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The zebra sh (*Danio rerio*) has gained in popularity as a uniquely important animal model for the study of vertebrate development and genetics. The species typically is used to analyze how the vertebrate nervous system is regulated at the cellular, genetic, and molecular levels.[25] The rapid expansion of zebra sh research in North America necessitates better education and resources on the part of the Institutional Animal Care and Use Committees (IACUCs), whose role it is to assure that animal research meets the minimum standards set forth in the *Guide for the Care and Use of Laboratory Animals* (the *Guide*).[18] Within the animal use program, centralized and satellite animal facilities must be inspected semiannually. The *Guide*, however, does not provide criteria speci c for establishing or evaluating sh programs, and therefore each IACUC must construct standards of its own.[13]

Performing inspections of investigator-managed satellite facilities that use any number of different housing styles can be particularly challenging for IACUC inspectors, especially those with little background in aquaculture. IACUC inspectors will be more equipped to ask pertinent questions by obtaining a good understanding of the principles of zebra sh facility operations. In addition, zebra sh facility managers can be better prepared for inspections by establishing standard operating procedures (SOPs) and complete facility operating records, thereby enabling inspectors to easily evaluate facility function, preparedness, and zebra sh health. By introducing a potential IACUC inspector to the basics of sh husbandry and by sharing with sh facility operators 1 paradigm of recordkeeping that was central to

systems. In recirculating systems, a proportion of the water exiting the system is recycled.

. A static water system is one that does not receive a continuous supply of new or recirculated water. It has very low inherent biological carrying capacity and relies mostly on partial-volume water changes. Water quality can be improved with the addition of devices that create water movement, such as pumps or air stones.[10] A sponge lter can aid in mechanical collection of debris and is gentle enough to be used in static tanks when rapid growth of fry is desired. These lters also provide a suitable substance and increased surface area for nitrifying bacteria to grow. Algae will grow in static tanks and should be kept under control but not eliminated. A minimum of algae should be removed regularly to keep the nitrogen cycle moving. Water chemistry testing is recommended as often as necessary to determine the proper tank cleaning and water change schedule for this type of system. Water changes of 75% or more of the volume usually are required once or twice weekly. Ammonia toxicity is an important concern in static tanks and must be measured daily. Although the goal is to keep ammonia levels below 0.02 ppm and nitrite levels below 1 ppm,[9] zebra sh can acclimate and survive in suboptimal water conditions, but this may impact negatively on growth, gamete production and immune function.[20] The IACUC inspector will observe static water systems and review SOPs for care and check maintenance and water quality records. Algae growth should not prevent visibility of the sh when looking through the side of the tank.

. A *flow-through system* requires a continuous supply of water either from a natural source (river, lake or ocean), a municipal source (tap water) or a well. Water is pumped separately into individual tanks, while the runoff water exits the tanks into a drain without recirculation. Because no holding tank is present typically, the incoming source must be good-quality water that is pretreated or ltered before it

enters the tanks. This goal can be accomplished by mechanical, chemical, or biological ltration or some combination thereof. Fluctuations in water condition at the source will be re ected

access to emergency power outlets is not available within the facility, standby generators may be necessary in case of a power outage. The IACUC inspector will check for electrical safety and inquire about emergency electricity supply.

Noise and vibration. Machinery within the zebra sh housing areas such as pumps and blowers should not be creating excessive noise or vibration that can disturb the sh.[21] Machinery should be easily accessible for servicing. The IACUC inspector will consider vibration and noise level of machinery in the proximity of sh. If questionable, it is the responsibility of the zebra sh facility staff to assure that the equipment is operating properly.

Light. Light in uences physiologic and behavioral processes including growth, development, and reproduction in sh.[26] If windows are present, they should be covered adequately to prevent light from passing through. A 14:10-h light:dark cycle typically is recommended because it nearly mimics the natural zebra sh environment and can be programmed with room timers.[21,29] Recommended light levels are between 5 and 30 fc or 54 to 324 lx at the surface of the water.[21] Fluorescent lights usually are mounted in the ceiling. Optimally, lights should be phased on and off to minimize the startle re ex of sh upon activation or deactivation. The IACUC inspector will review the light cycle program and logs (if available) and con rm that windows are covered (if present).

