

University of Washington  
April, 20 2017 IACUC Meeting Minutes

Members Present:	AB	EC	JS
	CG	JB	ML
	CG	JM	MSB
	CH	JPVH	RC
			SH
Members Absent:	KL	NK	SL
	MG	PB	

\*MSB Alternate for AS

\*RC Alternate for TB

\*EC Alternate for JFI

### **Opening Business**

The IACUC Chair called the meeting for order at 2:30pm.

### **Approval of the IACUC Meeting Minutes**

The IACUC Chair called for the approval of the March 16, 2017 IACUC meeting minutes.

Motion: A motion to approve the minutes as written was made and seconded.

Discussion: None

Vote on the Motion: The meeting minutes were approved with 13 members voting in favor and 5 abstentions.

### **The Benefits of Research – JS**

This month's benefit story comes from the Rubel and Raible labs [Stawicki, Esterberg, Hailey, Raible & Rubel, 'Using the zebrafish lateral line to uncover novel mechanisms of action and prevention in drug-induced hair cell death' *Front Cell Neurosci* 9:46 (2015)]. These two groups have been working collaboratively to figure out why certain antibiotics and cancer treatments cause permanent hearing loss in humans, using tiny zebrafish as a model system. Aminoglycoside antibiotics, such as streptomycin, and platinum-based chemotherapies are important treatment options for a broad range of serious health problems, including infectious diseases and cancer, but an unfortunate side-effect is that they can be toxic to structures in the inner ear—and drug-induced damage can lead to permanent hearing loss, tinnitus and dizziness. To better understand why these therapeutic compounds kill off cells in the ear that are responsible for detecting auditory vibrations in humans, the Raible and Rubel groups are using zebrafish, which have very similar cells embedded in their skin to detect vibrations in the water. The zebrafish cells show similar susceptibility to the toxins as mammalian cells. Simply by introducing the antibiotics or chemotherapy agents into the fish tank water, the researchers can study the underlying mechanisms that are causing the death of these cells. The researchers can also rapidly screen hundreds to thousands of drug compounds for their ability to protect against this damage. These experiments hold great promise for treatments that can protect against hearing loss when patients need to take antibiotics or chemotherapy agents.

## Protocol Review

- Dr. Leache, 4367-02 “Fence Lizard Adaptation”

MSB summarized the protocol and the reason it was being called for a Full Committee Review.

We have a full committee review of a new protocol from Dr. Adam Leache’s group, The fence lizard project. I will provide a basic overview of the project and the reasons for it being FCR.

The aims of this project are to determine the relative contributions of genetics and plasticity to lizard phenotypes. Individual phenotypes of organisms in nature are a product of both the environment and genetics. The study is to determine how much plasticity exists in the fence lizard that could predict how well the lizards adapt to change in their environment including climate change.

Wild-caught Adult Gravid lizards will be caught in Yosemite National Park. Up to 40 gravid female lizards will be collected and temporarily housed in the Sierra Nevada Research Station/Yosemite Field Station. Lizards will be caught at both high and low elevation sites. The goal of this experiment is to obtain the eggs collected from females lizards originating from different elevations and safely transport eggs from CA to WA. They are keeping the option of transporting adult gravid females via overnight carrier from CA to WA, there is the possibility that the eggs could be damaged, if transported the long distance, so transporting the mom lizards here to WA may avoid damage (shaking) of the eggs. The mom lizards will be euthanized after laying the eggs.

Once here at UW the eggs will be hatched. The lab will raise young lizards in lab conditions that are designed to mimic high and low elevation sites in the Sierra Nevada mountains. By simultaneously monitoring traits, such as color, growth, and feeding rates, and keeping track of population and family membership, they aim to understand how differences in lizard traits arise. Plastic, or non-genetic, changes in traits are hypothesized to be important for coping with climate change.

The main reason this is being called for FCR is the use of toe clipping both to identify lizard and to provide tissue/bone samples for DNA analysis. The adult lizards will be toe clipped post-euthanasia, new born will be toe clipped following hatching, they will have up to 400 newborn lizards to track.

Toe-clipping is the standard and the least stressful method for permanently marking reptiles. In general bleeding is non-existent. However if bleeding does occur pressure will be applied to the cut site. Additional antiseptic will be applied to the cut site before reintroducing the lizard to its individual tank. Marked animals will be carefully monitored for obvious distress after the procedure for up to 1 week. Hatchlings are expected to heal completely in less than 1 week.

