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HM-43A: JHU Guidelines and Standards for Research Animal Housing in Satellite Facilities: Supplement for Aquatic Vertebrate Species

This document supplements the JHU policy regarding satellite animal housing facilities, focusing specifically on requirements for housing and care of aquatic species. A satellite facility is defined as any area outside of JHU's centrally-managed housing facilities where animals are maintained for more than 24 hours (12 hours for species covered by the Animal Welfare Act Regulations).[1] The vast majority of vertebrates, measured by total number of individuals, biomass, and numbers of species, are aquatic. The dynamics of living in aquatic environments, as determined by basic physical principles, are categorically different from living in air. Further, aquatic environments allow for far greater differences in habitat, as measured by chemical composition, temperature, and other critical factors for life, than occurs in terrestrial habitats. These features represent a special challenge for development of guidelines for the care and use of aquatic species.

Some aquatic vertebrates, in particular the zebrafish (*Danio rerio)* and *Xenopus sp.*, have become much more widely used in recent years, but accurate information about husbandry and disease has not necessarily kept pace with their increased popularity. This document attempts to summarize the best currently available information about their care, but there is no single resource with standard information for all species. At the end of the document, some links are provided to online resources for the most commonly used species.

In brief, the elements critical to our success include a laboratory point (or points) of contact (POC) with direct or delegated responsibility for the satellite facility; physical plant and environmental considerations; appropriate choices of housing, husbandry procedures, and sanitation procedures for equipment used with animals; management of housing and supplies; arrangements for daily animal care; and veterinary care.

I. POLICY

[1] References for the definitions and criteria in this document include the *Public Health Service Policy on the Humane Care and Use of Laboratory Animals,* the National Research Council's 2011 *Guide to the Care and Use of Laboratory Animals,* and/or the U.S. Animal Welfare Act Regulations (enforced by the U.S. Department of Agriculture, USDA). Species covered by the Animal Welfare Act (i.e., "USDA species") include all mammals and wild-caught birds. Rats, mice, and birds that were bred for use in research are specifically excluded from coverage by the Act.

JHU is committed to a uniform standard of excellence in all aspects of its animal care and use program. The entire program is based on the recommendations of the *Guide for the Care and Use of Laboratory Animals* (*Guide*). At JHU, Research Animal Resources (RAR) is responsible for the animal housing and veterinary care programs.

The operating standard at JHU is to maintain terrestrial vertebrates within its centralized animal housing facility program managed by RAR whenever possible. However, due to the special considerations required for these aquatic animals, and because the relative size of the program that uses aquatic animals is small, RAR does not provide primary animal care and husbandry for aquatic vertebrates at Johns Hopkins University in most cases. Therefore, most protocols using aquatic vertebrates will be characterized as outside, or satellite, housing to indicate that the primary responsibility for animal care and husbandry will be provided by the investigator. Nevertheless, the aquatic animal care programs established in each of these laboratories will be monitored and evaluated via mechanisms that are similar to those developed for the satellite facilities used for terrestrial animals in the Johns Hopkins University animal program.

The policy of the JHU ACUC is to provide the RAR Director of Laboratory Animal Management copies of protocol and amendment applications involving satellite housing to enable RAR (1) to determine whether the activity can be accommodated within central facilities and (2) to provide consultation on the written plan for care in satellite housing that has been submitted to the ACUC. For first-time satellite housing location requests, ACUC evaluation of the request also requires a consultative inspection of the location by the Director or a designee.

Regardless of the length of time an animal is housed in an ACUC-approved satellite facility, the principal investigator (PI) on the ACUC-approved protocol is responsible for seeing that its housing and care are consistent with JHU Policy. To facilitate this, RAR is responsible for maintaining contact with the satellite facility to assure appropriate support, guidance, and oversight of the care of animals housed there.

By their very nature, physical plant, housing, and procedures in aquatic facilities will vary among sites. Adherence to the general principles of the *Guide* and to an overarching focus on animal welfare consistent with the needs of the research procedures approved by the ACUC is a basic tenet of this policy. The *Guide* itself sets forth an emphasis on a "performance approach," as preferable to an "engineering approach." That is, "Performance standards define an outcome in detail and provide criteria for assessing that outcome, but do not limit the methods by which to achieve that outcome" (*Guide*, 2011.) Given the wide range of research at JHU, it is possible that this document does not adequately address potential variations on housing and procedures that will arise. We anticipate that such variations will be discussed with RAR as they arise and incorporated in the laboratory standard operating procedures as appropriate.

