STANDARD OPERATING PROCEDURES

Fisheries Investigative Science House (FISH) Laboratory
School of Natural Resources, UNL
Reviewed and Revised 01/2023 by Israt Jahan and Lindsey Chizinski
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1. Standard Operating Procedure – Field Collection/ Receiving /Quarantine

1.1.0 Purpose

This document describes the general procedures on arrival of study animals in the facility. The study animals are either 1) collected in the field (Nebraska) and transported to the lab or 2) shipped by approved vendors.

1.2.0 Scope/Responsibility

The Principal Investigator has direct responsibility for the implementation/delegation of this procedure. Revision of the procedure, when necessary, is the responsibility of the Principal Investigator. These procedures are to be followed by all Laboratory staff and volunteers present at the facilities coming into direct contact with live animals.

1.3.0 Materials

Quarantine tank

Cooler

1.4.0 Procedures

1.4.1 All permits and importing paperwork must be completed prior to collecting/shipping fish,

1.4.2 If collected from field the transporting water must be well water, treated tap water or dechlorinated water,

1.4.3 Fish are Inspected for any signs of stress, trauma or mortality that may have occurred while in transit,

1.4.4 Fish are acclimated to laboratory conditions by: 1) placing bags (if applicable) into water for temperature acclimation, 2) slowly mixing laboratory quarantine tank water with field water over the course of 2-3 hours prior to transfer into laboratory tanks,\

1.4.5 Fish arriving with health concerns, or found sick are isolated from healthy fish by placing them in their own tanks. The Principal Investigator and clinical veterinarian are notified immediately and the fish will be medicated as recommended by the veterinarian,

1.4.6 Individual shipments are identified by source, date of arrival, and any pertinent information, by marking directly on the tank with a sharpie pen,

1.4.7 Animal numbers are recorded at time of arrival by the Principal Investigator and lab manager both on the census logs and the inventory sheet,

1.4.8 Fish arriving from the field or in a new shipment from approved vendors will be separated from existing stocks of fish (quarantine) so that signs of stress, health, and behavior can be monitored for 2 weeks prior to use for experimentation,

1.4.9 Note: any animals, no matter what the source, being brought into the facilities for the first time should automatically be suspect for pathogenic infection. The immediate application of prophylactic/preventative medication is highly recommended: ectoparasites can be treated with formaldehyde, ~150 mg/l, 3-5 treatments of 1 hour static each, 24 hours apart CAUTION – formaldehyde is a carcinogen DO NOT APPLY WITHOUT PI or Facility Manager AUTHORIZATION), and bacterial infections can be treated with oxytetracycline medicated feed (2.0 grams/pound, at the appropriate feeding rate, for 10-14 days).

1.4.10 Fish that develop sicknesses in the lab (section 7 Fish Health) will immediately be separated from stock fish or will be re-located away and individually housed. The veterinarian will be contacted immediately by the Principal Investigator, and medication will be applied as recommended followed by a request that the veterinarian visit our laboratory. Fish in both the stock and sick tanks will be monitored twice daily for signs of new infections, recovery or worsening of conditions. Fish will be euthanized if deemed necessary by the veterinarian.

2. Standard Operating Procedure – Daily Observations

2.1.0 Purpose:

This document describes the general procedures for regular monitoring of aquatic research animals.

Basic guidelines include:

- The aquatic animals are critically dependent on the maintenance staff for their very existence – they exist in an extremely delicate and artificial environment.

- Not only must the animals themselves be monitored, but the systems (pumps, heaters, chillers, valves, feeders, plumbing, etc.) producing and controlling their environment must be continuously checked as well.

2.2.0 Scope/Responsibility:

The Principal Investigator has direct responsibility for the implementation/delegation of this procedure. Revision of the procedure, when necessary, is the responsibility of the Principal Investigator. These procedures are to be followed by all Laboratory staff and volunteers present at the facilities coming into direct contact with live animals.

