



June 12, 2018

To Whom It May Concern:

The following application, # 18-05, was reviewed and approved by the University of Oregon Animal Care and Use Committee.

Title of Protocol Application: Zebrafish International Resource Center

Name of Applicant: Monte Westerfield, Zoltan Varga

Name of Institution: Institute of Neuroscience

Sponsoring Agency	Title of Grant Application	Sponsored Grant #	UO Grant #	EPCS #
NIH	Competing continuation of Zebrafish International	P40 0D011021-16	215450	19427

Protocol Expiration Date: **6/12/21**

Approved with the following minor modifications required:

This institution has an Animal Welfare Assurance on file with the NIH Office of Laboratory Animal Welfare (OLAW). The UO PHS Assurance number is D16-00004.

IACUC Member Designated to Verify Acceptance

Animal Use Protocol

Administrative Information

Title: Zebrafish International Resource Center

Protocol Status: AUP-18-05 "Zebrafish International Resource Center", v.5.0, Approved/Approved

Continuation of Expiring Protocol
15-05

Original Approval and Expiration
Dates: 03-18-2015 - 03-12-2018

Protocol Type

Please check all that apply.

Research Project

Colony Health Surveillance Project

Funding Source/ Grant Title	Funding Status ("New", "Continuation", "Renewal", "Revision")	Funding Status ("New", "Continuation", "Renewal", "Revision")	Grant #	Index #	EPCS #
NIH/Zebrafish International Resource Center	Continuation		P40 OD11021-16		24999
NIH/Development of Germplasm Resources for Preservation of Aquatic Models	Continuation		1 R24 RR023998-01A1		25538

Funding Information

Was this project originally funded or previously reviewed without the use of animals, or are there new significant changes in scope that now involve animals which were not previously outlined in the grant proposal?

No

What is your Funding Type?

Extramural

Cooperative Research

Is this a cooperative research project (are there principal investigators from more than one institution involved)?

Yes

Is any of the animal work and animal housing being conducted at the cooperating institution?

**No, all live animal work will be conducted here on UO campus
Please describe the nature of the collaboration.**

For additional guidance, please see UO Cooperative Research policy.

NIH/Development of Germplasm Resources for Preservation of Aquatic Models: Software and 3-D printed accessories/tools for zebrafish sperm cryopreservation will be developed, engineered, and produced by our collaborators at LSU. These tools will be shipped to ZIRC and evaluated for their utility in standardizing, optimizing, and refining cryopreservation. Fish will be anesthetized and sperm or oocytes harvested as outlined in the protocol section of this application.

Peer Review of Un-sponsored Research

Is this a teaching protocol or a protocol involving un-sponsored research, such as departmental or start up funds?

The IACUC requires that all proposals to use animals in research projects receive scientific peer review before being submitted to the IACUC. Most sponsored proposals receive this review during the funding submission process.

Rationale and Purpose

Rationale and Purpose

What are the study objectives or aims of the study?

Although there are no specific requirements to list separately all of the benefits and harms of the proposal, there is an expectation that the IACUC “will weigh the objectives of the study against potential animal welfare concerns” (NRC 2011, 27). The information provided below should be written in lay terms that a non-scientist would understand, and should not be a copy and paste of the aims from the grant. The information provided in this section could be used for possible press release.

Provide study objectives or aims of the study:

1. To serve as a central repository for zebrafish genetic stocks and research materials. ZIRC maintains healthy stocks of fish and frozen sperm of identified genotypes and makes them widely available to the research community. We continue to expand our collection by obtaining carriers of mutations and transgenes from the research community and breed them to produce new generations. We freeze and store sperm from all these lines. We acquire the most widely used wild-type lines and maintain them in a manner that preserves their genetic diversity. We receive and store antibodies, gene probes and markers used to identify and analyze wild-type and mutant stocks. Upon request, we ship fish and materials to domestic and international research laboratories. We also provide online information about the stocks, materials, ordering procedures and methods

(<http://zebrafish.org/zirc/home/guide.php>) in collaboration with ZFIN, the zebrafish model organism database (<http://zfin.org>). By providing these services in an efficient manner, ZIRC saves laboratories time and expense that can be better used for their research goals.

2. To provide consultation and pathology services. ZIRC provides diagnostic services and health status testing for laboratory zebrafish. We use histopathology, bacteriology, necropsy, and virology to analyze specific or suspected disease problems. We provide routine sentinel or quality control testing of zebrafish from healthy laboratory colonies for early detection of problems and to fulfill institutional animal care and use committee, IACUC, health status monitoring requirements. We continue to develop methods to detect and control disease in laboratory colonies. We continue to investigate the transmission and pathology of the microsporidian parasite, *Pseudoloma neurophilia*, and *Mycobacterium chelonae*. We maintain and update our online manual for the prevention, diagnosis and treatment of diseases affecting zebrafish: <http://zebrafish.org/zirc/health/diseaseManual.php>

3. To develop improved zebrafish husbandry methods. We establish standards and procedures for generating healthier and more vigorous colonies. We study the influence of stress, diet, water conditions, housing, and husbandry on larval and juvenile growth, adult fecundity, reproductive longevity and disease. We develop and improve standardized methods for cryopreservation and reconstitution of genetic lines.

How are the procedures and design of the study relevant and important for human or animal health, the advancement of knowledge, or the good of society?

See US Government Principle II online at

Remember, our Animal Welfare Program is required to follow the US Government Principles as outlined in the Public Health Service Assurance:

"This Institution is guided by the 'U.S. Government Principles for the Utilization and Care of Vertebrate Animals Used in Testing, Research and Training'."

Provide relevance and importance for human or animal health, the advancement of knowledge, or the good of society:

Our understanding of human development, hereditary medical conditions and disease has been tremendously augmented, by the research performed with genetic model organisms. The zebrafish is the newest of these model organisms. Because the basic genetic principles of embryonic development are very similar for vertebrates, insights gained from the research with zebrafish embryos and larvae have implications for human health and disease. Moreover, research on this organism meets the intent of the Animal Welfare Act because higher vertebrate models can now be replaced with this 'lower' vertebrate.

In the past decades, there was a dramatic increase in the number of laboratories using this organism to study the basic mechanisms of vertebrate development. Laboratories have generated several thousand transgenic fish lines, identified over 20,000 genetic mutations, and due to the recent completion of the zebrafish genome sequencing project, plans are underway to produce a mutation in every gene of the zebrafish genome (ca. 25,000 genes). Even more recently, genome editing methods have become available that enable scientists to target previously not mutagenized genes by conventional forward genetic methods. Most of the genetic stocks are distributed among more than 900 laboratories in more than 28 countries. To make room for new mutants, laboratories must discontinue some of their current stocks. Although mutations can be preserved as frozen sperm, not all laboratories are proficient with this technique. Thus, discontinued stocks may be permanently lost unless a central site serves as a repository to preserve and redistribute them for future research.

The Zebrafish International Resource Center acquires and maintains wild-type, transgenic, and mutant zebrafish stocks and makes them available to the international research community.

Principal investigator

Westerfield, Monte

Co-Investigators

Varga, Zoltan

Research personnel

Bauer, Justin

Clark, Renee

Core, Keely

Hwang-Shum, Jen-Jen

Lains, David

Loucks, Eryn

Marston, Dagmara

Matthews, Jen

Murphy, Joy

Murray, Katrina "Katy"

Nasiadka, Andrzej

Quinn, Erin

Smith, Calvin

Varga, Zoltan

Westerfield, Monte

Williams, Evan

Student Research Personnel

Black, Robin

Conway, Dylan

Schulze, Catherine

Wolfe, Jacklyn

Teaching Personnel

Not applicable

Animal Care and Veterinary Support Staff

Murray, Katrina "Katy"

Pre-Reviewers

Not applicable

Signers

Varga, Zoltan

Westerfield, Monte

Request addition of a person to the database

Collaborative Research Personnel

Provide Names and Job Titles for Collaborative Research Personnel

Provide Information:

Dr. Terrence R. Tiersch, Professor; Louisiana State University Agricultural Center, Louisiana Agricultural Experiment Station, Baton Rouge, Louisiana 70820

Collaborative Research Personnel

Not applicable

Emergency Contact

Varga, Zoltan

Animal Subjects Information

fish: Zebrafish

Total number of animals

Species	Min	Max
fish: Zebrafish	0	333694

USDA Pain Category

USDA Pain Category	Species	Procedure or Experimental Group	Procedure or Experimental Group
D	fish: Zebrafish	Shipping and Breeding Colony	0
D	fish: Zebrafish	Food/Diet Study	0
D	fish: Zebrafish	15 alpha-methyltestosterone	0

Animal Numbers Justification

For all animals in all Aims provide the scientific justification for the number of animals in each experimental group.

Provide justification narrative.

The requested animal numbers for ZIRC are based on actual shipping and breeding database entries for the year 2017, inflated by 10% (as buffer). In contrast to regular laboratories, colony breeding and distribution is *the* key part of ZIRC's mission, whereas "research activities" have somewhat lower priority. Hence, we list breeding colony and distribution animal numbers also in the research section, because that's what the NIH supports us for.

Provide common basis for animal numbers, if applicable. Check all that apply.

Numbers are based upon a statistical analysis or analyses.

In 2017, ZIRC produced 136,860 embryos specifically for distribution to laboratories. In addition, we bred 192,784 fish for stock propagation. Some of these adults were also used for distribution of adult fish. Hence the two numbers encompass the total number of animals for Stock Center functions.

The statistical assumptions for sample sizes for the Food/Diet study have been described in the procedures section. Briefly, we use a sample size of 40 in a test population of 80 fish. We set alpha to 0.05, beta to 0.2, and we expect a variance of 15% based on previous size and weight measurements. These values should allow us to resolve a difference of 10% between two means with a confidence level between 95-98 percent.

fish: Zebrafish

Animal Characteristics

For the current species (see highlighted subtab above), provide animal gender information. Check all that apply.

Female

Male

For the current species (see highlighted subtab above), provide animal age/developmental stage information. Check all that apply.

Embryonic

Larval (4 dpf through 7 dpf)

Larval (8 dpf through 30 dpf)

Juvenile

Adult

STRAIN and BREEDING LINE Information

Are there strains or breeding lines you will use that are not available via the checkboxes below in this section?

Yes

<http://zebrafish.org/fish/lineAll.php/>

Animal Source

For the current species (see highlighted subtab above), what is the source of the animals you will use? Check all that apply.

In-house Breeding Colony

Transfer from other UO PI(s)

ZIRC imports novel zebrafish lines from any submitting zebrafish laboratory at the UO if the line has scientific value for the research community. Specific PIs to be determined.

Other Source

ZIRC imports novel zebrafish lines from any submitting zebrafish laboratory from other institutions, if the line has scientific value for the research community. Specific PIs to be determined.

Animal Health Status

For the current species (see highlighted subtab above), describe the health status of the animals. Check all that apply.

Conventional

Acclimation Period

Acclimation upon arrival at University of Oregon:

Policies on acclimation and quarantine will be followed for this species.

Not applicable

Breeding and Genetic Information

For the current species (see highlighted subtab above), will animals be bred?

Yes

Are the animals bred under this protocol available from a commercial supplier?

No

Provide source(s) for animals that will be bred. Check all that apply.

Colony at Other Institution Managed by non-UO Investigator

Zebrafish Research Community, national and international

What is the final disposition of unwanted adult breeders and unneeded offspring? Check all that apply.

Euthanized using Approved Procedure(s)

List euthanasia methods under the "Animal Procedures/Experimental Design" tab.

What is the breeding scheme?

Fish

Will the IACUC-approved species-specific fish SOP for breeding be used?

Yes

Will offspring require special care or special monitoring?

No

Will genotyping be required?

