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Special Topic Overview

Aquatic Environment, Housing, and Management in the Eighth Edition of the *Guide for the Care and Use of Laboratory Animals*: Additional Considerations and Recommendations

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The eighth edition of the *Guide for the Care and Use of Laboratory Animals* recognizes the widespread use of aquatic and semiaquatic research animals by including, among other references, an entire section on aquatic animals in its chapter on environment, housing, and management. Recognizing the large number of aquatic and semiaquatic species used in research and the inherent diversity in animal needs, the *Guide* refers the reader to texts and journal reviews for specific recommendations and suggests consultations with persons experienced in caring for aquatic species. Here we present considerations that may add to the basic information presented in the *Guide* and offer some recommendations that may be useful for aquatic animal model caregivers and researchers.

Published in 2011, the eighth edition of the *Guide for the Care and Use of Laboratory Animals* is expanded in scope and now includes a separate section on aquatic animals, which is found in Chapter 3, *Environment, Housing, and Management*. Recognizing the "variety of needs for fish and aquatic or semiaquatic reptiles and amphibians is as diverse as the number of species considered,"[13] the *Guide* refers its readers to texts and journal articles for further information and to advice from experienced caregivers. Although numerous texts and articles exist,[3,7,11,12,14,17,27,30,31,34,37] we here hope to provide additional information that will prove useful. The considerations we present are derived, in many cases, from experiences in the care and use of zebrafish (*Danio rerio*) acquired after more than 25 y of fish husbandry at the University of Oregon, although the principles can generally be applied to other aquatic species as well. Although the present article does not touch on every section or passage in the *Guide*, we follow the general outline of the Aquatics section, provide some points to consider, and recommend various actions intended to improve the care and wellbeing of aquatic animals used in research.

Aquatic Environment, Housing, and Management

Aquatic environment. *Water quality.* By definition, aquatic life must have water to inhabit, and semiaquatic life must have water to frequent.[23] In the care of aquatic animal models, the importance of water quality cannot be overstated and, as seen in the *Guide*, water composition is essential to the wellbeing of aquatic life.[13] The *Guide* also notes that "different classes, species, and ages in a species may have different water-quality needs and sensitivities to changes in water-quality parameters."[13] Rapid changes in water quality can lead to stress and even death in some aquatic life."[27] Water quality is one of the first things to check when aquatic animals show signs of abnormal behavior or distress.[27] Water-quality tests to perform can include temperature, pH, nitrogen waste products (ammonia, nitrite, and nitrate), phosphorus, chlorine, oxidation-reduction potential, conductivity or salinity, hardness, alkalinity, dissolved oxygen, total gas pressure, ion and metal content, and others[8,11,12,13,18,22] The water-quality requirements for a given aquatic or semiaquatic species and the water system design dictate the appropriate tests. For example, concern for dissolved oxygen is much greater when keeping fish intolerant of low levels (for example, rainbow trout [*Oncorhynchus mykiss*]) than when keeping fish adapted to low levels (for example, goldfish [*Carassius auratus*]).[4,27] Whether for periodic checks or for emergency problem-solving, water-quality tests should be conducted with instruments that are calibrated and test kits that are within expiration dates.[18,22] Importantly, when troubleshooting an issue, parameters measured by automated systems should be verified with separately calibrated systems or fresh test kits. Records of regular maintenance and calibrations on process instruments should be kept with other husbandry records.[12] Before its use, any compound used to condition the water (for example, aragonite to raise water pH) should be evaluated with respect to its safety for the aquatic or semiaquatic species and its potential for compromising biosecurity.[18]

Filters and filter media used to treat water and maintain water quality should be monitored regularly, and media should be changed prior to the end of its useful life.[15] The same attention to monitoring and maintenance required for filters applies to disinfection units, and the bulb(s) and bulb sleeve(s) of a UV disinfection unit should be changed well in advance of UV dose degradation.[11,12,15] Regularly assessing the effectiveness of the

