EMORY UNIVERSITY

Institutional Animal Care and Use Committee
Policy, Guidelines, and
Standard Operating Procedures

Policy 371 Social and Environmental Enhancement for Non-primate Research Animals

371.1 Prologue

371.1.1 To enhance animal wellbeing, minimize animal stress, promote consistency and fulfill regulatory obligations, the IACUC has a standing policy for minimum (or "required") enrichment standards, specifically defining housing conditions and socialization opportunities, for all species used in research at Emory University with the exception of nonhuman primates (addressed separately, see: http://www.iacuc.emory.edu/documents/ee_nhp.pdf).

371.2 Introduction

371.2.1 The environment may influence the validity, reliability and replicability of experiments by introducing abnormal animals into studies, increasing variability within the population, and altering the number and type of individual animals between laboratories, respectively.[1] In the context of the research animal, the environment consists of both physical attributes (e.g., lighting, temperature, cage design and complexity) as well as the nature of social interactions. In the proper care and use of research animals, the availability or suitability of enrichment must be considered in the provision of the environment and specifically with opportunities to exhibit species-typical behaviors and activity.[2]

371.2.2 When animals are unable to perform species typical behaviors and control their environment, unintended stress results and may proceed to adversely affect physiology and distress manifested as abnormal behaviors, both maladaptive and malfunctional.[1] Altered physiology and abnormal behaviors in a population are similarly potentially confounding to science because they are usually expressed inconsistently across the spectrum of the group.[1] Their expression varies by genetic background and developmental experience and may not mitigate with aging.[1] As such, successful enrichments reduce or prevent the occurrence of physiological dysfunction and malfunctional and maladaptive behaviors through appropriately lifelong (e.g., from development onward) social and environmental enrichment, and/or increase the occurrence of species-typical behaviors.[1,3]

371.2.3 An important enrichment caveat is that social, environmental, dietary and other supplementations intended for improvement of animal well-being may alter important aspects of an animal's physiology and development in ways not easily predictable based on what is already known.[4,5] Enrichment is a research variable that must be sensibly managed and must be biologically relevant.[1,6] In essence, enrichment enables "good welfare" to equal "good science"[48].

371.2.4 In most cases, principles of training (e.g., operant conditioning or classical conditioning) may be employed to elicit voluntary cooperation with procedures. Habituating animals to routine husbandry, veterinary or experimental procedures is encouraged as it may assist the animal to better cope with a captive environment by reducing stress associated with those procedures . The type and duration of habituation or training needed will be determined by the individual species as well as by the complexity or frequency of the procedure.[2]

371.2.5 The social and environmental enrichments prescribed in this document have been demonstrated to promote animal wellbeing while enabling valid research. The IACUC assumes that investigators have sufficient knowledge of the species used and their model systems to take into account their associated needs and to scientifically justify any exceptions or enhancements from the minimum.

371.3 Abbreviations and Definitions

371.3.1 "Animal" – for purposes of the default enrichment program, "animal" is defined as to comprise all vertebrate species used in research at Emory University with the exception of nonhuman primates covered by a separate policy and program.

371.3.2 AWAR – Animal Welfare Act Regulations. Detailed regulations and standards for implementing the federal Animal Welfare Act of 1966 and subsequent revisions. Found in Title 9 of the Code of Federal Regulations (9CFR), Chapter 1, Subchapter A, Parts 1, 2 and 3.

371.3.3 Social Animals – Animals are considered to be social if during the adult stages of ontogeny they are found in groups of two or more individuals under natural ecological conditions. All plans for socialization should take into account the needs and natural history of the species at hand.

371.3.4 Enrichment – Animal management principles that attempt to enhance the quality of care by identifying and providing the environmental stimuli necessary for psychological and physiological wellbeing meets the definition of "enrichment".[7] Effective enrichment is based on the species' natural history and activity budgets, encourage beneficial species-specific behaviors, and are driven by specific goals (e.g., increase foraging behavior, decrease aggression, etc.).[8]

371.4 General Principles

371.4.1 Social animals will be housed in compatible pairs or groups, rather than individually, provided such housing is not contraindicated by the protocol in question and does not pose undue risk to the animals in question.[2] When individuals of social species are housed in a solitary arrangement, auditory, visual and/or olfactory contact to compatible individuals must be provided.