Yes

Fish

Select genotyping method(s):

Fin Clip

Visual Phenotype in Experimental Animals

We carry >10,500 distinct lines with > 36,000 alleles among them. Embryonic, larval, or adult phenotypes (or gene expression) will be assessed at appropriate stages, according to published, or submitter-provided information to determine the genotype of parents (recessive traits) or individual fish (dominant). In addition, ZFIN records will be looked up and used to identify genetic carriers, as needed (or as available) for each individual fish line. Molecular genotyping is more efficient, therefore morphology/phenotype will only be assessed if molecular characterization protocols do not exist, or if additional confirmation is needed (e.g. for transgenes that have been detected by PCR, but functional expression of the gene needs to be determined).

When is the phenotype visible?

0 dpf through 7 dpf

8 dpf or later

Visual Phenotype in Offspring Used to ID Parent(s)

As provided by ZFIN, submitting laboratory, or publication.

When is the phenotype visible?

0 dpf through 7 dpf

8 dpf or later

For the current species (see highlighted subtab above), will genetically engineered animals be

used?

Genetic Engineering is directed modification of the gene complement of a living organism by such techniques as altering the DNA, substituting genetic material by means of a virus, transplanting whole nuclei, transplanting cell hybrids, etc.

Yes

Important Reminder: Complete the "Use of Genetically Engineered Animals" questions under the "Safety, Hazards, and IBC" tab.

For the current species (see highlighted subtab above), what type of animal welfare documentation will be used?

Standard IACUC-Approved Checklist(s)

Multiple Protocol Animal Use

Will the individual animal(s) in this subtab be used on other protocols?

No

Animal Care, Housing and Transportation

Husbandry

Will animals need care at UO?

Yes

For the all species on this protocol, select the animal type. Check all that apply.

Fish

Will UO standard species-specific water quality parameters be used?

Yes

Will UO standard species-specific feed types and feeding frequency be used?

Yes

Will UO standard species-specific illumination and photoperiod be used?

Yes

Will UO standard species-specific noise/sound ranges be used?

Yes

Will UO standard species-specific temperature ranges be used?

Yes

Will UO standard species-specific tank/cage change schedule be used?

Yes

Will the animals need any other type of non-standard care?

No

Housing

Will animals be housed at UO?

Yes

For the all species on this protocol, select the animal type. Check all that apply.

Fish

Will UO standard species-specific tank/cage type(s) be used?

Yes

Will UO standard species-specific environmental enrichment be used?

Yes

Will it be necessary to house animals individually other than for fin clips?

Yes

Why must animals be housed individually?

If it is necessary to house animals singly—for example, when justified for experimental purposes, for provision of veterinary care, or for incompatible animals—this arrangement should be for the shortest duration possible. If single animals are housed in small enclosures, an opportunity for periodic release into larger enclosures with additional enrichment items should be considered, particularly for animals housed singly for extended periods of time. (Guide, p. 60)

No Reliable Method for Tagging or Labeling Individual Animal Exists

Other

We isolate individual fish mainly for fin-clipping and house them in crossing cages until PCR results have been obtained. Typically 2 animals are stored in a crossing cage with a central divider. Animals remain up to 2x2 days in static water conditions. They receive food on the second day, followed by a fresh water change. They are returned to the recirculating water system as soon as possible, after 4 days maximally, and are housed in groups of up to 20 fish.

For some lines, for which we do not have molecular identification protocols, we identify the carriers of genetic modifications by breeding them, and observing the embryonic or larval phenotypes of their offspring. This identifies the genotype of the parents and these adults are also housed individually until the genetic identification based on embryo/larval phenotypes is possible. However, fish stay never longer than 4 days in static conditions, and they receive food and water change on the second day in static water tank isolation.

Will the fish be outside the main vivarium for more than 24 hours?

For aquatic animals "main vivarium" includes the Zebrafish Facility, Stickleback Facility, and Zebrafish International Resource Center.

No

Location Assignments

Site	Building	Floor	Section	Room	Room Type
Main Campus	ZIRC	First (Ground)	Conventional	118 Housing & Procedure	Housing and Procedure
Main Campus	ZIRC	First (Ground)	Quarantine	103 Quarantine	Housing and Procedure

Location Assignments

HOUSING: Will you need to house animals in rooms not listed in the table above?

No

Will any of the Procedure Rooms contain or house animals for longer than 12 hours?

No

EXPERIMENTS: Will you need to perform experiments on animals in rooms not listed in the table above?

No

Transportation

Will you need to transport animals outside the main vivarium? (Not Field Studies)

Yes

Movement of animals within or between sites or institutions should be planned and coordinated by responsible and well-trained persons at the sending and receiving sites to minimize animal transit time or delays in receipt. Shipping should be coordinated to ensure that animals arrive during normal business hours or, if delivery occurs outside of this time, that someone is available to receive them. Defining and delegating responsibility to the appropriate persons, who are knowledgeable about the needs of the species being shipped, will help ensure effective communication and planning of animal transport (AVMA 2002). (Guide pp. 107-108)

What type of animal transport will be used? Check all that apply.

Intraintitutional

Careful planning for all types of transportation should occur to ensure animal safety and well-being. The process of transportation should provide an appropriate level of animal biosecurity while minimizing zoonotic risks, protecting against environmental extremes, avoiding overcrowding, providing for the animals' physical, physiologic, or behavioral needs and comfort, and protecting the animals and personnel from physical trauma. (Guide, p. 107)

Transport Between Main Campus Locations

Interinstitutional

Institutions should contact appropriate authorities to ensure compliance with any relevant statutes and other animal transportation requirements that must be met for animals to cross international boundaries, including those not of the country of final destination. The NRC publication Guidelines for the humane Transportation of Research Animals provides a comprehensive review of this topic (NRC 2006). (Guide, p. 107)

Commercial Transport Between Vendor(s) and Laboratories

Why will animals be transported outside the main vivarium? Check all that apply.

Collaboration(s) Between Laboratories

Acting as Vendor or Supplier to other Labs

Will animals return to the main vivarium?

No

Will the animals pass through public spaces or patient areas while in transit?

No

What method(s) will be used to transport the animals? Check all that apply.

REMINDER: Vehicles are Animal Facilities according to PHS Policy and, as such, are subject to IACUC inspection.

B. Animal Facility - Any and all buildings, rooms, areas, enclosures, or vehicles, including satellite facilities, used for animal confinement, transport, maintenance, breeding, or experiments inclusive of surgical manipulation. (PHS Policy, III. Definitions)

Non-Vehicle Transport
Insulated Container ("Cooler")

Vehicle Transport
Vendor Vehicle Transport

Will you follow the UO species-specific SOP on animal transportation?

Yes

Who will be responsible for the animal transport? Check all that apply.

Principal Investigator
Laboratory Personnel

approved personnel listed in personnel tab.

Main Vivarium Personnel

approved ZIRC personnel listed in personnel tab.

Where will the animals be transported?
Provide departure and arrival locations:

various locations, depending on request.

How long will the animals be in transit?
Provide travel duration estimate:

travel duration will vary based on the location. Transit time will always be attempted to get minimized.

International Shipments <4 days transit (Monday - Friday arrival)

Domestic Shipments: Over night

Animal Procedures/Experimental Design

Experimental Design / Animal Procedures	Animal Subjects Details
Genetic Repository and Distribution	fish: Zebrafish
Diagnostic Health Service	fish: Zebrafish
Cryopreservation	fish: Zebrafish
Electrosedation	fish: Zebrafish

Genetic Repository and Distribution

Animal Species Use

fish: Zebrafish

Experimental Design and Procedures Summary

Procedures Summary

For the current aim (see highlighted subtab above), explain the experimental design and provide a clear, concise and sequential description of the procedures involving the use of animals.

This narrative should explain the overall design and include things like experimental animal groups, group sizes, and group use.

IMPORTANT: Provide a clear and concise sequential description of the procedures (especially those that are listed in the procedures tab) involving the use of animals that is easily understood by all members of the IACUC. Details of any of the procedures should be described in the "Procedures" tab. This description should allow the IACUC to understand the experimental use of an animal from its entry into the experiment to the endpoint of the study.

NOTE: You may attach diagrams, flowcharts, etc., by using the paperclip icon at the top or bottom of this page.

Provide Experimental Design (see IMPORTANT help text above):

1. To serve as a central repository for zebrafish genetic stocks and research materials.

1. ZIRC acquires live males or cryopreserved sperm samples from submitting laboratories, after the health status of the submitting fish facility has been obtained. If not available all materials are treated a contagious. Live fish undergo stringent quarantine and additional health monitoring procedures in the quarantine room. Healthy males are bred and surface sanitized embryos are introduced into the main fish facility nursery. Testes are dissected and sperm samples are cryopreserved and stored in a designated vapor phase liquid nitrogen freezer. We continue to expand our collection by obtaining carriers of mutations and transgenes from the research community and breed them to produce new generations. We freeze and store sperm from all imported lines. We also acquire the most widely used wild-type lines and maintain them in a manner that preserves their genetic diversity. We receive and store antibodies, gene probes and markers used to identify and analyze wild-type and mutant stocks.

2. ZIRC propagates healthy stocks of fish (2.1) and maintains frozen sperm (2.2) of identified genotypes.

- 2.1 Live fish are maintained, identified and propagated in a manner that preserves their genetic characteristics and makes these available to requesting researchers. Live fish are also generated and maintained to amplify cryopreserved stocks.
- 2.2 Cryopreserved stocks are thawed if the sample count reaches a low threshold, and fish are raised, identified, and gametes are harvested to amplify the resource. Cryopreserved samples are also thawed upon request for shipment. After invitro fertilization, embryos are shipped to clients, and/or introduced to the main fish facility nursery to raise a new generation and reamplify the frozen stocks. Popular lines that are requested frequently or needed for teaching are maintained alive as long as needed and are retired to the sperm bank when requests reach a low annual threshold.

3. We make frozen and live stocks widely available to the research community. Upon request, we ship fish and materials to domestic and international research laboratories using commercial courier services. Animal shipments are packaged according to IATA regulations plus a heat pack if destinations or seasons require it. Live fish are bred and their offspring is sorted, cleaned, packaged and shipped as larvae, juveniles or young adults. Cryopreserved stocks are thawed, used for in vitro fertilization, and

embryos, juveniles or young adults are packaged and shipped to requesting laboratories. We also provide online information about the stocks, materials, ordering procedures and methods (<http://zebrafish.org/zirc/home/guide.php>) in collaboration with ZFIN, the zebrafish model organism database (<http://zfin.org>).

Please see attached SOP collection of ZIRC's routine operations for import, maintenance, preservation, and distributioof fish lines.

Animal Biosafety Level

Animal Biosafety Level

For the current aim (see highlighted subtab above), will the risks associated with the experiments exceed Animal Biosafety Level 1 (ABSL-1)?

Where biologic agents are used, the Centers for Disease Control and Prevention (CDC) and National Institutes of Health (NIH) publication biosafety in Microbiological and biomedical Laboratories (BMBL; DHHS 2009) and the USDA standards (USDA 2002) should be consulted for appropriate facility design and safety procedures. These design and safety features are based on the level of risk posed by the agents used. Special facilities and safety equipment may be needed to protect the animal care and investigative staff, other occupants of the facility, the public, animals, and the environment from exposure to hazardous biologic, chemical, and physical agents used in animal experimentation. (Guide, p. 19)

No, ABSL-1 is the risk level associated with the animal use in these experiments.

Humane Endpoints

Humane Endpoints

For the current aim (see highlighted subtab above), what are the humane endpoints?

Humane Endpoints adapted from information online.

Select Animal Type(s) Used in this Aim. Check all that apply.

Fish

Types of Humane Endpoints (Check all that apply.)