This document has been written to outline policies applying to all fish and other aquatic species, and is written to be very general. It is anticipated that a more detailed policy will be developed for each facility in consultation with RAR staff.

II. STANDARDS

A. The Laboratory Point of Contact (POC)

Accountability for the care of animals housed in satellite facilities is established formally in the satellite housing application through a laboratory POC. This person may be either the PI or another qualified person with delegated responsibility to ensure that the care given to laboratory animals in the satellite facility will be consistent with this policy. When delegated by the PI to a JHU staff member, POC responsibility should be listed as a major element on the individual's job description and annual performance evaluation. In some laboratories, depending on the continuity of animals in satellite housing, it is desirable to appoint a secondary POC as well.

The POC may operate by directing and overseeing others in the laboratory who are performing hands-on care, may perform laboratory-based care as a primary function, or may provide RAR technical personnel with specialty guidance to enable them to care for animals with unique experimental requirements in the satellite facility. The POCs are required to be familiar with and to follow the provisions of this policy and other institutional standards (e.g., RAR policies and ACUC-approved policies and guidelines) in support of the institution's mission to provide high quality care to all laboratory animals, regardless of housing location.

Laboratories are encouraged to create a simple task list for animal care, to have a posted list of the trained personnel who will provide back-up care in the absence of the primary care-giver(s), and to clearly establish the mechanism for arranging coverage by back-up personnel. Regardless of the approach to providing care, the POC is seen as responsible for assuring that all animals housed in the satellite receive adequate care every day.

Each satellite facility must have a copy of the *Guide* readily available, and the POC should be familiar with its contents. The *Guide* may be obtained free of charge from the ACUC office. Training and orientation sessions on satellite housing are arranged periodically by the ACUC office and/or RAR for POCs and others who are interested in this topic. In addition, RAR personnel make regular visits to each satellite facility, which provides the opportunity for addressing emerging issues relevant to each location. The frequency of the visits will be determined for each site, based on the husbandry requirements of the organism the needs of the investigators and other personnel.

B. Daily Checklist

Most of the critical elements of a basic program of laboratory-based care should be included in a daily checklist. This checklist provides a means for recording basic required elements of animal maintenance, as well as annotation that all animals were visually inspected seven days a week. The program for aquatic animals differs from the rest of the outside housing program in that no single, universal "Daily Animal Room Checklist" nor standard operating procedure (SOP) can be used. Samples of checklists in use by individual aquatic housing facilities are available from RAR; however, the checklist must be individually tailored for each facility, depending on the organism and type of equipment. Items that should be included are regular checks of water temperature and quality; the frequency and type of feeding; and performance of water filter changes and other regular equipment maintenance. The checklist and SOP will be part of the animal protocol, and will be subject to review and approval by the John Hopkins University IACUC. The IACUC will be alerted to the use of aquatic animals via a checkbox on the front

page of the protocol form. Daily entries will be made on these checklists to record actions performed in support of the animals. These sheets maintained at the site of care and subject to review by RAR and/or the IACUC upon request.

Because laboratories will provide primary care and oversight for the aquatic animals, per diem forms will not be distributed or collected.

C. Physical Plant and Environmental Conditions in Aquatic Housing Areas

Construction specifications are laid out in the *Guide* for animal housing, with an emphasis placed on maintaining a high level of sanitation; appropriate ventilation and air quality; isolation from other lab areas; and safety for laboratory personnel. The principles are outlined primarily with mammalian and avian housing in mind; while specific requirements may not apply to housing of aquatic organisms, the basic tenets of providing appropriate and sanitary housing clearly do.