371.4.2 The structural habitat will include objects that increase opportunities for the expression of species-typical postures and activities that enhance the animals' well-being.[2]

371.4.3 The minimum requirements in this policy for singly-housed animals of social species is above and beyond that for group housed animals to help compensate for the lack of social contact.

371.4.4 Exemptions from some or all of the requirements of the enrichment program for scientific reasons must be documented in the protocol and specifically approved by the IACUC. [9]

371.4.5 Qualified institutional veterinarians have the authority to exempt specific animals from inclusion in the enrichment program for reasons related to health, condition or well-being. The exemption and rationale must be documented in a medical record.

371.4.6 This program will be revised with the addition of new species to the census or in the case where significant new information emerges or approaches to enrichment come to be the norm.

371.4.7 An animal may be housed in social isolation due to being the remaining animal in an experimental cohort and a lack of conspecifics of an equivalent health status. Where there is a likelihood of a single animal remaining on census at a single site, every effort will be made to limit social isolation to the minimum period necessary. Animals in isolation will receive additional enrichment daily as described below under its species-specific section (e.g., additional foraging opportunity, positive interaction with human). The daily enrichment provided will be documented. A veterinarian will assess the animal's welfare at regular intervals and determine whether isolation can continue without undue harm. If the animal remains in isolation for more than 60 days, further approval from the IACUC will be required.

371.5 Default Enrichment Program Description by Species

371.5.1 Aquatics (Hagfish, Lamprey, Zebrafish)

- Minimum Requirements: **Zebrafish** must be housed at densities that promote normal feeding and other behaviors and minimize aggression. For **Hagfish** a retreat space, either a darkened tank or sand in which to burrow, must be provided. The **lamprey** larval form requires sand in which to burrow and sustenance to filter feed. The pre-spawning adult form is parasitic and requires fish to feed. Post-spawning lampreys do not feed.
- Additional Approved Recommendations: **Sea lamprey and hagfish** may be found as individuals or in groups in the wild – wherever a suitable habitat is found. Group housing is recommended, but not required. In the wild, **zebrafish** are found amid dense plant foliage, and also have been shown to prefer structure, e.g., plant foliage, PVC pipes in captivity.[10] The provision of structure is recommended, in low-density housing and breeding tanks, but not required.[11]

371.5.2 Birds (Passeriformes):

- Minimum Requirements: Individuals of social species must be housed in appropriate group sizes with compatible individuals.[12, 13] A shelter (e.g., shelves or nest/roost boxes) must be provided to allow birds to nest and roost (Association of Avian Veterinarians 1999) and obtain refuge from aggression. Additional perches that allow for proper footing and minimization of foot trauma must be provided to allow Passeriformes the option to stay above the ground. Space within the enclosure must allow for exercise (i.e. flapping of wings) and the ability to express a range of natural behaviors.[13] Nesting material in the form of hay, shredded paper or other appropriate material should be provided for species that construct nests during breeding periods.
- Additional Approved Recommendations: Housing birds in pens or aviaries versus cages is suggested as it provides more space for flight.[13] A water (or sand) bath should be available at least weekly to promote bathing activity. As foraging and feeding are two of the most common behaviors of birds, the complexity of food acquisition should be manipulated (scattering seed on floor, placing produce between mesh, hanging feeders from ceiling, providing feed in nature form such as millet sprays) and selections of food varied to satisfy
these needs.[13] For birds in social housing, multiple food portions should be presented to reduce hoarding by dominant individuals. Grit should be provided when appropriate.[14,15] Human interaction is encouraged and training birds to perform tasks by hand-feeding improves habituation to people.[13]

371.5.3 Galliforme Chicks:

- Minimum Requirements: Recently-hatched chicks and those up to 250 grams body weight (ca. 2-3 weeks of age) must be housed in brooders with a space requirement of 0.25 square feet per chick. [2] Substrate must be used to help the chicks maintain body temperature. Food and water must be provided in low profile linear troughs for ease of access and also to encourage foraging behavior. Any chicks remaining past 1.5 kg must be transferred to cages or pens with perches, litter and dust bath with a minimum of 1 square foot per bird.