2.3.0 Materials:

Record sheets

Thermometer

Flow meter

Pressure gauge

2.4.0 Daily Procedure

2.4.1 All animals and systems will be checked at least once daily on normal workdays; abnormal conditions will be reported immediately to the appropriate investigator; if the appropriate investigator is not on-site, contact the Principal Investigator (Posted on lab doors emergency contacts),

2.4.2 On non-working days (weekends and holidays), all animals and systems will be checked at least once daily,

2.4.3 Animals will be observed for normal swimming, behavior, and abnormal colorations.

2.4.4 Temperatures of each tank will be checked daily and recorded on appropriate record sheet for the tank,

2.4.5 Water flow will be checked for proper function and recorded as having been checked,

2.4.6 Dissolved oxygen content of each tank will be measured using a YSI and recorded on appropriate tank record sheet,

2.4.7 The photoperiod is 12/12 (lights on 7 am to 7 pm).

2.5.0 Weekly Procedures

2.5.1 At least once a week the water flow direction will be altered, to flow in the opposite direction.

2.5.2 Ammonia, Nitrite, and Nitrate concentrations are measured in all systems at minimum once weekly and more if tanks are stocked at high densities.

2.6.0 Bi-Weekly Procedures

2.6.1 The pH of each tank will be measured and recorded at least every other week.

2.7.0 Monthly Procedures

2.7.1 Record sheets will be replaced with new, blank ones.

2.7.2 Completed record sheets will be entered into a binder to be stored in the laboratory.

3. Standard Operating Procedure - Identification

3.1.0 Purpose:

The purpose of this Standard Operating Procedure (SOP) is to define animal identification procedures.

3.2.0 Scope/Responsibility:

The Principal Investigator has direct responsibility for the implementation/delegation of this procedure. Revision of the procedure, when necessary, is the responsibility of the Principal Investigator. These procedures are to be followed by all Laboratory staff and volunteers present at the facilities coming into direct contact with live animals.

3.3.0 Materials:

Daily log sheets

Daily inventory sheets

3.4.0 Procedure:

3.4.1 All animal containers/tanks shall have identification labels.

3.4.1.1 Large tanks: large tanks shall be individually labeled with the following information – tank number, project's principal investigator, animal species, number of animals, animal use and care protocol number, date of birth, arrival date,

3.4.1.2 Small tanks: small tanks shall have labels with either the same information as large tanks, or the tank number only,

3.4.1.2.1 If labeled with tank number only, there must be a posted form (in the same area) describing all such labeled tanks that includes the additional information required for large tanks, plus a floor map to facilitate tank identification,

3.4.2 Changes in animal status shall be transferred to the tank identification label information within 72 hours of occurrence,

3.4.3 Individual animal identification shall be at the discretion of the individual lab's investigators and shall be described in detail in the appropriate animal use and care protocol.

3.5.0 Weekly Procedures:

3.5.1 Animal ID and inventory records will be recorded on inventory sheets by individual investigators and verified each week by the project personal investigator.

3.6.0 Monthly Procedures:

6.1 Records for each month will be filed into a binder located in the laboratory and new inventory sheets will replace old ones.

4. Standard Operating Procedure – Maintaining Tanks

4.1.0 Purpose:

The purpose of this Standard Operating Procedure (SOP) is to define the aquatic animal tank maintenance requirements.

4.2.0 Scope/Responsibility:

The Principal Investigator has direct responsibility for the implementation/delegation of this procedure. Revision of the procedure, when necessary, is the responsibility of the Principal Investigator. These procedures are to be followed by all Laboratory staff and volunteers present at the facilities coming into direct contact with live animals.

4.3.0 Materials:

Tanks

Filters

Filter media

Buckets

Brushes

4.4.0 Procedure:

4.4.1 Flow through systems: defined as single pass water flow i.e. water is used only once and then discharged to waste, not recycled

4.4.1.1 Occupied flow through tanks are only cleaned to control excess algae, carried out as needed – algae shall not impede visualization of animals, nor shall it collect solid wastes (filamentous algae)

4.4.1.2 Unoccupied tanks should be disinfected with dilute bleach or iodophor at least once between occupations

4.4.2 Closed/recirculating systems: defined as having at least some percentage (including 100% or static) of water re-use, utilizing filtration/water treatment of some kind (mechanical, biological, etc.)

