FISH ORDERING, ACQUISITION, AND EXPERIMENTATION
Standard Operating Procedures – Earley Laboratory

The following *Standard Operating Procedures* are to be followed by all members of the Earley laboratory (hereafter: MEL) without question or exception. MEL includes the PI, Post-docs, Technicians, Graduate Students, Undergraduate Students, and Visiting Scientists conducting work with the animals. The purpose of these procedures is to:

1. Provide the training to all MEL in the proper handling, maintenance, and experimental use of animals.
2. Generate a working and compliant relationship with our Institutional Animal Care and Use Committee (IACUC)
3. Establish a record of all animals coming into and out of the Earley laboratory
4. Ensure that all MEL are aware of the required procedures for ordering/acquiring animals
5. Ensure that the PI (Dr. Earley) consents to the acquisition of animals
6. Establish that IACUC approval has been received prior to the acquisition of, and any subsequent experimentation on the animals that are ordered/acquired.

These procedures apply to:

a. Working with existing animals in any capacity
b. Ordering fish from a commercial supplier
c. Ordering and/or obtaining fish from a pet store (local or otherwise)
d. Acquiring fish from an outside source (e.g., another laboratory)
e. Capturing fish in the field and relocating the animals to the laboratory

Step #1: IACUC Training

Before any MEL can perform maintenance or research operations with animals, they ***must*** do the following within 2 weeks of joining the laboratory. This is non-negotiable and the MEL will be asked to leave the laboratory if they cannot complete these required activities. All information about IACUC Training can also be obtained on the Earley Lab website (http://rlearley.people.ua.edu/lab-info.html).

1. Complete the IACUC Training Course(s) by registering at http://www.citiprogram.org. Access this website through University of Alabama's Institutional Animal Care and Use Committee (IACUC) webpage (http://osp.ua.edu/site/iacuc.html). The required courses (subject to change) are:

    a. Aseptic Surgery – Basic Course
    b. Working with Fish in Research Settings
    c. Working with the IACUC, Basic Course

2. Print off ***three*** copies of your training results, and provide ***two*** copies to Dr. Earley; one of these copies will be kept on record in the laboratory and the other will be forwarded to

our Animal Care Facility staff. The third copy should be kept in a secure location where the trainee (MEL) can access her/his own information (e.g., folder, notebook).

3. Complete the Animal Care Facility Registration Form (see Appendix I). Make ***three*** signed copies of this form; ***two*** copies should be provided to Dr. Earley, one that will be kept on record in the laboratory and the other that will be forward to our Animal Care Facility staff. The third copy should be kept in a secure location where the trainee (MEL) can access her/his own information (e.g., folder, notebook).

4. Complete the Health History Medical Evaluation Form (see Appendix II). Make ***three*** signed copies of this form; ***two*** copies should be provided to Dr. Earley, one that will be kept on record in the laboratory and the other that will be forward to our Animal Care Facility staff. *Strict confidentiality will be maintained – the documents will not be released to other laboratory members*. The third copy should be kept in a secure location where the trainee (MEL) can access her/his own information (e.g., folder, notebook).

5. Schedule a meeting with Dr. Earley to be trained in fish husbandry practices, maintenance operations in the laboratory, and identification of fish disease states.

6. If any MEL wishes to have a non-laboratory member enter the Animal Care Facility (Nott Hall) or the Satellite Facility (Biology 228), they ***must*** have the non-laboratory member fill out the Transient Worker Registration Form (see Appendix III). Dr. Earley (Biology 228) or Animal Care Facility staff (Nott Hall) ***must*** approve this form before the transient worker can enter the satellite facility (Biology 228) or Nott Hall Animal Care Facility, respectively. Two copies of this form must be filled out; one given to Dr. Earley and another provided to the Animal Care Facility staff.

Step #2: Conducting Research Compliant with IACUC Procedures

Documents available on Earley Lab website (http://rlearley.people.ua.edu/lab-info.html).

If any MEL has an idea for an experiment, they will discuss this idea with Dr. Earley. Together, they will determine whether the hypotheses to be addressed, the methods to be used, and the species to be used are within the purview of an existing IACUC protocol. This will be confirmed via close communication with the IACUC. If it is determined that the procedures fall outside the purview of an existing IACUC protocol or if the proposed research involves new species, the MEL will collaborate with Dr. Earley to generate either a **Proposal Modification** (see Appendix IV for an example; now administered through eProtocol) or a *new* **IACUC protocol** (see Appendix V for an example; now administered through eProtocol). Note that the Proposal Modifications apply only to minor protocol changes therefore, the MEL should anticipate submitting a new IACUC protocol. Thus, laboratory members should **plan well in advance** by completing the protocol in collaboration with Dr. Earley *at least 2 months prior to experimentation*; this will give IACUC the necessary time to review the application, provide comments, and help us to establish a compliant plan of experimental action.

Other considerations for generating experimental plans:

- *If the experimental plan involves the capture of animals from the field, proper permits need to be obtained (or, if the permit is pending, evidence that an application has been submitted needs to be provided) prior to IACUC review. Immediately upon receipt of the permit, it is to be copied and/or scanned and sent immediately to Dr. Earley so that it can be forwarded to the IACUC.*

- *If the experimental plan involves the transport of animals from another laboratory, the following must be done prior to generating an IACUC protocol:*

  1. *Obtain a copy of the IACUC approval notice from the 'sender' laboratory.*
  2. *Obtain a copy of any field collection permits (if applicable) from the 'sender' laboratory.*
  3. *Obtain a certification of health from the 'sender' laboratory's IACUC veterinarian; if there are questions about this, Dr. Earley will consult with our Animal Care Facility staff and University of Alabama's IACUC veterinarian to determine the best course of action.*

  *** If any of these relevant sources of information is unavailable, the animals should be seen as rogue and unapproved, i.e., there is absolutely no way that we will accept these animals for housing in the Earley laboratory under any condition. This is non-negotiable. Any attempt to have unapproved animals shipped to, or housed in, the Earley laboratory will result in immediate dismissal from the laboratory.*

Although the MEL is welcome to begin designing their experimental apparatuses (e.g., fitting partitions into tanks, securing and organizing space within the laboratory) immediately, ***absolutely no research is to be conducted until we receive final approval from the IACUC***. To avoid unnecessary work and expense, close consultation with Dr. Earley and the Animal Care Facility staff is encouraged at the drafting and pilot stages of setting up your experiment. If the MEL's ideas involve the acquisition or capture of new fish species to be held in the laboratory, ***absolutely no animals shall be obtained, collected in the field, or otherwise held in the laboratory under any circumstance until we receive final approval from the IACUC***.

Step #3: Ordering and Receiving the Animals

Documents available on Earley Lab website (http://rlearley.people.ua.edu/lab-info.html).

If the animals are to be purchased from a commercial supplier or a local/regional pet store, the following must be done:

1. Fill out the relevant Animal Acquisition Forms (Appendix VI, which includes both an Earley lab Acquisition form and an ACF acquisition form)

2. Obtain Dr. Earley's signature on the forms, and he will forward the forms to the Animal Care Facility staff (this is absolutely non-negotiable…do not attempt to move forward without Dr. Earley's approval).
3. Please allow at least one week for this paperwork to be forwarded through the appropriate channels (i.e., Animal Care Facility [ACF] Staff)
4. Coordinate with Animal Care Facility Staff and the IACUC veterinarian for animal arrival; this means that you ***must*** maintain contact with the ACF and IACUC staff about fish ordering, shipment, tracking, and arrival.
5. The fish will arrive at the ACF (unless we acquire approval to have the fish shipped to Biology) so you ***must*** be available to unpack and acclimate the fish to the laboratory ***immediately*** upon arrival. Failure to do so will certainly compromise the health of the fish, and is grounds for IACUC sanctions.
6. Print and fill out a census log (see Appendix IX). This census log should be placed in clear view for ACF/IACUC Staff (e.g., directly on the tank, on the wall directly above a tank, or on the shelf accommodating the tank), and ***must*** be updated ***whenever*** a change is made to the animal 'contents' of the tank.