371.5.4 Gerbils:

- Minimum Requirements**:** Gerbils will be socially housed. Minimum enrichment for group housed animals includes either two types of nesting material or nesting material and a shelter. Gnawing enrichment is required for singly housed animals in addition to the standard enrichment.
- Additional Approved Recommendations: Exercise opportunities such as running wheels are encouraged. Gnawing devices (nylon chewing blocks/bones, aspen blocks, manzanita wood sticks) should be considered for socially housed animals. Novel food and foraging opportunities with veterinary approved materials can also be used.

371.5.5 Guinea Pigs:

- Minimum Requirements**:** Group-housing of compatible individuals is the standard housing option.[16-18] Shelter must be provided and must provide sufficient canopy to accommodate each individual in the enclosure.[19-21] PVC tubes, transparent red huts or other approved structures can be used to meet this requirement. Hay and approved produce must be placed in the enclosure or present in devices approved for guinea pigs to promote foraging behaviors and increase diversity of food items. Hay and/or produce must be given on a daily basis as a nutritional supplement to the regular diet to decrease risk of alopecia.[22] Singly housed Guinea pigs must get additional food enrichment.
- Additional Approved Recommendations: If permissible, human contact with guinea pigs (social- and single- housing) is recommended and contributes to positive social interactions.[23]

371.5.6 Hamster:

- Minimum Requirements: Social housing is not required. Hamsters may be cohabitated if a stable group or pairing is established at a young ag.[55] Nesting female hamsters, together with their litters, must be housed without other hamsters[54]. Enrichment must include nesting material(s) and at least one other enrichment option.[6]
- Additional Approved Recommendations: Other enrichment options include: shelters, tubes, lofts/platforms; gnawing devices (nylon chewing blocks/bones, aspen blocks, manzanita wood sticks); novel food and foraging opportunities (seeds, seed hulls, cereal, treats, vegetables); feed on the cage floor to allow normal caching behavior; positive human animal interaction and training.[24] Devices encouraging locomotion such as running wheels are strongly recommended.

371.5.7 Mice:

- Minimum Requirements: Mice must be group or pair housed unless otherwise approved by the IACUC. However, the following exceptions may apply; (1) Adult male mice which are either unfamiliar or have been separated from stable social housing must be housed individually or with female mice for breeding; a male mouse, used as a stud for a few days, may be returned safely to the group if the cage has not been changed; (2) Mice may be held in cages as single pregnant females to prevent cage mate trauma or overcrowding in the case of harem or trio breeding;[25] and (3) the occasional finding of singly-housed mice because of the death of all others in the cohort or removal of cage mates at scheduled experimental time points. Minimum enrichment for socially housed mice: nesting materials in addition to the regular bedding .[26] Minimum enrichment for individually housed mice: extra nesting materials or shelter in addition to regular bedding and regular nesting materials. Options for nesting materials include nestlet pads, shredded paper or other approved nesting materials such as twisted paper products made for use in laboratory mice .[5,27]
- Additional Approved Recommendations: Other enrichment options for mice include: shelters, tubes, lofts/platforms; gnawing devices (nylon chewing blocks/bones, aspen blocks, manzanita wood sticks), foraging opportunities with laboratory grade diets and running wheels.[26] Shelters, in particular, are strongly encouraged for all mice.