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UV disinfection equipment through the use of microbiologic monitoring can help to confirm normal operating conditions and can be a part of the overall water-quality assurance strategy. In addition, histopathology results from sentinel programs can sometimes be an indirect measure of UV effectiveness, especially for pathogens that are difficult to culture. Records on filter and disinfection unit monitoring and maintenance should be kept with other husbandry records.[11,12,22]

Life-support system. In the *Guide*, 'life support system' refers to the "physical structure used to contain the water and the animals as well as the ancillary equipment used to move and/or treat the water."[13] The *Guide* further defines the life-support system by grouping systems into 3 categories, comprising recirculating systems, flow-through systems, and static systems.[13] The system(s) used in a research program will be decided by combining the research requirements with the requirements of the animal model. Large programs potentially could use all 3 types of aquatic species life support. For example, a large zebrafish (*D. rerio*) program may use a recirculating life-support system for its main colony and a flow-through life-support system for its quarantine racks. In addition, a program may use some form of static housing that accommodates close observation and isolation of individual subjects for animals after or between procedures; for breeding small groups of fish; for holding embryos, larvae, and fry; and for treating fish with medication. Each type of system is designed around the basic concept of consistent, oxygen-rich, and pathogen- and contaminant-free water for the aquatic or semiaquatic species used.

In recirculating water systems, stable water quality benefits both the nitrifying bacteria in the biobed and the aquatic animals used in research.[18] Medications, especially antibiotics, should not be allowed to infiltrate the water in a recirculating system, because these compounds can be extremely harmful to nitrifying bacteria.[5,20,21] Treatments for sick aquatic or semiaquatic animals should happen in treatment or 'hospital' tanks that are separate from the main recirculating system, or the system should be designed so that tanks can be removed and isolated while undergoing treatment.[21] An alternative is to treat the entire system and risk harming the beneficial nitrifying bacteria.

A well-designed life-support system will have an arrangement of traps or catches, so that animals that may have escaped primary enclosures are kept out of the filtration loop and do not pose a threat to the overall health profile and pathogen-free status of the system.[26] In systems that use sumps or collection vessels to facilitate the water treatment and circulation, regular checks for escapees should be a standard part of the overall sump maintenance, and a procedure for removing and appropriately euthanizing escaped animals should be followed. Although data on age and genetic background are unknown, escapees can be fixed for histopathology examination and benefit the facility either by revealing disease conditions or by confirming a disease-free state. Systems that interface with surrounding environments or that discharge water into outdoor environments should use a combination of physical (lethal water temperature), mechanical (screens), and biologic (engineered sterility, triploids) barriers to prevent escaped aquatic animals from surviving outside the research facility.[24,26]

To provide for the periodic cleaning and maintenance of life-support system pipes and waterways, some design for cleanouts and a plan for draining effluent due to periodic maintenance away from animals and the main biofilter should be considered. Systems that use pumps to move water should monitor dissolved gases or incorporate degassing steps to eliminate the risk of gas supersaturation[18,27,40] As with all process monitoring equipment, probes should be calibrated regularly and replaced as necessary.[18,22]

Electrical power recommendations appear in the *Physical Plant* chapter in the new *Guide* and are not mentioned in the chapter on *Environment, Housing, and Management*.[13] However, in aquatic life-support systems, electrical power sources and backup or redundant power sources deserve careful consideration. Electrical power can be used to control water temperature, to circulate water through filters and tanks, and, for some species, to provide aeration. Water temperature is a critical factor for aquatic species,[11,27] and heaters or chillers must be operating to maintain species-specific water temperature requirements. In aquatic life-support systems that use pumps to move water, the power source itself and the consequences of even brief power outages should be considered. For example, some filtration types such as fluidized bed biofilters can experience collapse if water flow ceases.[36] As with other critical systems, a plan should be developed to detail actions to protect aquatic or semiaquatic animals when electrical power fails.[13]

All manufactured parts of life-support systems should be made of materials that are nontoxic and not harmful to aquatic and semiaquatic animals.[13,18] In addition, the life-support system should be made in a way that allows for disassembly and sanitizing.[18] During disinfection or sanitation procedures, animals may need to moved to another system.[39] Whenever possible, equipment that causes large vibrations or generates loud noises should be moved to a different room or away from the animals in the system,[11,12,18] although some species may be more tolerant than others with respect to noise.[2,7,33] Equipment that may wear or need to be replaced should be installed in a way that minimizes disruptions to the system when repairs or replacements are made. For example, system design can provide bypasses or alternate routes for water so sections of the water system can be shut down temporarily for repairs without affecting the health and wellbeing of animals.