Some aquatic housing facilities at JHU were constructed primarily for that use, while others are in laboratories constructed for other purposes. Both types of facilities should be acceptable, if the basic principles of the *Guide* and performance standards are kept in mind. Thus those arranging aquatic housing should direct their efforts to create an acceptable environment by implementing the following:

1) Place animals in a dedicated, secure area (e.g., separate lab, lab bay or portion thereof) and, as much as possible, away from other areas in the laboratory that do not involve laboratory animals. The door(s) to the room should be locked when responsible personnel are not present.

2) The physical plant in the area should be in good repair. Surfaces should be constructed of materials that can be easily sanitized to the standards required by the research. Remove or seal unsealed wood (e.g., shelves), unnecessary wall attachments, and replace damaged ceiling tiles. The floor, covering, and walls should be free of defects that impede sanitation. Where practical, materials should be water resistant to minimize damage from long-term exposure to water and high humidity. Electrical outlets used in wet areas should also be of the ground fault current interrupter-type.

3) While some aquatic species do not have a known requirement for a regular light-dark cycle, others do require a regular cycle to maintain normal behavior and for good breeding performance. In these cases, use a light timer and/or have a laboratory process to ensure that animals have a regular daily light cycle (unless an exception has been granted by the ACUC for scientific reasons).

4) Where temperature or humidity fall out of the *Guide* range for more than three consecutive days (or sooner if preferred), facilities management should be contacted to make adjustments. In some areas, humidity is difficult to maintain at greater than 30% in the winter in Baltimore, even by use of an appropriately placed room humidifier. If this is the case, consult with RAR and/or a veterinarian for appropriate methods to sample humidity at the cage level (for rodents) and/or to document whether the low humidity has an impact on the health of the animal.

D. General Housekeeping Provisions for Aquatic Housing Areas

1) The housing area must be maintained in a clean and orderly condition.

2) The placement of housing tanks should permit easy visualization of the animals contained within unless otherwise precluded by the requirements of the experimental protocol. While algae growth is normal and not harmful to the animals, it should be kept off of the tank walls to the extent necessary to permit visual inspection of the animals.

3) The housing area should be arranged to facilitate sanitation. However, the considerations are quite different for aquatic species than for mammals. Specifically, the risk of disease transmission from feral populations to laboratory animals is negligible, and there is no risk (in the case of fish) or little risk (in the case of frogs) of laboratory animals escaping and surviving outside the facility. Therefore, the emphasis should be on neatness and general cleanliness, and on a safe environment for animals and caretakers.

4) Potentially harmful chemicals should not be stored near animal cages, or in biosafety cabinets or fume hoods when animals are present. All risks that a chemical spill would injure or contaminate animals should be eliminated.

5) Bench space used for manipulation of animals or animal materials should be wiped down with a disinfectant/soap solution after each use.

E. Husbandry/Housing Requirements for Aquatic Species

For proper husbandry of any aquatic species, water temperature and quality are the most critical parameters, and every effort should be focused on maintaining these within parameters appropriate for the species. The design of the individual aquatic housing systems will vary greatly, in party because of the specific requirements of different species. However, while there are several commercial vendors of aquaculture systems, there is no single standard design, and some laboratories with smaller requirements will choose to construct their own systems. Therefore, the guidelines presented here are by necessity very general, and a detailed set of protocols must be developed for each laboratory.

1) Proper salinity is critical for survival of many aquatic vertebrates, and for others can contribute to optimal health and breeding. Protocols should specify the importance of salinity for the species, ranges, chemical composition, and methods for monitoring salinity. For example, in some cases specific gravity is measured and in other cases conductivity is a more useful measure.

2) In most aquatic systems, wastes accumulate in the water in which the animals live. Protocols must specify the how wastes will be handled, commonly done using water changes. Methods for management of evaporation may also be required, depending on the system design. Methods for delivering air, air exchange, and removal of carbonic wastes should also be described.

3) In recirculating systems with large numbers of animals, an effective biofilter is critical to maintaining water quality. The biofilter collectively refers to bacteria colonizing the system, that process nitrogen-containing waste and prevent the build-up of harmful levels of ammonia and nitrite in the water. Water quality testing for, at minimum, ammonia, nitrites, nitrates and pH should be regularly performed, at a frequency determined by the system maturity, stocking density, species housed, and aquatic system utilized (ex. recirculating vs flow through vs static). The acceptable levels of ammonia and nitrites vary according to species and age of the animals, and should be determined for each individual facility. In an established system, build up of ammonia and nitrite is generally not a concern, and infrequent (every two weeks or less) testing of these parameters should be sufficient. In a new system, care should be taken to not exceed the capacity of the biofilter, either by introducing animals too rapidly or by overfeeding. In these new systems, more frequent monitoring will be required until conditions stabilize.