4.4.2.1 Recirculating systems shall be maintained properly, including but not limited to cleaning filters, changing UV light bulbs, periodic water changes/flushing, monitoring water quality, etc.

4.4.2.1.1 Algae shall be controlled as in occupied flow through tanks

4.4.2.2 Closed recirculating systems generally operated over extended periods of time, but individual tanks may be added or removed from the system –such tanks should be disinfected with dilute bleach or iodophor; when entire systems are deactivated, and the various components should also be disinfected.

5. Standard Operating Procedure – Enrichment

5.1.0 Purpose

The purpose of this Standard Operating Procedure (SOP) is to define the aquatic animal environmental requirements.

5.2.0 Scope/Responsibility

The Principal Investigator has direct responsibility for the implementation/delegation of this procedure. Revision of the procedure, when necessary, is the responsibility of the Principal Investigator. These procedures are to be followed by all Laboratory staff and volunteers present at the facilities coming into direct contact with live animals.

5.3.0 Materials

Tanks/holding units

Refuge material e.g. PVC pipe, plants, rocks

5.4.0 Procedure

5.4.1 Environmental enrichment for aquatic animals is not only species specific, but season and life-stage specific as well, and enrichment designs should be incorporated into individual project populations (e.g. newly hatched sturgeon and salmonids are generally negatively phototropic, and should be either protected from strong light or provided with protective cover inside the tank; active schooling fish are strong swimmers, and should be provided with a relatively strong current/flow and tanks with corners avoided; during the breeding season males are often very aggressive towards females and sub-dominant males – protection should be provided, or the most aggressive fish isolated if not needed for spawning; etc).

5.4.2.1 Individual investigators must be familiar with their subject species' live histories and enrichment requirements or consult lab personnel with prior experience in handling said species.

6. Standard Operating Procedure – Fish Husbandry

6.1.0 Purpose

The purpose of this Standard Operating Procedure (SOP) is to define the aquatic animal husbandry requirements.

6.2.0 Scope/Responsibility

The Principal Investigator has direct responsibility for the implementation/delegation of this procedure. Revision of the procedure, when necessary, is the responsibility of the Principal Investigator. These procedures are to be followed by all Laboratory staff and volunteers present at the facilities coming into direct contact with live animals.

6.3.0 Materials

Restraint/transfer equipment

Inventory, feeding, and water quality record sheets

Tanks, water, water chillers/heaters, appropriate water quality monitoring equipment

Nets, buckets, stretchers, salt, MS-222/TMS (sedative/anesthetic), CO_2 gas, dosing tank, knives/saws, plastic bags

Plastic bags, oxygen cylinders, transport tanks/trailer

Appropriate feed, automatic feeders, feed storage space/containers, feed weighing/transfer equipment

Gloves, disease observation/treatment forms, chemo-therapeutant

6.4.0 Procedure

6.4.1 Initiate required record keeping of inventory, feed, and water quality

6.4.1.1 Fill in tank label (if appropriate) with all required information

6.4.1.2 Notify and instruct individuals directly responsible for daily maintenance care of animals.

6.4.2 Housing: different species have different requirements: fish exhibit significant variation in their environmental requirements, including temperature, light intensity, flow rates and patterns, tank shapes, etc. Different life stages of a single species may also have different requirements. All species require adequate water quality including but not limited to minimum dissolved oxygen concentrations, maximum dissolved ammonia concentrations, and temperatures appropriate for the species. Individual investigators must be familiar with their subject species' housing requirements, or consult lab personnel with prior experience in handling said species.

6.4.2.1 Basic water quality requirements by general fish groups:

6.4.2.1.1 Cold water species (e.g. salmonids) - temperature: 5-15^{o} C; dissolved oxygen: >7.0 mg/l; dissolved ammonia: < 300 ug/l.