Temperature. Water temperature will equilibrate to room temperature and should be monitored continuously. The common housing temperature for zebra sh is 28 °C.[29] A comfortable range is 22 to 30 °C,[21] however zebra sh have been shown to tolerate a wide temperature range.[11,20] Rapid water temperature changes can cause unnecessary stress and increase susceptibility to pathogens.[4] Temperature should not change more than 5 °C in a 24-h period[22,31]. Handheld thermometers should be used to check the accuracy of electronic monitors. The IACUC inspector will review the temperature logs and question any temperatures out of optimal range.

Housing. Rooms where sh are housed should be clean and orderly and separated from personnel areas. Food and beverage intended for human consumption should not enter the animal rooms. Fish should be separated from each other by species, age, and disease status. Protocol numbers and the names of principal investigators should be posted in each room (preferably on each tank), with contact information readily available.

Walls, doors, ceilings, and oors should all be clean and well-maintained. There should be little evidence of corrosion or water damage. Surfaces should be made of impervious, smooth, material that is sealed and easily sanitized. Floors should be made of nonskid material and be clear of standing water. Unless it is well-sealed and impermeable to water, wood should not be present in areas of constant dampness.

Main oor drains should be oversized to handle a transient large ow of water and should be kept clear of debris. Floors should be sloped toward the drains. Mesh of various sizes can be incorporated somewhere in the drainage system to prevent escaped sh from entering. Managers must be familiar with the regulations of the city, state, and federal agencies with regard to the discharge of live animals in the municipal sewer system. The IACUC inspector will note area cleanliness, facility structural maintenance, and check that contact information is available for each principal investigator.

Food storage. Zebra sh typically are fed a mixture of live prey and commercially prepared diets. Storage areas for commercially packaged, dry feed (such as pelleted diet and sh akes) should be cool [less than 20 ºC (68 °F)], dry (<75% humidity),

away from direct sunlight, and free of vermin. When received, original packaging should be labeled with the date of receipt and expiration date (sometimes stated as 'best used by'). When opened, contents remaining in the package should be sealed in waterproof containers protected from heat, humidity, and light and labeled with the product name and expiration date.[21] Shelf life of zebra sh feed varies, and each manufacturer should be consulted for appropriate use and storage guidelines. Pelleted feed can last 2 to 3 y when stored correctly, whereas sh akes have a shorter shelf life (up to 12 mo). Opened and unopened packages of artemia cysts (brine shrimp eggs) will last 3 to 4 wk if refrigerated at or below 4 ºC (40 °F). Packages must be resealed tightly to prevent moisture from entering. Freezing will increase the shelf life of *Artemia* cysts markedly.[8] Other live prey such as rotifers and paramecium used to feed fry must be propagated continually and cannot be stored.

Chemical storage. Drugs, biologics, and hazardous materials must be identi ed, and stored separately from feed, according to the manufacturer's speci cation. The IACUC inspector will check feed storage areas, shelf life of feed, labels on storage containers, and storage of other materials.

Waste. Waste material must be discarded appropriately. After euthanasia, zebra sh carcasses should be frozen until they are disposed of according to facility SOPs. Biohazard waste must be discarded according to local, state, and federal regulations. The IACUC inspector will inquire about the handling of waste material.

Microenvironment

Identification. According to the *Guide*, "means of animal identi cation include room, rack, pen, stall, and cage cards with written or bar coded information …. Identi cation cards should include the source of the animal, the strain or stock, names and locations of the responsible investigators, pertinent dates and protocol number when applicable."[18] This goal can be accomplished with individual tank or rack cards, room signs, or a coding system where information regarding each tank or rack can be viewed in a database easily accessible in each sh housing room. The IACUC inspector will check tank and rack identi cation cards. Identi cation methods should be consistent throughout TJ T38(oughout TJ2onon.)bg([( )63(O)1(t)1(h)72(m))9(u7(sa)7( )onm]1J 1* t 1J2oJ 138(ou(1)9(d)9(e)2(118(o1TJ2oJ 13e ste)-38Aum

for a healthy system has been reported to be between 500 and 800 ppm in striped bass.[3,23] In juvenile sh, best results are expected when nitrates do not exceed 200 ppm.[3,23] Completely eliminating nitrates from the water is ideal but dif cult to achieve.