If the animals are to be purchased or otherwise acquired (e.g., gifted) from a collaborator's laboratory, the following must be done:

1. Fill out the relevant Animal Acquisition Forms (Appendix VII, which includes both an Earley lab Acquisition form and an ACF acquisition form)
2. Obtain Dr. Earley's signature on the form, and he will forward the form to the Animal Care Facility staff (this is absolutely non-negotiable…do not attempt to move forward without Dr. Earley's approval).
3. Make sure that you have documentation of IACUC approval from the 'sender' laboratory and make sure that the 'sender' laboratory is acquiring the necessary documentation of fish health from their IACUC veterinarian prior to shipment.
4. Please allow at least one week for this paperwork to be forwarded through the appropriate channels (i.e., Animal Care Facility [ACF] Staff)
5. Coordinate with Animal Care Facility Staff and the IACUC veterinarian for animal arrival; this means that you ***must*** maintain contact with the ACF and IACUC staff about fish shipments, tracking, and arrival.
6. The fish will arrive at the ACF (unless we acquire approval to have the fish shipped to Biology) so you ***must*** be available to unpack and acclimate the fish to the laboratory ***immediately*** upon arrival. Failure to do so will certainly compromise the health of the fish, and is grounds for IACUC sanctions.
7. Print and fill out a census log (see Appendix IX). This census log should be placed in clear view for ACF/IACUC Staff (e.g., directly on the tank, on the wall directly above a tank, or on the shelf accommodating the tank), and ***must*** be updated ***whenever*** a change is made to the animal 'contents' of the tank.

If the animals are to be collected in the field, the following must be done:

1. Fill out the relevant Animal Acquisition Form (Appendix VIII)

2. Obtain Dr. Earley's signature on the form, and he will forward the form to the Animal Care Facility staff (this is absolutely non-negotiable…do not attempt to move forward without Dr. Earley's approval).
3. Coordinate with Animal Care Facility Staff and the IACUC veterinarian for animal arrival; this means that you *must* maintain contact with the ACF and IACUC staff about fish arrival, and quarantine (see Appendix XII for quarantine procedures).
4. Print and fill out a census log (see Appendix IX). This census log should be placed in clear view for ACF/IACUC Staff (e.g., directly on the tank, on the wall directly above a tank, or on the shelf accommodating the tank), and *must* be updated *whenever* a change is made to the animal 'contents' of the tank.

*Regarding field caught specimens: most permits require annual or periodic updates about the animals caught and retained, and the eventual disposition of the animals (what were they used for? How were they cared for? Were they terminated? How were they terminated?). All MEL are required to keep meticulous records of animal captures and the use/disposition of those animals. They must download the Excel file for field collection records, which is available at http://rlearley.people.ua.edu/lab-info.html (see Field Collections Section), and this form must be updated within 48h of **EACH** collection and forwarded to Dr. Earley via e-mail (rlearley@as.ua.edu). If the disposition of the animals is unknown at the time, please enter the anticipated disposition of the animals. If this changes, the form can be modified in the "NOTES" section (do not delete your initial evaluation of disposition).*

Step #4: Maintaining your animals

We (the MEL) are committed to the proper maintenance and care of our animals – after all, how can we possibly do good science without happy animals! Therefore, we have instituted a daily checklist, which can be downloaded ("weekly_fish_room_checklist.doc") under the *Easy Access to Fish Maintenance Documents* section at:

http://rlearley.people.ua.edu/lab-info.html (see Appendix XI).

This checklist *must* be filled out each day for each room that contains animals.

One critical component of this checklist is water quality (for all housing units that are *not* the Tupperware *Rivulus* tubs). Given that we often work with a variety of fish species, it is necessary that all MEL understand the requirements of their species (see "earleylab_sop_fishcare2.doc" under *Easy Access to Fish Maintenance Documents* section at http://rlearley.people.ua.edu/lab-info.html see also Appendix XII). And, it is critical for everyone to know how to measure water quality and evaluate whether the tanks require maintenance (see "water quality.doc" under *Easy Access to Fish Maintenance Documents* section at http://rlearley.people.ua.edu/lab-info.html; see also Appendix XIII).

Feeding is also critical, and instructions for properly feeding the animals (without compromising water quality) are available in the "Feeding Fish.doc" document under the *Easy Access to Fish*

Maintenance Documents section at *http://rlearley.people.ua.edu/lab-info.html;* see also Appendix XIV).

Step #5: Dealing with fish health problems - What if your fish become diseased?

See "earleylab_sop_fishcare2.doc" under *Easy Access to Fish Maintenance Documents* section at *http://rlearley.people.ua.edu/lab-info.html* see also Appendix XII) *and read the following….*

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!! ***DO NOT BLEACH THE TANKS IN AN AREA WITH LIVE FISH – REMOVE THE TANK FROM THE AREA FOR DECONTAMINATION!***

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.

Important phone #'s

1. University Police (205-348-5454) – if you need to get in touch with facilities over weekends or holidays

2. Facilities (Heating, Air Conditioning: 205-348-4616)
3. Jimmy Ramage (Animal Care Facility: 205-339-2243; call in emergency; cell: 205-862-5730)
4. Ryan Earley (559-451-6800 or 205-348-1827)

ANIMAL CARE FACILITY REGISTRATION FORM

PART I: GENERAL INFORMATION

NAME: _____DEGREE(s) HELD:_____
(last/first/middle initial)
POSITION: _____WORK PHONE: _____ FAX:_____
DEPARTMENT: _____EMERGENCY PHONE: _____
E-MAIL ADDRESS: _____
CAMPUS ADDRESS: _____
NAME OF PRINCIPAL INVESTIGATOR: _____
PROTOCOL NUMBERS: _____
ANIMAL SPECIES USED: _____

PART II: TRAINING

☐ Introduction to Animal Care and Use (Check one of the following)
- ☐ Laboratory Animal Science Course
  - ☐ BSC 555/455
  - ☐ Other: Name /Institution/Dates _____
    _____
- ☐ Online (http://www.citiprogram.org : Working with the IACUC (non VA version))
  Attach certificate of completion

☐ Instruction in the proper use of the Animal Facilities in which he/she will work.

_____ _____
Animal Facility Endorsement Date

☐ I have read the material on the Risk Assessment Website (http://ehs.ucdavis.edu/animal/risk/index.htm) and understand that there are potential risks associated with working with animals and further have read the procedures described for minimizing those risks.

_____ _____
Registrant's Signature Date

☐ Protocol specific training completed: Protocol #: _____ Date: _____

Trainer:_____ Trainer's Signature: _____

PART III: CONTACT WITH ANIMALS OUTSIDE OF FACILITY

☐ I come into regular contact with animals outside of the animal care facilities (for example keeping pets, raising or caring for any animals, hunting, etc).
Please describe type of animal(s), type of contact(s), and frequency.
_____
_____
_____

☐ I do not come into regular contact with animals outside of the Animal Care Facility.

PART IV: NOTICE OF OHS

☐ I am participating in the Occupational Health and Safety Program for Animal Care and Use and have completed the Health History/Medical Evaluation Form.

_____ _____
Registrant's Signature Date

Return form to Animal Care Facility

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UNIVERSITY OF ALABAMA ANIMAL CARE AND USE PROGRAM (UAACUP)
HEALTH HISTORY MEDICAL EVALUATION FORM

An important element of the UAACUP Occupational Health and Safety Program is medical evaluation and preventive medicine. A component of the medical evaluation is a health history oriented toward the environment in which animals are used in research. Your answers will direct the health team in determining if any special training, accommodation or diagnostic testing may be necessary. A component of preventive medicine is in providing immunization. Specific immunizations will depend upon specific exposures. Return the completed form to the Animal Care Facility.

Please fill out completely (Print or type)

_____
Name (Last, First, Middle Initial)
Date of Birth

_____
Position
Faculty Advisor

_____
Current Address

_____
City State
Zip Sex

_____
Home Telephone Campus Telephone
SS#

_____
Family Physician Name
Telephone

_____
In case of an emergency contact (name, address, phone)

_____

PART 1: EXPOSURE TO ANIMALS

Do you have any indoor pets? Yes _____ No _____
Do you work with or come in contact with any animals on a routine basis? Yes _____ No _____ If yes to either question, which animal and for how long?

Animal	1-2 years	2-3 years	3-4 years	Over 4 years
Dogs				
Cats				
Birds				
Rodents				
Reptiles				
Other (type)				

PART 2: SPECIAL NEEDS:

Answer the following questions as they relate to your anticipated work within the Animal Care Facility

1. Will you or do you perform functions that will involve aerosolization of toxic chemicals?
   Yes ____: Protocol #_____ No____ Don't know _____

2. Will you or are you required to lift animals, supplies or equipment exceeding 50 pounds?
   Yes ____ No____ Don't know _____

3. Do you have a previous illness/injury that will require special accommodations?
   Yes ____ No____ Don't know _____

4. Are you immunocompromised?
   Yes_____ No_____ Don't know_____

PART 3: HEALTH HISTORY AS IT RELATES TO EXPOSURE TO RESEARCH

Direct any questions regarding this form or how your health history might be affected by your work at UA, to your personal physician.

