perform housekeeping duties for the room and record in the animal holding room logs binder (white binder) on the room log form.

6.2.2.1. Mop floors

6.2.2.2. Restock supplies and empty trash

6.2.2.3. Disinfect nets by replacing RO water and Net Soak

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6.2.2.4. Perform weekly water quality checks, referring to the parameters listed above, following the steps outlined in SOP 74 (Maintenance of Aquatic Life Support Systems).

6.2.3. Replace the mechanical filter every week and rinse the carbon filter every week.

6.3. Monthly Husbandry

6.3.1. Replace the carbon filter every month

6.3.2. To replace these filters, review the instructions in the following SOP 74: Maintenance of Aquatic Life Support Systems.

6.4. Annual Health Monitoring

6.4.1. Swabs will be sent to IDEXX for PCR evaluation for the following pathogens at a minimum: *B. dendrobatidis, M. chelonae, M. gordone, M. marinum, P. xenopi, Ranavirus*.

6.4.2. Results will be reported to the AV who will make any treatment/management determinations in consultation with the PI/s.

7. Forms

7.1. Fill out daily husbandry and care logs in the white "Animal Holding Room Logs" binder.

7.2. In the blue "Animal Health and Husbandry Records" binders, record the date and time when the carbon and mechanical filters were replaced.

8. Records Management

None specific

Approval and Dates
Attending Veterinarian: [redacted] April 2021
MSU IACUC: 19th May 2021

SOP Number: **22**
ARC SOP Title:
Daily Husbandry, Health, Life Support System Checks for *Xenopus tropicalis*
Established: March 2021

Daily Husbandry, Health, and Life Support System Checks for *Xenopus tropicalis*

1. Purpose and Scope

This SOP details the procedures for the daily, weekly, and monthly husbandry tasks for *Xenopus tropicalis*.

2. References

2.1. Regulations

The contents of this document will conform to the most current editions of all institutional, local, state, and federal regulations, policies, and guidelines for working with animals used in scientific research projects.

3. Roles & Responsibilities

ARC Staff are responsible.

4. Special Requirements

Equipment and Supplies

In room thermometer, flashlight, pest traps, system screen, squeegee, salt, bicarbonate, measuring cup, reverse osmosis (RO) water

4.1. Safety Requirements

Gloves must be worn to handle frogs. Other personal protective equipment (PPE) as appropriate.

5. Applicable Locations

[redacted]

6. Procedures and Instructions

6.1. Daily Husbandry

6.1.1.Parameters

- 6.1.1.1. Room Temperature: 70-75°F
- 6.1.1.2. Humidity: 30-70%
- 6.1.1.3. Water Temperature: 74-77°F
- 6.1.1.4. pH: 6-8
- 6.1.1.5. Conductivity: 800-1200 µS
- 6.1.1.6. Housing: 1 adult per liter of water, 1 juvenile (froglet) per 0.25 liters of water
- 6.1.1.7. Feeding: Monday, Wednesday, Friday at 2 food pellets per frog

6.1.2. Record information in the animal holding room logs binder (white binder) on the room log. For the following steps refer to parameters in 6.1.1. If readings are not within parameters, report to operations manager.

- 6.1.2.1. Record the room temperature and humidity.

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6.1.2.2. Record the water temperature, pH, and conductivity, which can be found on the *Pro-face* screen located on the side of the tanks
6.1.2.3. Check all animals for general health. Check all animals in every tank; use a flashlight with red filter if necessary to complete check.
6.1.2.4. Ensure enrichment is provided; use a flashlight when tanks are obscured by darkness.
6.1.2.5. If an animal health case arises, record the case in the animal health and husbandry records log (blue binder) then notify operations manager and the attending veterinarian (AV).
6.1.2.6. Use siphon to remove any excess debris from tanks by inserting hard plastic tube end of the siphon into the tank, lowering the pump end, and priming the pump by squeezing the bulb repeatedly until water flows freely.
6.1.2.7. Check pest traps. If a pest is found in the trap, report to operations manager and create a new trap.
6.1.2.8. Replace pre-filter by cutting a filter to fit in the holder.
6.1.2.9. If floor is excessively wet, use squeegee to push water into drains.
6.1.2.10. Check the salt and/or bicarb tanks.
6.1.2.11. If they are low, refill with RO water and measure out to appropriate ratio of 30 g per liter of salt for low conductivity, and 30 g per liter of sodium bicarbonate for low pH.

6.2. Weekly Husbandry

6.2.1.Parameters
6.2.1.1. Water Temperature: 74-77°
6.2.1.2. pH: 6-8
6.2.1.3. Conductivity: 800-1200 µS
6.2.1.4. NH3: <0.5 mg/L
6.2.1.5. NO3: <50 mg/L
6.2.1.6. NO2: <0.5 mg/L
6.2.1.7. Chlorine: 0 mg/L

6.2.2. Perform housekeeping duties for the room and record in the animal holding room logs binder (white binder) on the room log form.
6.2.2.1. Mop floors
6.2.2.2. Restock supplies and empty trash
6.2.2.3. Disinfect nets by replacing RO water and Net Soak
6.2.2.4. Perform weekly water quality checks, referring to the parameters listed above, following the steps outlined in the SOP: “Maintenance of Aquatic Life Support Systems.”
6.2.2.5. Rinse carbon filter every week.

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6.3. Monthly Husbandry

6.3.1. Replace the mechanical filter every 2-3 weeks

6.3.2. Replace the carbon filter every month

6.3.3. To replace these filters, review the instructions in the SOP: "Maintenance of Aquatic Life Support Systems."

6.4. Annual Health Monitoring

6.4.1. Swabs will be sent to IDEXX for PCR evaluation for the following pathogens at a minimum: *B. dendrobatidis, M. chelonae, M. gordone, M. marinum, P. xenopi, Ranavirus*.

6.4.2. Results will be reported to the AV who will make any treatment/management determinations in consultation with the principal investigator/s.