Monitoring water-quality parameters with chemical test kits is more labor-intensive than using electronic monitoring devices. Various electronic devices are available for continuously tracking a variety of water parameters. When using electronic devices to measure water quality parameters, the sensor probes may require routine calibration or adjustments according to the manufacturer's recommendations. A chemical test can be used as a method of checking accuracy of the electronic system.

The use of an automatic pH and salt dosing unit is bene cial to any automated sh system. The doser housing is calibrated and programmed according to the system's needs. The buffer and concentrated salt water are stored in nearby holding tanks, with hoses or pipes connecting them to the system sump tank. Without an automatic pH–salt dosing system, additional time will be required to adjust the system's water quality for these 2 parameters. Furthermore, conductivity and pH will need to be checked and recorded once or twice daily. Although automatic, the salt and pH dosing unit requires monitoring to ensure correct functioning.

Time allocated to monitoring water quality applies to each system in place. Setting up a single system decreases the overall time required for water quality management but increases the risk of heavy losses if that system fails or becomes contaminated. Dedicating additional resources to operating a redundant system likely will save time in the long run by preserving sh. The IACUC inspector will inquire about water quality monitoring systems and reliability.

Algae. Algae growth is expected in zebra sh tanks.[2] The conditions that contribute to the growth of algae are the accumulation of nitrates and phosphates in the water and the presence of light.[2] Phosphates can originate as a byproduct of the mineralization of decomposing dead matter[14] or enter the system from the sh food or water (including RO water). Nitrate is the aerobic endproduct of the biological nitri cation process, without which algae would not be able to survive. Nitrate accumulates until it reaches equilibrium with processes that allow elimination such as water changes, plant or algae growth and removal, and chemical ltration.[14] A moderate amount of algae growth demonstrates evidence of nitrogen cycle completion. Excessive growth of algae can be indicative of increased nitrate levels, which can be detrimental to the sh. Because algae are photosynthetic eukaryotes, they produce oxygen during the light period but use oxygen at night and when decaying. When more algae decay than grow, the result is a net decrease in dissolved oxygen, which is stressful for sh.[14] Improving tank visibility is another bene t of minimizing algae load in the tank. The amount of algae that can be removed from the tank will depend on the system design. Tanks on systems with a separate biological lter substrate that is loaded with bene cial bacteria can be wiped completely clean. Cleaning the pipes and reservoirs of large systems is best done in a staggered manner a few times a year to prevent excessive bacterial death. Removing algae from a small, independent tank with a separate biological lter located adjacent to the tank (that is a self-contained 30-gallon tank typical of home aquariums) is also safe. To avoid removing the nitrifying bacteria living among the algae, the sides of static tanks should not be wiped down thoroughly with water changes. The amount and frequency of water changes in static tanks are based upon stable water chemistries.

Table 1. An example of an action log specific to the system described in this paper and organized by frequency

			Date							
Action	Frequency		1-Mar	2	3	4	5	6	7	. . .
Refill 50-gal salt carboy	2XM									
Refill rotifer concentrated saltwater	2XM									
Change rotifer water	2XW									
Change RO filters	2XY									
Water-test rotifers	2XW									
Send email of laid eggs	4XW									

Remove dead and sick fish. Sick fish can be isolated for treatment or euthanized with an overdose of MS222 (M-aminobenzoic acid ethyl ester; tricaine methanesulfonate; Sigma, St Louis, MO);[1,29] the use of MS222 is discussed in more detail later in the current article. Describe and record associated clinical signs of affected fish and note disposition (treated, euthanized, submitted for diagnostic testing, and so forth) Records of data regarding dead and sick fish will be very helpful to both the facility manager and the veterinary staff when evaluating system-wide problems. For rapid assessment of mortality trends,

habits. The water should be changed, but the algae layer does not need to be removed completely (see *Water quality*).

When growing rotifers in static tanks, the water-change frequency will vary depending on conditions. Testing the ammonia level in the rotifer water will help determine the need for water replacement. A water sample can be ltered through a sieve for capture of rotifers prior to testing, in an effort to avoid subjecting rotifers to chemicals. The IACUC inspector will check water quality records for sh and live food. Water in static tanks must be changed according to protocol.

V V). Zebra sh larvae can be reared in a variety of ways. A recirculating system set at a slow drip is ideal. However,

population, and overt signs of illness. Assure that sh are being euthanized humanely according to protocol.

10. Canadian Council on Animal Care (CCAC).