ALLERGIES

ARE YOU WORKING OR HAVE YOU WORKED WITH LABORATORY ANIMALS?

Animal	**Yes, current**	**Yes, past**	**No**	**Approx. contact hrs./day & past yrs. of experience**
Rats				
Mice				
Rabbits				
Guinea Pigs				
Monkeys				
Cattle				
Dogs				
Cats				
Swine				
Hamsters				
Birds				
Other				

If you are currently working with animals do you use or wear any of the following items:

PPE	**Yes**	**No**	**NA**
Protective Eye Wear			
Face Shield			
Mask/Respirator			
Lab Coat			
Shoe Covers			
Gloves			

ALLERGIC SYMPTOMS

Do you believe that you are allergic to any of these animals? Yes _____ No _____

Check all that apply:

Rats	Mice	Rabbits	Dogs
Cats	Monkeys	Cattle	Guinea Pigs
Swine	Birds	Hamsters	Reptiles
Other (specify)			

Do you regularly have any of the following symptoms? Yes _____ No _____
Please indicate if the symptom is present and the year of onset. Also check the location where symptoms are present.

Symptom	**Yes/No Present**	**Year of Onset**	**At work**	**At home**
Cough				
Sputum production				
Shortness of breath				
Wheezing				
Chest tightness				
Asthma				
Nose congestion				
Runny nose				
Sneezing				
Itchy eyes				
Sinus problems				
Hay fever				
Frequent colds				
Hives				
Skin rash				
Swelling of eyes/lips				
Eczema				
Difficulty swallowing				

Were you ever told by a doctor that you had allergies? Yes _____ No _____

If yes, which allergy? _____

Have you ever been skin tested or had serological tests for allergies? Yes _____ No _____

If yes, to which of the following substances were you determined to be allergic? Check all that apply.

Ragweed	Grass	Trees	Mold
Dust	Cats	Dogs	Rodents
Other (Specify)			

Have you ever received allergy (desensitization/immunotherapy) shots? Yes _____ No _____

OTHER ALLERGIES
Do you have a history of allergies to latex? Yes ____ No____ Don't know _____

Do you have a history of allergies to chemicals? Yes ____ No____ Don't know _____
If yes, specify _____

ASTHMA
Has a doctor ever said you have asthma? Yes ____ No _____

If yes, when did your asthma start? _____(year)

Are you currently taking medication (either over-the-counter or prescription) to control your asthma?
Yes _____ No _____

Do you regularly use "over-the-counter" (non-prescription) nose drops or nose sprays, e.g. Afrin, Neosynephrine?
Yes _____ No _____

Appendix II – Earley Laboratory Standard Operating Procedures – Page 4

SMOKING

Do you smoke tobacco products? Yes ____ No _____ If yes, how much per day? _____ How many years? _____

If not presently smoking, did you ever smoke? Yes _____ No _____

If yes, when did you stop smoking? _____(year) How many years did you smoke? _____

IMMUNIZATIONS

Tetanus-diphtheria (dT) booster should be within the last 10 years. Most recent booster: _____(date)

Will you be working with primates? Yes_____ No_____

If you will be working with primates please answer the following questions:

Most recent tuberculin testing: PPD Date/year _____ mm induration _____

Have you had a chest X-ray related to a positive PPD tuberculin test? Yes_____ No_____
If yes: Date/year _____ Normal _____ Abnormal _____(Please attach copy of chest x-ray results)

Have you completed the Hepatitis B immunization series? Yes _____ No _____ Date _____

List any other immunizations you have received other than childhood series:

Immunization	**Year**

Signature: _____ Date: _____

Reviewed by: _____ Date: _____
(Print)

Title: _____ Signature: _____

Recommendations and Comments:

_____
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ANIMAL CARE FACILITY
TRANSIENT WORKER REGISTRATION FORM

NAME:_____

DEPARTMENT:_____

DATE:_____

As part of my duties I must enter the Animal Care Facility and understand that there may be some increased health risk in doing so beyond those normally associated with my work. These risks are primarily associated with allergies to animals and animal products and the potential of contact with organisms carried by animals that might make me sick. These risks can be greatly reduced by a few simple procedures. These include keeping all object out of my mouth, washing my hands, and wearing personal protection equipment (masks, gloves, face-shield, labcoat) as needed.

If I experience any allergy symptoms including runny eyes or nose, difficulty breathing, itching, I should leave the area immediately, remove protective clothing and leave it behind, and notify Animal Care Facility workers and my supervisor.

___ I know that I am allergic to the following animals:

_____
_____

Symptoms are: _____
_____
_____

___ In the past week, I have had contact with animals outside of the animal care facility. To protect my animal contacts both inside and outside the ACF, I understand that I may be asked to wear protective clothing or may be prevented from entering the facility. Please describe types of animals and the contact that you have had. Example: I have a pet cat. I went hunting and carried out a deer. I visited a neighbor who has a pet parrot.

_____
_____
_____
_____
_____
_____

Signature: _____ Date:_____

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THE UNIVERSITY OF ALABAMA
INSTITUTIONAL ANIMAL CARE AND USE COMMITTEE

ONS: Please complete and submit this form (typed) to the Office for Research at 152 Rose Administration. If you have any questions, please contact the Research Compliance Officer at 348-5152.

PROTOCOL MODIFICATION FORM

General Information

otocol # ______ **Original Approval Date** ______

nvestigator ______ **Dept.** ______

Phone (Office) ______

le ______

ate ______ **Funding Agency** ______

A. Nature of Requested Changes

... the PHS require you to submit changes regarding the care and use of animals in ongoing protocols for IACUC review and approval ...enting the changes. **Failure to obtain IACUC approval of a modification to your protocol could result ... of the study that was previously approved, if the IACUC determines that the activity is not being ... accordance with the original approved protocol.** If this occurs, you will be required to cease all activities ...used in your study until further reviewed by the IACUC and the Institutional Official. If your protocol is ...f you continue to use animals, this is considered a violation of federal regulations that govern the use of ...earch. Such violations must be reported to the Federal government and to University officials. Termination ...h and your funding by the government and the University may occur.

... the nature of your requested change (check all that apply)

- ...ange of project title
- ...ange of funding agency
- ...ange in number of animals
- ...ange of and/or addition of animal species
- ...ange in duration of project

...se changes above apply, please submit a new ... form.

B. Proposed Modification

C. Justification for Modification

D. Signature

Signature of Principal Investigator **Date**

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THE UNIVERSITY OF ALABAMA
INSTITUTIONAL ANIMAL CARE AND USE COMMITTEE (IACUC)

The institutional Animal Care and Use Committee is responsible for the welfare of "any live, vertebrate animal used or intended for use in research, experimentation, testing, training, or related purpose" if these animals are maintained at The University of Alabama Animal Care Facility (ACF) or used under funds administered by The University of Alabama.

PROPOSED ANIMAL USE REVIEW FORM (revised 12/15/03)

For Office Use Only:

IACUC #: _____ Date Received: ____/____/____ Biohazard _____

Approval Date: ____/____/____ Radiation _____

Veterinary Consultation: _____ Date: ____/____/____

(PLEASE PRINT OR TYPE ALL ANSWERS)

PRINCIPAL INVESTIGATOR: _____

TITLE OF GRANT/CONTRACT/PROPOSAL OR COURSE NUMBER AND TITLE:

_____

FUNDING AGENCY/DEPARTMENT & ID NUMBER (if applicable):

PERIOD OF THE PROPOSED ANIMAL USE ACTIVITY: [_____/_____/_____ - _____/_____/_____]

As specified in the Animal Welfare Act, the attending veterinarian or his designee must be consulted in the planning of your animal use activity. The attending veterinarian will provide medical care as necessary.

Name of veterinarian consulted: _____ Date: _____/_____/_____

Certification: I will comply with the procedures and methods descried in the NIH Guide for the Care and Use of Laboratory Animals (Pub. 85-23) and with PHS policy, the Animal Welfare Act, and Applicable University of Alabama policies. As Principal Investigator, I acknowledge responsibility for this project and assure that the faculty, staff, and students who participate in it are qualified (or will be adequately trained) to conduct it in a humane manner.

_____ _____/_____/_____
Signature, Principal Investigator Date

_____
Name (typed)

I have reviewed this proposal and concur with its submission.

_____ _____/_____/_____
Signature, University Department Chairperson Date

A. **Description and Objectives:**

1. Write an abstract of the teaching/research project in **layman's language** (100 words or less). Stress relevance of the proposed teaching/research activity to the general public.