Clinical or Behavioral

Abnormal Color Change

Abscess

Distended Abdomen

Emaciation

Eye Bulging (Exophthalmos)

Fungal Growth on Body or Fins

Gas Bubbles

Hemorrhage or Redness

Masses or Swellings

Skeletal Deformity

Tumor Formation

Twirling

Swimming in circles

Ulcer

Wound

--- PLACEHOLDER for FUTURE FEATURE ---

Placeholder

Final Disposition and Euthanasia

Not applicable

Euthanasia / Final Disposition

For this aim, what will be the final disposition of the animals? Check all that apply.

Euthanasia

METHODS of EUTHANASIA and CONFIRMATION of DEATH

Select Animal Type(s) Used in this Aim. Check all that apply.

Fish

What method(s) of euthanasia will be used? Check all that apply.

IACUC-Approved Methods

(zebrafish, adult) Immersion in solution of buffered tricaine methanesulfonate (MS-222), 0.03% (w/v)

Finfish should be left in the anesthetic solution for a minimum of 10 minutes after cessation of opercular movement.

(AVMA Guidelines for the Euthanasia of Animals (2013), p. 72)

(zebrafish, all developmental stages) Rapid Chilling (Hypothermic Shock)

Transfer from acclimatized temperatures to water associated with a 2° to 4°C ice slurry must occur rapidly with as little transfer of warmer water as possible.

(AVMA Guidelines for the Euthanasia of Animals (2013), p. 73)

How will you confirm animal death? Check all that apply.

Antibody Production

Antibody Production

Will you produce antibodies as part of this Aim?

No

--- PLACEHOLDER for FUTURE FEATURES --- (Use of Visual Recordings, Photography, or Audio Recordings)

Surgery

Surgery

Will you be performing surgery as part of this Aim?

Yes

What type of surgery will be performed? Check all that apply.

MAJOR SURGERY

As a general guideline, major survival surgery penetrates and exposes a body cavity, produces substantial impairment of physical or physiologic functions, or involves extensive tissue dissection or transection (Brown et al. 1993).

MINOR SURGERY

Minor survival surgery does not expose a body cavity and causes little or no physical impairment; this category includes wound suturing, peripheral vessel cannulation, percutaneous biopsy, routine

agricultural animal procedures such as castration, and most procedures routinely done on an “outpatient” basis in veterinary clinical practice.

Major Surgery

Minor Surgery

What surgery category is part of this Aim? Check all that apply.

Surgical procedures are categorized as major or minor and, in the laboratory setting, can be further divided into survival and nonsurvival. As a general guideline, major survival surgery (e.g., laparotomy, thoracotomy, joint replacement, and limb amputation) penetrates and exposes a body cavity, produces substantial impairment of physical or physiologic functions, or involves extensive tissue dissection or transection (Brown et al. 1993). Minor survival surgery does not expose a body cavity and causes little or no physical impairment; this category includes wound suturing, peripheral vessel cannulation, percutaneous biopsy, routine agricultural animal procedures such as castration, and most procedures routinely done on an “outpatient” basis in veterinary clinical practice. Animals recovering from these minor procedures typically do not show significant signs of postoperative pain, have minimal complications, and return to normal function in a relatively short time. When attempting to categorize a particular surgical procedure, the following should be considered: the potential for pain and other postoperative complications; the nature of the procedure as well as the size and location of the incision(s); the duration of the procedure; and the species, health status, and age of the animal. Laparoscopic surgeries and some procedures associated with neuroscience research (e.g., craniotomy, neurectomy) may be classified as major or minor surgery depending on their impact on the animal (Devitt et al. 2005; Hancock et al. 2005; NRC 2003; Perret-Gentil et al. 1999, 2000). For example, laparoscopic techniques with minimal associated trauma and sequelae (e.g., avian sexing and oocyte collection) could be considered minor, whereas others (e.g., hepatic lobectomy and cholecystectomy) should be considered major. Although minor laparoscopic procedures are often performed on an “outpatient” basis, appropriate aseptic technique, instruments, anesthesia, and analgesia are necessary. Whether a laparoscopic procedure is deemed major or minor should be evaluated on a case-by-case basis by the veterinarian and IACUC.

(Guide, p. 117-118)

Survival Surgery

Nonsurvival Surgery

Was the veterinarian involved in the presurgical planning?

Presurgical planning should include input from all members of the surgical team (e.g., the surgeon, anesthetist, veterinarian, surgical technicians, animal care staff, and investigator). The surgical plan should identify personnel, their roles and training needs, and equipment and supplies required for the procedures planned (Cunliffe-Beamer 1993); the location and nature of the facilities in which the procedures will be conducted; and perioperative animal health assessment and care (Brown and Schofield 1994). A veterinarian should be involved in discussions of the selection of anesthetic agents and doses as well as the plan for perioperative analgesic use. If a nonsterile part of an animal, such as the gastrointestinal tract, is to be surgically exposed or if a procedure is likely to cause immunosuppression, preoperative antibiotics may be appropriate (Klement et al. 1987); however, the routine use of antibiotics should never be considered a replacement for proper aseptic surgical techniques. Presurgical planning should specify the requirements for postsurgical monitoring, care, and recordkeeping, including the personnel who will perform these duties. The investigator and veterinarian share responsibility for ensuring that postsurgical care is appropriate. (Guide, p. 116)

Yes

Will surgery be performed outside of the central animal facility?

Unless an exception is specifically justified as an essential component of the research protocol and approved by the IACUC, aseptic surgery should be conducted in dedicated facilities or spaces. When determining the appropriate location for a surgical procedure (either a dedicated operating room/suite or an area that provides separation from other activities), the choice may depend on the species, the nature of the procedure (major, minor, or emergency), and the potential for physical impairment or post-operative complications, such as infection. Most bacteria are carried on airborne particles or fomites, so surgical facilities should be maintained and operated in a manner that ensures cleanliness and minimizes unnecessary traffic (AORN 2006; Bartley 1993). If it is necessary to use an operating room for other purposes, it is imperative that the room be returned to an appropriate level of hygiene before its use for major survival surgery. (Guide, p. 116-117)

No

Which personnel will be involved?

Which personnel will perform non-survival surgery?

Keely Core (Testes dissection) Jen Matthews (Testes dissection) Joy Murphy (Testes dissection) Eryn Loucks (Testes dissection) Dagmara Marston (Testes dissection) Evan Williams (Testes dissection) Zoltan Varga (Testes dissection)

Which personnel will perform survival surgery?

Jen-Jen Hwang Shum (Fin Clips) Keely Core (Fin Clips) Eryn Loucks (Fin Clips) Dagmara Marston (Fin Clips) Evan Williams (Fin Clips) Robin Black (Fin Clips, student)

Which personnel will provide training of lab-specific surgical procedures to new lab members?

Jen-Jen Hwang Shum (Fin Clips); Jen Matthews (VMD; testes dissections)

Other than veterinary health reasons, have animals been surgically manipulated in other studies prior to your use?

No

Surgical Procedure Considerations (Check All)

Preoperative Considerations

Which preoperative steps (not including anesthesia) will be used to prepare animals for surgery? Check all that apply.

Physical Exam

For nonsurvival surgeries, will the surgery be performed using aseptic technique, clean technique or a combination of both?

In nonsurvival surgery, an animal is euthanized before recovery from anesthesia. It may not be necessary to follow all the techniques outlined in this section if nonsurvival surgery is performed but, at a minimum, the surgical site should be clipped, the surgeon should wear gloves, and the instruments and surrounding area should be clean (Slattum et al. 1991). For nonsurvival procedures of extended duration, attention to aseptic technique may be more important in order to ensure stability of the model and a successful outcome. (Guide, p. 118)

Clean Technique

For survival surgeries, will the surgery be performed using aseptic technique and in accordance with the Guide?

Aseptic technique is used to reduce microbial contamination to the lowest possible practical

level (Mangram et al. 1999). No procedure, piece of equipment, or germicide alone can achieve that objective (Schonholtz 1976): aseptic technique requires the input and cooperation of everyone who enters the surgery area (Belkin 1992; McWilliams 1976). The contribution and importance of each practice varies with the procedure. Regardless of the species, aseptic technique includes preparation of the patient, such as hair or feather removal and disinfection of the operative site (Hofmann 1979); preparation of the surgeon, such as the provision of appropriate surgical attire, face masks, and sterile surgical gloves (Chamberlain and Houang 1984; Pereira et al. 1990; Schonholtz 1976); sterilization of instruments, supplies, and implanted materials (Bernal et al. 2009; Kagan 1992b); and the use of operative techniques to reduce the likelihood of infection (Ayliffe 1991; Kagan 1992a; Lovaglio and Lawson 1995; Ritter and Marmion 1987; Schofield 1994; Whyte 1988). While the species of animal may influence the manner in which principles of aseptic technique are achieved (Brown 1994; Cunliffe-Beamer 1983; Gentry and French 1994), inadequate or improper technique may lead to subclinical infections that can cause adverse physiologic and behavioral responses (Beamer 1972; Bradfield et al. 1992; Cunliffe-Beamer 1990; Waynforth 1980, 1987) affecting surgical success, animal well-being, and research results (Cooper et al. 2000). General principles of aseptic technique should be followed for all survival surgical procedures (ACLAM 2001). (Guide, p. 118)

No

Provide Explanation

Fin clips are performed under MS-222 anesthesia with a sterile, disposable blade on a clean piece of parafilm to avoid genetic cross contamination between specimen. Personnel is wearing sterile gloves. Infections have not been observed at the amputation site with this technique.

What types of surgeon preparation will be used and what equipment will the surgeon employ? Check all that apply.

Sterile Gloves

What types of animal patient preparation will be used and what equipment will the animal patient be given? Check all that apply.

Other

Parafilm

What method(s) will be used to sterilize the instruments? Check all that apply.

Specific sterilization methods should be selected on the basis of the physical characteristics of the materials to be sterilized (Callahan et al. 1995; Schofield 1994) and sterilization indicators should be used to validate that materials have been properly sterilized (Berg 1993). Autoclaving and plasma and gas sterilization are effective methods most commonly used to sterilize instruments and materials. Alternative methods, used primarily for rodent surgery, include liquid chemical sterilants and dry heat sterilization. Liquid chemical sterilants should be used with appropriate contact times and instruments should be rinsed with sterile water or saline before use. Bead or dry heat sterilizers are an effective and convenient means of rapidly sterilizing the working surfaces of surgical instruments but care should be taken to ensure that the instrument surfaces have cooled sufficiently before touching animal tissues to minimize the risk of burns. Alcohol is neither a sterilant nor a high-level disinfectant (Rutala 1990) but may be acceptable for some procedures if prolonged contact times are used (Huerkamp 2002). (Guide p. 119)

Autoclave

Will preoperative anesthesia, sedation, or tranquilization be provided to the animals?

Yes. Preoperative drugs will be used to calm the animals.

MS-222

Postoperative Considerations

An important component of postsurgical care is observation of the animal and intervention as necessary during recovery from anesthesia and surgery (Haskins and Eisele 1997). The intensity of monitoring will vary with the species and the procedure and may be greater during the immediate anesthetic recovery period. During this period, animals should be in a clean, dry, and comfortable area where they can be observed frequently by trained personnel. Particular attention should be given to thermoregulation, cardiovascular and respiratory function, electrolyte and fluid balance, and management of postoperative pain or discomfort. Additional care may be warranted, including long-term administration of parenteral fluids, analgesics, and other drugs, as well as care of surgical incisions. Appropriate medical records should also be maintained. After recovery from anesthesia, monitoring is often less intense but should include attention to basic biologic functions of intake and elimination and to behavioral signs of postoperative pain, monitoring for postsurgical infections, monitoring of the surgical incision site for dehiscence, bandaging as appropriate, and timely removal of skin sutures, clips, or staples (UFAW 1989). (Guide, p. 119-120)

Describe immediate postoperative recovery monitoring procedures, including duration and frequency.

