UF myIACUC

Section: 1. Study Identification Information

Date: 12/18/2019, 4:12:28 PM Print

Reviewer Notes

Filter by Type Go Clear Advanced

Type	Reviewer	Modified
Public Note Please note that all listed team members have completed and passed their Pain Management in Laboratory Animals AALAS course. I have attempted to address all reviewers comments.	Jamie Foster	12/11/2019 10:59 AM
Public Note The grant and protocol correlate. The grant hold is removed.	Alan Eldred	11/18/2019 3:30 PM
Public Note This protocol is on grant hold until it can be correlated.	Alan Eldred	10/18/2019 1:36 PM
Coor **IACUC Change Request** Jamie Foster - Change Request Completed - 11/27/2019 12:46 PM A risk assessment form was completed by the PI and all team members listed in the IACUC protocol on 10/7/19. As of 11/27/19, however, completed risk assessment does not appear in Gator Tracs Latch website suggesting it is still in review	Caroline Schomer	10/15/2019 2:55 PM
Public Note Jamie Foster, Natasha Sng, and Madeline Vroom must register with the EH&S Risk Assessment, also known as the Animal Contact Program. Form is located at http://www.ehs.ufl.edu/programs/bio/a...	yaneisy reyes	10/14/2019 1:23 PM

1-5 of 5

1.1 Study Identification Information

1. * **Responsible Faculty (PI):**
   Jamie Foster

2. * **Protocol Type:**
   A clinical study is defined as a study involving privately-owned animals in which the procedures performed have the potential to benefit the animal's health or well-being.
   - (●) **Research Protocol**
   - ( ) Teaching Protocol
   - ( ) Clinical Study Protocol

a. Is this an Observation Only Protocol?	Section: 1. Study Identification Information
Date: 12/18/20 Yes No PM	Print

3. * Cadaver Study: Is this a Cadaver/Tissue Only Protocol?
   Yes No

4. * Previous Study: Is this a triennial, major revision or PI change from a previous Protocol?
   Yes No

5. * Monoclonal Antibody Study: Is this a request to ONLY produce a monoclonal antibody?
   Yes No

6. Study Coordinator:

7. Study Staff:
   Please list the PI and Study Coordinator in this Study Staff question.
   Also note that removing someone from the list below removes them from all other sections.

	Last Name	First Name	UFID	Email	Phone	Animal/Tissue Contact	Study Roles	Experience
View	Foster	Jamie	98663033	jfoster@ufl.edu	321-525-1047	yes	Breeding, Euthanasia, Administrative, Husbandry, Anesthesia, Tissue Harvesting, Imaging (MRI, X-Ray, etc.)	The PI has 27 years of working with the care, husbandry, anesthesia, and surgical dissections of the cephalopod bobtail squid (species Euprymna scolopes). The PI was formerly the lead caretaker and handler for the Euprymna scolopes at the University of Hawaii and University of Southern California and has maintained an Euprymna scolopes breeding colony at the University of Florida Space Life Sciences lab for the past 10 years.
View	Sng	Natasha	01117678	nsng@ufl.edu	857-225-6244	yes	Euthanasia, Husbandry, Anesthesia, Tissue Harvesting, Imaging (MRI, X-Ray, etc.)	Natasha Sng is a postdoctoral fellow and has been trained by the PI Jamie Foster in all aspects of caring for the bobtail squid species Euprymna scolopes.

Section: 1. Study Identification Information

Date: 12/18/2019, 4:12:28 PM Print

	Last Name	First Name	UFID	Email	Phone	Animal/Tissue Contact	Study Roles	Experience
View	Vroom	Madeline	89042177	mmvroom@ufl.edu	321-485-6337	yes	Euthanasia, Husbandry, Anesthesia	Maddie has been trained by the PI in the animal care and husbandry of the bobtail squid.

8. * **Project Identification:** ***If the project is supported by a grant/contract the title provided must match that of the grant. Multiple titles may be listed.***
For Teaching Protocols, include the course number if appropriate.

	Project Title	Funding Source	Newly Funded Research	Grant Number
View	Effects of modeled microgravity on the induction of bacteria-induced apoptosis during animal development	Other	yes	80NSSC18K1465
View	Impact of spaceflight on beneficial animal-microbe interactions	Other	yes	80NSSC19K0138

9. **VA Protocol: Is this a VA Protocol?**
If this is a VA Protocol which was approved by the VA IACUC, and it needs secondary approval from the UF IACUC, and you don't need to use the UF IACUC form, then please answer Yes to question 1.1.9 and upload the VA Protocol to question 1.1.9a.
○ Yes ◉ No

Reviewer Notes

Type	Reviewer	Modified

There are no items to display

0-0 of 0

1.2 Researcher Training Summary

1. **Study Staff Training:**

Training

Jamie S Foster

Course ID	Name	Completed	Course Due
AALAS-03982	Pain Management in Laboratory Animals	11/27/2019	11/22/2039
AALAS-04155	Working with the IACUC	10/5/2019	10/4/2022

Natasha J Sng

Course ID	Name	Completed	Course Due
AALAS-03982	Pain Management in Laboratory Animals	12/1/2019	11/26/2039
AALAS-04155	Working with the IACUC	10/11/2019	10/10/2022

Madeline M Vroom

Course ID	Name	Completed	Course Due
AALAS-03982	Pain Management in Laboratory Animals	12/4/2019	11/29/2039
AALAS-04155	Working with the IACUC	10/9/2019	10/8/2022

Section: 2. Project Overview

Reviewer Notes

Filter by Type Go Clear Advanced

	Type	Reviewer	Modified
Coor	**IACUC Change Request** In section 2.1.2 please clarify euthanasia while staining.	Alan Eldred	12/18/2019 2:19 PM
	Jamie Foster - Change Request Completed - 12/18/2019 3:57 PM I have changed section 2.1.2. to indicate that all paralarvae are anesthetized and euthanized prior to all staining activities.		
Coor	**IACUC Change Request** In section 2.1.2 please discuss husbandry and water quality for the paralarvae.	Alan Eldred	12/18/2019 2:19 PM
	Jamie Foster - Change Request Completed - 12/18/2019 3:59 PM In section 2.1.2, I have expanded on the description of the seawater used for the paralarvae to indicate that it is filtered with a 0.22 um filter to remove background bacteria that may unintentionally alter the development of the host animal.		
Coor	**IACUC Change Request** you indicate "Therefore, as anesthetizing the paralarvae may take up to 5 min to fully incapacitate the animal, for RNAseq experiments no anesthesia will be used as it may alter the RNA sequencing results and interp...	Colette St. Mary	11/19/2019 3:26 PM
	Jamie Foster - Change Request Completed - 11/26/2019 4:30 PM No, placing the paralarvae in RNAlater takes 1-2 seconds to kill the animal. It has been the standard protocol, which I and the Euprymna research community, have been using for the past 10 years for RNASeq experiments. Publications of this protocol can be provided if needed. The most ideal situation would be to flash freeze the paralarvae in liquid nitrogen, but this capability is not available on the International Space Station. RNAlater is the only NASA approved alternative for rapid preservation of the total RNA. Also, as all of our previous RNAseq datasets from a prior NASA grant have been generated this way, we feel this: 1) is the fastest way to terminate the experiment aboard the space station; 2) would correspond to all the previously collected RNAseq datasets on the symbiosis by my lab and the larger Euprymna research community; and 3) correspond to the approved NASA flight experiment procedure.		
Coor	**IACUC Change Request** study 6- more details are needed on all of these to clarify animal experience.	Colette St. Mary	11/19/2019 3:22 PM
	Jamie Foster - Change Request Completed - 11/27/2019 11:15 AM I am not sure what "Study 6" is referring to, but if it has to do with exposure to the reagents and antibodies, then these		

Section: 2. Project Overview

reagents are exposed to the euthanized animal that has been subsequently dissected and fixed.

The one exception is the addition of a caspase inhibitor, which has now been added to specific agents in section 2.2.

Coor **IACUC Change Request** Colette St. Mary 11/19/2019 3:19 PM

Study 4- is this study looking at the effects of microgravity on the establishment of symbiosis? it's not clear from the title or the study description. please clarify.

Jamie Foster - Change Request Completed - 11/26/2019 3:43 PM

I am not sure what "Study 4" means but, in the 4th paragraph of the project overview, the goal of the project is to look at beneficial host-microbe interactions in simulated and natural microgravity.

The experiment is looking at the impact of microgravity on the development of the symbiosis. Previous published work by the PI has shown that microgravity has no effect on the establishment of the symbiosis.

I have, however, added a sentence to section 2.1.1 to indicate we are examining the colonization, persistence, and maintenance of the symbiosis under microgravity conditions.

1-5 of 9

2.1 Project Overview - Research Protocol

1. * **Rationale and significance: (i) Describe in lay, non-technical terms (i.e. as in a newspaper article) that a non-scientist can understand and include the goal(s) and significance that justify animal use as part of the project (ii) Contrast the benefits of the project's expected results on the well-being of humans, animals, or society with the potential adverse events that could have a negative effect on the welfare of experimental animals (iii) The description should be no more than 500 words.**
If more than one grant supports the work in this protocol, describe the Rational and Significance in a sequential manner, first one grant then the second grant and so on.

Formatting Hint: Click the down facing arrow in the top right of the text box below to expand the Formatting Toolbar

For more than 30 years, the Hawaiian bobtail squid has served as a model animal system for studying beneficial relationships with symbiotic microbes. The bobtail squid has a specialized organ called the light organ, which is the site of the symbiosis and houses a beneficial microbe, called *Vibrio fischeri*, which has the ability to luminesce. The squid uses the light from the bacteria to camouflage itself from potential predators in a behavior called counter illumination.

The bobtail squid is an ideal model organism to understand symbiosis for four primary reasons: 1) the symbiosis is binary in that there is only one symbiont and one host, thereby enabling the effects of the bacterium on the host development to be more easily studied; 2) the animals have a very short developmental timeline (~20 days) enabling rapid turnover of experiments; 3) adult female squid will lay approximately 1000-3000 eggs in her lifetime enabling numerous experiments from a minimal number of adult animals; and 4) the paralarvae (i.e., newly hatched juveniles) are small (~3 mm) in size helping to reduce payload weights associated with microgravity experiments.