Running performance, endurance, stress level, and survival are unaffected by toe clipping in lizards, but climbing ability might be negatively impacted. These lizards cannot be released back into the environment as such any possible side-effect of climbing ability is moot. A long-term mark-recapture study of Australian geckos and skinks concluded that neither juvenile nor adult survival, recapture probability, stress level, or performance were negatively impacted by toe clipping. However, the climbing ability of arboreal lizards appears to be affected by toe clipping. Marking lizards by toe clipping is permanent and practical, as it can be used to identify large numbers of individuals without

University of Washington  
April, 20 2017 IACUC Meeting Minutes

extensive training of the researcher performing the procedure or healing time of the lizard. The protocol provides the specific citation for the publication.

Alternatives to toe clipping include Passive Integrated Transponder (PIT) tag implantation, Visible Implant Elastomer Tags (VIE), and other implants, surgical attachment of colored beads at the base of the tail, tattooing/branding, and photo identification. Surgical implantation of PIT tags and attachment of colored beads would severely impact the survival of hatchling lizards. Both of these methods should only be used in lizards > 40 mm snout-vent length (SVL). Effective implementation of visual implant elastomers requires an experienced researcher's, otherwise the misidentification rates of individuals are high.

Branding/tattooing reptiles does not appear to be an effective means of identification, as the brands take months to heal and rapidly fade through skin shedding. Photo identification is non-invasive, though for hundreds of juvenile lizards it is infeasible. Color patterns of juveniles could change dramatically in a few weeks and identifying an individual would require processing and comparing hundreds of photos. Another non-invasive method would be to paint the dorsal surface with a unique pattern of colored lines or dots. However, juveniles undergo rapid growth in the first few months of life and this would require repeated handling to reapply the unique marker. Repeated handling outside of momentary weighing's or measurements would increase stress and discomfort and negatively affect an experiment whose goal is to track growth in an approximately natural setting.

The benefits of using toe-clipping far outweigh the costs. Metadata will be collected to link mothers to hatchlings, and ultimately the metadata will be inducted into the online museum database Arctos. However, it will be impossible to physically identify mothers and hatchlings without an external mark. By taking toe clips from the adult females (post-euthanasia), the lab would be able to physically link mothers and hatchlings using the code and to determine the age of the mothers.

We have a (2) member(s) of the lab, available to address any concerns that the IACUC may have and at this point I turn it over to the IACUC to ask any questions they may have.

Discussion: The Committee and group members discussed at length the rationale for toe clipping as a method of identification as well as a number of possible alternatives for identification.

Motion: A motion was made and seconded to approve the Significant Change as written.

The Committee requested the group be asked to document their method for toe clipping and sterilization as described by the lab members during discussion. Additionally, the Committee asked the group to consider a pilot study in parallel to their current study to determine if identification by painting the toes is a viable method.

Vote on the Motion: The protocol was approved with 10 members voting in favor and 3 members opposed.

- Dr. Kwon, 4306-01, Significant Change "Neuromuscular Regulation of Bone in Zebrafish"

MSB summarized the significant change and the reason it was being called for a Full Committee Review.

University of Washington  
April, 20 2017 IACUC Meeting Minutes

We have a full committee review of an amendment to Dr. Kwon's IACUC protocol, Neuromuscular Regulation of Bone in Zebrafish. I will provide a basic overview of the project and the reasons for it being FCR.

This project studies Neuromuscular dysfunction as it is associated with impaired osteogenesis. The lab exploits the experimental flexibility of zebrafish to identify novel pathways mediating osteogenesis.

In this amendment they are employing *in vivo* chemical screening strategies in the regenerating zebrafish fin to identify novel chemical modulators of bone growth and mineralization. In this change, we seek to increase the efficiency of our assay by performing pilot experiments to explore the potential to do the following:

- 1) to increase our time of dosing to up to 4 hours per day
- 2) to repeatedly use the same volume of water/drug in the dosing tray -- reusing the same water/drug solution that was exposed to fish in previous days -- for up to 7 days, with the cumulative time of dosing no more than 4 hours for a single water/drug volume.
- 3) to withhold feeding for up to 7 days at a time while fish are on experiment

The overall goal of this change is to increase the efficiency of our screening assay. The specific rationale for each of our objectives is:

- 1) to increase our time of dosing to up to 4 hours per day

We are currently approved to dose for 2 hours per day. Increasing to 4 hours per day would potentially allow for greater drug accumulation in the fish, for the same amount of drug concentration in the water.