2. Research or Instructional *Objectives*. Briefly state, using easily understandable **Lay Terms**, the *objectives* and the *specific aims* of the research or teaching activity (as for a federal research grant or a course syllabus) and their relevance to advancing scientific knowledge and/or benefits to human/animal health (Note—A section from your grant application, using highly technical terms, is *not acceptable*):

B. **Personnel:**

1. Principal Investigator: _____

Title/Department: _____

Campus Mailing Address: _____

Telephone: Office: _____ Home: _____

Co-Investigator (s): _____

Mailing Address: _____

Telephone: Office: _____ Home: _____

Laboratory Contact, if any (name): _____ Telephone W: _____

H: _____

Technical Staff (name/s): _____ Telephone: _____

_____ Telephone: _____

_____ Telephone: _____

2. Qualifications. Describe the RELEVANT (species/procedures) training of investigators and technicians enabling them to conduct the procedures described in the proposal and to use the animal species chosen. If personnel will be trained for the study, please indicate how and by whom.

(a) Principle Investigator:

(b) Co-Investigator(s):

(c) Technician(s):

C. **CLASSIFICATION BY ANIMAL USE:**

1. Animal Requirements: This information is requested for University record keeping related to USDA and other records.

a. List animal species used in this protocol by scientific and common name. Indicate those that are endangered or threatened. If more space is needed attach list as an addendum.

(1) _____ (2) _____ (3) _____ (4) _____

b. Check the one most appropriate description under each category (b. 1, 2, 3, and 4) that apply to this protocol. *For multiple species, list each species after descriptors that apply to them*.

(1) *Classification*
____ Research_____
____Training_____
(2) *Organ System*
____Cardiovascular_____
____Digestive_____
____Endocrine_____
____Eye_____
____Hemato/Lymphatic_____
____Integument_____
____Musculoskeletal_____
____Nervous_____
____Respiratory_____
____Reproductive_____
____Urinary_____
____All Systems (e.g., Pathology)

(3) *Procedure/Study Area*
_____Anatomy/Developmental Research_____
_____Antibody Production_____
_____Behavioral Studies Research_____
_____Disease Induction_____
_____Immunologic Research_____
_____Oncologic Research_____
_____Pharmacologic Research_____
_____Physiologic Research_____
_____Molecular Biologic Research_____
_____Nutritional/Chemical Research_____
_____Toxicology_____
(4) *Surgery*
_____Applicable_____
_____Not Applicable_____

2. Classification by stress levels:

a. Non-Painful/Non-Stressful
Studies, experiments, and tests causing no pain or distress (e.g., routine procedures causing only transitory discomfort, such as venipuncture, injection, and the use of non-inflammatory adjuvants; cell, fluid, and/or tissue harvest from euthanized animals).

b. Painful/Stressful WITH Analgesia/Anesthesia/Tranquilizers
Painful and/or stressful procedures carried out with the use of appropriate anesthetics (e.g., for surgery), analgesics (e.g., for inflammation), and tranquilizers (e.g., for prolonged restraint) that will prevent and alleviate pain and distress.

c. Painful/Stressful WITHOUT Pain and Stress Relieving Measures
Painful and/or stressful procedures performed without the use of analgesic, anesthetic, and tranquilizing drugs or other measures that will prevent and/or relieve pain and distress; or those procedures not amenable to relief by therapeutic measures (e.g., infectious disease, carcinogen, or toxicity studies in which natural death is the end point; addictive drug withdrawal without treatment; noxious stimulation with escape).

(1) Non-Painful/Non-Stressful - NUMBER OF ANIMALS:

SPECIES	YEAR 1	YEAR 2	YEAR 3	YEAR 4	YEAR 5	TOTAL

(2) Painful/Stressful, with analgesia/anesthesia/tranquilizers - NUMBER OF ANIMALS:

SPECIES	YEAR 1	YEAR 2	YEAR 3	YEAR 4	YEAR 5	TOTAL

(3) Painful/Stressful with no relief of pain and/or stress - NUMBER OF ANIMALS:

SPECIES	YEAR 1	YEAR 2	YEAR 3	YEAR 4	YEAR 5	TOTAL

D. **Assurances:**

1. Lack of non-painful, non-stressful alternatives: The Principal Investigator **must consider alternatives** to procedures that may cause pain or distress to animals. For Class (2) and (3) studies (Item C.2) provide a statement that non-painful, non-stressful alternatives are not available and the methods and sources used to determine this. If a computer assisted literature search was conducted, provide the name(s) of the data base(s) searched and the date(s) of the search(es) and keep copies of the search results.

2. Research Duplication: The PI is required by law (CFR9, AWA para.2.31d) to provide a statement that alternative research models or teaching alternatives are not available and that the proposed research or teaching activity does not *unnecessarily* duplicate previous work. If a computer assisted literature search was conducted, provide the name(s) of the database(s) searched and date(s) of the search(es) and keep copies of the results.

3. Rationale for species: Enter a brief statement explaining why the *species described* and the *number(s) requested* must be used. Address reasons such as pertinence to previous work, statistical significance, etc.

E. **RESEARCH OR INSTRUCTIONAL PROCEDURES:**

1. Describe, in general terms, the *study or instructional design* and *all animal procedures*. Include information on the *numbers* and *frequencies* of the *procedures* and the *eventual disposition* of the *animals*. Specific details of surgery, anesthetics, etc., are requested later and are not required here. For field collection protocols, note any requirements for permits and copies of the needed permits must be on file before work can begin. Continue on page 7 if necessary.

E. **RESEARCH OR INSTRUCTIONAL PROCEDURES (cont.):**

2. Where will procedures be performed (building and room if known)?

3. Where will animals be housed?

4. *Exceptions* to standard Practices:

   (a) Unless otherwise approved, ALL DOGS, AND CATS WILL BE HOUSED AND HANDLED SO AS TO PROVIDE FOR ENVIRONMENTAL ENRICHMENT. If this is NOT compatible with the proposed research, state what exceptions are required and provide justification:

   (b) Describe special caging, care, diets, or housing required.

   (c) Unless otherwise approved, all dogs will be provided exercise. If this is not compatible with the proposed research, state what exceptions are required and provide justification.

F. **MONITORING ANIMALS FOR WELL-BEING** (other than post-operative care requested in paragraph G.9 and G.10).

1. Indicate *known potential painful/stressful effects* on animals listed in Item C.2, Classes (2) and (3).

2. *All animals will be visually inspected at least daily by ACF staff, and all animal rooms are continuously electronically monitored for temperature, with automatic calling of the UA Dept. of Public Safety if the temperature varies from the desired range*. If *additional* monitoring is needed, as for painful/stressful effects from experimental procedures, *how*, *by whom*, and *how often* will animals be monitored?

3. If, due to experimental procedures, pain/stress occurs in animals, how will it be treated?

G. **SURGICAL PROCEDURES (*if no surgery will be performed, check "not applicable" below and skip to H*):**

1. Describe, in a separate paragraph for each animal species, details of each surgical procedure. **Attach description as an Appendix**. *Appendix attached* ☐ *Not applicable* ☐

   Total number of surgical procedures to be performed each year:

2. Anesthesia: Complete table below related to anesthesia:

	Animal Species	Drug Used	Dose/frequency	Route
Induction Preanesthetic				
Anesthesia Maintenance				
Paralytic Agents				
Post-Operative Analgesic				

3. How will depth of anesthesia be monitored?

4. Surgeon's name:

   Telephone - Office: _____ Home: _____

5. Will animals be allowed to recover from anesthesia? Yes ☐ No ☐ (check one)

6. If the previous answer was YES, will more than one procedure be conducted on each animal? If so, how many?

7. If the previous answer was YES, provide justification for more than one survival procedure. If the previous answer was NO, please enter N/A.

8. Enter site of Operating Room: _____

   Enter site of Recovery Room: _____

   Animal Study Area, if not Operating Room: _____

9. Describe Post-operative Care, including surveillance and treatment for pain:

10. Person(s) Responsible for Post-operative Care: _____

Name: _____ Telephone: Office: _____ Home: _____

Name: _____ Telephone: Office: _____ Home: _____

H. **ANIMAL TRAINING:**

Describe completely the methods to be used for training of animals (if applicable). Otherwise enter "N/A."

I. **ANIMAL RESTRAINT:**

Describe completely the methods, frequency, and duration of restraint, *other than routine caging and handling*. If none, so state.

J. **EUTHANASIA:**

If euthanasia is the end point of the study, indicate the method to be used. INCLUDE agent and dose for each species. Euthanasia shall be performed in accord with methods approved by the AVMA guidelines, subject to prior consultation with the investigator.