When the bacteria colonize the juvenile light organ they trigger a normal, healthy developmental remodeling of the squid light organ through activation of the host's innate immune response. Many of the immune and developmental pathways in the bobtail squid exist in mammals, including humans, but unlike vertebrates, the symbiotic association in the bobtail squid light organ is much less complex in that there is only one host and one bacterial species. It is, therefore, much easier to understand the contributions of each partner to the onset of beneficial symbiosis rendering the bobtail squid and its vibrio symbiont a more simplified model system for the study of bacteria-induced animal development.

Specifically, the Foster lab uses the squid-vibrio symbiosis to examine how various environmental stresses, in particular, reduced gravity (or microgravity), impacts the normal healthy symbiotic interactions that occur between microbes and animals. The Foster lab studies these processes during the

colonization, persistence, and maintenance of the symbiosis up to 96 h. To conduct these experiments, the juvenile squid, known as paralarvae, are examined within the first 96 h after they have hatched from their eggs and been exposed to their natural symbiont, *Vibrio fischeri.* In the Foster lab, after exposure to simulated or natural microgravity, the animals are sacrificed to examine the molecular changes that occur to the animal's immune system in the presence and absence of the symbiotic bacteria. As it is important to use model systems to explore how environmental stresses affect the healthy microbiome of animals, the outcomes of this work will be conducted to minimize the negative experience for the paralarvae. As humans continue their exploration of space and missions become longer, using simplified model systems that are amenable to spaceflight will help improve our understanding of how long-duration space travel impacts astronaut health. The lessons learned from these reduced-gravity experiments can provide new insights into how gravity affects animal-microbe interactions here on Earth.

2. Research Study Description

If this is a research study, please provide, in general terms, a step-by-step and/or chronological-order (if appropriate) description of all experiments to be carried out for the next three years (provide a flow-chart if helpful). This section should include: (a) a brief, clear and concise overview of all experimental manipulations and treatments performed on living animals; and (b) a description of the experimental design, treatment groups and appropriate control groups. Details of specific procedures (e.g. surgical procedures, genotyping methods, drug dosages, etc.) are not required in this section if they appear in later sections of the protocol. Define any abbreviations or technical terms.

If this is an Observation Only study, please provide a brief overview of the observation and location(s) of where the study will be carried out.

If this is a triennial update, please provide a brief description of your progress for the last three years. Then describe all experiments to be carried out for the next three years.

Animal breeding colony: Adult animals are collected by the PI from the shallow sand flats around Oahu, Hawaii and shipped to the Space Life Science Lab located in Merritt Island, FL where they are maintained as a breeding colony. The adult animals are maintained in a recirculating aquarium and mated approximately once every two weeks and are not used in any subsequent experimental protocols.

Egg Incubations: The adult females lay egg clutches on 4" PVC pipes cut in half called "caves". The caves are removed from the adult tank and incubated in an aquarium for their 20-day developmental cycle. No additional experiments are done on the squid embryos.

Paralarvae experiments: When the bobtail squid hatch, they are in a juvenile state known as paralarvae. The paralarvae feed off of an internal yolk sack for the first week post-hatching. All experiments conducted in the Foster lab for both the NASA-funded projects examine the early stages of development during the first 96 h post-hatching when the paralarvae are feeding off of the yolk sac.

For all paralarvae experiments, within 30 min of hatching paralarvae are transferred to sterile Pyrex bowls where they are rinsed in seawater that has been filtered using a 0.22 micron filter to remove background bacteria. The water source is the same source for the adult breeding colony (which has the salinity, ammonia, nitrate, and nitrite measured weekly). The paralarvae are rinsed twice in this Filtered SeaWater (FSW) before being moved to the experimental treatment. In the Foster lab, all animals treatments and experiments are conducted on the paralarvae squid in FSW as described below:

1. *Aposymbiotic controls:* For all experiments conducted, a cohort of paralarvae are maintained without symbiotic *Vibrio fischeri* in 5 mL of FSW for all treatments.

2. Inoculation with symbiotic bacteria: Hatchlings used in colonization experiments are transferred to 20 mL scintillation vials containing 5 mL of FSW inoculated with between 1,000-1,000,000 CFU/mL of wild-type *Vibrio fischeri strain ES114* (concentrations depends on the experimental question). Strain *V. fischeri* ES114 was isolated from an adult squid and is not genetically modified. In the wild, *V. fischeri* concentrations range from 500-1000 cells/mL in the water column and 10,000-100,000 cells/mL in the sand in which they bury. Scintillation vials are placed in cardboard racks and loosely covered with shrink-wrap plastic film to limit evaporation. Racks are stored in a Percival growth incubator maintained at a constant 24°C incubation room, so that the hatchlings experience the same light-dark cycle and temperature as they did during development.

3. Monitoring the onset of symbiosis: On a daily basis, or until the experiment is complete, hatchlings are checked for colonization and luminescence. As the symbiont is bioluminescence, once the squid is colonized by the symbiont the squid effectively glows. The scintillation vial containing the 3 mm paralarvae in FSW is put into an ATP photometer (Promega GloMax), and the light produced by the bacteria that have colonized animal's light organ is recorded without harm or stress to the animal.

4. Simulated microgravity experiments: Paralarvae are placed in sterile 50 mL High Aspect Ratio Vessel (HARV) bioreactors, which are used to simulate the low shear environment of microgravity. The squid are added through a 1 cm port in the HARV using a wide-mouth glass pipette. The HARVs are rotated at 13 rpm in either a vertical position (to mimic microgravity) or in a horizontal position (as a gravity control). Between 1 and 5 paralarvae are co-incubated in a single HARV vessel. The Foster lab owns 16 HARVs, therefore the maximum number of animals per simulated microgravity experiments is n = 80 paralarvae. Typically, animals are incubated for between 2 and 96 h in the HARVs then examined for colonization state using the non-invasive ATP photometer and then undergo daily seawater changes. The animals are removed from the

Section: 2. Project Overview

HARVs by unplugging the 1 cm stopper and gently pouring the water (and paralarvae) out of the HARV into a sterile 50 mL beaker. The animals are then transported between vessels using a wide-mouth glass pipette.

After exposure to the simulated microgravity experiments, the animals are anesthetized and euthanized using a 1:1 ratio of 0.37M Magnesium Chloride ($MgCl_2$) and FSW and then undergo one of the following treatments: 1) immediate staining with nucleic acid stain (e.g. Acridine Orange or DAPI) to observe the onset of bacteria-induced development (e.g., apoptosis) using epifluorescence microscopy; 2) Flash freezing in liquid nitrogen to immediately inhibit transcription and translation and to preserve tissue samples for RNASeq and biochemical experiments; or 3) preservation in RNAlater to immediately inhibit transcription and translation and to preserve tissue samples for RNASeq experiments.

5. Spaceflight experiments: The funded NASA spaceflight experiment will use the Techshot Fluid Processing Cassette (FPC) maintained in the ADvanced Space Experiment Processor (ADSEP) (images provided in section 3). Paralarvae will be incubated in 32 mL cell culture bags with a Luer lock. Animals will be added to the bags using a 3-cc syringe. Due to their small size (3 mm), the paralarvae easily pass through the Luer lock syringe without damage. Preliminary experiments have shown that five paralarvae can be co-incubated for up to 72 h with no loss of life. There will be a total of 12 replicate cell culture bags of squid paralarvae for a total of 60 animals for the flight experiment.

After launch, the astronauts will transport ADSEP flight hardware to the ISS Express rack, which will automatically initiate the experiment. The astronauts will have no direct contact with the animals as the ADSEP hardware is fully automated and sealed. A cohort of animals n = 30 (i.e., 3 culture bags) will be inoculated with FSW containing 10,000 cells/mL of the symbiotic *V. fischeri* and incubated for 12 h. The second cohort of animals maintained in three separate culture bags will be inoculated with FSW without bacteria to maintain an aposymbiotic control.

At 2h, 6h, and 12h post-inoculation RNAlater will be automatically injected into the bags automatically to terminate the experiment and euthanize the paralarvae.

At the end of the experiment, astronauts will remove the culture bags containing the preserved paralarvae and store them in the MELFI at -80°C until the tissues can be returned to Earth for RNASeq transcriptome and biochemical analysis.

6. Additional experiments that paralarvae may be exposed after bacterial inoculation or microgravity exposures include:

a) Microscopic imaging of developmental progress (anesthesia used);

b) Incubation of paralarvae with caspase apoptosis inhibitors (i.e., nonhazardous peptides) for up to 24 h and subsequent anesthesia;

c) Chemical reagents and antibodies in FSW on anesthetized animals;

d) Preservation of tissue ultrastructure in 4% paraformaldehyde (anesthesia used); or

e) Rapid preservation of tissues to capture molecular (e.g., transcripts, proteins, metabolites) changes of treatment in RNAlater or liquid nitrogen.

a. Describe all EXPERIMENTAL ENDPOINTS for animals in your study.
Experimental endpoints are the point(s) in the study when you will achieve the results you require - time on experiment, disease state, tumor size or spread, endpoint score, physiologic or behavioral measurement (e.g. groups of animals are euthanized at 1, 3 and 6 months; groups of animals are euthanized after blood glucose exceeds 300mg/dl; groups of animals euthanized after a series of behavioral tests; animals transferred to another protocol; etc).

If this is covered in 2.1.2, just say "see above"
The most common means of euthanasia for cephalopods is an overdose of anesthesia (either ethanol or magnesium chloride ($MgCl_2$)) as per the guidelines for cephalopod research (pg 57).

Fiorito, G. et al. (2015) Guidelines for the care and welfare of cephalopods in research – a consensus based on an initiative by CephRes, FELASA and the Boyd group. *Laboratory Animals* 49(52) 1-90.

Adult animals in the breeding colony: All adults (both male and female) are allowed to live out their natural lives in the breeding colony. If, however, an animal is severely incapacitated and exhibits severe distress and senescence the animal will be euthanized. The observed behaviors for severely distressed or senescent bobtail squid include not feeding, significant whitening of skin (i.e., no longer able to control chromatophores), or the inability to control movement and right itself. At this point, adult animals are removed from the aquarium and euthanized by an overdose of anesthesia incubated in seawater containing increasing concentrations up to 2% ethanol on ice for 1 h or until no evidence of life exists. The tissues of euthanized adult animals are then disposed of as biological waste at the Space Life Science Lab.