4) In systems with a high rate of water exchange, rapid fluctuations of conditions, particularly water temperature or dissolved gas, can present a danger to a large number of animals. In these systems, water temperature should be monitored daily, and the caretakers alert to signs of problems such as unusual behavior or unexplained death.

5) Depending on the design of the system, water temperature is most often not determined by ambient conditions, although extremes of air temperature can affect water temperature. In cases where room air temperature is making the maintenance of proper water temperature difficult, facilities management should be contacted to make adjustments.

6) To ensure that food remains fresh and uncontaminated and does not attract vermin, it must be stored in a container that is kept tightly closed and clearly labeled with the type of food. Many aquatic species are ideally maintained with some component of live food in their diet (*Artemia* for zebrafish, blackworms for frogs, etc.). In those cases, there should be established protocols, clearly documented, for culturing, preparing, and feeding of live foods.

F. Standards for Sanitation and Specific Practices for Laboratory-Based Animal Care and Use Activities

As much as possible, equipment used for research animal housing, transport, or experimentation should be designed and constructed to allow efficient cleaning. A sound, comprehensive sanitation program for aquatic species care and use involves the following components:

1) In determining the schedule of tank changing and cleaning for an individual aquatic housing system, several factors should be considered. Multi-tank equipment or facilities are generally designed to use a common water supply, filtered and treated as required for the specific species. Although these may have a flow-through water supply, most often some portion of the water recirculates from individual tanks back to the common supply. A critical component of a recirculating system, in particular one housing large numbers of animals, is an effective biofilter. Therefore, it is not necessary to sterilize all components of an aquatic housing facility, and in fact it would be harmful to the animals. An additional consideration is that changing and cleaning tanks inevitably disturbs the animals and increases the chance for mixing up or losing valuable stocks. Therefore, a balance should be reached in the schedule of tank changes, to control

buildup of algae and waste products while minimizing disturbance to the animals. While tank changes in any system are unlikely to be required more than twice a month, the optimal schedule will vary greatly among systems and must be determined individually.

2) Tanks that can be sanitized (or sterilized) in a cage washer are automatically afforded the benefit of existing quality assurance measures (i.e., temperature-time monitoring, microbiological monitoring). Since detergents should not be used on tanks for aquatic species, the cage washer must be prepared to eliminate chemical residues and run in a mode that does not dispense detergents or other chemical treatments. RAR offers this capability, either on a routine basis for a larger facility, or in case of an epidemic or elevated level of opportunistic organisms, when a large number of tanks need to be sanitized. For tanks that must be sanitized by hand, laboratories should have written SOPs that describe the process for sanitation, appropriate to the tank materials and species. However, as noted above, complete sterilization of tanks is neither necessary nor likely to be effective, since the tanks will be returned to the common water supply, which is not sterile.

3) Other equipment used for housing or to support animal care or in vivo experimental activities must be cleaned at appropriate intervals. Pertinent equipment may include, but is not limited to, tanks used for temporary housing outside the main system, containers for transport or shipping, or surgical equipment. Apparatus that is only used in non-survival applications should be cleaned immediately after use, but documentation of effective sanitation is not required.

4) Equipment that can withstand treatment in the RAR cage washers should be scheduled for routine sanitation. Equipment that cannot withstand cage washing should be sanitized by appropriate methods devised in the laboratory, which will need to be verified as efficacious by RAR.

5) Mechanisms (e.g., schedules, equipment identification codes) should be established so that a record can be kept of equipment that needs to be sanitized, personnel who perform the sanitization, and of the dates on which it is sanitized.

6) Laboratories should give high priority to the replacement of equipment that may not be easily sanitized if improved versions that are easy to sanitize are available.