SPECIES	METHOD	AGENT	DOSE	COMMENTS

K. **ENDPOINT**

For those experiments without a defined endpoint, list criteria to be used to remove an animal from the protocol and how these conditions will be monitored. For example: Breeders will be euthanized if a.) the litter size drops below X, b.) there is an infection that does not respond to treatment, c.) stops eating, or d.) the animal shows other objective signs of pain or distress. If these conditions are observed the investigator and attending veterinarian will be notified and they will determine if the animal should be removed from the protocol.

L. **OTHER DRUGS & TISSUE COLLECTION:**

1. Blood/body fluid collection:

SPECIES	FLUID	AMOUNT	FREQUENCY	SITE/METHOD

2. Tissue Collection:

a. Tissue to be harvested:

b. Is this a post-mortem harvest? YES ☐ NO ☐ (If No, complete c. below)

c. Minor or non-surgical procedures (e.g., biopsies, endoscopies) to obtain tissue or fluid from live animals:

3. Tissue/Fluid/Drug Administration*

Describe agents to be administered not listed above:

SPECIES	AGENT	ROUTE	DOSE (mg/kg)	AMOUNT	POSSIBLE COMPLICATIONS

***** *The use of **Freund's Complete Adjuvant** requires adherence to the University Recommendation for that agent. Justify below any need for deviation from the written recommendation.*

M. **BIOSAFETY:**

Is there any *in vivo* use of infectious agents or biological or chemical toxins? YES ☐ NO ☐

If YES, specify agent(s): _____

_____

Date of approval from the Biosafety Research Committee: _____/_____/_____

Is there any *in vivo* radioisotope use? YES ☐ NO ☐

Date of approval from the Radiation Safety Committee: _____/_____/_____

(a) Labeled compound(s): _____

_____

(b) radioisotope(s): _____

_____

(c) Dose per animal: _____

_____

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ANIMAL ACQUISITION FORM - Commercial Suppliers or Pet Stores

Name of Commercial Supplier/Pet Store:

Contact Information for Commercial Supplier/Pet Store (name of contact, phone number, etc.):

Species Requested: Date Requested:

Common Name –
Genus/Species Name –
Other information if relevant (particular strains, sexes, etc.) –

Quantity Requested (explicitly specify needs with respect to sex, strain, etc.):

Price per fish: Total Cost (including shipping, if relevant):

Purpose of Requisition (please fill this section out in collaboration with Dr. Earley):

Animals to be held as 'dither' fish? *Yes* _____ *No* _____

IACUC Protocol # _____ *Approval Date* _____

Animals to be held for breeding purposes? *Yes* _____ *No* _____

IACUC Protocol # _____ *Approval Date* _____

Animals to be held for experimental purposes? *Yes* _____ *No* _____

IACUC Protocol # _____ *Approval Date* _____

Brief (and Informative) Description of experiment (a few sentences):

Fund to be used for animal purchase (please complete the Departmental Transfer Authorization (DTA) on the Department of Biological Sciences website [http://bsc.ua.edu/site/resources.htm], or ask Dr. Earley for the DTA):

Name of Funding Source:

DTA form filled out (to the best of your ability)? Please do so.
Census logs prepared (see Appendix IX)? Please do so.
OK for fish to be shipped to the Animal Care Facility (Nott)? If not, have accommodations been made to ship to Biology, and has the necessary approval been obtained?

Requester Name (print): Requester Signature: Date:

Principal Investigator (Dr. Earley; print): PI Signature: Date:

ACF Staff Acknowledgement of Receipt (print): ACF Staff Signature: Date:

ACF Staff: Please forward the final, signed hard copy of this document to Dr. Earley at Box 870344 or a scanned version via e-mail to rlearley@bama.ua.edu

ACF Animal Order Form

Investigator:

_____

Department:

_____

Protocol Number: _____ Approval Dates: From _____ To _____

Protocol Name:

_____

Account to be charged:

_____

Quantity	Species to be ordered	Strain designation if any	Catalog Number if any	Date Needed By

Vendor: _____

Vendor: Address:_____

_____

_____

Vendor Phone: _____ Fax: _____

Animals will be shipped to the ACF in Nott Hall unless otherwise requested.

Please ship to the following address:

_____

_____

_____

Animals will be housed in Nott Hall unless noted below.

Animals will be housed in: _____ IACUC approval:_____

Animals will be housed under standard conditions unless noted below.

Special handling instruction upon arrival.

_____

____

Date submitted: _____

PI signature: _____

Date Ordered: _____ Order placed by: _____

Confirmation # if available: _____

Expected Delivery Date: _____ Carrier: _____

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ANIMAL ACQUISITION FORM- Fish Acquired from Collaborator Laboratory

Name of Collaborator and Institution:

Contact Information for Collaborator (name of contact, phone number, etc.):

Species Requested: Date Collaborator Lab Contacted:

Common Name –
Genus/Species Name –
Other information if relevant (particular strains, sexes, etc.) –

Quantity Requested (explicitly specify needs with respect to sex, strain, etc.):

Are these fish gifted to the Earley lab (no associated cost)? Yes _____ No _____ (fill out next section)

Price per fish (if relevant): Total Cost (including shipping, if relevant):

Purpose of Requisition (please fill this section out in collaboration with Dr. Earley):

Animals to be held for breeding purposes? Yes _____ No _____

IACUC Protocol # _____ Approval Date _____

Animals to be held for experimental purposes? Yes _____ No _____

IACUC Protocol # _____ Approval Date _____

Brief (and Informative) Description of experiment (a few sentences):

Fund to be used for animal purchase, if applicable (please complete the Departmental Transfer Authorization (DTA) on the Department of Biological Sciences website [http://bsc.ua.edu/site/resources.htm], or ask Dr. Earley for the DTA):
Name of Funding Source:

- *DTA form filled out (to the best of your ability)? Please do so.*
- *Census logs prepared (see Appendix IX)? Please do so.*
- *OK for fish to be shipped to the Animal Care Facility (Nott)? If not, have accommodations been made to ship to Biology, and has the necessary approval been obtained?*

Documentation of IACUC protocols from the collaborator's laboratory attached? Yes _____ No _____ (fill out below)

You are required to append evidence of contact for IACUC-related information.
You are required to append a declaration of health for the fish from the sender's (collaborator's) IACUC veterinarian

Requester Name (print): Requester Signature: Date:

Principal Investigator (Dr. Earley; print): PI Signature: Date:

ACF Staff Acknowledgement of Receipt (print): ACF Staff Signature: Date:

ACF Staff: Please forward the final, signed hard copy of this document to Dr. Earley at Box 870344 or a scanned version via e-mail to rlearley@bama.ua.edu

ACF Animal Order Form

Investigator:
_____
Department:
_____
Protocol Number: _____ Approval Dates: From _____ To _____
Protocol Name:
_____
Account to be charged:
_____

Quantity	Species to be ordered	Strain designation if any	Catalog Number if any	Date Needed By

Vendor: _____
Vendor: Address:_____
_____
_____
Vendor Phone: _____ Fax: _____

Animals will be shipped to the ACF in Nott Hall unless otherwise requested.
Please ship to the following address:
_____
_____
_____

Animals will be housed in Nott Hall unless noted below.
Animals will be housed in: _____ IACUC approval:_____
Animals will be housed under standard conditions unless noted below.
Special handling instruction upon arrival.
_____
____
Date submitted: _____
PI signature: _____
Date Ordered: _____ Order placed by: _____
Confirmation # if available: _____
Expected Delivery Date: _____ Carrier: _____

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ANIMAL ACQUISITION FORM- Fish Acquired from the Field

Proposed Location of Collections:

Proposed Method of Collections:

Species to be collected: Proposed Collection Date:

Common Name –
Genus/Species Name –
Other information if relevant (particular strains, sexes, etc.) –

Proposed Quantity Collected:

Purpose of Collection (please fill this section out in collaboration with Dr. Earley):

Animals to be held for breeding purposes? Yes _____ No _____

IACUC Protocol # _____ Approval Date _____

Animals to be held for experimental purposes? Yes _____ No _____

IACUC Protocol # _____ Approval Date _____

Brief (and Informative) Description of experiment (a few sentences):

Permit Agency: Permit #:

Census logs prepared (see Appendix IX)? Please do so.

You are required to append evidence of an up-to-date Excel file into which you will document this collection. The excel file is available at An updated collection log (in Excel) must be submitted within 48 hours of collection to Dr. Earley via e-mail (rlearley@bama.ua.edu). By signing this form, you acknowledge this requirement.