6.4.2.1.2 Warm water species (e.g. Tilapia) - temperature: 16-28^{o} C; dissolved oxygen: > 6.0 mg/l; dissolved ammonia: < 600 ug/l.

6.4.2.2 Flows: flows must be appropriate for the size of animal (generally the larger the animal, the greater the flow, but feeding and swimming behaviors will also determine flow rates; flow is also a function of tank size and "turn-over" rate - the larger the tank, the greater the flow required to flush the tank adequately.

6.4.2.3 Tank size/stocking density: tank size will increase with individual animal size and increasing total biomass, but some species are behaviorally much more tolerant of crowding than others. Assuming that basic water quality requirements are being met, schooling fish generally like to be crowded, more solitary animals do not. During certain times of the year, particularly during spawning season, more belligerent fish like trout and channel catfish should be segregated by sex at lower densities.

6.4.3 Animal handling

6.4.3.1 Anesthesia: - all handling/restraint of aquatic animals causes severe stress, particularly when carrying out a long or invasive sampling procedure - this stress must be kept to a minimum by sedating (light anesthesia) or fully anesthetizing the animals where appropriate. When it is necessary to kill an animal for any purpose, it must be done is such a way as to minimize, if not eliminate, any potential pain and suffering. Animals undergoing anesthesia must be monitored continuously to avoid letting them get to deep or too light; they must also be monitored continuously during recovery until the animal is not only moving its opercula on its own, but has regained its equilibrium/balance and is no longer ataxic

6.4.3.1.1 Animals may be sedated or fully anesthetized with MS222 (Tricaine Methanesulfate or TMS); a dosing tank not connected to the facility drainage system should be used.

MS-222 for in-laboratory procedures:

Rapid Anesthesia

Salmonids	80-140 mg/l	5-10 min **max.**
Cyprinids	150-200 "	5-10 min
Ictalurids	150-200 "	5-10 min
Centrarchids	250-330 "	3-5 min
Acipenserids	150-250 "	5-10 min
livebearers	90 "	12 hours
egg layers	80 "	12 hours

Sedation

Salmonids	15-30 mg/l	up to 6 hours
Ictalurids	20-40 "	up to 6 hours
Cyprinids	20-30 "	up to 6 hours
Centrarchids	25 "	up to 12 hours
Acipenserids	15-30 "	up to 6 hours
Tropicals	40-60 "	up to 48 hours

MS222/TMS comes as a dry powder should be handled carefully wearing PPI; gloves should be worn to avoid direct skin contact with the powder; left over solutions are considered to be **hazardous waste**, and must be disposed to specific collection barrel and when filled contact EHS at UNL to accordingly dispose that (campus Safety Services/EHS).

6.4.3.2 Capture/transport: the time required for handling animals out of water during capture/transport must be kept to an absolute minimum; if the handling of animals out of water will exceed 10 seconds, special accommodations must be made to ensure the wellbeing of the animal; at the absolute minimum, the gills and skin must be kept wet.

6.4.3.2.1 - animals may be restrained in water with an appropriate sized net (two nets may be required - one for herding). In large tanks a preliminary "crowding" or condensing of numbers into a reduced volume may be appropriate. There are crowding nets already available for some sizes of circular tanks, but it may be necessary to make new ones for a given tank size.

6.4.3.2.2 If animals are to be transferred from one volume of water to another, and it will take more than 10 seconds to travel the required distance, a suitably sized, water filled container must be used. This container should be filled with water from the tank of origin, particularly if using sedation. Care must be taken to avoid both temperature and osmotic shock when releasing into the receiving tank.

