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**\*\*\* Procedures \*\*\***

**Surgery**

Resources: IACUC Website | OAR Website

1. Procedure Type: Surgery
2. Brief Description: Axolotl Limb Amputation
3. Species: axolotl (Sherman Fairchild)
4. USDA Pain/Distress Category: D
5. Approximate number of animals to be used in this procedure at this location: 6
6. Building or Facility: Sherman Fairchild
7. Room Number: SF B23
8. Please click "save" and then address all of the questions for each tab that will appear below. Incomplete or missing details will prevent the protocol from being submitted to the IACUC for review.

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**\*\*\* Surgery Info \*\*\***

**Multiple Survival Surgery Information**

**Note:** One Survival surgical procedure followed by a non-survival procedure, regardless of types (major or minor), need not be included in this section.

1. Surgery Type: T-Terminal  
NOTE: A major operative procedure is any surgical intervention that penetrates and exposes a body cavity or produces substantial impairment of physical or physiological functions.
2. Will this project include Multiple Survival Surgery? N  
If "yes", identify the surgical procedures, the time between them, and describe the criteria used to determine the potential impact on the animals' well-being.
3. Are there Multiple Major Survival Surgeries(MMSS)? N  
Multiple Major Survival Surgery Justification:  
Describe why it is necessary to perform multiple major surgical procedures on the same animal.

**\*\*\* Procedure Description \*\*\***

**Procedure Description**

**1. Detailed Procedure Description**

This procedure will be performed to film a methods portion of a JOVE manuscript.

All procedures will be performed using modified aseptic technique. In this case, animals will be placed in new containers with fresh water post-procedure. All tools will be cleaned autoclaved prior to use and we will use a hot bead sterilizer in between procedures. Animals will be anesthetized by placing them in a 0.1% Tricaine solution. Full anesthetization will be assessed by pinching the tail with forceps. An animal will be placed on its side in a Petri dish under the dissecting microscope. The amputation should take approximately 30 seconds, during this time the animal is constantly washed with 0.1% Tricaine solution to keep it moist. The limb will be held with forceps and surgical scissors will be used to cut through the limb. The bone will be trimmed by holding the limb and pushing back the muscle surrounding the remaining bone to expose the end of the bone. Curved iridectomy scissors will be used to cut off the end of the bone. (Note: If the bone is not trimmed, the muscle contracts around the remaining bone, which then protrudes from the healing limb and retards regeneration due to disruption of the wound epidermis.) Fine iridectomy scissors will be used to cut the epidermis around the limb circumference so that a flat area of internal tissues is exposed. Without this step, the mature skin can heal together and limit the surface area from which a blastema may form. The animal is then placed back into clean water.

This procedure does not require analgesic care afterwards. Previous literature has suggested that newts and salamanders secrete high levels of beta endorphins after amputation and throughout the duration of limb regeneration (Vethamany-Globus et al. 1983).

**2. Please list and describe any clinical effects or changes from the normal health and behavior of an untreated animal which may occur as a result of this procedure.**

All animal went through this procedure will be used for the skin flap surgery.  
This is a terminal procedure.

**3. Describe post procedure monitoring, observation schedules, and treatment that will be performed.**

All animal went through this procedure will be used for the skin flap surgery.  
This is a terminal procedure.

**4. What criteria will be used to determine if animals exhibiting clinical or behavioral changes should be euthanized? (see IACUC website for guidance regarding body condition scoring and human endpoints)**

All animal went through this procedure will be used for the skin flap surgery.  
This is a terminal procedure.

**\*\*\* Personnel Details \*\*\***

**Personnel Details**

**Personnel Details**

**Personnel Name**

Tsai, Stephanie

**\*\*\* Anesthetic Regimen \*\*\***

**Anesthetic Regimen**

**1. Parameters used to monitor and ensure appropriate anesthetic depth.**

Animals will be anesthetized by placing them in 0.1% Tricaine. Full anesthetization will be assessed by pinching the tail with forceps.

**Anesthetic Agents**

Agent Name	Dosage (in mg/kg if possible) AND Volume of Administration (when applicable)	Route
Tricaine	.1%	Immersion (IMM)

**\*\*\* Perioperative Care \*\*\***

**Perioperative Care**

**Post-operative Monitoring**

**Note:** A minimum of 24 hours of post-operative analgesia must be provided for minor surgical procedures and a minimum of 48 hours of post-operative analgesia must be provided for major operative procedures. All animals must be monitored for 96 hours (4 DAYS) following surgery regardless of when analgesic administration ceased.

1. Is the surgical model a USDA-covered species?      N
- 1.a. Recovery Location - Facility or Building Name
- 1.b. Room Number
2. What parameters are monitored to assess recovery?
3. Recovery – What is the duration and frequency of the monitoring for recovery from anesthesia?
4. Post-recovery – What is the duration and frequency of the monitoring following recovery from anesthesia?