371.5.8 Rabbits:

- Minimal Requirements: Rabbits that are compatible must be socially housed in pairs or groups. Attempts should be made to order compatible littermates when starting a study so that animals can be socially housed. Manipulanda such as toys must be provided. Food enrichment in addition to pelleted diet is required. Singly housed rabbits must be provided with the ability for visual, auditory and olfactory association with conspecifics. In addition, singly housed rabbits must have a regular rotation for manipulanda.
- Additional Approved Recommendations: Enrichment devices should be rotated to ensure novelty, and elevated shelves should be provided to provide an area for resting.[2] Huts or boxes should be provided as a means for visual retreat in social housing situations. Handling and positive social interaction with animal care staff is encouraged particularly for singly housed rabbits. Rabbits housed in isolation should be provided mirrors, additional enrichment devices and additional positive human interaction.

371.5.9 Rats:

Minimal Requirements: Rats must be socially housed unless otherwise approved by the IACUC.[3, 26, 28-30] However, the following exceptions may apply; (1) socially incompatible animals, such as in the case of sexually-mature, unfamiliar males (2) pregnant females (3) the occasional finding of singly-housed rats because of the death of all others in the cohort or removal of cage mates at scheduled experimental time points. The minimum requirement for socially housed rats are either chewing or nesting material. The minimum enrichment for an individually housed rat is to provide shelter and/or additional nesting material[5,27, 31-36,56]Tunnels, sections of PVC pipe, some type of nesting material[34] or shelters best serve this purpose. The quantity of the nesting material must be enough to cover the entire individual. When supplemented with nesting material, rats prefer long-fiber materials such as crinkled paper over nesting materials such as compressed cotton square.[32]

Additional Approved Recommendations: As rats are highly adaptable and readily acclimate to human handling and research procedures, acclimation and handling programs may be beneficial and

should be considered.[37] Some research procedures may qualify as positive human animal interaction. Shelter and nesting material as described above can also be used for socially housed animals, provided it still allows for animals to move normally. Other enrichment options for rats that scientists may elect include increasing cage complexity by using lofts/platforms gnawing devices (nylon chewing blocks/bones, aspen blocks, manzanita wood sticks), foraging opportunities with laboratory grade diets,[38] and positive human-animal interaction and training.[37]

371.5.10 Sheep:

Minimum Requirements: Sheep must be socially housed in stable, compatible pairs or small groups. If sheep are individually housed for approved research purposes, they will be positioned in such a way so that they can see at least one other conspecific, because visual isolation is stressful for sheep[49]. If other sheep are unavailable, a mirror will be used as it may alleviate isolation stress. However, because some sheep appear to treat their own reflection as a strange individual, a mirror may cause social stress[39, 40], careful monitoring and adjustments of mirror placement are necessary. Where there is likelihood of a single sheep remaining on census at a single site, experimental plans must account for the timely use of the remaining animal. Sheep are particularly susceptible to isolation stress and those not coping with social isolation will be subject to IACUC endpoints including veterinary interventions (e.g., tranquilization) or euthanasia. Sheep must be provided a diet high in roughage, such as hay, to allow species-typical feeding and rumination, and to reduce the likelihood of abnormal behaviors.

- Additional Approved Recommendations**:** Species-appropriate bedding such as straw or wood shavings is recommended and should be provided to enclosures when possible[50]. When feeding supplementation is provided using objects that can be manipulated by licking or pushing with the head is preferred. An undesirable behavior called wool biting may develop in sheep confined indoors for prolonged periods due to a paucity of roughage or other environmental stimulation. Strategies used to prevent or decrease this behavior include access to outdoor pastures, hanging chains from above the surface of the pen or adding objects to the pen (e.g., basketballs, plastic bottles, or chewing bars), playing music and altering the diet.[41] Stressful research manipulations (e.g., venipuncture, drug application) should be accomplished within the presence of a familiar conspecific. Care personnel should habituate animals that are subjected to experimentation or any new, stressful or fear-inducing situations.[40] Emphasizing positive reinforcement training techniques to encourage voluntary cooperation during common procedures such as blood collection and injection have been described in other ungulates and are recommended techniques for sheep whenever possible.