Temperature, humidity, and ventilation. Along with water quality, water temperature should be monitored and maintained in an effort to avoid sudden changes in temperature that may adversely affect certain aquatic or semiaquatic species.[11,27] To induce breeding in some aquatic species, temperature changes that mimic seasonal changes may be necessary.[29] In all cases, some method to allow for adjustment, monitoring, and daily logging of temperature should be used to avoid undo stress or even death in cases when the temperature falls below or exceeds critical thresholds. As with water-quality equipment, temperature probes, heaters, and chillers should be maintained properly and checked periodically to verify accuracy and effectiveness, and all maintenance records should be saved for review.[18,22]

As noted in the *Guide*, the aerosolization of water can lead to spread or transfer of pathogens.[13,20,32,38,39] However, for some aquatic environments, in-tank aeration is important for animal health and wellbeing, and the concern for pathogen control must be weighed against the benefit provided by in-tank aeration. For example, fish housed in hospital tanks that are isolated from a main filtration system can benefit from in-tank aeration. Regular checks on all aeration devices will help to ensure that setpoints are not higher than normal and leading to unwanted aerosolization. If possible, aquatic facility equipment necessary for water quality that may increase water spray (for example, degassing columns and trickle towers) should be placed away from animal housing or at least designed with water spray containment in mind.

Any equipment kept in the aquatic or semiaquatic environment should be checked regularly for signs of abnormal wear that might be caused by temperature, humidity, or compounds used to sanitize the equipment. In addition, aquatic or semiaquatic environments that require brackish or salt water should use equipment designed to withstand marine environments, and the equipment should be monitored for signs of degradation associated with high-salt environments.

Illumination. The *Guide* outlines several specifics for illumination. In addition, some aquatic and semiaquatic species require artificial changes in seasonal photoperiod to induce breeding behaviors. To facilitate these changes, photoperiod controls should be easy to adjust, monitor, and allow for daily logging of dawn and dusk times.

Aquatic housing. *Microenvironment (primary enclosure).* The *Guide* provides an excellent list of characteristics that help to define the appropriate primary enclosure for the aquatic or semiaquatic animal. Some of the points merit additional description, however. For example, the *Guide* indicates "in research settings acceptable primary enclosures are free of sharp edges and/or projections that could cause injury."[13] It should be noted in addition that the interior of the enclosure can be completely safe for the animal, but the exterior of the cage or tank may have features that, although they allow for easy stacking and storage, protrude and might cause injury to workers. Similarly, the subject of toxic materials that could leach into an aquatic system and affect research is a complicated one. Certainly there are toxicants that can leach from a primary enclosure that should never enter an aquatic system because of the animal mortalities that would result (for example, silicone sealant containing antimicrobial compounds or fungicides).[18] Toxicity testing should be performed before wide-scale adoption of new or potentially harmful substances or equipment. In some cases, the life-support system can be fitted with filters that can mitigate the deleterious effects of some toxicants (for example, activated carbon filters).[18]

Space. The species and its requirements for postural adjustments[13] and its responses to increased and decreased housing densities (for example, stress and aggression)[16] should dictate the space requirements for its housing and containment. As for terrestrial animals, when evaluating a tank or cage for any aquatic or semiaquatic species, it should be remembered that some animals are nocturnal,[28] and although movements (for example, postural adjustments or feeding behaviors) can better be observed when the environment is illuminated, they may be are slow or subdued; the animal's activity level can increase greatly and movements may require more space when the light level is diminished. For example, the spotted gar (*Lepisosteus oculatus*) is much more active at night,[35] and facilities using spotted gar should recognize that fact when observing animals to better design housing and when performing behavioral checks. Because technologies are advancing and methods for producing oxygen-rich, pathogen-free, and contaminant-free water are improving, concerns over water quality that previously affected space issues have been lessened. Importantly, when considering space issues, behaviors like aggression and hiding may affect the ratio between the space available compared with that actually used.[34] As stated in the *Guide*, "Institutions, investigators, and IACUC members should evaluate the appropriate needs of each species during program evaluations and facility inspections and continue to review ongoing research in these areas."[13]