6.4.3.2.3 If the transfer of animals is off-site, there are transport tanks available to be used in conjunction with the program vehicle/truck. Supplemental oxygen should be used if conditions warrant (high animal density, extreme temperatures, extended travel duration). **NOTE: the use of supplemental oxygen, as supplied by high pressure cylinders, can be extremely hazardous; anyone not familiar with the use of the safety regulator and the proper cylinder handling and storage must be accompanied by someone who is properly trained by their immediate supervisor (see Environmental Health and Safety); it is also necessary to undergo training by laboratory staff for the proper use of the hauling tank/trailer and compressed gas equipment, with appropriate documentation.**

6.4.3.2.4 All transfer nets and containers, including transport tanks and trailer, used for transfer/transport of animals must be properly disinfected with iodophore-detergent solution

6.4.4 Feeding: a consistent supply of high quality fresh food is essential for all aquatic animals; the feed type, size, and method of delivery must be adjusted to fit the species and stage of development; cleanliness in and around the animal holding and feed storage areas is essential. Individual investigators are responsible for coordinating feeding regimens with lab personnel.

6.4.4.1 Establish required feed type, ration, and method of delivery: do not commence feeding for at least 24 hours after arrival in the facility, unless fingerlings or less in size - then wait 6-8 hours

6.4.4.2 Feed of suitable size and composition will be administered to animal containing tanks either by hand as a single meal or by automatic feeders as multiple or continuous feedings.

6.4.4.2.1 Hand feeding will occur at least once daily on regular work days, and at least once daily on non-working days (weekends and holidays); automatic feeders will be loaded at least once daily.

6.4.4.2.2 Automatic feeders and feed transfer equipment will be brushed clean of leftover food particles before each loading; they will be cleaned at least once weekly by degreasing with an alcohol solution

6.4.4.3 Any spilled feed on tank covers/edge or the ground must be cleaned up immediately.

6.4.4.4 At least once a week automatic feeders will be cleaned of excess feed particles.

6.4.4.5 Non-frozen dry artificial feed stocks will be kept for a maximum of 6 months only; all storage containers should be labeled with at least the brand, type, particle size and milling/toss date (if no milling date is available, the purchase date may be used)

6.4.4.5.1 Artificial dry feeds for general maintenance (e.g., SilverCup salmonid formulation, regular and medicated; Rangen soft- or semi-moist salmonid formulations, non-medicated only) will be administered by lab staff. All lab staff using feed will sign out the amount in the feed log book to track usage. Non-maintenance diets (purified research diets, etc.) will be the responsibility of the respective individual investigator to supply. All dry diets, both maintenance and non-maintenance, will be stored at temperature not to exceed 70^{o} F.

6.4.4.5.2 Artificial feed for individual tanks will be weighed out immediately prior to administration in individually labeled feed transfer containers.

6.4.5 Health: animal health is of critical concern both for the animal's sake and the validity of research data. Diseases of aquatic animals are difficult to recognize and progress very rapidly in the aqueous medium. Infected animals must remain isolated to prevent the spread of any pathogens to non-infected populations, and to optimize the treatment of the diseased animals. Euthanasia of the entire population may be required if a disease outbreak has progressed far enough. Fish health is evaluated by carefully observing the physical appearance and behavior of fish in every tank. Oftentimes, if we catch fish health issues soon enough, we can implement procedures in collaboration with the IACUC veterinarian that can help to resolve (or at least quarantine) the issue before it gets out of hand. We will employ, at the veterinarian's discretion, whichever treatment we feel is best for the fish.

6.4.5.1 The health status of all tanks/populations will be monitored at least once daily.

6.4.5.2 If an animal is observed to be acting abnormally, **immediately** inform the project's Principal Investigator AND the facility's Principal Investigator, as specified by the Animal Use and Care Protocol, of the species, location, and abnormal behavior.

6.4.5.2.1 common symptoms of sick fish include but are not limited to: body shape emaciated ("skinny") or bent, bloating with raised scales, resulting in a fuzzy appearance, limp appearance, fins held close or folded rather than spread, eye bulging, open sores, internal hemorrhaging, torn or abnormally truncated fins, damaged or missing operculum (gill covering), flared, red gills and rapid breathing, erratic swimming (head-up, twirling, etc.), prolonged resting on tank bottom or floating at the surface

6.4.5.2.2 The facility's Principal Investigator will then contact the IACUC veterinarian.

6.4.5.2.3 Record initial disease observations on the designated diagnosis/treatment form, including any treatment schedule, while the disease is present and/or treatment is ongoing, the Disease and Observation form should be attached to the contaminated tank on a visibly red clipboard; upon resolution of the disease problem, the Disease Observation and Treatment form should be transferred to the centralized records/files kept by the lab/facility.