Requester Name (print): Requester Signature: Date:

Principal Investigator (Dr. Earley; print): PI Signature: Date:

ACF Staff Acknowledgement of Receipt (print): ACF Staff Signature: Date:

ACF Staff: Please forward the final, signed hard copy of this document to Dr. Earley at Box 870344 or a scanned version via e-mail to rlearley@bama.ua.edu

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CENSUS LOG

Tank/Shelf #: **Species:** **IACUC Protocol #:**

You are required to contact Dr. Earley (rlearley@as.ua.edu; 205-348-182; 559-451-6800) immediately if there are any fish deaths, even if these deaths result from old age.

Date	*# Added*	*Reason Added (e.g., collected)*	*# Removed*	*Reason Removed (e.g., death, experiment)*	*TOTAL #*

Place this table in a plastic sheet and append to the tank or shelf in question immediately upon fish acquisition. Any time a fish is added or removed from the tank or shelf, it must be recorded on this census log, no questions asked. Any violations of this procedure will be reported to IACUC immediately and are grounds for immediate removal from the laboratory.

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DECLARATION OF FISH CARE & MAINTENANCE UNDERSTANDING
Earley Laboratory

I (student name) _____ hereby affirm that I have discussed the new laboratory fish acquisition, care, and maintenance protocols with Dr. Ryan Earley on Month _____ Day _____ Year _____. In addition, Dr. Earley has addressed all questions that I have had about the new laboratory protocols, and I am aware that all relevant documentation is available online at http://bama.ua.edu/~rlearley/earleyIACUC.html. I affirm that I will follow these protocols and that I will immediately address any subsequent issues directly with Dr. Earley so that he can contact University of Alabama's Institutional Animal Care and Use Committee to rapidly alleviate any problems related to the fish facilities.

Student Signature: Date:

PI (Dr. Earley) Signature: Date:

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APPENDIX XI (WEEKLY CHECKLIST) ON THE FOLLOWING PAGES

MONDAY - DATE: _____

Have you signed in to the fish room security log? PLEASE DO SO!

ROOM #: 228 Complete all tasks relevant to the room and initial; if not relevant to the room, put NA.

Done?	Initial		
❑	_____	Individually housed killifish fed?	Already Fed?: 1 3 4 5 6 7 8 9 M P Other Feedings: 11 (Freshwater) 12 (Kristy) 2 & 10 (Liz) 245 246
❑	_____	Killifish saltwater reservoir filled (if at fill line) and salinity checked at 25 ppt?	
❑	_____	Brine shrimp water (for hatching the shrimps) filled (if below half-way) and salinity checked at 39-40 ppt or 25 ppt depending on which brine shrimp we need)?	
❑	_____	Brine shrimp hatchery, including tubing rinsed to be egg-free?	
❑	_____	Brine shrimp incubation buckets cleaned, airlines and airstones cleaned, lids cleaned?	
❑	_____	New brine shrimp (2 large scoops per container) made?	
❑	_____	Killifish hatchlings checked and logged both in the Earley lab notebook and inventory log?	
❑	_____	Empty hatchling 'pee cups' are cleaned out and the labels are removed?	
❑	_____	Killifish hatchlings placed in individual containers with NEATLY WRITTEN, appropriate labels (see the sticker color legend to the right of the workbench), and taped on (scotch tape)?	
❑	_____	Photoperiod controls checked and aligned?	
❑	_____	In need of supplies? If so, note below and contact Dr. Earley	
❑	_____	Are all census logs updated and placed properly?	
❑	_____	Trash removed	
❑	_____	Check the floor – is it dirty? If so, spray down and squeegee.	
❑	_____	Are the drains on the floor covered?	
❑	_____	Room temperature (ºC): Front _____ Back _____ Tank (Ambient) _____ Tank (Water) _____	

Please note any maintenance and fish health issues below and <u>CONTACT DR. EARLEY IMMEDIATELY</u>:

TUESDAY - DATE: _____

Have you signed in to the fish room security log? PLEASE DO SO!

ROOM #: 228 Complete all tasks relevant to the room and initial; if not relevant to the room, put NA.

Done?	Initial		
❑	_____	Individually housed killifish fed?	Already Fed?: 1 3 4 5 6 7 8 9 M P Other Feedings: 11 (Freshwater) 12 (Kristy) 2 & 10 (Liz) 245 246
❑	_____	Killifish saltwater reservoir filled (if at fill line) and salinity checked at 25 ppt?	
❑	_____	Brine shrimp water (for hatching the shrimps) filled (if below half-way) and salinity checked at 39-40 ppt or 25 ppt depending on which brine shrimp we need)?	
❑	_____	Brine shrimp hatchery, including tubing rinsed to be egg-free?	
❑	_____	Brine shrimp incubation buckets cleaned, airlines and airstones cleaned, lids cleaned?	
❑	_____	New brine shrimp (2 large scoops per container) made?	
❑	_____	Killifish hatchlings checked and logged both in the Earley lab notebook and inventory log?	
❑	_____	Empty hatchling 'pee cups' are cleaned out and the labels are removed?	
❑	_____	Killifish hatchlings placed in individual containers with NEATLY WRITTEN, appropriate labels (see the sticker color legend to the right of the workbench), and taped on (scotch tape)?	
❑	_____	Photoperiod controls checked and aligned?	
❑	_____	In need of supplies? If so, note below and contact Dr. Earley	
❑	_____	Are all census logs updated and placed properly?	
❑	_____	Trash removed	
❑	_____	Check the floor – is it dirty? If so, spray down and squeegee.	
❑	_____	Are the drains on the floor covered?	
❑	_____	Room temperature (ºC): Front _____ Back _____ Tank (Ambient) _____ Tank (Water) _____	

Please note any maintenance and fish health issues below and <u>CONTACT DR. EARLEY IMMEDIATELY</u>:

WEDNESDAY - DATE: _____

Have you signed in to the fish room security log? PLEASE DO SO!

ROOM #: 228 Complete all tasks relevant to the room and initial; if not relevant to the room, put NA.

Done?	Initial		
❑	_____	Individually housed killifish fed?	Already Fed?: 1 3 4 5 6 7 8 9 M P Other Feedings: 11 (Freshwater) 12 (Kristy) 2 & 10 (Liz) 245 246
❑	_____	Killifish saltwater reservoir filled (if at fill line) and salinity checked at 25 ppt?	
❑	_____	Brine shrimp water (for hatching the shrimps) filled (if below half-way) and salinity checked at 39-40 ppt or 25 ppt depending on which brine shrimp we need)?	
❑	_____	Brine shrimp hatchery, including tubing rinsed to be egg-free?	
❑	_____	Brine shrimp incubation buckets cleaned, airlines and airstones cleaned, lids cleaned?	
❑	_____	New brine shrimp (2 large scoops per container) made?	
❑	_____	Killifish hatchlings checked and logged both in the Earley lab notebook and inventory log?	
❑	_____	Empty hatchling 'pee cups' are cleaned out and the labels are removed?	
❑	_____	Killifish hatchlings placed in individual containers with NEATLY WRITTEN, appropriate labels (see the sticker color legend to the right of the workbench), and taped on (scotch tape)?	
❑	_____	Photoperiod controls checked and aligned?	
❑	_____	In need of supplies? If so, note below and contact Dr. Earley	
❑	_____	Are all census logs updated and placed properly?	
❑	_____	Trash removed	
❑	_____	Check the floor – is it dirty? If so, spray down and squeegee.	
❑	_____	Are the drains on the floor covered?	
❑	_____	Room temperature (ºC): Front _____ Back _____ Tank (Ambient) _____ Tank (Water) _____	

Please note any maintenance and fish health issues below and <u>CONTACT DR. EARLEY IMMEDIATELY</u>:

THURSDAY - DATE: _____

Have you signed in to the fish room security log? PLEASE DO SO!

ROOM #: 228 Complete all tasks relevant to the room and initial; if not relevant to the room, put NA.

Done?	Initial		
❑	_____	Individually housed killifish fed?	Already Fed?: 1 3 4 5 6 7 8 9 M P Other Feedings: 11 (Freshwater) 12 (Kristy) 2 & 10 (Liz) 245 246
❑	_____	Killifish saltwater reservoir filled (if at fill line) and salinity checked at 25 ppt?	
❑	_____	Brine shrimp water (for hatching the shrimps) filled (if below half-way) and salinity checked at 39-40 ppt or 25 ppt depending on which brine shrimp we need)?	
❑	_____	Brine shrimp hatchery, including tubing rinsed to be egg-free?	
❑	_____	Brine shrimp incubation buckets cleaned, airlines and airstones cleaned, lids cleaned?	
❑	_____	New brine shrimp (2 large scoops per container) made?	
❑	_____	Killifish hatchlings checked and logged both in the Earley lab notebook and inventory log?	
❑	_____	Empty hatchling 'pee cups' are cleaned out and the labels are removed?	
❑	_____	Killifish hatchlings placed in individual containers with NEATLY WRITTEN, appropriate labels (see the sticker color legend to the right of the workbench), and taped on (scotch tape)?	
❑	_____	Photoperiod controls checked and aligned?	
❑	_____	In need of supplies? If so, note below and contact Dr. Earley	
❑	_____	Are all census logs updated and placed properly?	
❑	_____	Trash removed	
❑	_____	Check the floor – is it dirty? If so, spray down and squeegee.	
❑	_____	Are the drains on the floor covered?	
❑	_____	Room temperature (ºC): Front _____ Back _____ Tank (Ambient) _____ Tank (Water) _____	

Please note any maintenance and fish health issues below and <u>CONTACT DR. EARLEY IMMEDIATELY</u>:

FRIDAY - DATE: _____

Have you signed in to the fish room security log? PLEASE DO SO!