Paralarvae: Juvenile paralarvae used in both microgravity and gravity (i.e., ground-based) experiments will have a range of termination points between 0 and 96 h post-hatching. Unless otherwise indicated, experiments with paralarvae are conducted in Filtered Sea Water (FSW).

Paralarvae incubated in vials and HARV bioreactors in the presence or absence of symbiotic bacteria are terminated between 0 and 96 h post-hatching and is dependent on the experimental question. At the end of an experimental incubation, the paralarvae are either:

Section: 2. Project Overview

1) anesthetized with a 1:1 ratio of FSW and 0.37 M Magnesium Chloride ($MgCl_2$) for 5 min. Depending on the final experimental question, the intact animal is then incubated in fixative for downstream microscopic examination or immediately dissected to remove the outer mantle and funnel to expose the light organ and then visualized with epifluorescent or confocal microscopy. All tissues from these experiments are disposed of as biological waste at the Space Life Science Lab;

2) For paralarvae preserved for transcriptome, proteome and metabolome research, the animals will be incubated in either HARV bioreactors or in the ADSEP spaceflight hardware and at the end of the experiment between 0 and 96 h the paralarvae will be immediately flash-frozen in liquid nitrogen or immediately preserved in RNAlater.

One of the key research questions examined by my funded NASA Space Biology proposals is to understand the impact that spaceflight has on host-microbe interactions over time. A key methodology to examine the molecular mechanisms in which animals respond to spaceflight is RNASeq. RNAseq examines all of the expressed genes at a given moment in time by sequencing mRNA transcripts.

It has been recently published in 2017 that the half-life of mRNA molecules (i.e., expressed gene transcript) is two minutes. That means potentially half of the transcripts will be degraded or turned over within 2 min of the end of a particular treatment. The citation for this recent publication, which used several different methods to assess mRNA turnover in eukaryotes, is listed below:

Baudrimont, Antoine, et al. "Multiplexed gene control reveals rapid mRNA turnover." *Science advances* 3.7 (2017): e1700006.

Therefore, as anesthetizing the paralarvae may take up to 5 min to fully incapacitate the animal, for RNAseq experiments no anesthesia will be used as it may alter the RNA sequencing results and interpretation of the effects of spaceflight on bacteria-induced animal development.

b. **Animals showing any of the following signs/symptoms will require veterinary interventions, euthanasia or scientific justification to remain on the protocol. The following are approved Humane Endpoints that should be followed.**
The Humane Endpoint is the point at which pain/distress is terminated, minimized or reduced by taking actions such as euthanizing the animal, giving the animal treatment to relieve pain and/or distress, or terminating a painful procedure.

Click here for the Body Condition Score Guideline

- **>= 15% weight loss from baseline weight or age-matched controls if the animals are still growing during the study**
- **Body Condition Score of 2 or less for rodents**
- **Inability to reach food or water**
- **Impaired mobility beyond experimental endpoints (impaired mobility requires a scientific justification and a description of the mobility endpoints for the protocol in section 2.1.2a)**
- **Tumors (spontaneous or induced for the study): Any tumor that become ulcerated or are over 1.5cm in mice or 2.5cm in rats, measured in any one dimension or a cumulative measurement**
- **Labored breathing, respiratory distress or cyanosis (blue tinged color)**
- **Tremors, convulsions or seizures lasting more than 1 minute or occurring more than once a day**
- **Dehydration lasting over 24 hours that is unresponsive to treatment**
- **Moribund - unable to right itself**

Any exception to the above listed Humane Endpoints or any additional Humane Endpoints should be described here:
no exceptions

3. **Upload multiple documents or charts that help explain the project. For complex experimental designs it is recommended that you upload a detailed scientific description such as a flow chart, diagram or table which depicts the experiments, sequence of events, time lines and numbers of animals in each group.**

Name	Modified	Version
Section 2.3_ charts and figures.pdf	10/8/2019 2:35 PM	0.01

Section: 2. Project Overview

Reviewer Notes

Filter by Type Go Clear Advanced

	Type	Reviewer	Modified
Coor	**IACUC Change Request**	Karl Andrutis	10/16/2019 8:20 AM

Please add all agents to which live animals will be exposed: RNAlater, LN2, etc.

Jamie Foster - Change Request Completed - 11/26/2019 3:58 PM

I have added liquid nitrogen and RNAlater to the list.

As I used them as a method of termination, I didn't realize they were considered a medical agent.

1-1 of 1

2.2 Project Overview

Listed Medical Agents/Drugs

1. **Please use the Add or Update buttons below to list all substances administered to animals as part of the experimental protocol. Be sure to answer the required pharmaceutical grade question for each listed substance.**
   List Medical Agents/Drugs you plan to use on this study. If none are used, Continue.
   Do not include Biological Agents (viruses, cells, recombinant DNA, bacteria, etc). These should be included in section 21.
   Also, do not include antigens for antibody production. These should be included in sections 8 and/or 9.
   (NOTE: Specific sections used will be summarized in section 24.2).

	Agent Name	Agent Is Pharmaceutical Grade	# Sections Used
View	Caspase Inhibitors - peptides	yes	0
View	Ethanol	no	0
View	Liquid Nitrogen	no	0
View	Magnesium Chloride	no	2
View	RNAlater	no	0
View	Vibrio fischeri - wild-type	no	1

2. **If you are using an anesthetic gas how will it be scavenged?**

   I will use one of the following approved methods:

   - **Use of a chemical fume hood**
   - **Use of the gas scavenging canisters as outlined below:**
     - **Gas scavenging canisters will be positioned to ensure the exhaust ports at the bottom of the canister are not blocked or occluded in any way.**
     - **At installation, and immediately after each use, the gas scavenging canister will be removed and weighed to evaluate the remaining absorption capacity. The weight will be recorded and dated on the side of the canister. Canisters that exceed 30 grams (F/Air) or 100 grams (Enviro-Pure) of total accumulated weight must be removed and placed in a sealed plastic prior to disposal in regular trash.**
     - **The induction chamber will be thoroughly cleaned immediately after each use to avoid residual waste anesthetic gas being released into the facility.**

   If another method is being used please specify:
   Anesthetic gas will not be used.

Section: 3. Animal Species and Subject Information

Reviewer Notes

Filter by Type Go Clear Advanced

Type	Reviewer	Modified
Public Note	Colette St. Mary	11/19/2019 3:28 PM
I'll just say for the record that using Cat C for breeders that will be euthanized would appear to make a breeder category completely obsolete. Should these animals be Cat B?		
IACUC Change Request	Karl Andrutis	10/16/2019 8:26 AM
Please change the adult squid used for breeding to Category C since they will eventually be euthanized.		

Jamie Foster - Change Request Completed - 11/26/2019 4:26 PM

I have changed to Cat C, but can change to Cat B in response to the additional reviewer's comments.

Typically, the breeders die naturally of old age.

1-2 of 2

3.1 Animal Species

1. *

	Species	Genus	Strain/Breed	Mutant	Sex	Age/Size/Wt	Special Conditions	Reused	Animal Location	Scientific Justification	Permit#	Pain Levels & Animal#
View	Other	Euprymna scolopes			Both	Adult/ 4 cm/ 28 g			Other	For 30 years, the bobtail squid has served as a model organism to understand symbiosis for four primary reasons: 1) the symbiosis is binary in that there is only one symbiont and one host, thereby enabling the effects of the bacterium on the host development to be more easily studied; 2) the animals have a very short developmental timeline (~20 days) enabling rapid turnover of experiments; 3) adult female squid will lay approximately 1000-3000 eggs in her lifetime enabling numerous experiments from a minimal number of animals; and 4) the paralarvae (i.e., newly hatched juveniles) are small (~3 mm) in size helping to reduce weights associated with spaceflight.	not a protected species no permits are required to catch squid	C 45

Total bobtail squid : 36045

All Category C : 45

All Category D : 35840

All Category E : 160

Section: 3. Animal Species and Subject Information

	Species	Genus	Strain/Breed	Mutant	Sex	Age/Size/Wt	Special Conditions	Reused	Animal Location	Scientific Justification	Permit#	Pain Levels & Animal#
View	Other	Euprymna scolopes			Both	paralarvae/ 3 mm/ 1 mg			Other	For 30 years, the bobtail squid has served as a model organism to understand symbiosis for four primary reasons: 1) the symbiosis is binary in that there is only one symbiont and one host, thereby enabling the effects of the bacterium on the host development to be more easily studied; 2) the animals have a very short developmental timeline (~20 days) enabling rapid turnover of experiments; 3) adult female squid will lay approximately 1000-3000 eggs in her lifetime enabling numerous experiments from a minimal number of animals; and 4) the paralarvae (i.e., newly hatched juveniles) are small (~3 mm) in size helping to reduce weights associated with spaceflight.	not a protected species	D 35840 E 160

Total bobtail squid : 36045

All Category C : 45
All Category D : 35840
All Category E : 160

2. Please provide a <u>scientific justification</u> for withholding anesthesia, analgesia, treatment, or timely intervening euthanasia for unalleviated pain and/or distress - not just pain:
During spaceflight and simulated microgravity experiments that require RNAseq analysis (i.e., monitoring the effects of the treatment on the transcriptome) the paralarvae will be placed immediately in liquid nitrogen or RNAlater. No anesthesia will be provided for these cohorts of

paralarvae. Although pain is likely to occur, the process would take only 1-3 seconds to euthanize the paralarvae by direct submersion in liquid nitrogen or RNAlater.

One of the key research questions examined by my funded NASA Space Biology proposals is to understand the impact that spaceflight has on host-microbe interactions over time. A key methodology to examine the molecular mechanisms in which animals respond to spaceflight is RNASeq. RNAseq examines all of the expressed genes at a given moment in time by sequencing mRNA transcripts.

It has been recently published in 2017 that the half-life of mRNA molecules (i.e., expressed gene transcript) is two minutes. That means potentially half of the transcripts will be degraded or turned over within 2 min of the end of a particular treatment. The citation for this recent publication, which used several different methods to assess mRNA turnover in eukaryotes, is listed below:

Baudrimont, Antoine, et al. "Multiplexed gene control reveals rapid mRNA turnover." *Science advances* 3.7 (2017): e1700006.