- 2) to repeatedly use the same volume of water/drug in the dosing tray -- reusing the same water/drug solution that was exposed to fish in previous days -- for up to 7 days, with the cumulative time of dosing no more than 4 hours for a single water/drug volume.

By reusing the same volume of water/drug for either multiple fish (dosed one a time), or the same fish (dosed over several days), we will be able to conserve drug quantities.

- 3) to withhold feeding for up to 7 days at a time while fish are on experiment

Our rationale for this change is twofold. First, we believe that the most likely adverse effect of reusing water/drug solutions during dosing is the accumulation of unionized ammonia. In this case, we believe that withholding food may reduce ammonia exposure. Second, there is significant variability in the amount of food eaten by individual fish, and we believe that this may be a confounding variable that increases inter-individual variability in regrowth. By reducing this variability, we can reduce the number of fish that we need to attain sufficient statistical power.

The reason for the FCR is due withholding food for 7 days in experiment 004. Osteoactive Compound Screening in the Regenerating Zebrafish Fin.

Post distribution a minor edit was made to specify the maximum number of fish this fasting would apply to where the fasting is first mentioned in the experimental description. Specifically stating in bold "**no more than 25 fish will be fasted up to 7 days**". It is and was specified later in the description when they supply the specific of the experimental design.

Two members of the lab were present to address any concerns that the IACUC may have.

University of Washington  
April, 20 2017 IACUC Meeting Minutes

Motion: A motion to approve the Significant Change was made and seconded.

Discussion: The Committee discussed with the group the implication of fasting the fish, the possibility of providing some food and the rationale for fasting them and whether or not the drug concentration would change over the 7-day period.

Motion: A motion to approve the Significant Change was made and seconded.

Vote on the Motion: The Significant Change was approved with 12 members voting in favor and 1 abstention.

**Hover Board (HB) update.** SS updated the Committee on the following.

1. Follow-up DMR questions. If you are the DMR of an item and you had committee questions, we will send those to the PI for you. If you have follow-up questions to the original questions, after you add them using the 'Reviewer Notes' functionality, you need to 'Request Clarification by DMR' to send the questions to the group otherwise the group doesn't receive the questions and have the ability to edit the protocol. This is because once you are assigned as the DMR, the item only shows up in your inbox and it becomes hard for our office to track.
2. Ancillary review by subject matter expert: Sometimes you might be assigned as an Ancillary Reviewer of an item in HoverBoard. When this happens there will be an explanation as to what it is we are asking of you. Usually someone is hoping that as an expert in a specific field can weigh in on the item in question and shed some light on a specific part of the submission. You can mention that SL (Steve) just went through this with an amendment and it seemed to work just fine. Ask him to talk a little about it.

**OAW Director's Report** - Refer to meeting folder

**Adverse event(s):**

The IACUC reviewed and discussed a non-compliance that occurred on protocol 4283-01. It was explained that a graduate student performed a tail snip for genotyping outside of the normal age range allowed on the protocol. This occurred because she was used to performing tail bleeds for the malaria studies and she got confused about the age limitations for tail clipping and is deeply apologetic for the incident.

The IACUC Rodent Genotyping and Analgesic policies state that if mice older than 28 days are tailed, the use of anesthesia and analgesia is expected. The protocol was not approved for tailing of mice older than 28 days.

The veterinary staff checked the mice after this occurred and reported that all of the mice appeared to be in good clinical condition and their tails appeared to be healing well with no obvious bone exposure or signs of inflammation. The mice did not appear painful on palpation of the tails and as it was a small amount of tail removed, the mice were not treated with analgesics as they appeared stable and non-painful.

Motion: The Committee voted to send the PI a letter of counsel to remind her that all animal work must be done in accordance with her approved IACUC protocol. The Committee requested that she reply to the

University of Washington  
April, 20 2017 IACUC Meeting Minutes

letter indicating the steps that she will take to prevent future occurrences of this nature. This event was reported to OLAW.