7. Forms

7.1. Daily husbandry and care logs in rooms.

8. Records Management

None specific

Approval and Dates
Attending Veterinarian: [redacted] 10th May 2021
MSU IACUC: 19th May 2021

SOP number: ARC SOP 41.4
Title: **Routine Health Monitoring of Rodents**
Established: June 2011
Revision Date: November 2021

Routine Health Monitoring of Rodents

1. Purpose and Scope

The purpose of this SOP is to describe the process for routine health monitoring of ARC rodent colonies.

2. References

2.1. Regulations

The contents of this document conform to the most current editions of all institutional, local, state and federal regulations, policies and guidelines for working with animals used in scientific research projects.

2.2. Supplementary

Idexx Bioanalytics is the commercial laboratory used for sample analysis. See Environmental Diagnostics Information for details of screening panels: https://www.idexxbioanalytics.com/edx

3. Roles & Responsibilities

3.1. ARC staff carry out the procedures described in this SOP.
3.2. The Attending Veterinarian (AV) reviews results and determines actions for positive results.

4. Special Requirements

4.1. Equipment and Supplies

4.1.1. Exhaust Air Duct Monitoring equipment: *Tecniplast Interceptor*
4.1.2. *Fecal samples from colony mice*

4.2. Safety Requirements

4.2.1. None Specific

4.3 Personal Protective Equipment (PPE)

4.3.1 Standard PPE for the appropriate biosafety level. A minimum of a surgical mask should be worn when removing interceptor devices from exhaust air ducts.

5. Applicable Locations

5.1. Include all rodent housing rooms in the ARC facility with the exception of Germ Free and BLS2 colonies.
5.2. Rats and guinea pigs may be exempt from testing based on time in the facility and needs of the protocol. Testing need will be evaluated by the AV at each testing interval.

6. Procedures and Instructions

6.1. Health Monitoring Information

- ARC mouse breeding rooms [redacted] exclude Mouse Norovirus (MNV) and Helicobacter sp.
- ARC mouse rooms [redacted] accept MNV and Helicobacter positive status.
- Except in special cases approved by the AV, mice are housed in Tecniplast Individually Ventilated Cages. A small number of mice may be housed in static cages as needed based on experimental requirements. ARC breeding rooms do not accept incoming animals unless they have cleared a comprehensive quarantine process or arrive directly from approved supplier. Once animals are transferred from breeding rooms they must not return.
- ARC experimental rooms generally consist of animals transferred from breeding rooms or those sourced from approved commercial suppliers with clean health monitoring backgrounds. Animals introduced to experimental rooms from other sources must complete a quarantine period.

Approval Dates
Attending Veterinarian: 15th December 2020
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- Animals in experimental rooms, generally have a higher turnover rate and do not stay in the facility as long. Screening in these rooms is less intensive than the breeding rooms.
- Sampling may be carried out at any time under veterinary guidance if there is reason to suspect a pathogen introduction or outbreak.

6.1.1. Environmental samples collected from exhaust air ducts (EADs) are used for routine health monitoring along with fecal pellets collected at random from colony animals. In some cases, such as in quarantine, samples may be collected directly from animals. Samples collected from animals may include fur swabs, fecal pellets or occasionally sentinel animals may have blood samples collected.

6.1.2. Sampling Schedule

- **Breeding colonies**: Perform IDEXX Mouse Opti-XXpress EDx **"G**lobal panel" one quarter and "Prevalent panel" three quarters.
- **Experimental colonies**: Perform IDEXX Mouse Opti-XXpress EDx "Prevalent panel" for all quarters.

6.1.3. Environmental Testing via EAD and Fecal samples

- *Tecniplast Interceptor* units must be in place (in EADs) for a minimum of 8 weeks prior to sampling.
- Two *Tecniplast Interceptor* units should always be in place.
- Remove the oldest *Interceptor* to send for sampling, replace with a new interceptor with the replacement date written the date clearly on the front.
- Collect a fecal pellet from 10 cages per room. If the room has more than one rack, divide the number of racks by 10 so that a representative sample is collected from each rack and pooled for the room (e.g., if a room has 2 racks, collect a fecal pellet from 5 cages per rack). Select samples from different strains if multiple strains are on a rack. Combine (pool) samples for each room and place in one microcentrifuge tube. Label tube with room number. Submit along with Tecniplast EAD Interceptor samples that are also labeled with the room number.
- Inform AV of the results. If test results are positive, consult with the AV regarding replacement of the 2nd interceptor which was in place at the time of the positive result.

6.1.4. Sample Collection & Handling Procedures - *Tecniplast Interceptor*

- To insert/remove the interceptor device, open the exhaust panel and remove the metal prefilter. Place the interceptor into the metal frame, replace the prefilter, slide interceptor to the last line so the filter is exposed and replace the panel. Reverse the process to remove.
- Once removed, fold the housing over the filter, complete information on the card, and place in the provided resealable bag for analysis.
- Additional resources and procedure video can be found at: https://www.tecniplast.it/en/product/interceptor.html#
- Place all samples Interceptor EADs and fecal samples in microcentrifuge tubes in a padded envelop and ship to IDEXX Bioanalytics.
- Include a copy of the online submission forms in a plastic bag.

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7. Forms

7.1. Submit forms online via Idexx submission portal (https://www.idexxbioanalytics.com). Include instructions in the comments section of the submission form requesting that the fecal samples and filter sample be extracted as separate samples and then pooled before testing. Indicate that this testing method as well as the no pooling fee has been discussed with [redacted].

8. Records Management

8.1. Health Monitoring Results are kept on file for a minimum of 3 years.

Approval Dates
Attending Veterinarian: 15th December 2020
MSU IACUC: 16th December 2020

SOP number: ARC SOP **46.3**
Title: **Quarantine Procedures for Rodents**
Established: November 2011
Revision Date: 7th April 2021

Quarantine Procedures for Rodents

1. **Purpose and Scope**
   This SOP details the procedures for importation of animals from non-commercial animal sources.

2. **References**
   2.1 Regulations
   The contents of this document will conform to the most current editions of all institutional, local, state and federal regulations, policies and guidelines for working with animals used in scientific research projects.