ROOM #: 228 Complete all tasks relevant to the room and initial; if not relevant to the room, put NA.

Done?	Initial	
❑	_____	Individually housed killifish fed? Already Fed?: 1 3 4 5 6 7 8 9 M P Other Feedings: 11 (Freshwater) 12 (Kristy) 2 & 10 (Liz) 245 246
❑	_____	Killifish saltwater reservoir filled (if at fill line) and salinity checked at 25 ppt?
❑	_____	Brine shrimp water (for hatching the shrimps) filled (if below half-way) and salinity checked at 39-40 ppt or 25 ppt depending on which brine shrimp we need)?
❑	_____	Brine shrimp hatchery, including tubing rinsed to be egg-free?
❑	_____	Brine shrimp incubation buckets cleaned, airlines and airstones cleaned, lids cleaned?
❑	_____	New brine shrimp (2 large scoops per container) made?
❑	_____	Killifish hatchlings checked and logged both in the Earley lab notebook and inventory log?
❑	_____	Empty hatchling 'pee cups' are cleaned out and the labels are removed?
❑	_____	Killifish hatchlings placed in individual containers with NEATLY WRITTEN, appropriate labels (see the sticker color legend to the right of the workbench), and taped on (scotch tape)?
❑	_____	Photoperiod controls checked and aligned?
❑	_____	In need of supplies? If so, note below and contact Dr. Earley
❑	_____	Are all census logs updated and placed properly?
❑	_____	Trash removed
❑	_____	Check the floor – is it dirty? If so, spray down and squeegee.
❑	_____	Are the drains on the floor covered?
❑	_____	Room temperature (ºC): Front _____ Back _____ Tank (Ambient) _____ Tank (Water) _____
❑	_____	**CLEANED LIDS AND AIR LINES ON BRINE SHRIMP HATCHERY RIG?**
❑	_____	**FISH ROOM BENCH ORGANIZED AND FREE OF DEBRIS?**
❑	_____	**RUNNING LOW ON RIVULUS FOOD? # LEFT: SHRIMP EGGS _____**

Please note any maintenance and fish health issues below and CONTACT DR. EARLEY IMMEDIATELY:

Version #3– August 16, 2014

WEEKEND!! **DATE: _____**

Have you signed in to the fish room security log? PLEASE DO SO!

ROOM #: 228 Complete all tasks relevant to the room and initial; if not relevant to the room, put NA.

Done?	Initial	
❑	_____	Individually housed killifish fed? Already Fed?: 1 3 4 5 6 7 8 9 M P Other Feedings: 11 (Freshwater) 12 (Kristy) 2 & 10 (Liz) 245 246
❑	_____	Killifish saltwater reservoir filled (if at fill line) and salinity checked at 25 ppt?
❑	_____	Brine shrimp water (for hatching the shrimps) filled (if below half-way) and salinity checked at 39-40 ppt or 25 ppt depending on which brine shrimp we need)?
❑	_____	Brine shrimp hatchery, including tubing rinsed to be egg-free?
❑	_____	Brine shrimp incubation buckets cleaned, airlines and airstones cleaned, lids cleaned?
❑	_____	New brine shrimp (2 large scoops per container) made?
❑	_____	Killifish hatchlings checked and logged both in the Earley lab notebook and inventory log?
❑	_____	Empty hatchling 'pee cups' are cleaned out and the labels are removed?
❑	_____	Killifish hatchlings placed in individual containers with NEATLY WRITTEN, appropriate labels (see the sticker color legend to the right of the workbench), and taped on (scotch tape)?
❑	_____	Photoperiod controls checked and aligned?
❑	_____	In need of supplies? If so, note below and contact Dr. Earley
❑	_____	Are all census logs updated and placed properly?
❑	_____	Trash removed
❑	_____	Check the floor – is it dirty? If so, spray down and squeegee.
❑	_____	Are the drains on the floor covered?
❑	_____	Room temperature (ºC): Front _____ Back _____ Tank (Ambient) _____ Tank (Water) _____
❑	_____	**SUNDAY ONLY: All eggs get water change**
❑	_____	**CHECK WHITE TABLE FOR FISH – IF PRESENT, CULL!**

Please note any maintenance and fish health issues below and CONTACT DR. EARLEY IMMEDIATELY:

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Appendix XII – Standard operating procedures – fish care and maintenance – Page 1

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

Appendix XII – Standard operating procedures – fish care and maintenance – Page 3

*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 <u>each</u> 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.

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Appendix XIII – How to measure water quality – Page 1
http://bama.ua.edu/~rlearley/WaterQuality.pdf

Water Quality

On Mondays and Fridays, we are **required** to check the water quality of all large tanks (i.e., any tank that's not a Tupperware) in the fish room…and any tanks in Nott Hall.

The tanks are designated with a number, which can be found on the sheet of paper sticking either to the tank itself or to the wall near the tank (Census logs).

You will test pH, ammonia, nitrate, nitrite for all tanks. You will also test GH and KH for the freshwater tanks (i.e., Nott Hall cichlid tanks, shiner tanks, large sunfish tank, 'strange' killifish tank)

Here's what you do:

1. Go to Room 245 and find the clear Tupperware containers (similar to what we house the killifish in)
2. Fill these Tupperwares with water from each tank that you'll test and make sure to keep them straight (might want to label them, or arrange them in order on the bench).
3. Using the small syringe (on bench in Room 245), suck up 5ml from the container and squirt into 3-5 glass vials (depending on whether you'll test GH/KH for that tank; if not, you'll have 3 vials).
4. Follow the directions for the test kits (see next couple of pages), and compare against the colored cards hanging from the shelf (or look at the GH/KH worksheet to calculate the value from the # of drops you added to the tube before it changed color)
5. Record both the tank number and the values on the daily checklist.
6. If ammonia or nitrite are > 0 ppm…or if nitrate is > 10 ppm, you need to do a water change.
7. *Please get with Dr. Earley or graduate students if you are unfamiliar with how to do the water changes! We MUST siphon the gravel (i.e., sucking a bunch of water out of the tank isn't enough)*
8. Add appropriate chemicals (e.g., colonize, stress coat, freshwater salt) as needed (again, get with Dr. Earley or graduate students if you have questions about this)

Appendix XIII – How to measure water quality – Page 2

Directions for Testing Ammonia Levels

1. Fill a clean test tube with 5 ml of water to be tested (to the line on the tube).
2. Add 8 drops from Ammonia Test Solution Bottle #1, holding the dropper bottle upside down in a completely vertical position to ensure uniformity of drops added to the water sample.
3. Add 8 drops from Ammonia Test Solution Bottle #2, holding the dropper bottle upside down in a completely vertical position to ensure uniformity of drops added to the water sample.
4. Cap the test tube and shake vigorously for 5 seconds. Do not hold finger over the open end of the tube, as this may affect the test results.
5. Wait 5 minutes for the color to develop.
6. Read the test results by matching the test solution against the Ammonia Test Color Chart. The tube should be viewed against the white area beside the color chart. Color comparisons are best made in a well-lit area. The closest match indicates the ppm (mg/L) of ammonia in the water sample. Rinse the test tube with clean water after each use.

Directions for Testing Nitrite

1. Fill a clean test tube with 5 ml of water to be tested (to the line on the tube).
2. Add 5 drops of Nitrite Test Solution, holding dropper bottle upside down in a completely vertical position to ensure uniformity of drops added to the water sample.
3. Cap the test tube and shake the tube for 5 seconds. Do not hold finger over the open end of the tube, as this may affect test results.
4. Wait five minutes for the color to develop.
5. Read the test results by matching the color of the solution against those on the Nitrite Test Color Chart. The tube should be viewed against the white area beside the color chart. Color comparisons are best made in a well-lit area. The closest match indicates the ppm (mg/L) of nitrite in the water sample. Rinse the test tube with clean water after each use.