Therefore, as anesthetizing the paralarvae may take up to 5 min to fully incapacitate the animal, we are requesting that for RNAseq experiments only no anesthesia be used as it may alter the RNA sequencing results and interpretation of the effects of spaceflight.

Reviewer Notes

Filter by Type Go Clear Advanced

	Type	Reviewer	Modified
Coor	**IACUC Change Request**	Colette St. Mary	11/19/2019 3:36 PM

for the RNA studies just do the final math for us to say how many total animals this study will need. thanks!

Jamie Foster - Change Request Completed - 11/27/2019 11:13 AM

I have revised that section and just listed the number we will need to complete the spaceflight RNAseq experiments.

1-1 of 1

3.2 Animal Species - Animal Number Justification

1. **Justification of your animal numbers, for research submissions:**
   For Research Protocols, justify and provide assurance that you are not asking for too many or too few animals for your study. If animals are needed for training indicate those numbers. Sufficient detail must be provided so that the exact number of animals requested can easily be understood by the IACUC. Unless already described in section 2.1.2, explain the animal groupings and the number per group so that actual numbers in the experiment can be justified.

   A power analysis or other statistical program may be used to justify the numbers and should include the method of analysis, measurement of variance, and magnitude of effect size deemed important to detect. If statistical power analysis is not applicable, you may indicate numbers are based on experience or information obtained from similar studies in the literature (a reference should be included).
   Typically, we maintain 8-12 female squid at a given time, as not all of the females will lay eggs. When we collect animals in the wild we do not know the age of the animals and therefore, we try to collect cohorts of between 8-12 females and 1-3 males each collection trip to try and ensure that we collect a few females that are of reproductive age (~ 3-6 months old). Females older than 6 months typically no longer lay viable eggs. We typically collect animals twice a year to maintain an active breeding colony throughout the year.

   For experiments involving hatchling squid, also known as paralarvae, a typical simulated microgravity experiment uses 30-80 hatchlings per technical replicate; therefore, our maximum egg production would generate 12,000 hatchlings each year enabling approximately 200 experiments annually. If excess paralarvae are generated they are exposed to simulated microgravity and cached as a tissue repository.

   The number of biological and technical replicates used in this project will be partially constrained by logistics associated with working in the space environment. As a result of the flight and ground experiments, sample size will be limited to 10 biological replicate animals spread across two technical replicates for each of the treatments. In the spaceflight experiment, six of the animals for each treatment will be used for RNA-Seq and four animals used for metabolomics. Previous research in the Foster lab has indicated that these sample sizes can generate robust statistical analyses using the proposed techniques described in the proposal and in previously funded projects (e.g. Foster et al., 2013; Heath-Heckman et al., 2016; Casaburi et al., 2017).

   Foster, JS, CLM Khodadad, SR Ahrendt and ML Parrish (2013) Impact of simulated microgravity on the normal developmental timeline of an animal-bacteria symbiosis. Scientific Reports 3:1340.

   Heath-Heckman, EA, JS Foster, MA Apicella, WE Goldman, MJ McFall-Ngai (2016) Environmental cues and symbiont MAMPs function in concert to drive the daily remodeling of the crypt-cell brush border of the Euprymna scolopes light organ. Cellular microbiology. doi: 10.1111/cmi.12602

   Casaburi, G, I Goncharenko-Foster, AA Duscher and JS Foster (2017) Transcriptomic changes in an animal-bacterial symbiosis under modeled microgravity conditions. Scientific Reports 7:46318

RNA-Seq – To enable enough biological and technical replicates we try to have a minimum of 3-6 paralarvae per biological replicate for accumulate enough RNA for sequencing analysis. Therefore, we estimate needing 60 paralarvae for the spaceflight experiment.

Section 3 Animal Species and Subject Information

2. Justification of your animal numbers, for wildlife submissions:
For wildlife and fisheries the following may be listed: "This is an Observational/tag and release/survey study, and the number of animals requested cannot be exactly determined."

For fisheries and wildlife studies that include faunal inventories, surveys, and monitoring programs, for which the species potentially encountered and the number of individuals are unknown, please provide estimates of likely maximum numbers. You do not need to list all the potential species that may be encountered. Instead, you may group species in the same Families or Orders, such as snakes, frogs, rodents, sunfish, perching birds, etc.
not applicable

3. Does this project duplicate previously performed experimental work?

○ **Yes** ◉ **No**

4. Upload any documents or charts that may help describe/justify animal numbers:

Name	Modified	Version
There are no items to display		

Section: 4. Location of Animal Use

Reviewer Notes

Filter by Type Go Clear Advanced

Type	Reviewer	Modified
Public Note	Uli Pamedar	12/6/2019 2:04 PM
Space Life Sci. Lab has been inspected.		
Coor **IACUC Change Request**	Alan Eldred	10/18/2019 1:47 PM
Please contact Steven Butler to arrange for an inspection of your rooms @352-273-9534		

Jamie Foster - Change Request Completed - 11/27/2019 2:07 PM

An inspection of my off-campus facility was conducted on 11/27/2019 by Skype.

Please note the inspector had a question regarding the Magnesium Chloride I used for anesthesia and asked whether pharmaceutical-grade MgCl2 was available.

I have looked with Sigma Aldrich and they only make the molecular grade, which is what I used.

1-2 of 2

4.1 Locations of Animal Use

1. * **Please select the building(s) where animals will be housed, or areas where located (i.e. field, pasture, park, wildlife area, etc). If housing is within ACS managed facilities, ACS will assign room numbers. All animals are to be cared for/monitored/inspected every day including holidays and weekends by a member of the research team or with an agreement by ACS personnel. If animals are housed outside of ACS managed facilities, complete and upload the non-ACS housing form.**
Searching Hint: % is a wildcard. Try % followed by your room number (%235). If ACS Assigned, type %acs

Animal Housing

	Species	Location	Location Other
View	bobtail squid	OTHER (Bldg #OTHER) Room OTHER	University of Florida labs at the Space Life Science lab, Merritt Island, FL

2. **Attachments:**
Upload non-ACS housing form here.
Foster_SLSL_Non-ACS-Housing-Facilities-Req.docx 10/11/2019 11:22 AM 0.02

3. **For animals normally housed in ACS facilities will those animals be housed anywhere outside of that facility for a period of >12 consecutive hours (USDA covered species), or a period of >24 hours for rats of the Genus Rattus, mice of the Genus Mus, or any other animal not covered by the USDA regulations?**
○ Yes ● No

4. * **Will animals be transported from one housing location to another housing location?**
(If this is a wildlife study, answer NO and answer 22.11.1)
○ Yes ● No

5. * **Procedures Performed: Please select the building(s) or areas (pastures, private farms/ranches, wildlife areas, etc.) where procedures, treatments or observations on animals will be performed. If location is ACS Assigned, please also select the corresponding building.**
Each room location is listed separately. Indicate all that apply

	Species	Location	Other Location	Procedures	Other Procedure

Section: 4. Location of Animal Use

	Species	Location	Other Location	Procedures	Other Procedure
View	bobtail squid	OTHER (Bldg #OTHER) Room OTHER	Space Life Sciences Lab - Room 233/234	Non-Survival Surgery Euthanasia Imaging (MRI, X-Ray, etc.) Tissue Harvesting	

Section: 5. Animal Care

Reviewer Notes

Type	Reviewer	Modified

There are no items to display

0-0 of 0

5.1 Animal Care

1. * **As a result of this protocol, will animals experience disease, injury or other clinical condition (e.g. diabetes, chronic seizures, infections with disease agents, etc...)?**
   ○ Yes ◉ No
2. * **Will the experiments involve procedures that may lead to a moribund condition, state of dying (e.g. administration of compounds with potential toxic effects, etc...)?**
   ○ Yes ◉ No

Reviewer Notes

Type	Reviewer	Modified

There are no items to display

0-0 of 0

5.2 Animal Care - Genetically Modified Animals

1. * **Will you be using genetically modified animals (GMA)?**
   ○ Yes ◉ No

Reviewer Notes

Filter by Type Go Clear Advanced

	Type	Reviewer	Modified
Coor	**IACUC Change Request**	Alan Eldred	12/18/2019 2:19 PM

Please answer No in section 5.3.

Jamie Foster - Change Request Completed - 12/18/2019 4:05 PM

I have changed the response to no for section 5.3

1-1 of 1

5.3 Animal Care - Exclusion of Enrichment

1. * **Will animals housed in ACS facilities be excluded from the ACS environmental enrichment or dog exercise program?**
   ○ Yes ◉ No

Section: 6. Rationale and Alternatives to the Use of Animals and Relief of Pain and Distress

Reviewer Notes

Filter by Type Go Clear Advanced

Type	Reviewer	Modified
Coor **IACUC Change Request**	Alan Eldred	10/18/2019 1:43 PM

6.2 - AWIC is not a database and is not suitable for this search section. Please select a suitable database for your searches.

Jamie Foster - Change Request Completed - 11/27/2019 11:00 AM

I have removed this notation regarding AWIC and added PubMed database.

Also, please note I have contacted the Health Center Library's reference department and requested assistance to generate a comprehensive search on current best practices to minimize pain or distress in cephalopods.

1-1 of 1

6.1 Search For Rationale And Alternatives To The Use of Animals (Replacement And Reduction)

Please conduct a literature search of two or more relevant databases for each search indicated below (one each for 6.1.1a and 6.1.1b) to reflect your attempt to achieve the 3R's, a refinement of techniques, procedures or methods, a reduction in number of animals used, and alternatives to the use of live animals in the research. The search should have been conducted within the past 3 months. For keyword and search strategy assistance, contact the Health Science Center Library's reference department at reference@library.health.ufl.edu or 352-273-8414 (http://library.health.ufl.edu/help/literature-search). Discuss the findings of your search (the narrative) that less sentient species were not found suitable and alternatives to live animals are not available:

Refinement
Alternative techniques or procedures to minimize potential pain, distress, or discomfort to the animals used.
Reduction
Alternatives or methods which allow you to minimize the number of animals used to obtain significant results.
Replacement
Alternatives to the use of live animals for this research. Absolute replacements (i.e., replacing animals with inanimate systems such as computer programs) as well as relative replacements (i.e., replacing animals such as vertebrates with animals that are lower on the phylogenetic scale).