3. **Roles & Responsibilities**
   ARC Staff are responsible knowing and following relevant SOPs.
   ARC Staff are responsible for placing signage to indicate that quarantine room has animals and any specific pathogen information.
   Only ARC staff may enter quarantine rooms to perform animal care duties.

4. **Special Requirements**
   4.1 Equipment and Supplies
   PPE listed in SOP 65 for routine entry and exit of quarantine rooms.
   Additional supplies as needed.
   4.2 Safety Requirements
   None Specific

5. **Applicable Locations**
   Rooms in ARC designated as quarantine holding rooms: [redacted].

6. **Procedures and Instructions**
   Rodents from colonies of **approved** commercial animal vendors are housed in the ARC without a period of quarantine.
   Animals may not be imported from non-commercial sources without the express permission of the Attending Veterinarian and the Facility Manager.
   Rodents from non-approved sources are subject to a quarantine period per the recommendations of the Attending Veterinarian. The rodent quarantine period is a minimum of 28days with testing and treatment procedures conducted according to advice from the AV following health report review from incoming animals. Prior to animal importation, the AV will assess the health status of incoming animals and provide a quarantine plan to the ARC Facility manager.
   This SOP details standard quarantine procedures.

   Experimental Procedures: Experimental procedures are not permitted while animals are in quarantine. Breeding of rodents in quarantine is permissible. SPF 'clean' animals used for breeding may also be used as sentinels for health screening on completion of quarantine.

Approval and Dates
Attending Veterinarian: [redacted] 7th April 2020
MSU IACUC: 21st April 2021

6.1 Entering and working in rodent quarantine

6.1.1 Before entering quarantine, ensure that you have all the necessary supplies to complete intended tasks.

6.1.2 Follow the steps outlined in SOP 65 for entering and exiting rodent quarantine.

6.1.3 Dirty caging removed from the quarantine room must first be sprayed with appropriate disinfectant (e.g., Rescue) then moved directly to the dirty cage wash area.

6.1.4 The rodent quarantine room should be entered last during the day whenever possible.

6.1.5 Full change of scrubs and a shower is required to enter high health status mouse rooms after entering a quarantine room.

6.1.6 Full change of scrubs only is required to enter another mouse (non-high health status) room after entering a quarantine room.

6.2 Treatment procedures for mice in quarantine

6.2.1 Animals may be fed a commercial diet containing Fenbendazole or Ivermectin or receive either substance in pouches for the treatment of Pinworms. This may be for the duration of quarantine, depending upon background health reports and AV advice.

6.2.2 Advantage-multi (moxidectin/Imidacloprid) or other appropriate acaricide treatment may also be administered during quarantine according to AV advice.

6.2.3 Treatment with topical acaricides is given in 2 doses 21 days apart.

6.2.4 Dilute the Advantage-multi or other appropriate acaricide treatment to a working solution using sterile water. Mice should receive 0.5mg/Kg of Moxidectin and 2mg/Kg of Imidoclopride microliters of the solution onto the skin between the shoulder blades. If using Selamectin, mice should receive 10mg/Kg topically.

6.2.5 Working solutions of Advantage-multi or Selamectin diluted in sterile water may be stored in airtight tubes for up to 14 days.

6.2.6 A cage change should be performed following the second Advantage-multi treatment.

6.2.7 Other treatments may be undertaken on AV advice and depending upon health status review, purpose of animals etc.

6.3 Testing procedures for mice in quarantine

6.3.1 Prior to release from quarantine, animals including new litters must be tested for any pathogens excluded from the rooms they will move to.

6.3.2 All animals must show negative test results for fur mites and pinworms prior to release from quarantine.

6.3.3 Sentinel animals may be used, and blood/tissues taken for analysis after a minimum of 21days in contact with quarantined animals (via dirty bedding transfer or direct housing in the case of breeding animals)

6.3.4 Fur and feces samples may be collected from index animals for PCR evaluation of adventitious agents.

6.3.5 Environmental samples may also be used for PCR evaluation of adventitious agents.

6.3.6 Testing requirements prior to release should be outlined in the quarantine plan provided by the AV prior to importation of animals. This plan will define the number of individual animals to be evaluated to assure colony health.

6.4 Release from quarantine

Approval and Dates
Attending Veterinarian: [redacted] 7th April 2020
MSU IACUC: 21st April 2021

6.4.1 When all required treatments have been administered and test results received, the AV will assess the potential for release from quarantine.

6.4.2 Animals may be required to complete further treatments or testing as necessary.

6.4.3 Once the AV has carried out an assessment and deemed that animals safe to release from quarantine to a designated animal room, the AV will write to the ARC Facility manager and the PI to confirm.

6.4.4 The ARC Facility manager will make plans to relocate animal and decontaminate the quarantine room.

6.4.5 The PI will receive a notification once animals are relocated to standard housing and released from quarantine.

7. Forms

None Specific

8. Records Management

All treatments and testing will be documented in the animal health binder in the quarantine room. Records will be kept on file for a minimum of 3 years.

Revisions

7th April 2021 Revision: Update 6.2.2 and 6.2.5 to add other appropriate acaricide.

Approval and Dates
Attending Veterinarian: [redacted] 7th April 2020
MSU IACUC: 21st April 2021

SOP Number: **65.2**
ARC SOP Title:
Daily Husbandry, Health, and Life Support System Checks for Zebrafish
Established: 2011
Revision Date: February 2021

Daily Husbandry, Health, and Life Support System Checks for Zebrafish

1. Purpose and Scope

This SOP details the procedures for daily care and husbandry of Zebrafish at the ARC.

2. References

2.1. Regulations

The contents of this document will conform to the most current editions of all institutional, local, state, and federal regulations, policies, and guidelines for working with animals used in scientific research projects.

3. Roles & Responsibilities

ARC Staff are responsible for carrying out the procedures in this SOP.

4. Special Requirements

4.1. Equipment and Supplies

In room thermometer, flashlight, pest traps, system screen, squeegee, salt, bicarbonate, measuring cup, reverse osmosis (RO) water

4.2. Safety Requirements

Gloves must be worn to handle fish. Other personal protective equipment (PPE) as appropriate.

5. Applicable Locations

Zebrafish Room: [redacted].