What criteria will be used for postoperative monitoring of animal condition during recovery from anesthesia? Check all that apply.

Other

Fish are placed in transparent 1-gallon static water tanks, and are observed for successful and full recovery from MS-222 anesthesia immediately after fin clipping. Fin clipped fish are placed in a dedicated area of the facility and are observed for any signs of pain, distress, or discomfort (or other humane endpoints listed previously) at least once a day until genotyping results are obtained and identified fish can be placed into recirculating water tanks (2-4 days).

What postoperative method(s) of animal support will be used during immediate recovery? Check all that apply.

Mechanical Ventilation

Describe postoperative care support activities following surgery.

The selection of appropriate analgesics and anesthetics should reflect professional veterinary judgment as to which best meets clinical and humane requirements as well as the needs of the research protocol. The selection depends on many factors, such as the species, age, and strain or stock of the animal, the type and degree of pain, the likely effects of particular agents on specific organ systems, the nature and length of the surgical or pain-inducing procedure, and the safety of the agent, particularly if a physiologic deficit is induced by a surgical or other experimental procedure (Kona-Boun et al. 2005). Preemptive analgesia (the administration of preoperative and intraoperative analgesia) enhances intraoperative patient stability and optimizes postoperative care and well-being by reducing postoperative pain (Coderre et al. 1993; Hedenqvist et al. 2000). Analgesia may be achieved through timely enteral or parenteral administration of analgesic agents as well as by blocking nociceptive signaling via local

anesthetics (e.g., bupivacaine).
(Guide, p. 121-122)

Will analgesia be provided for relief of postoperative pain?

No

Wound healing is extremely fast and typically, fish never show signs of discomfort pain or distress

What is the estimated period of time to full recovery for the animal?

Provide Recovery Period

2-3 minutes

Where will the animal be located during postoperative recovery?

Provide Recovery Location

Main fish room, transparent static tank; Water is changed every other day after a feeding with flake food. If genotyping exceeds 4 days, animals are placed individually in 1-gallon tanks on the recirculating water system (over the weekend and until results are available).

Which personnel will be monitoring the animal during postoperative recovery?

Provide Names of Personnel

Jen-Jen Hwang Shum, April Freeman, Zoltan Varga, Keely Core, Eryn Loucks, Dagmara Marston, Evan Williams

What is the frequency of postoperative monitoring?

Provide Frequency of Postoperative Monitoring

Immediately after MS-222 anesthesia, then at least once daily, at feeding, and at water changes

What, if any, complications could reasonably be expected?

What parameters will be used to detect signs of pain, distress or discomfort? Check all that apply.

Wound Healing

IMPORTANT: This parameter requires checking the wound at least daily until suture removal.

extremely rare

Other

All humane endpoints described earlier for zebrafish

When observations indicate pain, discomfort, or distress, will the UO Emergency Veterinary Care SOP be followed?

No

A decision will be made by the ZIRC veterinarian whether or not to euthanize the fish.

What are the provisions for after hours, weekend, and holiday care for animals in recovery?

Provide Description of After Hours, Weekend, and Holiday Care for Animals in Recovery

If genotyping exceeds 4 days, animals are placed individually in 1-gallon tanks on the recirculating water system (over the weekend and until results are available). They are fed and monitored along with all other animals in the facility.

Unexpected Outcomes

Unexpected Outcomes

Will this Aim introduce novel experimental variables that may affect animal health and welfare?
No

Animal number calculation for experimental part Genetic Repository and Distribution

fish: Zebrafish			
	Min	Max	Procedure or Experimental Group
		68430	Shipping
+		68430	Shipping
+		96392	Stock Propagation and Adult shipping
+		96392	Stock Propagation and Adult shipping
	0	329644	fish: Zebrafish

	Min	Max	Procedure or Experimental Group
		2550	Diet/Feeding Study
	0	2550	

	Min	Max	Procedure or Experimental Group
	0	0	Animals
		1250	17 alpha-methyltestosterone
	0	1250	

Procedures, Pre-Defined and Manually Entered

Not applicable

Description of Procedures

Please see attached SOP collection of ZIRC's routine operations for import, maintenance, preservation, and distribution of fish lines.

Masculinization of fish stocks with 17 alpha-methyltestosterone

ZIRC fish populations are frequently skewed to increased female proportions. However, ZIRC depends particularly on males and the cryopreservation of sperm to turn over and manage its resources efficiently. Therefore, a high ratio of females poses a problem especially in small stocks, when a certain number of cryopreserved samples is required.

We exposed larvae to 17-alpha-methyltestosterone (17-AMT) as listed in the Zebrafish book by bath immersion. However, this method was difficult to implement in the nursery spaces of the main fish room (for practical reasons) and also did not produce consistent results. Therefore, we added 17-AMT to larval flakes to induce male-skewed stocks. Our preliminary analysis suggested that 20 mg 17-AMT/kg food produced 100% males, whereas at 1 mg/kg food results were inconsistent.

As a next step, we will perform a dose-response analysis to define the minimal dose at which male development is consistently induced. If successful, we will test whether the 17-AMT feeding method can be implemented for routine use in the ZIRC quarantine and/or main fish room nurseries.

To determine the optimal dose of 17 AMT, we will feed Larvae between 7 – 28 days post fertilization (dpf) 1mg/kg, 5 mg/kg, 10 mg/kg, 15 mg/kg, and 20 mg/kg 17-AMT. Each dose will be tested in a stock of 50 larvae, and we will replicate the test 3 times. 3x 50 larvae x 5 doses = 750 fish

Once an optimal feeding dose has been determined, we will introduce 5 stocks of 50 larvae each into the quarantine room and the main fish room and raise them on the optimal dose of 17-AMT diet. 2 x 50 larvae x 5 stocks = 500 fish

To determine successful masculinization of larvae, we will observe fish morphology for male traits, and breed 17-AMT reared males with regular AB wild-type females. We will score fertilization rates and successful breeding events. In addition, fish will be anesthetized by immersion in fish water with 168 mg/L MS-222 and sperm will be collected as outlined in the cryopreservation section. After sperm has been collected, fish will be returned to a recovery tank and observed until they have fully recovered from anesthesia. Sperm samples will be analyzed for cell density, live/dead cell proportion, and motility, using Computer Assisted Sperm Analysis Software.

Males that do not breed, or do not produce sperm for quality assessment, will be euthanized by overdose of MS-222 and dissected to assess gonad morphology. Alternatively, fish will be fixed, embedded in paraffin, and sectioned. Sections will be transferred on microscopy slides and stained with H&E for histochemical analysis of gonad tissue.

Food / Diet Testing

Several new diets have become available in the past years, which have not been tested for use in ZIRC’s colony. To better understand the qualities of these new diets, we will compare them with our current flake and live feed mixes and evaluate the well-being and performance of fish that have been reared on them over the course of 11 months. We will analyze two control groups and 4 test groups: Control Group 1 will receive our SOP diet, which includes an Artemia feeding (live food) in the AM, and two Juvenile- or Adult Flake Mix feedings (mainly Zeigler flakes) at noon and in the PM (3 feedings total). Control Group 2 will receive only the Zeigler-based flake mix, without Artemia. The adult flake mix is composed of Zeigler Zebrafish Adult Flakes : O.S.I. Spirulina Flakes : Golden Pearl 300-500 µm Flakes in a 3.6 : 1 : 1 ratio. The juvenile Mix contains 150-250 µm Zeigler Larval Diet (AP 100) : 250-450 µm Zeigler Larval Diet (AP 100) : 200-300 µm Golden in a 2 : 2 : 1 ratio. Juvenile and adult flake mixes differ in particle size to accommodate different gaps of juvenile and adult fish. Mixing different diets is meant to ensure a complete diet containing Vitamins, essential amino acids, trace elements, protein, and fiber. However, because the exact composition of commercially available diets is proprietary, the exact formulation is unknown, which makes laboratory formulated diets with a defined composition particularly interesting for a well-defined animal husbandry environment.

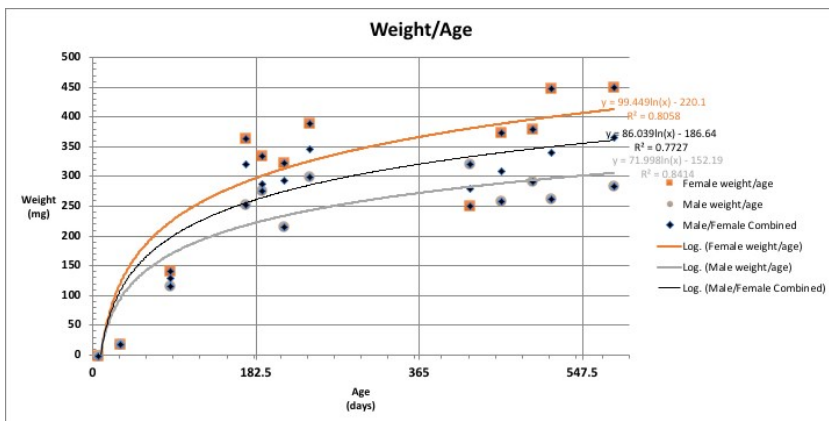
We will test 3 undefined, commercially available zebrafish diets and an experimental, laboratory formulated food; these test groups, like Control Group 2, will not receive any live food (Artemia) either:

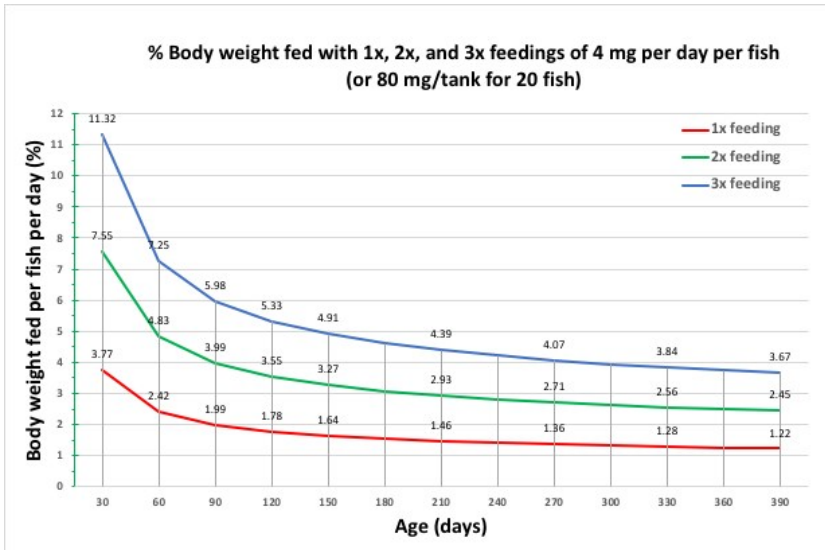
- 1) Skretting, Gemma flakes (<http://skretting.com/en/products/gemma/475103>);
- 2) Otohime, juvenile and adult flakes (http://reedmariculture.com/product_otohime_fish_diet.php#tab_tech);
- 3) Sparos, juvenile and adult flakes (<http://www.sparos.pt/index.php/en/produtos/zebrafeed>) and
- 4) Steve Watts’ laboratory-formulated (defined) juvenile and adult flake food.

The feeds will be stored in color-coded bags and food will be provided with color-coded 3-D printed spoons. The spoon volume will be adjusted to hold exactly 80 mg of flakes, based on the predetermined density of each of the 5 diets. For example, spoons for our current Zeigler diet will measure 0.198 ml for our juvenile (1-3 months) flake mix and 0.161 ml for our adult (3-12 months) flake mix. Two spoons are necessary, because of the slightly different particle sizes, composition, and density.