1. **Search for Alternatives to Use of Live Animals and Minimization of Numbers (Replacement and Reduction) Per Federal regulation and UF policy, each protocol, regardless of pain classification, must contain a description of methods and sources of information used by the investigator to determine that alternatives to the proposed animal model and procedures are not appropriate. Two or more databases must be searched for alternatives.**

   a. * **Database1:**

Database	Date Performed	Years Covered	Keywords	Search Findings

Section: 6. Rationale and Alternatives to the Use of Animals and Relief of Pain and Distress

	Database	Date Performed	Years Covered	Keywords	Search Findings
View	Google Scholars	10/10/2019	Any period	cephalopod, in silico, symbiosis, replacement, model, pain, distress, minimize	At this time, no virtual or non-animal model exists for these symbiotic relationships and the simplified squid-bacteria model is one of the best systems to study the influences of beneficial bacteria on animal development. Several articles came up in the search regarding the recent status on using cephalopods in research. A couple of recent papers that came up in the search are listed below. Vidal, E. A., Villanueva, R., Andrade, J. P., Gleadall, I. G., Iglesias, J., Koueta, N., ... & Albertin, C. B. (2014). Cephalopod culture: current status of main biological models and research priorities. In *Advances in marine biology* (Vol. 67, pp. 1-98). Academic Press. Fiorito, Graziano, et al. "Cephalopods in neuroscience: regulations, research and the 3Rs." *Invertebrate Neuroscience* 14.1 (2014): 13-36.

b. * **Database2:**

	Database	Date Performed	Years Covered	Keywords	Search Findings

Section: 6. Rationale and Alternatives to the Use of Animals and Relief of Pain and Distress

	Database	Date Performed	Years Covered	Keywords	Search Findings
View	Medline/PubMed	11/27/2019	Any period	cephalopod, in silico, symbiosis, replacement model, pain, distress, minimize	As keyword search of PubMed to minimize pain in cephalopods yielded a few results worth noting: 1. A leader in this field of how cephalopods sense pain is Robyn Crook and she recently published a paper that because to assess neural and behavioral correlates with different anesthesias and euthanasia in cephalopods. This study suggested ethanol and magnesium chloride caused loss of neurological pain signals and were recommended for both anesthesia and euthanasia. Butler-Struben, H. M., Brophy, S. M., Johnson, N. A., & Crook, R. J. (2018). In vivo recording of neural and behavioral correlates of anesthesia induction, reversal, and euthanasia in cephalopod molluscs. *Frontiers in physiology*, *9*, 109. 2. Crook's earlier work in 2011 primarily described the likelihood of molluscs feeling pain and suggested the use of magnesium chloride to relax muscles of cephalopods. Crook, R. J., & Walters, E. T. (2011). Nociceptive behavior and physiology of molluscs: animal welfare implications. *Ilar Journal*, *52*(2), 185-195. 3. In this 2018, paper below provides a nice review of the organization of neurological structures in molluscs. The paper discusses presence of nociceptors and that mollucs have the capacity to feel pain. The paper also reviews the current understanding of anesthetics on molluscs, including cephalopodsm but the conclusions state there is still a need for future studies and makes the recommendation for using magnesium chloride. Winlow, W., Polese, G., Moghadam, H. F., Ahmed, I. A., & Di Cosmo, A. (2018). Sense and Insensibility–An Appraisal of the Effects of Clinical Anesthetics on Gastropod and Cephalopod Molluscs as a Step to Improved Welfare of Cephalopods. *Frontiers in physiology*, *9*. 4. In 2019 the paper below discusses the challenges of detecting pain in cephalopods but again recommends using anesthesia such as magnesium chloride. This feature article talks about how there is a need for additional studies in these areas. Neff, E.P. (2019) Considering the cephalopod. *Lab Animal* 48, 19-22.

Reviewer Notes Section: 6. Rationale and Alternatives to the Use of Animals and Relief of Pain and Distress

Filter by Type Go Clear Advanced

Type	Reviewer	Modified
Coor **IACUC Change Request**	Alan Eldred	10/18/2019 1:43 PM

In section 6.2.1 you should use search terms to determine if there exists methods to refine techniques, procedures or methods to reduce the potential or intensity of pain or distress the animals may experience. Therefore, keywords such as pain, di...

Jamie Foster - Change Request Completed - 11/27/2019 11:22 AM

I have written to the UF Health Science Library reference department to provide assistance with a search in this area of reducing pain or distress in cephalopods. I am awaiting a response from the library staff.

1-1 of 1

6.2 Search for Alternative Methods to Minimize Pain and Distress, Category D & E only, (Refinement)

Per federal regulations and UF policy, each protocol must provide a narrative of methods and sources of information (http://library.health.ufl.edu/help/literature-search) used by the investigator to determine that alternatives to the methods and procedures that cause more than momentary pain and distress have been considered. Two or more relevant databases must be searched for alternatives (one each for 6.2.1a and 6.2.1b). If none, please indicate in the narrative that alternatives to painful procedures were not found suitable.

1.

a. * **Database1:**

	Database	Date Performed	Years Covered	Keywords	Search Findings
View	Google Scholars	10/10/2019	any	cephalopods, refinement, animal, model, research	Although there were several articles discussing best practices regarding refinement in cephalopod research in the past couple of years, such as the papers listed below, this seems to be an emerging area for cephalopods. In my research over the past few decades, I have restricted my research questions to examine early developmental events in the animals. Focusing on early development helps minimize the length of the paralarvae exposures to various experimental treatments (e.g. microgravity). Liguori, Gabriel R., et al. "Ethical Issues in the Use of Animal Models for Tissue Engineering: Reflections on Legal Aspects, Moral Theory, Three Rs Strategies, and Harm–Benefit Analysis." *Tissue Engineering Part C: Methods* 23.12 (2017): 850-862.

b. * **Database2:**

	Database	Date Performed	Years Covered	Keywords	Search Findings

Section: 6. Rationale and Alternatives to the Use of Animals and Relief of Pain and Distress

	Database	Date Performed	Years Covered	Keywords	Search Findings
View	Medline/PubMed	11/27/2019	any time	cephalopod, refinement, models research, pain, distress, minimize	As keyword search of PubMed to minimize pain in cephalopods yielded a few results worth noting: 1. A leader in this field of how cephalopods sense pain is Robyn Crook and she recently published a paper that because to assess neural and behavioral correlates with different anesthesias and euthanasia in cephalopods. This study suggested ethanol and magnesium chloride caused loss of neurological pain signals and were recommended for both anesthesia and euthanasia. Butler-Struben, H. M., Brophy, S. M., Johnson, N. A., & Crook, R. J. (2018). In vivo recording of neural and behavioral correlates of anesthesia induction, reversal, and euthanasia in cephalopod molluscs. *Frontiers in physiology*, *9*, 109. 2. Crook's earlier work in 2011 primarily described the likelihood of molluscs feeling pain and suggested the use of magnesium chloride to relax muscles of cephalopods. Crook, R. J., & Walters, E. T. (2011). Nociceptive behavior and physiology of molluscs: animal welfare implications. *Ilar Journal*, *52*(2), 185-195. 3. In this 2018, paper below provides a nice review of the organization of neurological structures in molluscs. The paper discusses presence of nociceptors and that mollucs have the capacity to feel pain. The paper also reviews the current understanding of anesthetics on molluscs, including cephalopodsm but the conclusions state there is still a need for future studies and makes the recommendation for using magnesium chloride. Winlow, W., Polese, G., Moghadam, H. F., Ahmed, I. A., & Di Cosmo, A. (2018). Sense and Insensibility–An Appraisal of the Effects of Clinical Anesthetics on Gastropod and Cephalopod Molluscs as a Step to Improved Welfare of Cephalopods. *Frontiers in physiology*, *9*. 4. In 2019 the paper below discusses the challenges of detecting pain in cephalopods but again recommends using anesthesia such as magnesium chloride. This feature article talks about how there is a need for additional studies in these areas. Neff, E.P. (2019) Considering the cephalopod. *Lab Animal* 48, 19-22.

Section: 7. Nature of Study

Reviewer Notes

Filter by Type Go Clear Advanced

Type	Reviewer	Modified
Coor **IACUC Change Request**	Karl Andrutis	10/16/2019 8:30 AM

Please answer YES to Wildlife Study and complete the section since the adults are wild caught.

Jamie Foster - Change Request Completed - 11/26/2019 4:22 PM

I have answered YES to this question.

1-1 of 1

7.1 Nature of Study

1. **Select "Yes" for ALL boxes that apply to experimental procedures used in your study. Selecting "Yes" will generate appropriate forms for your study while "No" will permit you to skip these sections.**

* Yes **No** Monoclonal Antibody Production

* Yes **No** Polyclonal Antibody Production

* Yes **No** Behavioral Research

Yes No Breeding of Other Species, Not Mice or Rats

* Yes **No** Non-surgical Collection of Blood, Body Fluids or Tissues from live animals including genotyping and terminal procedures

* Yes **No** Food and/or Water Restriction (other than *ad libitum* and fasting before surgery)

* Yes **No** Prolonged Physical Restraint (more than 15 min). This does not include standard agriculture practices (e.g. squeeze chute) or wildlife studies (e.g. trapping)

* Yes **No** Studies using Non-Standard Feed, Special Diets or Special Water. If only post-irradiation antibiotics are used, the information should be listed in section 17.4 in lieu of this section.

* Yes **No** Survival Surgical Procedures (defined as making an incision through the skin)

* **Yes** No Whole Body or Targeted Irradiation, Imaging (MRI, Radiography, Fluorescent, CT Scan, Microscopic, etc), Neuromuscular Blocking Agents or Electric Shock.