6. Procedures and Instructions

6.1. Daily Husbandry Tasks

6.1.1. Parameters

6.1.1.1. Room Temperature: 75-81°F
6.1.1.2. Humidity: 30-70%
6.1.1.3. Water Temperature: 79-81°F
6.1.1.4. pH: 7-8
6.1.1.5. Conductivity: 900-1200 µS
6.1.1.6. Housing: 5 fish per liter in flow-through system. 1-2 fish per liter in static tanks.
6.1.1.7. Feeding: 2× per day. Feeding should be fine-tuned so that very little to no food is left 5-10 minutes after food was added to the tank (care should be taken to not over feed and have excess food in the tank). Supplement fish food with artemia (brine shrimp) 1× per day.
6.1.1.8. Static Tanks: **after each feeding**, excess food should be siphoned off the floor of the tank **5-10 minutes** after addition of food (thus, 2× per day).

Approval and Dates
Attending Veterinarian: [redacted] 10th May 2021
MSU IACUC: 19th May 2021

Flow-through system: siphon off excess food before the end of the day.

6.1.1.9. Monitor the filters in tanks for fry or larvae. The filters can easily become clogged with the finer fry food.

6.1.1.10. Record information in the animal holding room logs binder (white binder) on the room log form. For the following steps refer to parameters in 6.1.1 above. If readings are not within parameters, report to operations manager.

6.1.1.11. Record the room temperature and humidity.

6.1.1.12. Record the water temperature, pH, and conductivity, which can be found on the *Pro-face* screen located on the side of the tank rack.

6.1.1.13. Check all animals for general health. Check all animals in every tank; use a flashlight with red filter, if necessary, to complete check. Check that all fish are swimming easily, at appropriate speeds, are upright, and have normal body shape and size.

6.1.1.14. Ensure enrichment is provided, such as artificial aquarium plants; use a flashlight with red filter when tanks are obscured by darkness.

6.1.1.15. If an animal health case arises, record the case in the animal health and husbandry records log (blue binder) then notify operations manager and the attending veterinarian (AV).

6.1.1.16. Use siphon to remove any excess debris from tanks by inserting hard plastic tube end of the syphon into the tank, lowering the pump end, and priming the pump by squeezing the bulb repeatedly until water flows freely.

6.1.1.17. Check pest traps. If a pest is found in the trap, report to operations manager and replace with a new pest trap.

6.1.1.18. Check the “sock” pre-filter (Fig. 1.) for debris. Replace when fabric appears clogged.

Approval and Dates
Attending Veterinarian: [redacted] 10th May 2021
MSU IACUC: 19th May 2021

Fig. 1. — Unopened "sock" prefilter for use in [redacted] zebrafish system.

6.1.1.19. If floor is excessively wet, use squeegee to push water into drains.

6.1.2. Check the salt and/or bicarb tanks.

6.1.3. If they are low, refill with reverse osmosis (RO) water and measure out to appropriate ratio of 30 g per liter of salt for low conductivity, and 30 g per liter of sodium bicarbonate for low pH.

6.2. Weekly Husbandry Tasks

6.2.1. Parameters

Water Temp	79-81°F
pH	7-8
Conductivity	900-1200 µs
NH_3	<0.02 mg/L
NO_3	<200 mg/L
NO_2	0 mg/L
Chlorine	0 mg/L

6.2.2. Fish need to be spawned 1× per week to prevent them from being egg bound.

6.2.2.1 Fish should be moved from the flow-through system tank into a spawning tank (Techniplast breeding tank - beach style design) containing system water, and marbles on

top of the perforated false bottom overnight, and then returned to the flow-through system tank the following day.

6.2.3. Perform housekeeping duties for the room and record in the animal holding room logs binder (white binder) on the room log form.

6.2.3.1. Mop floors

6.2.3.2. Restock supplies and empty trash

6.2.3.3. Replace RO water and Net Soak used for disinfecting nets, see SOP: "Maintenance of Aquatic Life Support Systems"

6.2.3.4. Perform weekly water quality checks, referring to the parameters listed above, following the steps outlined in SOP: "Maintenance of Aquatic Life Support Systems".

6.3. Monthly Husbandry Tasks

6.3.1. Replace the mechanical, carbon, and prefilter at most every fourth week. Refer to SOP: "Maintenance of Aquatic Life Support Systems".

6.3.2. Rinse filters as needed between changes.

7. Forms

7.1. Fill out daily husbandry and care logs in the white "Animal Holding Room Logs" binder.

7.2. In the blue "Animal Health and Husbandry Records" binders, record the date and time when the carbon and mechanical filters were replaced.

8. Records Management

None specific

Approval and Dates
Attending Veterinarian: [redacted] 10th May 2021
MSU IACUC: 19th May 2021

SOP number: ARC SOP 68.1
Title: Rodent Husbandry and Cage Changing Procedures for ABSL1 Animal Holding Rooms
Established: August 2020
Revision Date: 5th February 2021

Rodent Husbandry and Cage Changing Procedures for ABSL1 Animal Holding Rooms

1. Purpose and Scope

The purpose of this Standard Operating Procedure is to describe husbandry and procedures for cage changing using the Techniplast rodent (mouse) cages.

2. References

2.1. Regulations

The contents of this document will conform to the most current editions of all institutional, local, state and federal regulations, policies and guidelines for working with animals used in scientific research projects.

3. Roles & Responsibilities

ARC staff is responsible for carrying out the procedures described in this SOP.

4. Special Requirements

4.1. Equipment and Supplies

Adequate numbers of clean prepared rodent caging and/or spare supplies. Rodent Feed. Water pouches. Water sipper valves. Wire Lids. Cage tops. Nesting materials.

4.2. Safety Requirements

Animal cages must be opened in a Biosafety Cabinet (BSC).

4.3. Personal Protective Equipment (PPE)

Standard PPE for the ABSL 1.

5. Applicable Locations

All ABSL1 rodent housing rooms in the ARC facility.