We will breed fish (numbers see below) and raise them to 3-4 weeks according to our standard, IACUC-approved, nursery protocol. Test and control diets will be administered starting at 1 month through 12 months (360 days). Currently, adults receive two flake feedings per day averaging approximately 3 % body weight (1.5% per feeding). Artemia is considered extra and has not been included in the %-body weight calculation.

Each control and test group will be fed once (mornings only), twice (mornings and evenings), or three times (mornings, noon, evenings) a day with their respective diet. At each feeding, we will provide 4 mg food per fish. 20 fish will be housed in 1-gallon tanks; therefore, each tank receives 80 mg food per feeding. We determined 4 mg based on a standard growth curve of AB wild-types (determined during approved electrosedation testing), which weigh approximately 261 mg at 6 months of age (males and females combined); the data for a more differentiated evaluation based on gender is available but doesn’t change our calculations significantly). Based on these measurements, 4 mg food represents 1.5% of body weight. However, considering the changing weight of fish during their life-span 4 mg (1x feeding per day) is the equivalent of 3.77% body weight at 30 days and 1.25% body weight at 360 days (1x feeding/day). At 3 feedings per day, 12 mg is 11.32% body weight at 30 days, and 3.75% body weight at 360 days. Body weight values for 2x feedings per day (8 mg per fish) will lie between these curves. Thus, over the lifespan of a year, we will provide a percentage range of body weights between 11 % and 1% body weight fed each day.





At 1 month, 10 tanks of 25 juvenile AB (250 juveniles) will be placed over millimeter paper in a 40x40 cm photography chamber, photographed, and their standard length (snout to caudal peduncle) and width at the level of the pectoral fin will be determined and averaged. The juveniles will then be anesthetized with 168 mg/L MS-222, placed in a weighing dish, excess water will be gently removed with tissue paper, and the average weight per juvenile will be determined. Fish will be returned to their tanks after measurements and observed for recovery and vital signs. This data will provide an averaged body condition factor (BCF), averaged body weight, and standard length at 1-month of age, constituting the starting point for a growth- and BCF-curve.

At 3 months, equal ratios of males and females will be adjusted in each 1-gallon tank. To this end, we will overproduce embryos and juveniles (see animal numbers below). Body size and weight measurements will be determined on five randomly chosen males and females from each tank (50% of each tank population/test group). Fish will be anesthetized as described above, returned to their tanks after measurement, and observed.

At 6 months, size and weight measurements will be gathered as described at 3 months. In addition, we will set up 5 breeding pairs from each tank and score breeding success, the number of eggs, fertilization-, and hatching rates. We will assess embryo/larval morphology between 0-5 Days post fertilization (dpf) and keep score of any abnormal development that might be indicative of variations in egg quality. The five remaining males will be anesthetized and squeezed to collect sperm using approved IACUC protocols (see cryopreservation section), and cell density and motility will be determined. All fish will be returned to their tanks after testing and observed daily for recovery and vital signs.

The same measurements will be taken at 9 and 12 months of age. At 9 months, we will, in addition, evaluate the general health of 5 fish from each tank using histopathology. To this end, fish will be euthanized according to established protocols for in-house health monitoring. Fish will be embedded in paraffin blocks, sectioned, and stained with H&E for viewing under the microscope. The ZIRC veterinarian will evaluate the presence or absence of pathogens in the sampled fish and determine whether or not any diet has a more or less favorable outcome for overall fish health. The analysis will also include adipose cell deposits versus signs of emaciation. Lastly, at 12 months we will rear a new generation of fish derived from the control and test groups. These embryos/larvae will also be assessed for morphology but will be raised and analyzed as a new generation. Depending on the analysis of our data from the first year of the feeding study, we will decide what tests to conduct on these new generations. For this new phase, we will submit an amendment to our AUP to the IACUC.

Sample sizes have been determined using the sample size calculators at <http://clinical.com/stats/sampleize.aspx> and <https://www.surveysystem.com/sscalc.htm>. Previous experiments indicate approximately 15% variance in weight or body measurements in a population (stock). To ensure that a 10% difference in size effect is not due to a false positive, we chose alpha 0.05 (5% chance that a significant difference is due to chance), and to minimize the possibility that a significant difference is missed (false negative) we chose a beta value of 0.2 (statistical power 80%). For these values, we need population sizes of 70 and sample sizes of 35. We inflated these numbers to 80 and 40 to take into account the possibility that fish might die unexpectedly during a year of observation. A sample size of 40 out of 80 should yield a confidence level of almost 99%.

80 fish of each test group will be split in 4 replicate tanks (20 fish each). From each tank, we will sample 10 (5 males/5 females) for body measurements (40 per test group) and at least 5 fish per tank for gamete quality control and health assessments (20 per test group). To ensure an equal sex ratio per tank we will overproduce one extra tank of 20 fish for each test group, determine the gender ratio at 3 months and then adjust it in the four replicate test tanks in all test and control groups. Excess fish will be added back to our colony operations e.g. for distribution to the community). If excess fish need to be euthanized, it will be done according to the approved protocol (hypothermal shock).

We anticipate, that over the course of the study and also during sampling some fish will die (e.g. they do not wake up from anesthesia). To maintain a constant fish density in each tank, we will also breed 500 *nacre* mutant fish that carry a distinct pigment mutation and can be easily distinguished from their AB wild-type counterparts. Whenever AB fish perish accidentally in the 4 replicate tanks, we will adjust density and gender, using fish from the fourth replicate tank. Fish in the fourth replicate tank will then be replaced with *nacre* mutants. This way, we will maintain a wild-type AB population in 3 of the four replicates for each diet. In the fourth replicate, *nacre* fish will maintain a normal fish density, but will not be used for testing.

Animal breeding and space requirements:

We will feed 3 doses per day, test 6 diets in groups of 80 fish, but produce initially 100 per test group to adjust for potentially skewed gender ratios at 3 months. Breeding requirement:

- a) 3 doses x 6 diets x 5 replicate tanks of 20 fish = $3 \times 6 \times 5 \times 20 = 1800$ AB. After equalizing gender ratios at 3 months: 3 doses x 6 diets x 4 replicate tanks (20 fish each) = $3 \times 6 \times 4 \times 20 = 1440$ AB
- b) 250 fish (AB) will be bred for bulk measurements at 1-month for the starting point of a growth curve.
- c) 500 *nacre* will be bred as replacement fish to maintain a 20 fish/gallon stocking density, throughout test groups.

Space requirements: 2600 larvae (nursery); 90 tanks (AB), + 20 tanks (*nacre*, back-ups) = 110 adult 1-g tanks.

--- PLACEHOLDER for FUTURE FEATURE ---

Additional Procedure Details

Prolonged Restraint

Prolonged Restraint

Will any of the procedures listed above involve prolonged animal restraint?

No

Blood or Fluid or Tissue Collection

Blood or Fluid or Tissue Collection

Will any of the procedures listed above involve the collection of fluid from a live animal or the collection of tissues from a live animal?

Fluid can be blood, bile, spinal fluid, etc.

Do not include information about animal identification methods.

Yes

DETAILS

Provide Specimen Name

Gamete collection by abdominal massage (during standard anesthesia with MS-222)

Provide Collection Site

Urogenital opening

Provide Specimen Volume or Weight

Sperm <5 μ l

Eggs ~ 500 μ l

Provide Number of Samples

~ 5000 per year

How will specimen be discarded?

Not discarded. Sperm is added to cryogenic repository or used for in vitro fertilization; eggs are used for in vitro fertilization

Substance Administration

Substance Administration

Will you be administering any substances to animals?

"Substances" here does not include agents used for anesthesia, analgesia, or euthanasia. Nor does it include hazardous agents. Questions about these are asked in other areas of the eAPM system.

No

Use of Controlled Substances

Use of Controlled Substances

Will controlled substances be used for anesthesia, analgesia, restraint, animal management, testing, or euthanasia?

Yes

Controlled Substance Details

Provide Controlled Substance(s) Name(s)

17- α -Methyltestosterone (Schedule IIIN Controlled Substance)

Provide Authorization from EHS

IMPORTANT: Attach a copy of the Authorization from EHS by using the paperclip icon found at the top or bottom of this page.

DEA Registration #: RV0493104; 04/12/2017

Oregon Exception Request to Conduct Research - OAR 855-080-0095; 04/26/2017 valid through May 31, 2018

Provide Names of Authorized Individuals

Zoltan Varga

Jen Matthews

Joy Murphy

Provide Storage Details

17 α -Methyltestosterone is maintained at room temperature in a locked, unmarked drawer in Room 109, which serves as an auxiliary laboratory space. Access to this space requires the building access code (keypad and card). A camera monitors the outside entrance to the room. The drawer is equipped with a Schlage safety lock, to which the keys are stored in a wall mounted key-safe with combination code access. Only the DEA registrant and the registered experimenters have access to the code (memorized, not captured in writing). An annual inventory is conducted according to federal law, and use of the CS is tracked and logged every time.

Food or Fluid Regulation

Food or Fluid Regulation

Will food or fluid regulation be used in this study?

Yes

What is the justification for the use of food and/or water regulation?

Provide Justification

During the identification of individual carriers of a genetic modification, animals are held for up to 4 days in isolated, transparent, static water tanks (1.5 Liter). During this time they do not receive food for two days. Approximately 30 minutes after feeding (2nd day) animals receive a fresh water

change and are held for another 2 days in static, isolated conditions without food. If the time to obtain genotyping results exceeds 4 days individual fish are placed in 1-gallon tanks on the recirculating water system, where they receive regular feeding (twice daily) and health monitoring every day. Once genotyping results are obtained, carriers of genetic modifications are pooled into 1-gallon tanks and placed on the recirculating water system (max. density 20 fish/gallon) with regular feeding and monitoring schedules.

What alternative methods were considered, including positive reinforcement?

What is the nature, extent, and frequency of food and/or water regulation and how was this determination made?

What are the potential adverse consequences of the regulation on the animal's health and well-being?

What methods and signs will be used to assess the animal's health and well-being and how frequently will it be assessed?

Body weights should be recorded at least weekly and more often for animals requiring greater restrictions.

(Guide, p. 31)

Provide Details about Assessment of Health and Well-Being

Animals are monitored visually at least once a day for humane endpoints or any signs of discomfort, pain or distress. If the latter is observed water is changed, and monitoring continues to ascertain that symptoms have been alleviated. If humane endpoints are observed the veterinarian is consulted and a determination is made whether or not to euthanize the animal(s). Note: Humane endpoints as listed in this protocol include several/most signs of discomfort, pain or distress.

Where will written records be located?

Written records should be maintained for each animal to document daily food and fluid consumption, hydration status, and any behavioral and clinical changes used as criteria for temporary or permanent removal of an animal from a protocol.

(Guide, p.31)

Provide Location of Records

ZIRC breeding log (Database and excel spreadsheet). Hardcopy is maintained with animals during the 2x2 days of food and wather restriction

What criteria will be used to remove the animal from the study?

Provide Criteria Used to Remove Animals from Study

Fish that do not carry the genetic modification are euthanized according to IACUC sanctioned methods.