Discussion: The Committee discussed the situation and the fact that the individual fully understood that a mistake was made and felt very badly about it. The PI met with group, and reached out immediately to OAW when the issue was identified.

Vote on the Motion: The motion was approved with 11 in favor and 1 abstention. CG was not present for the vote.

The Committee felt that the veterinary technician who was providing care for other animals in the room should be commended for discovering the inappropriate tailing procedure very quickly and speaking up.

Motion: A motion was made and seconded to send the veterinary technician a letter of commendation.

Vote on the Motion: The motion was approved unanimously with 12 members voting in favor.

### **Update on Prior Non-Compliance**

STI reminded the IACUC of the non-compliance reported at the March IACUC meeting involving the tailing of mice that were older than 28 days of age. The PI replied to the letter of counsel as required and explained that following the occurrence a group meeting was held to discuss the issue and to reiterate the rules in place for this procedure and its appropriate performance. All individuals were reminded of the age restriction for performing this procedure. Re-instruction was also given on how to perform tail biopsy in a manner that is minimally distressful and with minimal bleeding. In addition, Miracle Care Kwik Stop Styptic Powder is provided to promote clotting and prevent bleeding following biopsy. To ensure that the appropriate age is designated for weanling mice to prevent errant tail biopsy beyond 28 days, bi-weekly assessment of breeder cages is performed to accurately record date of birth. This information is entered into a shared google doc with a designated wean-by and tag and tail-by date. In addition the individual responsible for the previous action is no longer employed by the lab. The PI thanked the Committee for bringing this issue to his attention and assured the Committee that the occurrence was dealt with swiftly and with the utmost care.

### **Attending Veterinarian's Report - ML**

#### **Facility issues:**

**Humidity:** Low humidity is still a problem in many facilities.

**Temperature and lights:** 3/20/17: Lighting problem in one rodent animal facility where due to a power failure, the lights did not come on as they should have. This was detected by the animal technicians, service was contacted and the problem was fixed.

#### **Protocol Monitoring:**

There are 14 active protocols on the veterinary monitoring program. At this time they are all going well.

University of Washington  
April, 20 2017 IACUC Meeting Minutes

**Adverse Events:**

On 4/1/17 a cage of three mice in a centralized facility was found without a water bottle. Two of the mice in the cage were found dead, the third was dehydrated though recovered with supportive care. These mice were not on study and the group says they had not handled that cage in about two weeks. Husbandry had last handled the cage on 3/24 to change the cage tops and had not since. Necropsy was not performed due to the significant degree of autolysis. While the cause of death cannot be known definitively without a necropsy, dehydration is the most likely cause. This event was reported to OLAW.

Discussion: The Committee discussed the frequency of monitoring and how this could have occurred.

**HBA Subcommittee:** - JS

The HBA Committee met this month. The topics of discussion were clinical and experimental issues of morbidity or mortality, a discussion of a non-human primate protocol that would like to increase the number of laparoscopic surgeries from 5 to 6. The decision of the committee was to refer that amendment to the full committee for discussion. We did discuss trying to establish a limit as to the number of surgeries that were appropriate for any given protocol, but a number was not determined. I would like to thank everyone for their attendance.

**SOPs/Policies/Guidelines**

- B.1071 Environmental Enrichment for Rats – ML summarized the changes.

Motion: A motion was made and seconded to approve the SOP.

Discussion: None

Vote on the Motion: The SOP was approved unanimously with 12 members voting in favor.

- Environmental Enrichment for Acomys (Spiny Mice) – ML summarized the SOP.

Discussion: EC noted a typo in part A, Section II, which said *provided* instead of *proved*.

Motion: A motion was made and second to approve with the typo corrected

Vote on the Motion: The SOP was approved unanimously with 12 members voting in favor.

**Cage Height Exemption for Infant NHP** – CH summarized the exemption and explained why it was necessary.

Motion: A motion to approve the exemption was made and seconded.

Discussion: None

Vote on the Motion: The exemption was approved unanimously with 12 members voting in favor.

University of Washington  
April, 20 2017 IACUC Meeting Minutes

**Closing Business:**

The Meeting was brought to a close at 4:37 pm. The floor was opened to public comment.