6. Procedures and Instructions

6.1. Procedures for cage changing

6.1.1. Rodent cage bottoms and wire grid tops are changed every 2 weeks at a minimum.
IVC Filter/Static Top Lids are changed monthly at a minimum.
Cage changing may be delayed for breeding females with very young pups, especially those who are poor breeders/mothers.

6.1.2. Animal health checks arecarried out at least once daily. At this time the cage is checked for food, water and visible soiling. Cages my be spot- changed at any time if required.

6.1.3. Set up the BSC for work.

6.1.4. Designate one side/area of the prepared BSC as **"clean"** where only clean cages and accessories are placed.

6.1.5. Designate a 2nd side/area of the BSC as **"soiled"** where only soiled cages and accessories are placed.

6.1.6. Prepare the dip and touch tray by filling approximately ¼ with appropriate disinfectant, position on the **clean** area of the hood.

6.1.7. Gloved hands must be dipped lightly in disinfectant regularly whilst manipulating cages. This is referred to as the "DIP & TOUCH" technique.

Note: Hands should not be dripping wet, using a finger to dip, and spread disinfectant over hands is a good way to prevent dripping wet hands. Additionally, the use of a paper towel in the BSC to blot hands is also helpful. The paper towel should be changed when soaked.

6.1.8. Set up the clean supply area on the side of the BSC designated as clean. This area should have all clean cage components on an easily accessible cart.
6.1.9. The clean cart should hold the hydropac pouches, food supplies and enrichment items.
6.1.10. Set up the used cages cart on the 'dirty' side of the BSC: designate areas for used cage components and other waste.
6.1.11. Dip and Touch gloved hands before setting up a clean cage in the BSC.
6.1.12. Dip and touch, then collect all cage components and accessories required for the clean cage and place in BSC. Dip and touch again before moving animals.
6.1.13. Remove the dirty cage lid and observe animals for health issues.
6.1.14. Transfer any remaining food over to the new cage.
6.1.15. Transfer cage cards(s) from soiled cage to clean cage, checking that information on card matches cage contents
6.1.16. Dip and touch.
6.1.17. Fill food hopper of clean cage up to full.
6.1.18. Replace Hydropac pouch with a new one if required.
6.1.19. Pre-place nesting materials, mouse houses/domes and enrichment materials in clean cage.
6.1.20. Transferring some nesting material from the dirty cage is advisable for groups of males and small groups of animals, young mice and breeding females.
6.1.21. Transfer each live animal from soiled cage to clean cage, visually observing as they are moved for signs health status and verifying sex.
6.1.22. For nests of pups, lift the entire nest and transfer.
6.1.23. Dip and touch.
6.1.24. Close up the clean cage and add appropriate clean lid with new water pouch if applicable or transfer over lid from pervious cage.

- Note: Whenever possible only use static lids (i.e. without clips) in breeding rooms. Cages in experimental rooms may need to be transferred and thus need to have filter tops (i.e. with clips) to prevent accidental escape during transport procedures.

6.1.25. Check that water is accessible from the hydropac sipper in the cage by gently squeezing the bag until water is visibly expressed. If this is not seen – pull out the water pouch assembly and insert a new one or re-configure until water can be expressed.
6.1.26. Place the clean cage assembly back on the rack and ensure that it is docked.
6.1.27. Dirty cage items are placed on the dirty cart.
6.1.28. Repeat cage changing steps until all cages are changed.
6.1.29. Clean the BSC.
6.1.30. Record Cage change in the room husbandry logs as well as any other relevant details or findings.

6.2. <u>Transporting soiled cages to cage wash</u>

Cover the soiled cage cart/s with the cloth autoclave bags in the AHR and spray liberally with appropriate disinfectant.
Transport the soiled cage cart/s directly to cage wash – these carts should not stay in the corridor.

Approval Dates
Attending Veterinarian: 5th February 2021
MSU IACUC: 17th February 2021

6.3. Spot check husbandry in the animal holding room

Animal health checks are carried out daily any husbandry issues addressed.

6.3.1. Move the cage requiring attention to BSC.
6.3.2. Use dip and touch technique for handling cages.
6.3.3. If the water pouch is less than ¼ full, replace it with a full one.
6.3.4. If the food hopper is less than ¼ full of pellets, fill until about half-full with fresh food.
6.3.5. If any cage is excessively soiled or wet, change the cage and record on the daily room sheet in health binder.
6.3.6. If animal health cases requiring non urgent attention are identified, record and notify a veterinarian or veterinary technician as necessary.
6.3.7. If animal health cases requiring urgent attention are identified, notify a veterinarian or veterinary technician immediately.

7. Forms

None Specific

8. Records Management

None specific.

Revision 5th February 2021: Note added at 6.1.24.

Approval Dates
Attending Veterinarian: 5th February 2021
MSU IACUC: 17th February 2021

SOP number: ARC SOP 72
Title: Quarantine of Aquatic Species (Fish and Frogs)
Established: February 2021

Quarantine of Aquatic Species (Fish and Frogs)

1. Purpose and Scope

This SOP details the procedures for quarantine of aquatics species (fish and frogs). All aquatic species must undergo a period of quarantine upon arrival at the ARC or may be isolated/quarantined due to health conditions.

2. References

2.1. Regulations

The contents of this document will conform to the most current editions of all institutional, local, state, and federal regulations, policies, and guidelines for working with animals used in scientific research projects.

3. Roles & Responsibilities

ARC Staff are responsible for providing daily health checks, feeding, any applicable treatments and testing, and following tank order.

4. Special Requirements

4.1. Equipment and Supplies

Static tanks with appropriate system water
System tanks
Nets
Net Soak
Reverse Osmosis (RO) water
Instant Ocean Salt
Stress Coat
Other supplies as needed

4.2. Safety Requirements

Appropriate Personal Protective Equipment (PPE) should be worn. Gloves are required for handling of aquatics species.

5. Applicable Locations

Aquatics rooms - [redacted]
Static frog room - [redacted]

6. Procedures and Instructions

Follow any additional instructions provided by the attending veterinarian (AV), facility manager, or Principal Investigator (PI).

6.1 Frog Quarantine (*laevis* and *tropicalis*)

Note: Always process quarantine tanks after handling the system tanks.