Imaging Studies

Imaging Studies

Post-Procedural Monitoring

Post-Procedural Monitoring

Procedures Training Verification

fish: Zebrafish

PROCEDURES TRAINING VERIFICATION TOOL MATRIX

	Bauer, Justin	Black, Robin	Clark, Renee	Conway, Dylan	Core, Keely
	Hwang-Shum, Jen-Jen	Lains, David	Loucks, Eryn	Marston, Dagmara	Matthews, Jen
	Murphy, Joy	Murray, Katrina "Katy"	Nasiadka, Andrzej	Quinn, Erin	Schulze, Catherine
	Smith, Calvin	Varga, Zoltan	Westerfield, Monte	Williams, Evan	Wolfe, Jacklyn

Diagnostic Health Service

Animal Species Use

fish: Zebrafish

Experimental Design and Procedures Summary

Procedures Summary

For the current aim (see highlighted subtab above), explain the experimental design and provide a clear, concise and sequential description of the procedures involving the use of animals.

This narrative should explain the overall design and include things like experimental animal groups, group sizes, and group use.

IMPORTANT: Provide a clear and concise sequential description of the procedures (especially those that are listed in the procedures tab) involving the use of animals that is easily understood by all members of the IACUC. Details of any of the procedures should be described in the "Procedures" tab. This description should allow the IACUC to understand the experimental use of an animal from its entry into the experiment to the endpoint of the study.

NOTE: You may attach diagrams, flowcharts, etc., by using the paperclip icon at the top or bottom of this page.

Provide Experimental Design (see IMPORTANT help text above):

ZIRC provides diagnostic pathology and consultation services.

- ZIRC provides diagnostic services and health status testing for laboratory zebrafish. We use histopathology, bacteriology, necropsy, and virology to analyze specific or suspected disease problems. We provide routine sentinel or quality control testing of zebrafish from healthy laboratory colonies for early detection of problems and to fulfill institutional animal care and use committee, IACUC, health status monitoring requirements. We continue to develop methods to detect and control disease in laboratory colonies. We continue to investigate the transmission and pathology of the microsporidian parasite, *Pseudoloma neurophilia*, and *Mycobacterium chelonae* and we continue to discover novel pathogens through the diagnostic service.
- The ZIRC veterinarian monitors the health status of the ZIRC fish colony and maintains biosafety during line exchanges. The ZIRC veterinarian assesses the presence and prevalence of pathogens in the facility by monitoring sentinel fish populations, or strategically and randomly selected fish, and proposes strategies to control or eliminate any pathogens identified in the colony. The ZIRC veterinarian is also in charge to put in place policies to prevent novel pathogens from entering the facility during import and provides health statements to clients upon fish exports. A quarterly colony health statement is prepared and made available online to the research community.

Please see attached SOP collection of ZIRC's *routine operations for the colony health monitoring procedures and Diagnostic Health Services*.

- **Research component I:** We will develop a platform, based on a panel of PCR assays, to screen for the most prevalent pathogens of laboratory zebrafish. We will use genomic sequence information from known zebrafish pathogens to design and test PCR primers for species-specific detection. We will validate the sensitivity of these molecular assays with standard health screening by histopathology. We will use these PCR assays and other diagnostic tools to establish and implement screening procedures for in-house detection and monitoring of zebrafish pathogens. We will establish a platform, through the ZIRC website, for sharing the ZIRC pathogen monitoring program and tools for diagnostic testing with the research community, and we will provide consultation and diagnostic services, using this new platform, to identify pathogens in research laboratories, through our Pathology Service.
- **Research component II:** Currently, ZIRC can only perform post-mortem diagnostics for most pathogens. We will therefore collaborate with the Kent laboratory at OSU to develop, test, and validate a *Pseudoloma neurophilia* Droplet DNA test. A Droplet DNA test will permit testing of tank water whether a population carries a pathogen, such as *P. Neurophilia*. We will use 8-month fish that have been designated for routine pathogen monitoring at ZIRC. If identified as positive carriers, we will also test the 3-month offspring of these populations, and if possible test the offspring later again (around 8-months) if an initial test was negative. This method will be more efficient to identify infected populations, and can also be more systematically used to detect the prevalence of a pathogen in a colony.

Animal Biosafety Level

Animal Biosafety Level

For the current aim (see highlighted subtab above), will the risks associated with the experiments exceed Animal Biosafety Level 1 (ABSL-1)?

Where biologic agents are used, the Centers for Disease Control and Prevention (CDC) and National Institutes of Health (NIH) publication biosafety in Microbiological and biomedical Laboratories (BMBL; DHHS 2009) and the USDA standards (USDA 2002) should be consulted for appropriate facility design and safety procedures. These design and safety features are based on the level of risk posed by the agents used. Special facilities and safety equipment may be needed to protect the animal care and investigative staff, other occupants of the facility, the public, animals, and the environment from exposure to hazardous biologic, chemical, and physical agents used in animal experimentation. (Guide, p. 19)

No, ABSL-1 is the risk level associated with the animal use in these experiments.

Humane Endpoints

Humane Endpoints

For the current aim (see highlighted subtab above), what are the humane endpoints?

Humane Endpoints adapted from information online.

Select Animal Type(s) Used in this Aim. Check all that apply.

Fish

Types of Humane Endpoints (Check all that apply.)

Clinical or Behavioral

Abnormal Color Change

Abscess

Distended Abdomen

Emaciation

Eye Bulging (Exophthalmos)

Fungal Growth on Body or Fins

Gas Bubbles

Hemorrhage or Redness

Masses or Swellings

Skeletal Deformity

Tumor Formation

Twirling

Swimming in circles

Ulcer

Wound

--- PLACEHOLDER for FUTURE FEATURE ---

Placeholder

Final Disposition and Euthanasia

Not applicable

Euthanasia / Final Disposition

For this aim, what will be the final disposition of the animals? Check all that apply.

Euthanasia

METHODS of EUTHANASIA and CONFIRMATION of DEATH

Select Animal Type(s) Used in this Aim. Check all that apply.

Fish

What method(s) of euthanasia will be used? Check all that apply.

IACUC-Approved Methods

(zebrafish, adult) Immersion in solution of buffered tricaine methanesulfonate (MS-222), 0.03% (w/v)

Finfish should be left in the anesthetic solution for a minimum of 10 minutes after cessation of opercular movement.

(AVMA Guidelines for the Euthanasia of Animals (2013), p. 72)

(zebrafish, all developmental stages) Rapid Chilling (Hypothermic Shock)

Transfer from acclimatized temperatures to water associated with a 2° to 4°C ice slurry must occur rapidly with as little transfer of warmer water as possible.

(AVMA Guidelines for the Euthanasia of Animals (2013), p. 73)

How will you confirm animal death? Check all that apply.

Cessation of Vital Signs

Antibody Production

Antibody Production

Will you produce antibodies as part of this Aim?

No

--- PLACEHOLDER for FUTURE FEATURES --- (Use of Visual Recordings, Photography, or Audio Recordings)

Surgery

Surgery

Will you be performing surgery as part of this Aim?

No

Unexpected Outcomes

Unexpected Outcomes

Will this Aim introduce novel experimental variables that may affect animal health and welfare?
No

Animal number calculation for experimental part Diagnostic Health Service

Min	Max	Procedure or Experimental Group
0	0	
0	0	

Procedures, Pre-Defined and Manually Entered

Not applicable

Description of Procedures

Please see attached SOP collection of ZIRC's *routine operations for the colony health monitoring procedures and Diagnostic Health Services*.

Research Component I: ZIRC Diagnostic Health Services Research:

Background. In recent years, a number of pathogens that infect zebrafish have been identified, many by our own studies. These pathogens have negative direct and indirect effects on research. Impacts include altered behavior, repressed fecundity, massive mortalities, and human infection with zoonotic pathogens. The research community currently lacks a platform for systematic health screening. Some commercial services are offered, but because they are for-profit, critical information about detection assays including sensitivity and validation are proprietary and not made available to researchers who use the service. If negative results are incorrectly reported to the user, this can provide a false sense of pathogen-free status that can lead to severe consequences. Similarly, false positives can result in unnecessary anxiety and culling of animals. ZIRC has already established successful health-monitoring programs. However, current monitoring is predominantly based on histopathology. We outsource molecular screening for pathogens to the Oregon Veterinary Diagnostic Lab (OVDL), and this testing is currently limited to only a few species. Our recent work in-house and our studies of cases submitted to our Pathology and Health Services have identified and characterized an increasing number of pathogens present in zebrafish research facilities (Table 1 below). Thus, it is critically important for us to develop a reliable, standardized platform for systematic pathogen detection within the ZIRC facility.

Pathogen	Type	Colony Risk / virulence	Zoonotic	Diagnosed in submissions to ZIRC pathology service 2005-2015
<i>Pseudoloma neurophilia</i>	Microsporidia	Low	no	160 cases (1212 fish)
<i>Mycobacterium</i> species:	Bacteria			134 cases (451 fish)
<i>M. marinum</i>		High	yes	-
<i>M. haemophilum</i>		High	yes	-
<i>M. fortuitum</i>		Low	yes	-
<i>M. chelonae</i>		Low	yes	-
<i>Myxidium streisingeri</i>	Myxozoan	Low	no	79 cases (276 fish)
<i>Pseudocapillaria tomentosa</i>	Nematode	High	no	39 cases (122 fish)
<i>Pleistophora hypohessobryconis</i>	Microsporidia	Low	no	8 cases (35 fish)
<i>Edwardsiella ictaluri</i>	Bacteria	High	no	3 cases (12 fish)
<i>Mycoplasma</i> spp.	Bacteria	Low	no	Not calculated

Table 1. Species we will include in the PCR-based platform.

We further propose that ZIRC will provide a complementary diagnostic service to the research community, by empowering individual laboratories and fish facilities to carry out their own pathogen monitoring, tailored specifically to their research programs. In doing so researchers will no longer need to rely on one-size-fits-all screening panels with uncertain detection sensitivities and unknown protocol validation, as are currently offered by commercial entities. To this end, we will develop a PCR panel for the most prevalent pathogens found in zebrafish research facilities (Table 1). We will provide the information on our web site, along with general health monitoring methods and the most typical screening strategies that can be applied in fish facilities of various sizes, from small to very large. To help set up user-specific health monitoring programs, we will also advise laboratories how to apply the screening panels to their specific needs. We will charge a consultation fee for this service.

We will design PCR assays to detect *Pseudoloma neurophilia*, *Mycobacterium* species (i.e. *M. marinum*, *M. haemophilum*, *M. chelonae*, and *M. fortuitum*), *Mycoplasma* spp. (Karen Guillemin, personal communication), *Pleistophora hypohessobryconis*, *Edwardsiella ictaluri*, *Myxidium streisingeri*, and *Pseudocapillaria tomentosa*. We will use DNA sequences from these pathogens for heat-shock protein and ribosomal RNA (rRNA) genes. Sequence information is available. As we identify new pathogens through our ongoing Pathology and Health Services, we will use the same strategy to add them to the platform.

Unlike ZIRC, most laboratories do not have Kompetitive Allele Specific PCR (KASP; LGC Genomics) genotyping capability. Therefore, we will design two types of assays in parallel. In the first, the PCR products will be analyzed by conventional gel electrophoresis and visualized with ethidium bromide and UV illumination. In the second, KASP technology will be used, which relies on fluorescent probes. This will alleviate the need to use ethidium bromide gels and will make pathogen detection and monitoring faster, more efficient, and safer. Because KASP amplicons are typically 50 base pairs or shorter, we will use commercially synthesized DNA oligonucleotides of pathogen-specific SNPs for assay testing.

Validation. We will first test our KASP assays with DNA oligonucleotides alone and then with added wild-type genomic DNA. We will also use synthetic DNA oligonucleotides as positive and negative controls for routine pathogen molecular screening. In addition to single-PCR product assays (utilized for general pathogen detection), we will also design RFLP, dCAPS, and KASP assays to identify *Mycobacterium* substrains. We will validate tested molecular assays and determine their sensitivity by performing parallel histopathology analyses. We will test a number of preparations including samples prepared from different tissues, and samples prepared from water, biofilms, and aquarium debris.