F. Enrichment Standards for Aquatic Species

Enrichment for aquatic species, including for those housed in satellite housing locations, is described in *HM-28 Downtown Enrichment Plan.* Please refer to this document to determine enrichment requirements for these species.

III. DISEASE CONTROL AND VETERINARY CARE

The program of veterinary care at JHU is housed in RAR, under the Director of Laboratory Animal Medicine. The responsibility for veterinary care rests exclusively with the RAR veterinary staff. The only exceptions to this policy are those specific elements of clinical care

that are associated with the maintenance of an experimental animal that have been defined and explicitly stated in the ACUC-approved animal care and use protocol. In particular, use of antibiotics that are not included in an approved protocol must be under the direction of a clinical veterinarian.

Comparatively little is known about disease prevalence, susceptibility, diagnosis, and treatments for aquatic species, particularly as applies to large laboratory colonies. For zebrafish, infectious diseases affecting significant numbers of animals are largely considered opportunistic, affecting older fish or those under stress for other reasons. Therefore, the best approach to disease control is to maintain ideal culture conditions, avoid overcrowding, and cull older stocks regularly. For all species, caretakers and laboratory personnel should knowledgeable be trained to recognize signs of illness. The most straightforward approach to dealing with diseased animals is to euthanize them, but in the case of valuable stocks or individuals, RAR veterinary staff should be consulted about possible treatments. However, it should be kept in mind that treatment of individual animals will have to be carried out in isolation from the main system, which may be difficult for any extended period of time.

If unexpected disease phenotypes or an increase in animal morbidity and mortality is noted, consultation with RAR veterinarians should be arranged.

Whenever possible, it is recommended that adult animals brought in from outside the colony be kept in an isolated system, for example a quarantine rack of tanks with a separate water supply. In the case of zebrafish, embryos from these adults can then be introduced into the main system after bleach treatment.

IV. WHAT ASSISTANCE WILL BE PROVIDED BY RAR TO SATELLITE FACILITIES?

To assure that standards and practices for aquatic laboratory animals housed in satellite areas conform to this policy, RAR provides the following assistance:

1) An RAR technician will visit each satellite facility on a regular schedule to review the provision of care in conformance with this and provide consultation; frequency of visits may increase or decrease as conditions warrant.

2) The RAR technician will review animal health and coordinate submission of samples for disease diagnosis as appropriate.

V. ONLINE RESOURCES AND ADDITIONAL INFORMATION

Zebrafish
http://zfin.org/zf_info/zfbook/cont.html#cont1
Link to an online version of “The Zebrafish Book”, a laboratory manual with information on standard husbandry, breeding, and rearing of larvae

http://zebrafish.org/zirc/health/diseaseManual.php

Link to an online disease manual, with procedures for necroscopy, pathology, and diagnosis and treatment of common diseases

Xenopus
http://www.xenopus.com/links.htm
Page with numerous links to information about *Xenopus* (primarily *laevis*), including many on husbandry and disease
http://tropicalis.berkeley.edu/home/husbandry/index.html
Richard Harland's home page at UC Berkeley, with detailed protocols for husbandry of *Xenopus tropicalis*, explanations of standard aquaculture systems, some information on diseases

Axolotl
https://ambystoma.uky.edu/education1/guide-to-axolotl-husbandry

Yandulskaya AS, Monaghan JR. Establishing a New Research Axolotl Colony. Methods Mol Biol. 2023;2562:27-39. doi: 10.1007/978-1-0716-2659-7_2. PMID: 36272066; PMCID: PMC10948202.

Commercially available aquaculture systems
Aquatic Habitats (http://www.aquatichabitats.com/)
Marine Biotechnology (http://www.marinebiotech.com/)
Aquaneering (http://www.aquaneering.com/)
Aquatic Solutions (https://www.aquaticsolutions.it/)
Iwaki Aquatic (https://iwakiaquatic.com/)

I acknowledge that I have read and understand the **"JHU Policy and Standards for Research Animal Housing in Satellite Facilities: Supplement for Aquatic Vertebrate Species"** and I will follow the procedure.

I agree to bring any deviation in this procedure to the attention of the Director of Lab Animal Management.

_____ _____
Name (Print) Date

_____
Signature