* Yes **No** Tumor Production or Injection of Cells into live animals

* **Yes** No Wildlife Study

* Yes **No** Non-Survival Surgery (includes whole body or organ perfusion studies on live animals)

Section: 11. Breeding of Mice, Rats or other Species

Reviewer Notes

Filter by Type Go Clear Advanced

Type	Reviewer	Modified
Public Note	Colette St. Mary	11/22/2019 5:02 PM

I believe once you've indicated this is a wildlife study there will be specific fields in which to put the information about adult collection etc. and they will not need to be included here, you will just indicate that they are field collected.

...

1-1 of 1

11.1 Breeding

1. * **Who will perform in-house breeding?**

- ○ Both My Lab & ACS
- ○ Just ACS only
- ◉ **Just My Laboratory**
- ○ Other

a. **Please select who will perform breeding.**
This list is compiled of those persons listed in section 1.1.7. If you do not see the appropriate person in this list make sure you have added them to the study staff in section 1.1.7. Or, describe in the text box for "Other" such as agricultural production personnel.

UFID	Last Name	First Name	Middle Name
98663033	Foster	Jamie	S

Reviewer Info:

Name	Roles	Role Other	Experience
Jamie Foster	Breeding, Euthanasia, Administrative, Husbandry, Anesthesia, Tissue Harvesting, Imaging (MRI, X-Ray, etc.)		The PI has 27 years of working with the care, husbandry, anesthesia, and surgical dissections of the cephalopod bobtail squid (species Euprymna scolopes). The PI was formerly the lead caretaker and handler for the Euprymna scolopes at the University of Hawaii and University of Southern California and has maintained an Euprymna scolopes breeding colony at the University of Florida Space Life Sciences lab for the past 10 years.

b. **Other**

2. * **Describe where you will get each of your adult breeders, by species or strain , if using rodents, to start your colony/herd/other. (Colony Management)**
Include the name of the source and include any contact information.
Adult bobtail squid (2-5 cm in size) are collected from the shallow sand flats in O'ahu, Hawaii by the PI at night. The animals are collected along the shore in approximately 1-2 feet of seawater. A flashlight is used to spot the animals and a dip net is used to scoop up the animals. Each animal is then transferred to an individual 1-gallon zip lock bag containing 1 liter of local seawater without touching them. The bags are then placed in a 5-gallon bucket and then transported to the Kewalo Marine Lab, which is part of the University of Hawai'i and located in Honolulu. The animals are released into running seawater tanks at the Kewalo Marine Lab for the duration of the collection field trip and fed local shrimp each day.

For shipment back to the University of Florida labs at the Space Life Science Lab (SLSL), each animal is transferred to a polypropylene bag containing 1.5 liters of seawater, topped with pure oxygen and then the bag is closed with elastic bands. A secondary containment bag is added and sealed. The bags of squid are then placed in a padded shipping crate and transferred to Delta Cargo where they are flown to Orlando airport and then transported to the SLSL by the PI.

The animals are acclimated to the closed recirculating aquarium system at the SLSL using the "drip method", which has had a 100% survival rate since it was implemented in the Foster lab in 2009. Using the "drip

	Section: 11. Breeding of Mice, Rats or other Species

method", animals are removed from the bags and placed in a Pyrex dish containing 1 liter of the water they were transported in. The ammonia levels are measured and are typically > 4 ppm upon arrival. Every 15 min, approximately 50 mL of water from the bowl is removed and 50 mL of new aquarium water is added to the Pyrex bowl containing the squid. This process is repeated until the ammonia levels are below 0.5 ppm (approximately 3 hours). The "drip method" occurs inside the environmental growth chamber so that the temperature of the water in the Pyrex dish containing the animal is also slowly acclimating to the temperature of the chamber. Once ammonia levels are below 0.5 ppm the animals are transferred to individual compartments within the closed aquarium system. The animals are closely monitored for typical behavior (swimming to the bottom and burying in the sand or feeding on shrimp).

If for some reason an animal shows signs of osmotic shock (flipping over on its mantle) the animal is squid is quickly returned to the Pyrex bowl and water is flushed across its gills using a plastic pipette. This response has happened only once in the past 10 years and the animals was revived and the drip method was continued for another hour until the animal behaved normally and acclimated to the aquarium seawater conditions.

Reviewer Notes

Type	Reviewer	Modified

There are no items to display

0-0 of 0

11.3 Breeding - Monitoring

All research animals are to be **CARED FOR/INSPECTED/MONITORED EVERY DAY INCLUDING HOLIDAYS AND WEEKENDS** by a member of the research team or with an agreement by ACS personnel. Describe the following:

1. * **Monitoring Schedule. Include provisions for health assessment and treatment:**
   All animals are monitored daily including holidays and weekends. Adult animals are fed daily as well.

   If an animal looks sickly. the information is recorded. If an animal has died the animal is removed immediately and treated as biological waste.

   Typically, lab personnel rotates weekly from Monday through Sunday.

2. * **Any specific/unique breeding characteristics inherent to each species/strain of animal. Include clinical signs, physiological or behavioral abnormalities and describe pain/distress, discomfort/condition that the offspring might experience prior to weaning/transfer to other protocols:**
   There are no unique breeding characteristics other than the squid are nocturnal animals and breed at night.

   There is no parental care. Once the females lay the eggs there are no further interactions between adult squid and eggs/paralarvae.

Section: 11. Breeding of Mice, Rats or other Species

Reviewer Notes

Type	Reviewer	Modified

There are no items to display

0-0 of 0

11.2 Breeding - Other Species

1. **Provide detailed information regarding your breeding management and methodology by species as appropriate.**

   a. * **Describe the criteria used to select and retire breeding animals.**
   When collecting animals in the field, only animals of reproduction age are collected (estimated to be 2-3 months old) and are typically between 2 and 3 cm.

   Only males and female squid that do not exhibit end-of-life behaviors (e.g. not feeding, not burying, whiten skin) are selected for breeding.

   The mantle lengths of all squid are measured in the first week of arrival after collection. A single female is matched with a similarly sized (or slightly smaller) male.

   b. * **Explain the scheme, arrangement, and management of breeding individuals, pairs or groups.**
   During each breeding event (once every two weeks for each female), the male is moved into the female's tank for 12 h overnight, and returned to its own tank the next morning for recovery. Each male has at least two days of recovery between mating events.

   c. * **Describe the procedures used to in the management of the breeding colony such as genotyping, artificial insemination, embryo transfer, hormonal manipulations, surgical procedures, etc.**
   There is no interference or alteration of the natural reproduction process. Mating is very stressful for the female and therefore it is limited to once every two weeks. Typically females are only mated once or twice during their captivity as each female typically will lay 12-15 healthy egg clutches and we take efforts to minimize the overproduction of eggs.

   d. * **Describe the final disposition of breeders and offspring.**
   Adult animals are allowed to live out their natural lives in the closed recirculating aquarium. Once the animals exhibit end-of-life-symptoms they are euthanized as previously described (for adults 2% ethanol on ice for 1 h).

   Egg clutches are transported to separate aquariums where they are incubated for 20-30 days for hatching.

   e. * **Describe the record keeping used to track breeding performance. A production index (PI) could be used in addition to other criteria such as litter size, weaning weight or other.**
   Electronic animal records are maintained in the Foster lab for ease of input and access. Facility records, such as water parameters and purchasing receipts, are maintained in paper and electronic form. Temperature is recorded daily. Salinity, nitrate, nitrite and ammonia levels are checked weekly or more often if animals display signs of distress.

   Animal identifiers are designated by number and monitored for the duration of the animal's life. Animal death dates are recorded, and if the animal is euthanized, that is recorded as well. Mean longevity in captivity can thus be calculated.

   Each egg clutch is assigned a unique identifier. For each clutch, the Day 20 anticipated hatching date, the approximate number of eggs, the female number, and the PVC cave identifier are recorded.

   From each clutch, the number of hatchlings is recorded. This information includes the number of paralarvae that hatched overnight and the number of paralarvae that hatched at known times. Any dead, sickly, or premature hatchlings are also noted. From these data, we can compute the number of hatchlings per clutch total, the number of hatchlings per time period, the number of hatchlings per female, and the clutch's range of incubation time.

Section: 11. Breeding of Mice, Rats or other Species

Reviewer Notes

Type	Reviewer	Modified

There are no items to display

0-0 of 0

11.4 Breeding - Weaning/Euthanasia

1. **Will pregnant females be euthanized and fetuses harvested before birth?**
   - ○ Yes ◉ No
2. **Will offspring be euthanized before the normal weaning age?**
   - ○ Yes ◉ No

Reviewer Notes

Type	Reviewer	Modified

There are no items to display

0-0 of 0

11.5 Breeding - Genotyping

1. * **Will you genotype animals?**
   - ○ Yes ◉ No

Section: 17. Other Non-Surgical Procedures

Reviewer Notes

Type	Reviewer	Modified

There are no items to display

0-0 of 0

17.1 Other Non Surgical Procedures - Anesthestized/Neuro Agents

1. * **Will the animals receive neuromuscular blocking (paralyzing) agents for any non-surgical procedures?**
   See ACS Guidelines
   ○ **Yes** ◉ **No**

Reviewer Notes

Type	Reviewer	Modified

There are no items to display

0-0 of 0

17.2 Other Non Surgical Procedures - Electric Shock without Anesthesia

1. * **Will Electric Shock without anesthesia be performed in this protocol?**
   ○ **Yes** ◉ **No**

Section: 17. Other Non-Surgical Procedures

Reviewer Notes

Filter by Type Go Clear Advanced

Type	Reviewer	Modified
Coor **IACUC Change Request**	Colette St. Mary	11/22/2019 5:05 PM

please add expected outcome even if it is "no adverse effects"

Jamie Foster - Change Request Completed - 11/26/2019 4:31 PM

Thank you, that was an oversight on my part.

1-1 of 1

17.3 Other Non Surgical Procedures - Imaging/Microscopy

1. Are there diagnostic imaging or microscopy procedures performed in this protocol?

Yes **No**

a. For each species, describe each procedure separately, the length of time of each procedure, the number of times they will be performed, and the frequency between procedures.

	Species	Description	Expected Outcome	# Of Times Performed	Frequency	Duration
View	bobtail squid	Microscopy	no adverse effects	once per animal	once in the lifetime of a paralarvae	10 -15 min maximum

For each species, describe the method of restraint or anesthetizing agent if used. Provide the dose (mg/kg or %), volume, route of administration, frequency and duration of administration, and type and length of monitoring.