371.5.11 Spiny Mice:

- Minimum Requirements**:** Spiny mice must be housed in social groups. Minimum enrichment for socially housed spiny mice: shelter and chewing enrichment. For spiny that are individually housed, nesting material must also be provided in addition to regular enrichment.

- Additional Approved Recommendations: Food enrichment such as mealworms and sunflower seeds can be provided, with consideration for the fact these animals are prone to obesity.

371.5.12 Swine:

- Minimum Requirements: Swine must be housed socially with compatible cage mates in suitable size runs or pens that allow them to turn around and move freely (unless justified, clinical, or in the case of a single animal which would receive special considerations). Within each enclosure, at least one toy per animal should be provided including one hanging and one freely movable toy. Provision of destructible or deformable materials provide an outlet for natural behaviors such as exploration, rooting, and chewing; reducing the likelihood that these behaviors will be redirected toward the bodies of pen-mates. Such enrichment materials can lower the risk of injuries and harassment from tail biting, ear chewing, and belly nosing, reduce aggressive behavior, increase activity and modulate the immune response[51]. Less destructible free-moving or hanging objects can offer short-term enrichment by attracting exploration and stimulating play. Singly housed animals must have positive human interaction and additional manipulanda.
- Additional Approved Recommendations: The use of visual barriers and separate feeding stations may help ameliorate aggression. Opportunities that encourage rooting, scratching and wallowing should be provided through the use of substrates (i.e. wood chips, ice blocks[53], wheat straw, or hay) and/or a scratching brush. Rubber mats, cotton ropes, and straw are preferred forms of enrichment and lead to improvements in well-being[52]. Acclimation and training to any research equipment is encouraged and can be readily achieved by using food rewards. Researchers are encouraged to order littermates when possible for early weaned piglets.

371.5.13 Voles:

- Minimal Requirements**:** Voles must be housed in social groups[42, 43]with males closely monitored for fighting. However, unfamiliar same-sex adult animals can be housed individually at the discretion of program because they could be socially incompatible. To maintain pheromone cues, part of the old nesting material must be transferred to the new cage at cage change.[44] Minimum enrichment for socially housed voles: nesting material in the form of hay, straw, paper products such as crinkled paper or nestlets must be provided in adequate amounts to fulfill burrowing needs in addition to the regular bedding.[45] For voles that are individually housed, chewing devices (nylon chewing blocks/bones, aspen blocks, manzanita wood sticks) must be provided in addition to regular enrichment.
- Additional Approved Recommendations: As additional shelter, tunnels, such as a PVC pipe section, can be used. Other enrichment options for voles: foraging opportunities with laboratory grade diets or hay (Timothy cubes).

371.5.14 *Xenopus laevis:*

- Minimum Requirements: Adult *Xenopus laevis* must be housed in tanks with a population density not exceeding one per 2 liters tank water volume and as otherwise stipulated by facility SOP. With respect to the latter, water treatment, circulation and quality may stipulate that greater volumes be accorded per head. For frogs housed with direct exposure to room light or housed in light colored containers refuges or retreats must also be provided in the form of pipes, flower pots, floating lily pads, or submerged plastic boxes unless the environment is already sufficiently dark. For tanks with young froglets who are typically weaker swimmers, small, floating balls must be placed in the tanks to provide structure to which froglets can cling to so that they can "hang" and rest at the surface.[46] Singly housed frogs at minimum must be provided a shelter and an enrichment object.

- Additional Recommendations: Although typically found solitary in the wild, African clawed frogs are often found socially housed in captivity. Maintaining *Xenopus* with familiar frogs in established, long- term housing cohorts is an advisable practice whenever possible.[46] An increase in aggression may be observed if the stocking density is too high or there is no refuge cover available for lower ranking animals.[47] Other enrichment may be used as a form of refuge, which may include: rocks, foam pieces, larger rocks, tiles, cups, and other plants.[46]

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