6.1.1 Fill static tanks with 8 L of system water, to the side bar line, or to 6 L. Adult *X. laevis* should be housed at 2 L of water per frog and adult *X. tropicalis* should be housed at 1 L of water per frog. Juvenile (froglet) *X. laevis* can be housed at 1 L per frog and juvenile (froglet) *X. tropicalis* at 0.25 L per frog.

6.1.2 Add 5 g of Instant Ocean Salt per L of system water and 1 drop of Stress Coat total per tank and let dissolve.

Approval and Dates
Attending Veterinarian: [redacted] 10th February 2021
MSU IACUC: 19th May2021

6.1.3 Wearing wetted gloves and gown place the affected animal or incoming animals into static tanks and if necessary, move to static frog rack in housing room or to room [redacted]. Nets used on affected or quarantined aquatics must be kept separate in their own bucket of Net Soak.
6.1.4 Once the appropriate number of animals are added to the tank, place the lid on the tank.
6.1.5 Affix cage card filled out with the relevant information and treatment sheets if necessary.
6.1.6 Cover tanks lightly with lab coat or gown when completed.
6.1.7 Generally, aquatics are not fed on day of arrival, check with manager or AV.
6.1.8 If feeding is necessary, feeding prior to tank change is optimal to ensure leftover feed does not foul the water.
6.1.9 Document activities in room logs or in static tank logs as needed and record in census if required.

6.2 Zebrafish Quarantine

Note: Always process quarantine fish tanks after handling the system fish.

6.2.1 Fish may be received directly onto the system or into a quarantine tank filled with system water. Ensure correct housing by consulting with the facility manager or AV. Zebrafish (adults and juveniles) should be housed at a rate of 1 L of water per 5 fish in the flow-through system and 1-2 fish per L in static tanks.
6.2.2 Fill quarantine tank(s) with system water.
6.2.3 Move affected or incoming fish into quarantine tank at appropriate density (see IACUC policy) using a quarantine dedicated clean net.
6.2.4 Affix cage card filled out with the relevant information and treatment sheets if necessary.
6.2.5 Record onto census and update room paperwork as needed.
6.2.6 Generally, aquatics are not fed on day of arrival. Check with manager or AV if unsure.
6.2.7 Feed fish normally after day one or follow any specific feeding treatments as directed.
6.2.8 Siphon any unfinished food out using a quarantine dedicated siphon and replace with system water.

6.3 Testing Quarantined Animals: Frogs

Note: The AV will review incoming animal health reports if available to determine quarantine scope, time frame, and testing requirements.

6.3.1 Frogs will be tested 5 days post arrival with pooled skin and tank swabs unless otherwise directed by the AV. Quarantined frogs from the facility will be tested as needed and directed by the AV.
6.3.2 Swabs will be sent to IDEXX for PCR evaluation for the following pathogens at a minimum: *B. dendrobatidis, M. chelonae, M. gordone, M. marinum, P. xenopi, Ranavirus*.
6.3.3 Once results are received the AV with either clear animals to be put on system or prescribe necessary health treatments.
6.3.4 Affected frogs should be isolated from other non-affected frogs and single-housed in static tanks.
6.3.5 All frogs are observed daily. Testing and treatment are done as needed and in coordination with the needs of the PI.

6.4 Testing Quarantined Animals: Zebrafish

Note: The AV will review incoming animal health reports if available to determine quarantine scope, time frame, and testing requirements.

6.4.1 There is no standard Zebrafish quarantine testing currently. Needs and testing will be determined by the AV on an as-needed basis.

Approval and Dates
Attending Veterinarian: [redacted] 10th February 2021
MSU IACUC:

7. Forms

7.1. Room logs
7.2. Census sheets
7.3. Treatment sheets
7.4. Cage cards

8. Records Management

Forms to be maintained in room binders or other as directed.

Approval and Dates
Attending Veterinarian: [redacted] 10th February 2021
MSU IACUC:

SOP Number: 81
ARC SOP Title: Fruit Bat Husbandry and Health Checks
Established: August 2021
Revision Date: N/A

Fruit Bat (*Artibeus jamaicensis*) Husbandry and Health Checks

1. Purpose and Scope

This Standard Operating Procedure (SOP) details the procedures for performing all bat husbandry and daily health checks in the ARC.

2. References

2.1. Regulations

The contents of this document will conform to the most current editions of all institutional, local, state and federal regulations, policies and guidelines for working with animals used in scientific research projects.

3. Roles & Responsibilities

Trained ARC Staff are responsible for performing husbandry and health checks as described in this SOP.

4. Special Requirements

4.1. Equipment and Supplies

Cage tray liners, shelf liners, food/water bowls. Appropriate disinfectant (e.g., Rescue), foamer, trashcans lined with biohazard waste autoclave bags, net for capture, leather gloves for handling, cloth bags for weighing.

4.2 Safety Requirements

Occupational Health Assessment and clearance must be obtained to work with bats.
Personal protective equipment (PPE) required for performing husbandry and procedural tasks in this location include use of lab coats, shoe covers, face shields, and a surgical face mask. Personnel who have not been vaccinated for SARS CoV-2 must wear an N95 face mask.

4.3 Stocking Density

No more than 8 adult bats may be housed per cage. Bats must be housed in pairs or groups except as required due to unavailability of other animals or as necessary to treat a medical condition under the supervision of the Attending Veterinarian. Adult male and female bats may not be housed together.

4.4 Animal Identification

Bats may be individually identified as described in the IACUC approved protocol. Cages will be labeled with a cage card documenting the Protocol number, PI, date of receipt, sex, and number of animals.

4.5 Environmental enrichment

Each cage will be provided with strips of shade cloth attached to the cage ceiling to provide for nesting sites. When possible, unpeeled fruit will be fed to give the bats an opportunity to manipulate the food item.

5. Applicable Locations

ABSL2 East Containment bat housing rooms - ARC

6. Procedures and Instructions

Follow the steps required to enter the ABSL2 housing area (see relevant SOP).