**\*\*\* Other Drugs Utilized \*\*\***

**Other Drugs Utilized**

Please list all drugs used in THIS procedure that have not already been individually listed.

Do NOT relist: anesthetics, analgesics, paralytics, or antibiotics/antimicrobials

DO list: fluids, anticoagulants, sedation and anesthetic reversal drugs, continuing antibiotics, and disease maintenance drugs such as insulin or glucose.

**Surgery**

Resources: IACUC Website | OAR Website

1. Procedure Type: Surgery
2. Brief Description: Full skin flap surgery
3. Species: axolotl (Sherman Fairchild)
4. USDA Pain/Distress Category: D
5. Approximate number of animals to be used in this procedure at this location: 6
6. Building or Facility: Sherman Fairchild
7. Room Number: SF B23
8. Please click "save" and then address all of the questions for each tab that will appear below. Incomplete or missing details will prevent the protocol from being submitted to the IACUC for review.

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**\* \* \* Surgery Info \* \* \***

**Multiple Survival Surgery Information**

**Note:** One Survival surgical procedure followed by a non-survival procedure, regardless of types (major or minor), need not be included in this section.

1. Surgery Type: T-Terminal  
NOTE: A major operative procedure is any surgical intervention that penetrates and exposes a body cavity or produces substantial impairment of physical or physiological functions.
2. Will this project include Multiple Survival Surgery? N  
If "yes", identify the surgical procedures, the time between them, and describe the criteria used to determine the potential impact on the animals' well-being.
3. Are there Multiple Major Survival Surgeries(MMSS)? N  
Multiple Major Survival Surgery Justification:  
Describe why it is necessary to perform multiple major surgical procedures on the same animal.

**\*\*\* Procedure Description \*\*\***

**Procedure Description**

**1. Detailed Procedure Description**

This procedure will be performed to film a methods portion of a JOVE manuscript.

Wound epithelium formation is critical to the initiation of limb regeneration. By preventing wound epithelium formation with this surgery, we can examine the mechanisms underlying the importance of this structure during the early stages of regeneration.

Prior to performing the surgery, the animals will be anesthetized in 0.1% tricaine solution. Once the animals have been checked that they are fully anesthetized, the limb will first be amputated at either the humerus/femur or radius/ulna/tibia/fibula. The skin will then be gently pulled back and the remaining underlying stump tissue will be trimmed back in order to ensure full coverage of the skin over the stump tissue. The skin will then be pulled over the exposed stump tissue and sutured with 4-0 size non-absorbable sutures. The animals will then be kept wrapped in a moist paper towel in a container with antibiotic water (0.5% sulfamerazine) that will be on ice for a total of 1 hour to allow for recovery from the surgery. After the recovery, all animals will be euthanized.

See Mescher et al. 1976 (Effects on adult newt limb regeneration of partial and complete skin flaps over the amputation surface.) for reference.

**2. Please list and describe any clinical effects or changes from the normal health and behavior of an untreated animal which may occur as a result of this procedure.**

This is a terminal procedure.

**3. Describe post procedure monitoring, observation schedules, and treatment that will be performed.**

This is a terminal procedure.

**4. What criteria will be used to determine if animals exhibiting clinical or behavioral changes should be euthanized? (see IACUC website for guidance regarding body condition scoring and human endpoints)**

This is a terminal procedure.

**\*\*\* Personnel Details \*\*\***

**Personnel Details**

**Personnel Details**

**Personnel Name**

Tsai, Stephanie

**\*\*\* Anesthetic Regimen \*\*\***

**Anesthetic Regimen**

**1. Parameters used to monitor and ensure appropriate anesthetic depth.**

Adult animals will be anesthetized in 0.1% tricaine solution for 15-20 minutes or until they no longer respond to physical stimuli such as a tail pinch.

**Anesthetic Agents**

Agent Name	Dosage (in mg/kg if possible) AND Volume of Administration (when applicable)	Route
Tricaine methanesulfonate (MS-222) (buffered)	0.1% tricaine	Immersion (IMM)

**\*\*\* Perioperative Care \*\*\***

**Perioperative Care**

**Antibiotics or Anti-Microbials**

Agent Name	Dosage and Volume	Route	Duration and Frequency of Administration
Sulfamerazine sodium salt	0.5% sulfamerazine	Immersion (IMM)	For 1 hour after the surgery has taken place during recovery time.

**Post-operative Monitoring**

**Note:** A minimum of 24 hours of post-operative analgesia must be provided for minor surgical procedures and a minimum of 48 hours of post-operative analgesia must be provided for major operative procedures. All animals must be monitored for 96 hours (4 DAYS) following surgery regardless of when analgesic administration ceased.

1. Is the surgical model a USDA-covered species? N

1.a. Recovery Location - Facility or Building Name

1.b. Room Number

2. What parameters are monitored to assess recovery?

3. Recovery – What is the duration and frequency of the monitoring for recovery from anesthesia?

4. Post-recovery – What is the duration and frequency of the monitoring following recovery from anesthesia?