We will determine the sensitivity of tested molecular assays by performing them in parallel with our routine in-house histopathology analyses that we have developed previously as part of our Pathology and Health Services. By using newly developed molecular and already established histopathology assays **side-by-side** for systematic in-house pathogen screening, we will validate results in the context of our own health program. Our molecular assays will also undergo independent testing and validation in external facilities, the OVDL and other laboratories. We will use the most optimal and reliable molecular assays for systematic in-house pathogen screening. No extra animals will be bred or used for this research, we will use cloned DNA provided by other laboratories, synthetic DNA, or DNA derived from tissue of routine in-house histopathology cases.

Animals obtained from other laboratories are usually submitted as fixed tissue. Whenever live animal are received, or whenever we will use live in-house fish for colony health monitoring to validate the PCR panel, we will euthanize fish according to IACUC approved euthanasia methods (MS-222 overdose or hypothermal shock, listed in the attached SOP collection). Freshly isolated tissue will be used for DNA isolation before the carcass is fixed for paraffin embedding, sectioning, and staining for histopathology analysis. The isolated fresh tissues will be enzyme-digested and DNA will be isolated using standard molecular laboratory protocols.

Research Component II: *Pseudoloma* Droplet DNA testing

Background. As part of their R24 grant aims, the Kent laboratory at OSU will be developing non-lethal PCR tests for prevalent zebrafish pathogens using digital droplet DNA testing of water. The laboratory has been developing such tests for salmon pathogens with excellent success, and now will be moving to adapt the technology for zebrafish pathogens.

The aim of the collaboration between the Kent laboratory and ZIRC is to validate a *Pseudoloma* Droplet DNA test, because 1) *Pseudoloma* occurs in our facility, 2) Our Veterinarian (Katy Murray) is already testing tanks with individual fish histology, and 3) such a test will be very valuable for the efficient diagnosis of live fish populations. We currently do not have a droplet DNA system, so the Kent Laboratory will provide replicate DNA samples for our in-house comparison with their own PCR test and the droplet test.

We routinely test all wild-type fish tanks when fish reach 8 months of age. On average, 20-gallon tanks house 150 to 250 fish. Presently, Katy is testing fish by histology with Luna stain and has recently started implementing in-house PCR in addition to histology (See Health Services Procedures). Tanks that are positive at 8 months for *Pseudoloma* are marked in the database and flagged for fish euthanasia and tank removal in the fish room. The fish are then euthanized according to approved hypothermal shock protocol as soon as possible. OSU will be provided euthanized fish from these tanks. We (Katy) will also freeze euthanized fish in Eppendorf tubes and save water following the Kent laboratory's filtering protocol (see below). Each month, approximately 10 tanks are routinely tested at ZIRC, and typically 2-3 are *Pseudoloma* positive.

Procedures

Pseudoloma samples from 8-month fish.

If a ZIRC tank with 8-month fish is tested positive for *Pseudoloma* during routine (histopathology) health monitoring, the Kent laboratory will test water from that tank by DNA droplet analysis. All fish from these tanks will be euthanized, frozen, stored in zip-lock bags (for incineration); however, 30 carcasses will be transferred to the Kent laboratory for subsequent *Pseudoloma* testing by PCR.

3 x 30 x 12 = 1080 fish/year

If all of the 8-month tanks are negative by histology, the water test is performed on up to 3 these randomly chosen tanks and 30 fish from these tanks are saved for and PCR (Kent and ZIRC tests). If 3 "negative by histology" tanks are not available on a given month, we will randomly test 3 different 8-month tanks to conduct this task.

3x30x12 = 1080 fish/year

Pseudoloma sampling of 3-month old offspring from positively identified stock populations

By 8 month of age, a new generation has already been bred and is growing up in our facility. These fish are typically 2-3-month old. A key advantage of the droplet DNA testing will be that we will be able to test live fish (i.e. their water) for pathogens much earlier, before we breed them. Because we will test specific populations directly, this method will significantly reduce maintenance time of fish that would have tested positive at 8 months. They are eliminated earlier, which reduces the potential of pathogen transmission by our husbandry procedures.

Each month, 2 tanks with 3-month old fish derived from *Pseudoloma*-positive (8-month) fish will be used for water testing. In addition, 30 3-month old fish will also be selected from these tanks for euthanasia and subsequent PCR testing.

2x30x12 = 720 fish/year

Each of these tanks is tested once at 3 months, and then later again at 8 months. If the 3-month old fish test *Pseudoloma*-negative, they continue to be used for the ZIRC program. If a 3-month old fish tank tests positive, all fish (up to 250) will be transferred to OSU, maintained in the Kent fish facility, and tested again at 8-10 months to follow increases in prevalence within a tank/stock. This may happen up to 12 times per year (depending on fish availability at ZIRC, and/or space availability in the Kent fish facility)

2x250x12 = 6000 fish/year (maximally). These fish will be transported to OSU, maintained, and euthanized according to Kent laboratory, OSU-IACUC approved procedures.

Total Fish used: 1080 + 1080 + 720 + 6000 = 8880 Fish

Pain Category: D

The 8880 fish used in this study are all part of ZIRC's routine breeding concept and health monitoring and are included in the animal numbers of the main protocol (Repository); Fish will be euthanized according to protocol before histopathology and PCR testing. Because fish may have other uses and may be anesthetized e.g. to collect gametes or for fin-clipping prior to diagnostic testing, we will list them in Pain Category D.

Fish Data for Collection:

1. Strain,
2. Age,
3. Number of Fish,
4. Time in Tank,
5. Tank Volume,
6. ZIRC tank location

Water Sampling Method

1) Turn water off for 6 h, provide aeration

2) At the end of the static period, water in the tank is stirred and 4 liters of tank water are filtered using a vacuum pump. 4 filters are exposed per aquarium, 2 for droplet- and 2 for ZIRC PCR analysis. Katy Murray will oversee filtering at ZIRC. Filters are folded and saved frozen for future processing.

3) **Prevalence of infection in fish:** At least 30 fish will be screened by qPCR at OSU. Fish will be preserved in groups of five and frozen. The Kent laboratory will thaw, dissect brain and spinal cord, and process in pools of 5 as described in the Sanders et al. publication. Prevalence will be estimated using the algorithm of Williams and Moffitt (2005).

Justification for 30 fish/tank sample

Dhand, N. K., & Khatkar, M. S. (2014) provides an online program for calculating the precision of prevalence results with a known subsample from a finite population <http://statulator.com/SampleSize/ss1P.html>

A low sample number of individuals will produce larger margins of error (standard deviation from the mean). However, this is acceptable to correlate water droplet scores (quantification of DNA by ddPCR) with a prevalence estimate in the fish population from the same tank, with a large number of tanks (over time) that show a range of prevalence of infection. Thus, although results have a broad range, with many tanks tested we will see how water testing and histopathology correlate

E.g. If 31% of the fish test positive in a population (based on histopathology of individual fish), and the average population size in 20-gallon ZIRC tanks is 200 fish, the study would require a sample size of 30 for estimating the expected proportion with 50% precision relative to the expected proportion (i.e. $0.5 \times 0.31 = 15.5\%$ absolute precision) and 95% confidence. This means, if 30 fish are sampled randomly from a tank, and if we determine that 31% of subjects test positive for *Pseudoloma*, there will be 95% confidence that between (31% +/-) 15.5% and 46.5% of subjects in the population have the factor of interest. This is sufficient to correlate histopathology and DNA droplet water testing

References

- Dhand, N. K., & Khatkar, M. S. (2014). Statulator: An online statistical calculator. Sample Size Calculator for Estimating a Single Proportion. Accessed 22 March 2018 at <http://statulator.com/SampleSize/ss1P.html>
- Sanders JL, Kent ML 2011. Development of a sensitive assay for the detection of *Pseudoloma neurophilia* in laboratory populations of the zebrafish *Danio rerio*. Dis Aquat Org. 96:145-156
- Williams and Moffitt. 2005. Estimation of pathogen prevalence in pooled samples using maximum likelihood methods and open-source software J. Aquat Animal Health 17: 386-391)

Personnel

Katy Murray and Evyn Loucks will perform all animal work at ZIRC. Kent laboratory staff will instruct ZIRC in the water sampling and Droplet DNA testing methods. No personnel need to be added.

--- PLACEHOLDER for FUTURE FEATURE ---

Additional Procedure Details

Prolonged Restraint

Prolonged Restraint

Will any of the procedures listed above involve prolonged animal restraint?

No

Blood or Fluid or Tissue Collection

Blood or Fluid or Tissue Collection

Will any of the procedures listed above involve the collection of fluid from a live animal or the collection of tissues from a live animal?

Fluid can be blood, bile, spinal fluid, etc.

Do not include information about animal identification methods.

Yes

DETAILS

Provide Specimen Name

Whole fish: 1) DNA Digest of Nervous tissue for PCR 2) Embedding of fixed carcass in paraffin and sectioning for H&E stain for histopathology

Provide Collection Site

ZIRC Facility

Provide Number of Samples

8880 Fish

How will specimen be discarded?

Not applicable - specimen are paraffin embedded and will be stored for resectioning.

Substance Administration

Substance Administration

Will you be administering any substances to animals?

"Substances" here does not include agents used for anesthesia, analgesia, or euthanasia. Nor does it include hazardous agents. Questions about these are asked in other areas of the eAPM system.

No

Use of Controlled Substances

Use of Controlled Substances

Will controlled substances be used for anesthesia, analgesia, restraint, animal management, testing, or euthanasia?

No

Food or Fluid Regulation

Food or Fluid Regulation

Will food or fluid regulation be used in this study?

No

Imaging Studies

Imaging Studies

Post-Procedural Monitoring

Post-Procedural Monitoring

Procedures Training Verification

fish: Zebrafish

PROCEDURES TRAINING VERIFICATION TOOL MATRIX

	Bauer, Justin	Black, Robin	Clark, Renee	Conway, Dylan	Core, Keely	
	Hwang-Shum, Jen-Jen	Lains, David	Loucks, Evyn	Marston, Dagmara	Matthews, Jen	
	Murphy, Joy	Murray, Katrina "Katy"	Nasiadka, Andrzej	Quinn, Erin	Schulze, Catherine	
	Smith, Calvin	Varga, Zoltan	Westerfield, Monte	Williams, Evan	Wolfe, Jacklyn	

Cryopreservation Animal Species Use

fish: Zebrafish

Experimental Design and Procedures Summary

Procedures Summary

For the current aim (see highlighted subtab above), explain the experimental design and provide a clear, concise and sequential description of the procedures involving the use of animals.

This narrative should explain the overall design and include things like experimental animal groups, group sizes, and group use.

IMPORTANT: Provide a clear and concise sequential description of the procedures (especially those that are listed in the procedures tab) involving the use of animals that is easily understood by all members of the IACUC. Details of any of the procedures should be described in the "Procedures" tab. This description should allow the IACUC to understand the experimental use of an animal from its entry into the experiment to the endpoint of the study.

NOTE: You may attach diagrams, flowcharts, etc., by using the paperclip icon at the top or bottom of this page.

Provide Experimental Design (see IMPORTANT help text above):

All fish lines acquired by the ZIRC are preserved as cryogenically frozen sperm samples. Currently, ZIRC preserves 78,581 samples of 34,181 distinct alleles (~2.3 samples/allele) in approximately 11,000 fish lines (~7.1 samples/line).