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Attending Veterinarian: [redacted]
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SOP Number: 81
ARC SOP Title: Fruit Bat Husbandry and Health Checks
Established: August 2021
Revision Date: N/A

Document completion of the following procedures on the Fruit Bat Monthly Room Log. Some procedures require additional documentation as described below.

6.1. Daily

6.1.1. Environmental Monitoring

- Record room temperature and humidity. Desired room temperature = 75+/- 2F. Desired room humidity: 30-70%. Light cycle: lights on at 7 am and off at 7pm. Report readings outside the acceptable range to ARC Manager.
- Check screens over air supply and exhaust vents and drop down door seals daily to ensure that they are intact. Repair as needed.

6.1.2. Sanitation

- Replace cage tray liners as needed depending on the number of animals housed per cage, minimally every other day. Pull out the tray, remove soiled liner, place a new pre-cut liner in the tray, and slide back under the cage. Dispose of used liner in a trashcan lined with both a biohazard waste autoclave bag and standard trash bag.
- Tie closed trash bags as needed to avoid odors and fruit fly infestation. Discard trash as necessary (but minimally at least twice weekly) to prevent infestation with fruit flies.

6.1.3. Feeding and Watering

- Diet items:
  - Fruit including the following items - watermelon, cantaloupe, honeydew, apples, pears, papaya, grapes, oranges, frozen mangos, frozen blueberries, bananas (can be frozen first, or not), frozen strawberries.
  - Mazuri Softbill Diet for Iron Sensitive Birds.
  - Mix the Mazuri diet into chopped fruit.
  - When possible, provide whole fruit (with peel) as enrichment. Wash fruit with peel prior to offering as food to remove pesticides and bacteria.
  - Feed approximately 1 cup of food per day per animal. Increase or decrease the amount of food offered based on the amount of food left uneaten each day.
- Transport food items on a disposable paper plate/bowl to the bat procedure room.
- Remove used water and food bowls from each cage. To avoid the need to clean dishes each day, disposable weigh boats may be used as food dishes as long as the animals do not knock them over.
- If reusable food dishes are used, scrape uneaten fruit into the trash and dump water down the sink drain. Remove all fruit from the food dishes so that it does not clog the drain during washing.
- Rinse bowls prior to reuse. Return bowls to the same cage unless they have been washed.
- Refill water bowls with fresh RO water.
- Refill food bowls with predetermined type and amount of feed based on number of bats per cage and daily food consumption.
- Hang food items on cage clips attached to the top or side of the cage.

6.1.4. Health Checks

- Check all animals in both the AM and PM. Record observations from the PM check as well as any other tasks performed at that time on the Animal Health Information form.
- Verify that all bats are accounted for and that no bats have escaped from the cages. If a bat has escaped, wearing leather gloves and using the net provided in the animal housing room,

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attempt to capture the animal and return it to its cage. Contact other ARC members for help as needed.

- Visually assess each animal for activity levels, normal behavior, skin and body condition. Check the cage tray waste pan for signs of any abnormalities.
- Report medical concerns (e.g., injury, disease, or abnormal behavior) immediately to the Attending Veterinarian or vet on call as appropriate and the PI.
- Document abnormal observations, timings and actions taken in the animal's individual medical record.

6.2. Weekly

6.2.1. Sanitization

- Disinfect the floors and door handles with appropriate disinfectant via mop or foamer.
- Collect trash from all waste containers.
- To process trash, tie the standard black trash bags containing soiled cage liners and animal waste and place in an autoclave bag.
- Close the bags using autoclave tape and process using the dirty side cage wash autoclave on the "Reg cages" setting per the applicable SOP.
- Sanitize water and food dishes in the sink in the procedure room. To sanitize, remove as much debris as possible using wetted paper towels, discard used paper towels in trash can, place dishes in the plastic tub, foam with Rescue, let stand for 10-15 minutes and then rinse well. Retreat excessively soiled areas as needed.

6.3. Every Two Weeks

- Sanitize all caging components including cages and water/ feed bowls. To sanitize, remove and discard the shade cloth, remove as much debris as possible using wetted paper towels, discard used paper towels in trash can, place caging components in the large plastic tub provided in the room, foam with Rescue, let stand for 10-15 minutes and then rinse well. Retreat excessively soiled areas as needed. Arrange plastic tub in room so that there is no risk of splashing the animals or caging in use.
- Wipe shelves with Rescue disinfectant.
- Research personnel will move the bats to a clean cage at least once every 2 weeks. This will reduce the stress of recapture of the animals specifically for the purpose of cage sanitation. In the event that bats have not been moved to clean cages within a 2 week time period, contact the lab to request that they move the bats to a clean cage.

7. Forms

7.1. Fruit Bat Monthly Room Log
7.2. ARC Animal Health Information

8. Records Management

Records are kept on file at the ARC for a minimum of 3 years.

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Environmental Enrichment Program

1. **Purpose**
   The purpose of this SOP is to describe the ARC/JRL environmental enrichment program.

2. **General**
   A. Per the NRC Guide for the Care and Use of Laboratory Animals "The primary aim of environmental enrichment is to enhance animal well-being by providing animals with sensory and motor stimulation, through structures and resources that facilitate the expression of species-typical behaviors and promote psychological well-being through physical exercise, manipulative activities, and cognitive challenges according to species-specific characteristics".
   B. ARC staff are responsible for implementing the environmental enrichment program in the ARC. The mission is to promote psychological well-being of all laboratory animals housed in the ARC. The goals of the program are to provide interaction and self-initiated behaviors, allow the animal a degree of control over its environment, and to increase species-typical behaviors while decreasing pathological maladaptive behaviors.
   C. Methods of enrichment may include but are not limited to: 1) social housing, 2) housing devices, 3) manipulative devices, 4) visual, auditory, and olfactory cues, and 5) food items.
   D. Whenever possible, animals should be housed in a manner that provides for visual, auditory, olfactory, and tactile exposure to conspecifics.
   E. Implementation of the environmental enrichment program as described on this SOP is required unless the PI has an IACUC approved exemption or as approved by the AV due to animal health concerns.