6.4.5.2.4 If a diagnosis of the problem is not possible, or there is no available treatment for the diagnosed disease, the infected population must be euthanized immediately following guidelines in section 7 of this manual, so as to avoid any additional suffering by the animals, as well as further contamination. The only time infected, untreatable animals may not be immediately killed is if there is an approved Animal Use and Care Protocol specifically describing such a need (e.g. research involving new disease challenges, new therapeutic drug tests, etc.).

6.4.5.2.5 Euthanized animal carcasses should be disposed of properly and all surfaces exposed to the carcasses disinfected.

6.4.5.2.6 After euthanizing a tank for a disease outbreak, it must it must be disinfected with bleach: expose all wetted surfaces to a 200 mg/l bleach solution (3.8 mls of 5.25% commercial bleach per liter of water) for at least 8 hours, followed by a 20 minute exposure to 10% iodophore (Wescodyne, Prepodyne, Betadyne, etc.), usually administered with a hose sprayer, and a final clean water rinse.

6.4.5.3 If an animal has died, **immediately** inform the project's Principal Investigator AND the facility's Principal Investigator, as specified by the Animal Use and Care Protocol, of the species and location.

6.4.5.3.1 Remove the animal from the tank; and subsequently submerge (entirely) whichever net, or other materials, you used to remove the fish in 10% Bleach solution for at least 24h,

6.4.5.3.2 Take a water sample from the tank (500 ml), cover, label with date, location and your initials, and store in the refrigerator,

6.4.5.3.3 Make an incision in the belly of the animal (gently, so as to not destroy internal organs)

6.4.5.3.4 Place the animal in at least 10X its weight 10% buffered formalin (contact lab manager for location). For instance, if the fish weighs 4 grams, place it in at least 40 ml of 10% buffered formalin,

6.4.5.3.5 Label the tube/container into which you placed the fish with the following information: 1) species, 2) date, 3) time, 4) your initials, 5)

estimation of how long the fish has been dead (you can usually tell this by the color of the body; the 'opaquer' the fish is relative to a 'normal' fish, the longer it has been dead), 6) tank #, and 7) IACUC protocol #.

6.4.5.3.6 Label the tank/system from which any sick or dead fish are removed with a "QUARANTINE" label until released by the veterinarian,

6.4.6 Carcass/Biological waste removal: all animal carcasses must be properly and safely disposed of.

6.4.6.1 Place carcass(es) in a freezer bag labeled with your initials, date, species identification, number of carcasses, and where the carcasses came from.

6.4.6.2 Store them in the freezer

6.4.6.3 where do they go from the freezer???

7. Standard Operating Procedure – Euthanasia

7.1.0 Purpose

The purpose of this Standard Operating Procedure (SOP) is to define the aquatic animal veterinary care program requirements.

7.2.0 Scope/Responsibility

The Principal Investigator has direct responsibility for the implementation/delegation of this procedure. Revision of the procedure, when necessary, is the responsibility of the Principal Investigator. These procedures are to be followed by all Laboratory staff and volunteers present at the facilities coming into direct contact with live animals.

7.3.0 Materials

Nets

Plastic bags

Gloves

MS 222

$NaHCO_3$

NaCl

7.4.0 Procedure

Common methods used to euthanize fish include noninhaler methods (ie, immersion and injection) and physical methods. Because of general differences in anatomy and application seen between finfish and terrestrial animals (especially about primary respiratory organs, and aqueous vs air environment), techniques involving addition of drugs to the fish's environment (i.e., the water), for purposes of this document, are considered noninhaler methods. Descriptions of methods used to euthanize fish follow and include 1-step and 2-step procedures.