	Species	Anesthetizing Agent	Method of Restraint	Dose (mg/kg)	Volume (ml/kg)	Route of Administration	Frequency of Administration	Type and Length of Monitoring
View	bobtail squid	Magnesium Chloride (non Pharmaceutical)	none	0.37M	1 mL	added to the seawater	1 dose	visual monitoring for 5-10 minutes

Listed Agents:
Ethanol (non Pharmaceutical)
Liquid Nitrogen (non Pharmaceutical)
Vibrio fischeri - wild-type (non Pharmaceutical)
Caspase Inhibitors - peptides
Magnesium Chloride (non Pharmaceutical)
RNAlater (non Pharmaceutical)

b.

Reviewer Notes

Type	Reviewer	Modified

There are no items to display

0-0 of 0

17.4 Other Non Surgical Procedures - Irradiation

1. * Are animals subjected to targeted (includes tumor irradiation) or whole body irradiation levels that are lethal or sublethal?

Yes **No**

Section: 19. Euthanasia or Disposition of Animals at the end of the project

Reviewer Notes

Filter by Type Go Clear Advanced

	Type	Reviewer	Modified
Coor	**IACUC Change Request**	Karl Andrutis	10/16/2019 8:37 AM

Please complete the "Agent" column with all possible agents- MgCl2, ethanol, LN2, RNA later...

Jamie Foster - Change Request Completed - 11/26/2019 4:17 PM

RNAlater and liquid nitrogen have been added as agents

1-1 of 1

19.1 Euthanasia Methods

1. * **Will euthanasia be performed in this protocol?**
   If euthanasia is ONLY performed as part of non-survival surgery (section 23), answer No to 19.1.1 and describe in section 23.

   Yes **No**

Describe the method(s) of euthanasia used in this protocol. Include the species, procedure, agent, dose and route of administration, and which physical method will be employed to assure death:

	Species	Procedure	Agent	Dose (mg/kg)	Route of Administration	Physical Method

Listed Agents:
Ethanol (non Pharmaceutical)
Liquid Nitrogen (non Pharmaceutical)
Vibrio fischeri - wild-type (non Pharmaceutical)
Caspase Inhibitors - peptides
Magnesium Chloride (non Pharmaceutical)
RNAlater (non Pharmaceutical)

Section: 19. Euthanasia or Disposition of Animals at the end of the project

	Species	Procedure	Agent	Dose (mg/kg)	Route of Administration	Physical Method
View	bobtail squid	Euthanasia is performed through over-anesthetization followed by a quick-freeze method. Anesthetization is performed by exposing the animal to either 2% ethanol or 0.37M $MgCl_2$, in filtered sterilized seawater (FSW). Typically, after an experiment, anesthetized paralarvae are placed immediately in a pouch containing liquid nitrogen. Within a second or two, the paralarvae is completely frozen and stored at -80˚ C. For RNAseq experiments only, animals are immediately immersed in RNAlater or flash-frozen in liquid nitrogen.	Magnesium Chloride (non Pharmaceutical)	0.37 M	addition to the surounding seawater	For paralarvae, the animals undergo surgical removal of the mantle, funnel and light organ in which the animals do not survive. For adults, the animals are placed on ice in addition to the anesthesia overdosing.

a.

b. If decapitation without anesthesia or cervical dislocation without anesthesia is performed for euthanasia, please list who will perform the procedure:

UFID	Last Name	First Name	Trained by ACS
There are no items to display			

	Section: 19. Euthanasia or Disposition of Animals at the end of the project

Reviewer Notes

Type	Reviewer	Modified

There are no items to display

0-0 of 0

19.2 Euthanasia - AVMA Compliance

1. . * **Are these procedures in compliance with the current AVMA recommendations and UF policies for euthanasia (either acceptable or acceptable with conditions)?**

(●) Yes ( ) No

Reviewer Notes

Type	Reviewer	Modified

There are no items to display

0-0 of 0

19.3 Euthanasia - Remaining Animals

1. * **Will any animals remain alive at the completion of the study, or transferred live to another protocol during the study?**

( ) Yes (●) No

Section: 20. Special Animal Husbandry Procedures

Reviewer Notes

Type	Reviewer	Modified

There are no items to display

0-0 of 0

20.1 Special Husbandry

1. * **Are special husbandry practices required for this protocol that may deviate from the Guide, USDA Regulations, Ag Guide or UF policies? Examples of special husbandry practices include temperature/humidity extremes, special housing/caging, metabolic caging (wire mesh flooring), modified light cycle, special health monitoring, unusual means of identification, non-standard bedding/litter change, non-standard feeding schedule, etc.?**

   ○ Yes ◉ No

Section: 21. Chemicals, Biologicals, Recombinant or Radiological Materials

Reviewer Notes

Type	Reviewer	Modified

There are no items to display

0-0 of 0

21.1 Toxic or Hazardous Chemical Agents

Using a hazard of any kind requires a review and potential registration by EH&S. Use of toxic chemicals, toxic pharmaceuticals, Complete Freund's Adjuvant (CFA), suspected or known mutagens, carcinogens, teratogens, antineoplastics, nucleotide analogs, or similar agents in animals who are handled by research or animal care staff, indicate by "yes" and identify in the appropriate TABLE.

1. **Using Toxic or Hazardous chemical agents?**
   ○ Yes ● **No**
2. **Using Nanoparticles?**
   ○ Yes ● **No**

Reviewer Notes

Type	Reviewer	Modified

There are no items to display

0-0 of 0

21.2 Biological Materials

1. * **Using Biological Materials <u>that require EH&S registration</u>:**

   Biological materials that do not require EH&S registration should be reported in section 24.1 (e.g. Rodent cecal slurry, Human fecal slurry and Citrobacter rodentium). Click here for EH&S Forms
   ○ **Yes** ● **No**

Reviewer Notes

Type	Reviewer	Modified

There are no items to display

0-0 of 0

21.3 Radioactive Agents or Irradiation

1. * **Will you be using radioactive agents or irradiation? (Do not include radiography, fluoroscopy, or imaging)**
   ○ **Yes** ● **No**

Section: 22. Description of Wildlife Studies

Reviewer Notes

Type	Reviewer	Modified

There are no items to display

0-0 of 0

22.1 Wildlife - Background

1. * **Does the work require State, Federal, or other permits?**
   - ○ **Yes** ◉ **No**

2. **This study will involve:**

Description
◉ **Capture Only**
○ Observation and Capture
○ Observation Only

Section: 22. Description of Wildlife Studies

Reviewer Notes

Type	Reviewer	Modified

There are no items to display

0-0 of 0

22.2 Wildlife - Animal Capture

Check the appropriate method(s) below and describe in the table the requested information.

1. **Does the study involve 'Live Trapping?'**
   ○ Yes ◉ No
2. **Does the study involve 'Netting?'**
   ○ Yes ◉ No
3. **Does the study involve 'Darting with Pharmacological Agent?'**
   ○ Yes ◉ No
4. **Does the study involve 'Other Methods' of animal capture?**
   ◉ Yes ○ No

   a. **Complete the following table:**

	Method	Season	Time of Day	Potential Adverse Effects	Rate of Injury	Method of Euthanizing Injured Animals
View	Collection with dip nets	All year	Night time	No adverse effects. The animals are gently scooped into a dipnet then transferred to a zip-loc bag containing seawater. The animals are never touched.	0%	not applicable as no squid has ever been hurt during collection

5. * **What are the potential health risks associated with this protocol, and what actions will you take to ensure personnel handling animals are protected from zoonotic diseases, physical hazards, and allergens?**
   If previously answered in Section 1, please disregard.
   There are no known potential risks. There is never direct contact with the animal and collector.
6. **Please describe methods used to exclude unwanted non-target species:**
   The targeted animal sits on a sand flat and is typically alone. A dipnet (a 12 inch circular net on a 3-foot wooden dowel) is used to scoop up the animal. The animal is kept in the net in the water and gently scooped up into a zip lock back without touching the targeted animal and no other animal is collected during this process.

Reviewer Notes

Type	Reviewer	Modified

There are no items to display

0-0 of 0

22.3 Wildlife - Animal Capture - Euthanasia

1. * **Will animals be euthanized as an intended component of the study?**
   If yes, complete section 19, Euthanasia or Description of Animals at End of Study
   ○ Yes ◉ No

Section: 22. Description of Wildlife Studies

Reviewer Notes

Type	Reviewer	Modified

There are no items to display

0-0 of 0

22.4 Wildlife - Animal Distress

1. * **Describe what steps will be taken to minimize potential pain and distress to animals during capture and manipulation:**
   The animals are never touched. Each collected animal is maintained separately in 1.5 L of local seawater in a zip-loc bag for the 3-4 hours time period that I am out collecting the animals. After the collection effort has ended, the animals are immediately transferred to running seawater tanks at the Kewalo Marine Lab in Honolulu, HI and fed.

   If there is distress (i.e., the animal inks), then the animal is placed in a new bag with clean seawater.

   In my 27 years collecting the bobtail squid no animals have died during this collection process.

Reviewer Notes

Type	Reviewer	Modified

There are no items to display

0-0 of 0

22.5 Wildlife - Animal Locations

1. * **Please list the geographical sites where you will observe or capture these animals:**
   Maunalua Bay, Honolulu, Hawaii

Section: 22. Description of Wildlife Studies

Reviewer Notes

Type	Reviewer	Modified

There are no items to display

0-0 of 0

22.6 Wildlife - Animal Restraint / Handling

1. **Describe any physical methods that will be used to restrain or handle animals after they have been captured:**
   One the animals have been scooped up in the dipnet, a 1-gallon zip-loc bag is used to come from under the animal and transfer the animal to the bag without touching and disruption to the animal. The animals are kept in the zip-loc until returning to the Kewalo Marine Lab

2. * **Are you using chemical agents to restrain or handle animals?**
   ○ **Yes** ◉ **No**

3. **Duration of the restraint/handling:**
   Typically, the animals are kept in the bags as we walk along the sand flats, which takes about 3-4 hours.

4. **Describe precautions that will be taken to avoid injury or stress to animals during handling:**
   Each animal is kept in its own bag, with ample headspace of air to enable oxygen exchange with the water. The animals are never touched during this process.