3. **Social Housing:**
   A. Emphasis is placed on social housing for social species whenever possible. Individual housing of social species is avoided. The reason that an animal is singly housed should be clearly communicated either through use of a single housing cage card or similar means.
   B. Exemptions to social housing of rodents include but are not limited to the following:

      1. Animals that exhibit social incompatibility (i.e., aggressive behavior) towards conspecifics or which, based on historical information, may be expected to exhibit aggressive behavior (e.g., adult breeding age male mice that are not siblings or cage mates at weaning). Male mice used as stud males for timed matings, or who are temporarily not being used for pair or harem breeding, are included in this exemption.

      2. Animals that are recovering from a survival surgical procedure. Exemption from social housing is limited to the post-operative time period.

      3. Animals that have surgically implanted cannula and/or other implants that may be disrupted by cage-mates.

      4. Animals involved in food or water consumption or restriction studies. Exemption must be approved in the IACUC protocol and is typically limited to the time period during which the food consumption or restriction study is being performed.

      5. Pregnant female rodents. Exemption is limited to the time period of pregnancy and until 14 days after the pups are weaned, to provide a rest period for the female prior to re-mating.

6. Animals that need to be separated in order to provide oral medication (in the case of mammals) or chemical treatment (in the case of fish) on an individual basis. Exemption is limited to the time period of treatment.
7. Animals remaining in a cage or tank after the other cage/tankmates were humanely euthanized or found dead (due to naturally occurring disease or experimentally related reasons). Efforts will be made to combine fish from the same strain or female mammals from the same strain to reduce the number of singly-housed animals as long as doing so does not result in aggression or interfere with scientific objectives.
8. Rodents weaned from a litter containing only one animal of that sex or animals that must be separated due to cage size constraints.
9. Efforts will be made to combine female mice of the same strain to reduce the number of singly-housed animals. However, male mice from different litters may not be combined in most circumstances unless that is done at the time of weaning.
10. Juvenile zebrafish may be singly housed for up to 48 hours to enable genotyping

C. Exemption to stable social groups includes female mice that are timed-mated. Once mated, female mice will be co-housed with other females if possible, but not necessarily with females from their original social group.

4. Rodent Environmental Enrichment

A. Mouse: aspen chip bedding, nesting material (Nestlets, crinkle paper, Environ-dri, unscented Kleenex), mouse houses (e.g., igloos).
B. Rat: aspen chip bedding, nesting material (e.g., paper towel), polycarbonate or pulp paper bio tunnels, wood blocks or tongue depressors.
C. Enrichment devices must be sanitized or discarded at least once every two weeks.

5. Guinea Pig Environmental Enrichment

A. Poly-carbonate huts/shelters inside the cage, wooden blocks for chewing and gnawing.
B. Small amount of fruit (apple) and/or vegetables (spinach) 3x per week (typically Monday, Wednesday, and Friday). Caution should be used to ensure that the amount of treats is limited to ensure that the animals eat their nutritionally balanced diet.

6. Jamaican Fruit Bat Environmental Enrichment

A. Provide a variety of fruit. Wash all fruit and when possible, leave the fruit unpeeled.
B. Hang shade cloth or 'nesting hides' from the ceiling of the cage to provide roosting areas.

7. Xenopus laevis Frogs Environmental Enrichment

A. Polycarbonate cubic tunnel in each tank.

8. Zebrafish Fish Environmental Enrichment

a. Artificial aquarium plants in stand-alone tanks.
b. Laminated gravel image under tanks.
c. Artemia (brine shrimp) 1x per day as live prey.

Receipt and Acclimation of Rodents from Approved Vendors

1. **Purpose**
This SOP explains the proper procedure for receipt and acclimation of mice, rats and guinea pigs from [redacted]. The program coordinator places all animal orders and provides a copy to the ARC staff who sort orders by the technician responsible for each researcher's animals and hangs the orders on the feed room door under each technician's name.

2. **Preparation for Rodents**
   A. Commercial vendor orders typically arrive Wednesday or Thursday
      1. Check incoming orders daily
      2. On the day you are expecting an order email the ARC team regarding order details.
      3. Prepare cage cards and cages
         a. Create cage card using info from the order form. Color code the cage card assigned to the researcher/species/strain. Record PI, IACUC protocol #, DOB, DOA, # of animals, vendor.
         b. Follow housing instruction on order, if none consult with PI if housing questions, otherwise:
            i. House all pregnant rodents one per cage, unless directed otherwise by the PI
            ii. Verify any social group instructions on the order form. House adult males 1/cage unless the animals are siblings or cage mates from weaning and <5 weeks old. Use single housing exemption card to indicate males cannot be co-housed due to fighting.
            iii. House up to 5 non-bred adult females/cage.
   B. Prepare cage, place card on cage.
   C. After animals are on site, update cage census log.

3. **Receiving Rodents**
   A. Rodents are delivered to the ARC clean dock and placed on a clean cart.
   B. Before accepting order, verify the shipment paperwork against the order form – cross reference for errors.
   C. Disinfect the outside of the box, including all surfaces (top, bottom and sides).
   D. Place cart of animals in the hallway outside of room [redacted].
   E. Remove packing slips and health certificates and give to Program Coordinator.
   F. Check shipping box for damage.
   G. Confirm room or area number from order form with packing slip. Notify Program Coordinator and ARC Director if order varies from order form.

4. **Unpacking and housing Rodents**
   A. Enter animal room adhering to room PPE requirements
   B. Place one box in BSC, open
   C. Disinfect gloves
   D. Examine each animal, evaluate the health, and place in ARC cages as per housing instructions. If any health issue is observed notify ARC Director and AV immediately. Repeat until shipping box is empty.
   E. Place cages on IVC rack
   F. Check shipping container for any extra animals
   G. Repeat B-G until all containers are empty
   H. Throw shipping containers away once confirmed empty
   I. Email ARC Director, PI or lab personnel to communicate and document that animals have arrived. Specify that the animals must be provided with an acclimation period of 3 days for breeding or experiments, or 7 days for surgery or behavioral testing. An exception to the acclimation period is made for timed pregnant rodents where researcher is harvesting tissue at a specific day of age.

Approval and Dates
Attending Veterinarian: [redacted]
MSU IACUC: