STANDARD OPERATING PROCEDURES – EARLEY LAB FISH FACILITY

PROCEDURES

1. Field Collection/Receiving/Quarantine

    a. Convict cichlids are either: 1) collected in the field (Nicaragua) and transported in Kordon ® breathing bags or 2) shipped by approved vendors. Killifish (*Rivulus* or *Kryptolebias*) are collected in the field (Florida) and transported in WhirlPaks or buckets (with seawater). Bluenose shiners and longear sunfish are collected in the field (Alabama) and transported in an aerated cooler with water from their native streams. Upon arrival, all bags are checked for damage (if applicable). All permits and importing paperwork must be completed prior to collecting/shipping fish.
    b. Inspect fish for any signs of stress, trauma or mortality that may have occurred while in transit.
    c. Fish are acclimated to laboratory conditions by: 1) placing bags (if applicable) into water for temperature acclimation, 2) slowly mixing laboratory stock tank water with field water over the course of 2-3 hours prior to transfer into laboratory tanks.
    d. Fish arriving with health concerns, or found sick are isolated from healthy fish by placing them in their own tanks. The facility manager and clinical veterinarian notified immediately and the fish will be medicated as recommended by the veternarian.
    e. Individual shipments are identified by source, date of arrival, and any pertinent information, by marking directly on the tank with a sharpie pen.
    f. Animal numbers are recorded at time of arrival by the PI and ACF manager both on the census logs and the inventory sheet.
    g. 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 (see *8. Fish Health)*.
    h. Fish that develop sicknesses in the lab (see *8. Fish Health*) will immediately be separated from stock fish (cichlids, shiners, sunfish) or will be re-located away from the colony (killifish; individually housed). The veterinarian will be contacted immediately, 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. Housing

    a. Cichlids should be housed in groups of 50-70 fish per 370-liter ‘pond’, or in breeding pairs (2 fish) in 37-liter or 75-liter tanks. Bluenose shiners and sunfish will be held separately in 150-liter ‘ponds’ with 2 sunfish and 20-25 shiners

maximum; or in a 560-liter tank with 8 sunfish and 50-75 shiners maximum. All tanks will be equipped with filtration, gravel substrates, aeration (cichlids), and plants (shiners/sunfish).

b. A net is utilized for catching fish that must be removed from a tank.
c. Water conditions and quality for the species are as follows:

Convict Cichlid Water Quality				
Parameter	Range	Preferred	Method	Frequency
Temperature	18-31 ^{0}C	25-28 ^{0}C	Temp Probe (ambient + tank)	Daily
PH Alkalinity	6.0-8.0 50-150 ppm	7.4 65-80 ppm	Pinpoint monitor Salifert test kit	Biweekly Biweekly
Hardness	4-20 dGH	8-14 dGH	Salifert test kit	Biweekly
Ammonia	0	0	API test kit	Biweekly
Nitrite	0-25 ppm	0	API test kit	Biweekly
Nitrate	0-40 ppm	10 ppm	Pinpoint monitor (saltwater), Photometer (freshwater)	Biweekly

Killifish (Rivulus/Kryptolebias) Water Quality – Large Tanks (not Tupperwares)				
Parameter	Range	Preferred	Method	Frequency
Temperature	16-33 ^{0}C	25-28 ^{0}C	Temp Probe (ambient + tank)	Daily
PH Alkalinity	6.0-8.0 50-200 ppm	7.0 170 ppm	Pinpoint monitor Salifert test kit	Biweekly Biweekly
Hardness	4-10 dGH	8 dGH	Salifert test kit	Biweekly
Ammonia	0	0	API test kit	Biweekly
Nitrite	0-25 ppm	0	API test kit	Biweekly
Nitrate	0-40 ppm	10 ppm	Pinpoint monitor (saltwater), Photometer (freshwater)	Biweekly

Bluenose Shiners/Sunfish Water Quality				
Parameter	Range	Preferred	Method	Frequency
Temperature	18-30 ^{0}C	25-28 ^{0}C	Temp Probe (ambient + tank)	Daily
PH Alkalinity	6.0-9.0 50-400 ppm	7.4 150 ppm	Pinpoint monitor Salifert test kit	Biweekly Biweekly
Hardness	4-8 dGH	6 dGH	Salifer test kit	Biweekly
Ammonia	0	0	API test kit	Biweekly
Nitrite	0-25 ppm	0	API test kit	Biweekly
Nitrate	0-40 ppm	10 ppm	Pinpoint monitor (saltwater), Photometer (freshwater)	Biweekly

*Water quality must be recorded on the **Earley Lab Daily/Weekly checklist**. Note that the frequency of water quality measures will increase for tanks that deviate significantly from the range/preferred values until the measures stabilize.*

d. Room light cycle, 12 hours light (7 AM till 7 PM) /12 hours dark cycle for tropicals (cichlids, killifish; sunfish/shiners during non-breeding period); 16 hours light (6AM till 10pm)/8 hours dark cycle for shiners and sunfish (breeding photoperiod). If necessary (i.e., sunfish/shiner breeding program begins), different photoperiod regimes will be maintained in Biology 228 by constructing a temporary enclosure in consultation with the ACF staff and IACUC to accommodate a different light cycle for sunfish/shiners. Otherwise, Dr. Earley will seek space at the ACF.
e. Housing access is limited to IACUC certified personnel only.

3. Feeding occurs once daily. *See also detailed feeding instructions in Appendix XV*
   a. *Kryptolebias marmoratus* (mangrove rivulus) – these fish are fed brine shrimp nauplii, which are hatched in our laboratory. Briefly, 2 tablespoons of brine shrimp cysts are placed in 1.5 L of 37-40ppt saltwater with aeration, bubbled overnight, and then the hatched cysts are separated from the live shrimp nauplii using a device that we constructed in the laboratory (consists of a large bottle flipped upside down with a piece of tubing fixed through the cap). Shrimp cysts float so, after ~30-40 min of settling time, we can extract only live shrimp nauplii and discard the cysts. We use repeating pipettes for feeding to ensure that each fish gets an equivalent amount of food; all fish > 2 months old receive 2ml daily; all fish < 2 months old receive 1ml daily.
   b. All remaining species – receive a daily mixture of Bio-Pure, Hikari® bloodworms (vitamin fortified), Bio-Pure, Hikari ® adult brine shrimp (gut loaded with vitamins), and TetraMin ® flake food (1.5 x 1.5 cm block of bloodworms; 1.5 x 1.5 cm block of brine shrimp; and a pinch of flake food). After the mixture is made, we use 2ml transfer pipettes to feed the fish. We add one pipette-full at a time (~1.5 ml), monitor fish feeding activity, and add more as appropriate (depending on how many fish are in the tanks). On average, we feed approximately 1 pipette-full for every 5 fish in the aquarium but, given the wide variety of fishes that we have in the lab (and their different feeding habits), we take care to monitor feeding so as to minimize accumulation of food in the gravel substrate.
   c. Record feeding on the **Earley Lab Daily/Weekly checklist**.

4. Daily duties – *Please also refer to the checklist in Appendix XI*
   a. Conduct health surveillance**.**
   b. Feed daily as outlined above.
   c. Assure no investigator's supplies or trash is left in the room.
   d. Check supplies (food, paper towels).

e. Complete **Earley Lab Daily/Weekly checklist** for each room
f. Assure all new arrivals are added to, and any animals sacrificed or found dead are documented with ACF manager.
g. Empty trash and make sure the floor has been mopped free of standing water
h. Record ambient and tank temperature from the dual temperature probe

5. Weekly room duties - *Please also refer to the checklist in Appendix XI*
   a. Maximum ¼ water change for tanks > 10 gallons plus addition of freshwater aquarium salts (for freshwater tanks only), Stress Coat ® and Cycle ®. Water change must be accompanied by siphoning of gravel to remove feces and debris.
   b. Send water quality analyses to ACF staff (until further notice)

6. Biweekly
   a. Water chemical analyses are conducted to determine water quality parameters.
   b. API test kits, Salifert test kits, and probes/photometers are used to monitor nitrite (NO_2), nitrate (NO_3), ammonia (NH_3), hardness, alkalinity, and pH levels.
   c. All tests are performed according to the manuals accompanying the kits and/or probes.
   d. Remove, squeeze, and rinse any sponge-based filters

7. Bimonthly
   a. Change carbon filtration in all tanks (rinse activated carbon before adding to the tank)

8. Quarterly (beginning October 1, 2010)
   a. Water will be collected from representative aquaria for water quality analysis by an approved outside vendor
   b. Water quality instrument calibration recorded in the **Earley Lab Daily/Weekly checklist**

8. Fish Health

Fish health is evaluated by carefully observing the physical appearance and behavior of fish in every tank.

Common symptoms of sick fish include but are not limited to:
1. body shape emaciated (“skinny”) or bent
2. bloating with raised scales, resulting in a fuzzy appearance
3. limp appearance, fins held close or folded rather than spread
4. eye bulging
5. open sores
6. internal hemorrhaging

7. torn or abnormally truncated fins
8. damaged or missing operculum (gill covering)
9. flared, red gills and rapid breathing
10. erratic swimming (head-up, twirling, etc.)
11. prolonged resting on tank bottom or floating at the surface

Dealing with fish health problems - What if your fish become diseased?

FIRST – contact Dr. Earley ***immediately*** and he will contact the IACUC veterinarian and ACF staff. ***Do not*** wait to contact Dr. Earley; do so immediately! Even if you think it is something you did that is making the fish sick, it is absolutely critical that we get the situation under control as quickly as possible. You will not be punished for bringing fish health issues to the attention of Dr. Earley *whenever* you suspect it might be an issue. In fact, Dr. Earley will be indebted to you for bringing it to his attention, and so will the IACUC staff. 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.

The fish tank containing dead animals must be quarantined immediately. This means that you are required to place anything that has touched that tank in 10% Bleach solution for at least 24h. Do not touch any other tank with anything that has come into contact with the potentially diseased tank, even your hands!!

If you find a fish that has recently died, you *must:*

1. Contact Dr. Earley immediately
2. 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.
3. Take a water sample from the tank (500 ml), cover, label, and store in the refrigerator.
4. Make an incision in the belly of the animal (gently, so as to not destroy internal organs)
5. Place the animal in at least 10X its weight 10% buffered formalin (contact Dr. Earley for location). For instance, if the fish weighs 4 grams, place it in at least 40 ml of 10% buffered formalin.
6. 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 more 'opaque' the fish is relative to a 'normal' fish, the longer it has been dead), 6) tank #, and 7) IACUC protocol #.
7. Label the tank from which any sick or dead fish are removed with a "QUARANTINE" label until released by the veterinarian.