Aquatic management. *Behavior and social management.* The reference to latex glove toxicity in the *Guide*[13] underscores the risk posed to aquatic or semiaquatic species by foreign materials that may enter the animal's environment. From husbandry implements like scrubbers and siphons to often-overlooked items like hand lotions, hand soap residues, and laboratory gloves, the list of potentially harmful items found in the aquatic research facility is lengthy.[11,13] Each item that is planned for use in water with aquatic or semiaquatic animals should be considered with respect to its potentially harmful effects, and those items found to be potentially harmful should be replaced with safe substitutes. In cases where replacement is not possible (for example, soap residue from hand washing), the risk from potentially harmful substances should be mitigated through training and education.

Husbandry. *Food.* Regarding the principles for feeding terrestrial animals as presented in the *Guide,* namely to feed animals "palatable, uncontaminated diets that meet their nutritional and behavioral needs at least daily, or according to their particular requirements, unless the protocol in which they are being used requires otherwise,"[13] note that considerations for feeding aquatic and semiaquatic animals go beyond those for terrestrial animals.[12,25] These considerations include any loss of nutrients if food consumption is delayed,[12,25] effects of uneaten feeds on water quality,[12,25] and the possibility that nutrients are available through naturally occurring cultures (for example, planktonic organisms).[25] Unless a specific nutritional profile for the kept species is known, fish researchers and managers are encouraged to use data on related species when designing diets.[12,22] Although processed feeds can be purchased, persons developing diets are encouraged strongly to check ingredients lists and guaranteed analysis data to ensure that essential dietary requirements including proteins, lipids, and carbohydrates at least closely approach the target profile.[11] Formulated feeds offer benefits including increased control over nutritional content and reduced risk of pathogens, but replicating the complete nutritional content of live food is difficult to achieve.[12] When using a formulated feed, caretakers should be trained and encouraged to observe signs of nutritional deficiencies (for example, skeletal deformities associated with inadequate intake of vitamin C).[6,19] In contrast, live foods can provide more complete nutrition than do processed feeds,[12] but concerns over pathogens and contamination from live feeds must first be resolved.[11,12,39] In all cases, appropriate storage and documentation for processed feeds, culture and documentation for live feeds, and distribution to animals should be in accord with the counsel found in the *Guide* and elsewhere.[12,13]

Substrate. When system design and species needs have been evaluated[13] and the determination to use a substrate is made, concerns for pathogen control should be resolved prior to the introduction of the substrate[18,39]. For example, some cichlid species fish are kept in tanks with substrate, including types of sand or gravel.[1,9,10] The substrate should be disinfected (for example, autoclaved) before use to eliminate risk from pathogens.

Emergency, weekend, and holiday care. Although many aquatic and semiaquatic species do not require food daily and although many life-support parameters can be automated, researchers and caretakers should remember the mandate for daily observation of research animals for signs of illness, injury, or abnormal behavior and provide trained personnel on weekends and holidays.[13,22] Notification procedures and emergency contact information for veterinarians and supervisors should be readily accessible, that is, "prominently posted."[13]

Conclusions

The new section on aquatics is a welcome addition to the *Guide.* Aquatic and semiaquatic animals are becoming more commonplace in animal care programs, and principles regarding their care and use are now readily available for reference by personnel, including IACUC members, researchers, and caretakers. Other chapters of the *Guide* also include recommendations applicable to aquatic and semiaquatic species, and the references at the end of each chapter of the *Guide* provide more detailed and recommended reading.[13] Although we have focused on the aquatics-specific additions found in Chapter 3, "the *Guide* is meant to be read by the user in its entirety."[13] Our hope is the considerations and recommendations presented herein will help program personnel further refine and improve the care and use of aquatic and semiaquatic animals used in research.

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