A recent study (Balko et al., 2018) demonstrated that use of buffered MS 222 in a 1-step immersion technique was inadequate for euthanasia of goldfish (*C auratus*), a hypoxia-tolerant species. Results from this study support the recommendation for use of a 2-step method for euthanasia of goldfish and some other hypoxia-tolerant species, including cichlids, with the first step of involving immersion to render the fish unconscious and the second involving application of an adjunctive method (such as decapitation, pithing, or freezing) to complete euthanasia. Fish that are too large for practical or cost-effective immersion in lethal doses of buffered MS 222 can be euthanized by applying the concentrated, buffered solution directly to the gills.

7.4.1 Failure to buffer the solution appropriately could result in discomfort/pain to the subjects. For poorly buffered water sources there should generally be a ratio of 1:2 (weight:weight) of MS222 : Sodium Bicarbonate powder.

7.4.2 A stock solution can be prepared for use in water bath or spray applications. Commonly used MS222 stock solutions are at 10g/L using aged tap water. Sodium Bicarbonate should be added (10-20g/L).

7.4.3 Containers must be **labeled** with the agent, concentration, and date of preparation.

7.4.4 Store preparation in a dark brown bottle or use tinfoil around the bottle. Store in freezer. Discard after one month or earlier if an oily film or discoloration of the stock solution develops.

7.4.5 Euthanized carcasses must be disposed of as outlined in section 6.4.6 of this manual.

7.4.6 Safety Precautions

7.4.6.1 It is the responsibility of the PI on the corresponding animal use protocol to assure the safe use of MS222 by laboratory staff. The PI is responsible for the appropriate training of research personnel for the safe use of this anesthetic in the species approved on the protocol.

7.4.6.2 Personnel using MS222 should be familiar with the MSDS, which should be readily available in the lab. Questions regarding safety practices should be directed to FAU Environmental Health & Safety.

7.4.6.3 MS222 is not regulated as a hazardous waste by the US Environmental Protection Agency under the Resource Conservation and Recovery Act. However, users should realize that it is considered an irritant to the eyes, respiratory system and skin and safety precautions need to be employed.

7.4.6.4 To avoid inhalation, an N95 mask is required while mixing in a well-ventilated area. To avoid skin and eye contact, goggles, gloves, and a lab coat/protective clothing should be worn while measuring the powder. Using a top loading balance with a clear plastic wind/breeze guard can help minimizing the risk of having the light powder blowing around by air currents.

7.4.6.5 In typical use, the mode of action is by absorption through the skin of aquatic animals. It can also permeate human skin although at a much lower rate, particularly when dissolved. Personnel should therefore minimize contact with medicated water whenever possible and should be aware and attentive to nausea or headaches after any prolonged exposure of even low concentrated MS222 solution.

References

Balko, J. A., Oda, A., & Posner, L. P. (2018). Use of tricaine methanesulfonate or propofol for immersion euthanasia of goldfish (Carassius auratus). *Journal of the American Veterinary Medical Association*, *252*(12), 1555-1561.

Bowker, J.D., J.T. Trushenski, M.P. Gaikowski, and D.L. Straus, Editors. 2012. Guide to Using Drugs, Biologics, and Other Chemicals in Aquaculture. American Fisheries Society Fish Culture Section.

8. Standard Operating Procedure – House Keeping

8.1.0 Purpose

The purpose of this Standard Operating Procedure (SOP) is to define the aquatic animal house keeping requirements.

- The primary goal is to provide a healthy environment for the animals, as well as a safe, aesthetically conducive and scientifically productive environment for the researchers.

8.2.0 Scope/Responsibility

The Principal Investigator has direct responsibility for the implementation/delegation of this procedure. Revision of the procedure, when necessary, is the responsibility of the Principal Investigator. These procedures are to be followed by all Laboratory staff and volunteers present at the facilities coming into direct contact with live animals.

8.3.0 Materials

Tank cleaning equipment (brushes, scrub pads, tubing)

Brooms

Cob web brushes

Hoses w/spray nozzles

8.4.0 Procedure

Buildings and grounds shall be kept clean and in good repair in order to protect the animals from injury and to facilitate the good fish husbandry practices

8.4.1 Facilities shall be kept free of clutter, unnecessary storage of equipment, and trash.

8.4.1.1 Animal areas, prep areas, sinks, and surgery areas shall not be used for storage. Only the items needed for the specific tasks required shall be available in these spaces while in use.

8.4.1.2 Designated storage areas shall store items off the floor on either mobile carts, cabinets, racks, or storage units, all of which must be waterproof. No cardboard storage is allowed outside of the office area.

8.4.1.3 Loose cords are not allowed on the floor of the facility.

8.4.1.4 Animal and support spaces must be cleaned and disinfected regularly to prevent contamination; cleaners and disinfectants must be used according to manufacturer's guidelines

8.4.1.5 Cleaning utensils/equipment and tank specific nets shall be labeled and assigned to designated areas and disinfected after every use.

8.4.1.6 Food and drink are not allowed in the lab facility but may be kept in the office area while present in the lab.

8.4.1.7 Personal storage spaces are available, but permission to use must be obtained by the lab manager.

5.0 Monthly Procedure

5.1 All cobwebs will be removed from area shelving structure, etc. at least once monthly.

5.2 Dust and leaves on overhead racks and plumbing will be removed at least once monthly

9. Standard Operating Procedure – Physical Plant

9.1.0 Purpose

The purpose of this Standard Operating Procedure (SOP) is to define the physical plant procedures.

- The primary goal is to provide a healthy environment for the animals, as well as a safe, aesthetically conducive and scientifically productive environment for the researchers.

9.2.0 Scope/Responsibility

The Principal Investigator has direct responsibility for the implementation/delegation of this procedure. Revision of the procedure, when necessary, is the responsibility of the Principal Investigator. These procedures are to be followed by all Laboratory staff and volunteers present at the facilities coming into direct contact with live animals.

9.3.0 Materials

General plumbing supplies, pumps, valves, etc

Central water pumps, air blowers/compressors, heaters, chillers, filters, UV disinfection chambers, monitoring/alarm systems, plumbing

9.4.0 Procedure

Adequate water supply and drainage are essential for the proper function of the overall facility, it is the responsibility of the facility manager to provide a reliable primary water source and drainage system; unauthorized dumping of any substances other than water and fish feed into the drainage system will cause immediate and serious consequences, physical plant equipment operation is critical to the wellbeing of all animals at the facilities, due to the artificial nature of their environment; the animals' physical requirements, as well as the experimental research needs, must be addressed in the system design and the equipment required to fulfill that design.

9.4.1 Water Supply/Drainage:

9.4.1.1 Staff shall ensure the adequate supply of water (defined here to be a minimum dissolved oxygen content of 7.0 mg/l, a total gas saturation level not to exceed 100.5%, and no generally adverse contaminants such as heavy metals, pesticides, etc.) to the facilities by monitoring the supply wells and primary delivery systems on a daily basis, particularly by observing function (including pumps, motors, modulating and shut off valves, flow meters, central control panel), delivery pipelines, valves and manifolds up to the labs.

9.4.1.2 The only constituents allowed in the facility drainage systems without prior approval are unadulterated water and fish food/fish metabolic waste; all other substances must have prior approval from the Facility manager or PI.

9.4.2 Plant Equipment Operation and Maintenance:

9.4.2.1 The management and maintenance of the primary physical plant equipment supplying basic inputs to the facility shall be the sole responsibility of the managing staff; this includes all equipment associated with the main well pump stations (pumps, valves, control panel, etc.), all water valves on supply lines that are outside the labs and buildings.

9.4.2.2 All building problems (lighting, electrical, HVAC, water, etc.) should be reported to the responsible staff for referral to the Campus Facilities department; if it is an emergency and the responsible staff is not immediately available, call Campus Facilities Services directly.