Directions for Testing Nitrate
Read thoroughly before testing. DO NOT allow Test Solutions to get into aquarium.
To remove childproof safety cap: With one hand, push red tab left with thumb while unscrewing cap with free hand.

1. Fill a clean test tube with 5 ml of water to be tested (to the line on the tube).
2. Add 10 drops from Nitrate Test Solution Bottle # 1, holding dropper bottle upside down in a completely vertical position to ensure uniformity of drops added to the water sample.
3. Cap the test tube and invert tube several times to mix solution. Do not hold finger over the open end of the tube, as this may affect test results.
4. Vigorously shake the Nitrate Test Solution Bottle # 2 for at least 30 seconds. This step is extremely important to insure accuracy of test results.
5. Now add 10 drops from Nitrate Test Solution Bottle #2, holding dropper bottle upside down in a completely vertical position to assure uniformity of drops to the water sample.
6. Cap the test tube and shake vigorously for one minute. This step is extremely important to insure accuracy of test results.
7. Wait five minutes for the color to develop.
8. Read the test results by matching the color of the solution against those on the Nitrate Test Color Chart. The tube should be viewed against the white area beside the color chart. Color comparisons are best made in a well-lit area. The closest match indicates the ppm (mg/L) of nitrate in the water sample. Rinse the test tube with clean water after each use.

Appendix XIII – How to measure water quality – Page 3

Directions for Testing Carbonate Hardness (KH):

- Read thoroughly before testing. Do not allow Test Solutions to get into aquarium.
- Remove childproof safety cap using one hand to push red tab while unscrewing cap with free hand.
- Rinse a clean test tube with water to be tested.
- Fill the test tube with 5 ml of aquarium water (to the line on the test tube).
- Add Carbonate Hardness Test Solution, one drop at a time. Hold dropper bottle upside down in a completely vertical position to ensure uniform drops. After first drop is added, solution will turn blue (If the water sample contains only 1°dKH, the solution will turn from clear to its yellow endpoint after the first drop is added).
- Cap the test tube and invert several times after each drop. Count the number of drops being added. Do not hold finger over open end of the tube, as this may affect the test results.
- The test is completed when the water in the test tube, after having been shaken, turns from blue to yellow. If you have difficulty discerning the color after the first drop of test solution is added, remove the cap from the test tube and while holding it over a white background, look down through the tube.
- The Carbonate Hardness value is determined by the number of drops of the reagent that must be added to turn the water in the test tube bright yellow. Each drop is equal to 1 °dKH or 17.9 ppm KH.

Directions for Testing General Hardness (GH):

- Read thoroughly before testing. Do not allow Test Solutions to get into aquarium.
- Remove childproof safety cap using one hand to push red tab left while unscrewing cap with free hand.
- Rinse a clean test tube with water to be tested.
- Fill the test tube with 5 ml of aquarium water (to the line on the test tube).
- Add General Hardness Test Solution, one drop at a time. Hold dropper bottle upside down in a completely vertical position to ensure uniform drops. After first drop is added, solution will turn orange (If the water sample contains only 1°dGH, the solution will turn from clear to its green endpoint after the first drop is added).
- Cap the test tube and invert several times after each drop. Keep count of the drops being added. Do not hold finger over open end of the tube, as this may affect the test results.
- The test is completed when the water in the test tube, after having been shaken, turns from orange to green. If you have difficulty discerning the color after the first drop of test solution is added, remove the cap from the test tube and while holding it over a white background, look down through the tube.
- The number of drops of the reagent that must be added to turn the water in the test tube green determines the General Hardness value. Each drop is equal to 1 °dGH or 17.9 ppm GH.

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Appendix XIV – Detailed Fish Feeding Instructions – Page 1

EARLEY LAB FISH FEEDING INSTRUCTIONS – WILL BE UPDATED SOON

1. In this room, you'll see two beakers 'brewing' brine shrimp. One is labeled "TWO" and one is labeled "ONE"; this refers to the number of days that the shrimp have been incubating.
2. There also is a shrimp 'separator' (i.e., an apple juice container that has been inverted with a tube stuck through the cap). Make sure that the white clip around the tubing is locked to prevent flow.
3. Dump the shrimp brewing beaker TWO into the shrimp separator, and wait about 30 minutes. In the meantime, rinse out beaker TWO and place under the tubing from the shrimp separator (just in case it starts to leak).

   Check to make sure the shrimp that you just poured are alive….believe me, you'll know. If it smells rancid or, once separated, looks milky orange, they're dead. If that's the case, rinse out the separator and try using the beaker labeled ONE.

4. Go to the research lab, obtain the freezer key from the drawer, open the freezer, and pull out some frozen brine shrimp and frozen blood worms. Take about a 1.5 x 1.5 inch square of each and place them in the 'cichlid food beaker' (located near the sink to the left of Room 212 (the side room of the lab).
5. From the same key drawer, obtain the key for Nott Hall ACF Facility.
6. Bring the food beaker back to the fish room, fill with about 150 ml of water, and add a pinch of flake food (located on the same shelf as the killifish egg containers in a small Tupperware). Wait about 5 minutes for the shrimp/worms to thaw, then mix thoroughly.
7. Feed the sunfish (the enormous gray plastic tank at the back of the room), shiners, and freshwater killifish (*Rivulus cryptocallus, Rivulus cylindraceus, Rivulus caudomarginatus*) the shrimp/worm/flake cocktail. It is important to watch the fish while you feed them. Put a squirt of food in….watch…..if they eat it all, give them another. If they don't, there's no need to give them more food.
8. After making sure you have the key to Nott Hall, walk over to the Nott Hall Animal Care Facility (ACF) with the shrimp/worm/flake cocktail. Unlock the loading dock door (should lock behind you), log in with your personalized key code, and make sure that the ACF door closes behind you. Go to Room 107 (the key is in the hanging folder on the door), sign in, feed the cichlids ~ 6-8 full squirts of the shrimp/worm/flake cocktail (again, watch them eat to make sure you aren't overfeeding). Check the water levels of the large blue tanks (shouldn't be much lower than one inch below the ridge that runs along the interior edge of the tanks) and the 10-gallon glass tanks on the shelves (shouldn't be much lower than 1 inch below the black lip). If water levels are low, refill to the appropriate level. There's a binder on the central table; please fill out the checklist. When finished, lock the door, place the key back in the hanging folder on the door, log out of the ACF with your personalized key code, **make sure the door closes behind you**, leave the ACF building and **make sure that the loading dock door is locked**.

Appendix XIV – Detailed Fish Feeding Instructions – Page 2

Now, the shrimp over in the Biology fish room should be ready.

9. **PUT THE NOTT HALL KEY BACK IN THE DRAWER**
10. Drain the first 30-40 ml of the 'separator' bottle into the small glass beaker (this will contain lots of shrimp eggs).
11. Drain the rest into the large beaker that you cleaned out.
12. *If necessary:* Pour 300ml of this into the fleaker for Amanda's fish.
13. Using the 25ppt water (the large container with saltwater at the back of the room), dilute Amanda's fleaker up to 600ml and put the airstone into the fleaker (*if necessary)*. Also, dilute the remaining shrimp in the large beaker up to about 1700-1800 ml with 25ppt water.
14. Using the repeating pipettes (with a yellow pipette tip attachment), feed the killifish in Tupperware containers. All fish that are **less than** 2 months old (see sticker with birthday on the tupperwares) should be given 1ml. All animals older than that should receive 2ml. I typically feed the large containers housing the LION clone about 4ml.
15. Feed the RHL communal tank (bottom shelf under the LK clone tupperwares) about 20ml in each of the 4 compartments. Feed the DAN2K communal tank (small tank, top shelf near the fish room door) 20ml total.
16. The remainder of the shrimp (should be very little) can be dumped. Do not try to re-use the shrimp or dump them into the existing 'brewing containers'…they will foul up the food, and make it unusable for the next day.
17. Clean out the beaker
18. Bring the beaker to the research laboratory, and find an open container of brine shrimp eggs in the refrigerator. Add 2.5-3 large scoops. Bring the beaker back to the fish room and fill the beaker (~2 inches from the top) with 40ppt water (located in small bucket with purple cover near the large saltwater reservoir). Bring to room 245, place an airstone into the beaker and stir. Place the foil labeled "ONE" onto this beaker and transfer the foil labeled "TWO" to the beaker of shrimp that you did not use.

    Note: if the shrimp in beaker TWO were dead and you had to use the shrimp in beaker ONE, refill both beakers with 2.5-3 large scoops of shrimp and re-incubate.