   If the animal inks, the water is changed.

Reviewer Notes

Type	Reviewer	Modified

There are no items to display

0-0 of 0

22.7 Wildlife - Animal Marking

1. **If animals will be marked, complete the table below:**

Method of Marking	Adverse Effects	Removed	Reasons for not using alternatives

There are no items to display

Reviewer Notes

Type	Reviewer	Modified

There are no items to display

0-0 of 0

22.8 Wildlife - Radiotelemetry

1. **If animals will be tracked using radiotransmitters, complete the table below:**
   If surgery is required to implant the transmitter, complete the table below along with section 16.

Method of attaching Transmitter and removal	Weight Compared to Animal	Adverse Effects	Removed

There are no items to display

Section: 22. Description of Wildlife Studies

Reviewer Notes

Type	Reviewer	Modified

There are no items to display

0-0 of 0

22.9 Wildlife - Proposed Procedures

1. **Describe any proposed procedures after capture and restraint that have not been previously described. Clealy indicate if these procedures will be repeated on recaptured animals.**
We do determine the sex of the animal right after capture in the dip net. We look for the presence of a hectocotylus arm (i.e., presence indicates a male squid) prior to returning to the lab. If the animal is not needed (i.e., we primarily collect females) then it is immediately released before returning to the lab.

   We do not recapture animals that have been released.

Reviewer Notes

Type	Reviewer	Modified

There are no items to display

0-0 of 0

22.10 Wildlife - Animal Recapture

1. * **Will wild animals be recaptured?**
   ○ **Yes** ◉ **No**

Reviewer Notes

Type	Reviewer	Modified

There are no items to display

0-0 of 0

22.11 Wildlife - Transport

1. * **Will captured animals be transported from the site of capture to another location?**
   ◉ **Yes** ○ **No**

   a. **Complete the table below:**

	Container Type	Extreme Temp Precautions	Food and Water	Method of Transport	Number per Container	Transit Time
View	Zip-loc bag	The animals are collected at night and maintained in the local ambient seawater. They are not left for extensive time periods and therefore the temperature flux is minimized.	no	Zip-loc bags containing the squid will be placed in 5-gallon buckets (about 3-4 bags per bucket) and driven to the Kewalo Marine Lab in Honolulu.	1	Maximum 1 h to drive from the collection site to the Kewalo Marine Lab.

Section: 24. Final

Reviewer Notes

Type	Reviewer	Modified

There are no items to display

0-0 of 0

24.1 Other Procedures or Agents

Other Non-Surgical Procedures

1. **For each species, provide a DETAILED description of any procedure or experimental manipulation(s) mentioned in Section 2.1 that is NOT described in detail in previous sections.**

Other Agents

Please list below all medications/drugs/agent/experimental compounds/chemicals/sedatives/tranquilizers/antibiotics/anesthetics/biological materials administered to animals as part of the experimental protocol but NOT previously listed in any other sections

	Species	Agent	Purpose	Dose (mg/kg)	Volume (ml)	Route of Administration	Frequency of Administration	Expected Effect
View	bobtail squid	Vibrio fischeri - wild-type (non Pharmaceutical)	To initiate symbiosis in host animal	500 to 10,000 cells per ml of seawater	10 ul for a 100 mg paralarvae	addition to the surounding seawater	once	Addition of the wild-type V. fischeri will initiate the natural symbiosis of the host squid.

Listed Agents:
Ethanol (non Pharmaceutical)
Liquid Nitrogen (non Pharmaceutical)
Vibrio fischeri - wild-type (non Pharmaceutical)
Caspase Inhibitors - peptides
Magnesium Chloride (non Pharmaceutical)
RNAlater (non Pharmaceutical)

2.

Reviewer Notes

Type	Reviewer	Modified

There are no items to display

0-0 of 0

Medical Agents/Drugs List

Please provide additional data for all Non-Pharmaceutical Grade Drugs listed below

- **Listed Agents:**

	Agent Name	Pharmaceutical Grade	Filtered	Vehicle	Vehicle Has Oils	Pharmaceutical Grade Available	Justification	# Sections Used
View	Ethanol	no	no	seawater	no	no		0
View	Liquid Nitrogen	no	no	there will be no vehicle.	no	no		0
View	Magnesium Chloride	no	yes	seawater	no	no		2
View	RNAlater	no	no	None	no	no		0
View	Vibrio fischeri - wild-type	no	no	seawater	no	no		1
View	Caspase Inhibitors - peptides	yes						0

Section: 24. Final

Medical Agents/Drugs Usage Summary

- **Other Non Surgical (Section 17.3)**

	Agent	Dose	Volume	Route	Frequency	Duration	Species
View	Magnesium Chloride (non Pharmaceutical)	0.37M	1 mL	added to the seawater	1 dose	5 -10 minutes	bobtail squid

- **Euthanasia Procedures (Section 19.1)**

	Agent	Dose	Volume	Route	Frequency	Duration	Species
View	Magnesium Chloride (non Pharmaceutical)	0.37 M		addition to the surounding seawater			bobtail squid

- **All other medications/drugs/agents/experimental compounds/chemicals/sedatives/tranquilizers/antibiotics/anesthetics administered (Section 24.1)**

	Agent	Dose	Volume	Route	Frequency	Duration	Species
View	Vibrio fischeri - wild-type (non Pharmaceutical)	500 to 10,000 cells per ml of seawater	10 ul for a 100 mg paralarvae	addition to the surounding seawater	once	between 2 and 96 h	bobtail squid

- # Endpoint Summary

- **2.1.2a Experimental Endpoints (Research):**
The most common means of euthanasia for cephalopods is an overdose of anesthesia (either ethanol or magnesium chloride ($MgCl_2$)) as per the guidelines for cephalopod research (pg 57).

  Fiorito, G. et al. (2015) Guidelines for the care and welfare of cephalopods in research – a consensus based on an initiative by CephRes, FELASA and the Boyd group. *Laboratory Animals* 49(52) 1-90.

  Adult animals in the breeding colony: All adults (both male and female) are allowed to live out their natural lives in the breeding colony. If, however, an animal is severely incapacitated and exhibits severe distress and senescence the animal will be euthanized. The observed behaviors for severely distressed or senescent bobtail squid include not feeding, significant whitening of skin (i.e., no longer able to control chromatophores), or the inability to control movement and right itself. At this point, adult animals are removed from the aquarium and euthanized by an overdose of anesthesia incubated in seawater containing increasing concentrations up to 2% ethanol on ice for 1 h or until no evidence of life exists. The tissues of euthanized adult animals are then disposed of as biological waste at the Space Life Science Lab.

  Paralarvae: Juvenile paralarvae used in both microgravity and gravity (i.e., ground-based) experiments will have a range of termination points between 0 and 96 h post-hatching. Unless otherwise indicated, experiments with paralarvae are conducted in Filtered Sea Water (FSW).

  Paralarvae incubated in vials and HARV bioreactors in the presence or absence of symbiotic bacteria are terminated between 0 and 96 h post-hatching and is dependent on the experimental question. At the end of an experimental incubation, the paralarvae are either:

  1) anesthetized with a 1:1 ratio of FSW and 0.37 M Magnesium Chloride ($MgCl_2$) for 5 min. Depending on the final experimental question, the intact animal is then incubated in fixative for downstream microscopic examination or immediately dissected to remove the outer mantle and funnel to expose the light organ and then visualized with epifluorescent or confocal microscopy. All tissues from these experiments are disposed of as biological waste at the Space Life Science Lab;

  2) For paralarvae preserved for transcriptome, proteome and metabolome research, the animals will be incubated in either HARV bioreactors or in the ADSEP spaceflight hardware and at the end of the experiment between 0 and 96 h the paralarvae will be immediately flash-frozen in liquid nitrogen or immediately preserved in RNAlater.

  One of the key research questions examined by my funded NASA Space Biology proposals is to understand the impact that spaceflight has on host-microbe interactions over time. A key methodology to examine the molecular mechanisms in which animals respond to spaceflight is RNASeq. RNAseq examines all of the expressed genes at a given moment in time by sequencing mRNA transcripts.

  It has been recently published in 2017 that the half-life of mRNA molecules (i.e., expressed gene transcript) is two minutes. That means potentially half of the transcripts will be degraded or turned over within 2 min of the end of a particular treatment. The citation for this recent publication, which used several different methods to assess mRNA turnover in eukaryotes, is listed below:

  Baudrimont, Antoine, et al. "Multiplexed gene control reveals rapid mRNA turnover." *Science advances* 3.7 (2017): e1700006.

  Therefore, as anesthetizing the paralarvae may take up to 5 min to fully incapacitate the animal, for RNAseq experiments no anesthesia will be used as it may alter the RNA sequencing results and interpretation of the effects of spaceflight on bacteria-induced animal development.

Section: 24. Final

- **2.1.2b Additional Humane Endpoints (Research):**

  Animals showing any of the following signs/symptoms will require veterinary interventions, euthanasia or scientific justification to remain on the protocol. The following are approved Humane Endpoints that should be followed.
  The Humane Endpoint is the point at which pain/distress is terminated, minimized or reduced by taking actions such as euthanizing the animal, giving the animal treatment to relieve pain and/or distress, or terminating a painful procedure.

  Click here for the Body Condition Score Guideline

  - **>= 15% weight loss from baseline weight or age-matched controls if the animals are still growing during the study**
  - **Body Condition Score of 2 or less for rodents**
  - **Inability to reach food or water**
  - **Impaired mobility beyond experimental endpoints (impaired mobility requires a scientific justification and a description of the mobility endpoints for the protocol in section 2.1.2a)**
  - **Tumors (spontaneous or induced for the study): Any tumor that become ulcerated or are over 1.5cm in mice or 2.5cm in rats, measured in any one dimension or a cumulative measurement**
  - **Labored breathing, respiratory distress or cyanosis (blue tinged color)**
  - **Tremors, convulsions or seizures lasting more than 1 minute or occurring more than once a day**
  - **Dehydration lasting over 24 hours that is unresponsive to treatment**
  - **Moribund - unable to right itself**

  Any exception to the above listed Humane Endpoints or any additional Humane Endpoints should be described here:
  no exceptions