Typically, live fish are imported into the quarantine room, acclimated, observed for health status, and either bred and cryopreserved, or cryopreserved only. When cryopreserved samples are imported in liquid nitrogen, samples are transferred into a vapor phase cryogenic freezer. When fish lines are rederived from frozen sperm to fulfil a request, to reamplify a stock, or to genetically identify carriers, the health status of the submitting laboratory will dictate what biosafety measures are taken with the live embryos and larvae. Frequently requested fish lines are maintained live in the fish facility, however once the number of requests drops below 9 per year, lines are cryopreserved and live propagation is discontinued. Similarly, frequent requests trigger the reactivation of a frozen line to the live maintenance status. In addition, when regular requests for frozen samples deplete a frozen resource, lines are reactivated to freeze more sperm samples and reamplify the resource. Hence ZIRC's genetic line management revolves around the constant freezing and thawing of fish lines based on user request frequencies and resource availability, minimizing the number of live lines as much as possible to manage the genetic resources as efficiently as possible.

An emergency back-up site exists in Fort Collins, Colorado at the National Center for Genetic Resource Preservation (ARS/USDA) for cryopreserved ZIRC samples that are not backed up reliably in other laboratories or Resource Centers.

Please see attached SOP collection of ZIRC's *routine cryopreservation procedures*.

Cryopreservation Research Component

The zebrafish research community has produced several tens of thousand mutations, transgenes, and continues to produce more novel modifications of the zebrafish genome. However, the success of zebrafish comes with a price: there are simply too many lines to maintain as live populations within laboratories. The preservation of these genetic resources is jeopardized by a systemic lack of reliability and efficiency of methods and the relatively high cost of acquiring cryopreservation-related equipment and devices that are needed to achieve standardized sample quality and cryopreservation success. Our goal in previous funding was achieved by establishing a central pathway for high-throughput cryopreservation, spanning the activities in the cryopreservation process from sample collection to *in vitro* fertilization. This pathway was specifically beneficial for the Zebrafish International Resource Center (ZIRC) for high-throughput sample loading, labeling, and freezing. The approach was to 1) optimize each step in the process individually and in relation to other steps, 2) assemble and streamline these steps after multiple iterations into a pathway, and 3) standardize the pathway to improve the overall efficiency of processing, sample usage and post-thaw quality and fertilization success. ZIRC and its collaborators at the LSU are now taking this step further by developing, engineering, testing, and distributing tools, small equipment, and software that will help laboratories follow the same standards and methods that have been so successful for ZIRC. Development of these tools will be performed at LSU with our collaborators in the Tiersch (aquatic Cryopreservation) and Monroe (Engineering) laboratories. Animals are not involved at LSU, however, our collaborators will send us 3-D printed parts and tools, or software, which will be tested at ZIRC by cryopreserving sperm obtained from ZIRC fish. ZIRC's feedback is used to refine the instruments and software before making them available for researchers in the community. The following parts, instruments/tools and software are planned to be tested at ZIRC:

1) A Microfabricated Enumeration Grid Chamber (MEGC); this is essentially a microfabricated counting chamber which in contrast to Mackler Chambers and Hemocytometers will be low cost and disposable. It will help research labs to quickly and easily (and at low cost) determine zebrafish sperm cell densities and adjust concentrations for optimal cryopreservation.

2) A Sequential Logarithmic Mixing Apparatus (SeLMA)/Microfabricated Activation and Motility Chamber (MAMC); This is a microfabricated microscope slide that allows

the controlled mixing of water and milt for the activation of sperm cells using microfluidic laminar flows and mixing chambers, before activated sperm enter a viewing chamber under the microscope objective. Activation of sperm and observation of motility will become a straightforward 1-step process and will help provide laboratories to implement computer assisted sperm analysis (CASA) to provide reproducible quality control measurements with other tools we develop.

3) A belt-driven device and a cryopreservation float, named Positional Cooling Platform Device (PCPD), for standardized (slow cooling rates 1-10 °C/min) freezing of single straws and vials. The former will move straws over liquid nitrogen and nitrogen vapor at a predetermined rate before dropping straws into liquid nitrogen. The latter will be floating on liquid nitrogen and suspend vials at predetermined distances above the liquid in the vapor phase. The distance for the liquid phase determines the freezing rate.

4) 3-D printed devices for standardized vitrification (ultra-rapid cooling). This includes a 3-D printed vitrification device (VSID) with a sample loop and a protective sleeve that can be used for the labeling (identification) of the vitrified sample. Loops with fresh samples are immersed at high speed into liquid nitrogen to achieve several 100 - 1000 °C/min cooling rates per minute. Actual sample vitrification will be verified visually/optically before long term storage.

5) A smart-phone/tablet holder that can be used to attach smart-devices to microscope oculars and align the cameras for microscopy/photography/movie recording. Short-sequence movies recorded of swimming sperm will be acquired by specialized app software, which will transmit sperm motility data to a server at LSU. Data will be analyzed in real-time, and results will be sent back to the user within seconds. Based on a growing database of evaluations and motility data, standards for sperm motility can be provided to laboratories that quality control their sperm samples before cryopreservation or after thawing of samples.

In combination with the MEGC and SeLMA/MAMC we hope to provide laboratories with low-cost, efficient, standardized, and reliable sample quality control capabilities, before they use the PCPC for cryopreservation of samples in their laboratory. Streamlined QC capability at a fraction of the cost (compared to obtaining professional grade cryopreservation equipment) will empower laboratories to bank their own fish lines more effectively and efficiently, thereby reducing maintenance costs and the number of animals that need to be maintained in their facilities to propagate precious research lines. At the same time, it will also allow for standardization of cryopreservation methods across the zebrafish and other aquatic research communities, which will aid to improve genetic line preservation in aquatic biomedical laboratories and fisheries.

Total of 3450 Animals

Animal Biosafety Level

Animal Biosafety Level

For the current aim (see highlighted subtab above), will the risks associated with the experiments exceed Animal Biosafety Level 1 (ABSL-1)?

Where biologic agents are used, the Centers for Disease Control and Prevention (CDC) and National Institutes of Health (NIH) publication biosafety in Microbiological and biomedical Laboratories (BMBL; DHHS 2009) and the USDA standards (USDA 2002) should be consulted for appropriate facility design and safety procedures. These design and safety features are based on the level of risk posed by the agents used. Special facilities and safety equipment may be needed to protect the animal care and investigative staff, other occupants of the facility, the public, animals, and the environment from exposure to hazardous biologic, chemical, and physical agents used in animal experimentation. (Guide, p. 19)

Humane Endpoints

Humane Endpoints

For the current aim (see highlighted subtab above), what are the humane endpoints?

Humane Endpoints adapted from information online.

--- PLACEHOLDER for FUTURE FEATURE ---

Placeholder

Final Disposition and Euthanasia

Not applicable

Euthanasia / Final Disposition

For this aim, what will be the final disposition of the animals? Check all that apply.

Antibody Production

Antibody Production

Will you produce antibodies as part of this Aim?

--- PLACEHOLDER for FUTURE FEATURES --- (Use of Visual Recordings, Photography, or Audio Recordings)

Surgery

Surgery

Will you be performing surgery as part of this Aim?

Unexpected Outcomes

Unexpected Outcomes

Will this Aim introduce novel experimental variables that may affect animal health and welfare?

Animal number calculation for experimental part Cryopreservation

fish: Zebrafish			
	Min	Max	Procedure or Experimental Group
		300	
+		300	
+		600	
+		2250	
	0	3450	fish: Zebrafish

Procedures, Pre-Defined and Manually Entered

Not applicable

Description of Procedures

Please see attached SOP collection of ZIRC's *routine cryopreservation procedures*.

Cryopreservation Research:

Males will be isolated from females at least two weeks before experimentation (up to 4 weeks), and maintained in 1-gallon tanks on the recirculating water system in rm 118 at a density of 9 fish per tank (9 fish per gallon; 2.5 fish / L). During isolation, fish will receive an extra feeding of Adult Master Mix flake feed at mid-day each day. The total number of feedings will be 3 per day for these fish. On the day of experimentation, fish will be transferred from 118 to the laboratory in 109. Fish will be sedated in 168 mg/L MS-222, transferred onto a slit sponge in dorsal recumbency, and sperm will be harvested with a 20 µl glass capillary and gentle abdominal massage with rubber padded forceps close to the urogenital opening. Once sperm is obtained, fish are immediately transferred into a recovery chamber with fresh system water, and their recovery is observed throughout the next 30-60 minutes. Fish are monitored again when they are placed back on the recirculating water system in 118, and then daily,

every evening when all fish are routinely monitored for their well-being and health status.

When sperm is collected, it is captured in 20 µl of E400 extender, which blocks premature cell activation. If necessary, cell density is determined and adjusted to an experimental **standard**, or a dilution series is prepared. Sperm samples are then tested for motility using CASA as indicated for each of the devices below, before freezing and/or after thawing. Samples will also be used for in-vitro fertilization to compare motility and fertilization capacity.

Zebrafish sperm will be tested using the following devices:

1) Microfabricated Enumeration Grid Chamber (MEGC); this microfabricated counting chamber will be compared to cell counting methods already used at ZIRC such as measuring the optical absorption of cells in extender at 400 nm with a nanodrop spectrophotometer. Cell densities are calculated from the A400 absorption value according to a predetermined second order binomial absorption curve for zebrafish sperm. Sperm density is also determined with a hemocytometer or a mackler counting chamber, which are similar to the MEGC, but come at considerably higher cost. Analyses will address material toxicity, functionality, practicality, and reproducibility. **300 Males (includes assumed 3 rounds of feedback and device refinements): 300 Animals**

2) Sequential Logarithmic Mixing Apparatus (SeLMA)/Microfabricated Activation and Motility Chamber (MAMC). Previously, CASA measurements required the mixing of activating solution and sperm on the slide, coverslipping the slide and placing it under the microscope for recording of short, 60-frame video sequences. The SeLMA/MAMC provides controlled, hands-free mixing of water and sperm sample under the microscope for the timed activation and immediate observation of sperm cells for CASA measurements. Activation of sperm and observation of motility will become a straightforward 1-step process and will help laboratories to implement computer assisted sperm analysis (CASA) for reproducible quality control measurements. **300 Males (includes assumed 3 rounds of feedback and device refinements): 300 Animals**

3) A belt-driven device and a cryopreservation float, named Positional Cooling Platform Device (PCPD), for the standardized freezing of single straws and vials will be tested ("standard" = slow-cooling rates of 1-10 °C/min). Zebrafish sperm will be harvested as described above, and cell density and quality will be determined by Nanodrop optical absorption and CASA analysis. Cell density will be adjusted for optimal cryopreservation density and the devices will be tested for the controlled cooling of the samples. A thermocouple in control cryovials and straws (immersed in extender) will provide reference temperature readings to control the belt-driven device's speed before it drops samples into liquid nitrogen. The height of samples floating above liquid nitrogen in the PCPD will also be adjusted based on these controls. Once samples have been frozen in liquid nitrogen they will be stored in a liquid nitrogen freezer over night, and thawed for analysis the next morning or within a few following days. Material toxicity, functionality, practicality, and reproducibility will be assessed by thawing samples and analyzing them by CASA and in vitro fertilization. **Two devices x 300 Males (includes assumed 3 rounds of feedback and device refinements): 600 Animals**

4) We will test 3-D printed loops (VSID), and optical control devices for standardized vitrification (ultra-rapid cooling 100 - 1000 °C/min cooling rates per minute). This includes a 3-D printed vitrification device (VSID) with a sample loop and a protective sleeve that can be used for the labeling (identification) of the vitrified sample. Loops with fresh samples are immersed at high speed into liquid nitrogen to achieve vitrification, the amorphous form of water crystallization. The vitrification of samples will be verified visually/optically before long term storage, by placing loops on a 3-D printed holder that permits the assessment of vitrification success with stripes imprinted under the sample. Perfectly vitrified samples will not obstruct the pattern/lines in any way, but will essentially behave like "glass". Opaque or non-transparent samples indicate crystal formation or lack of successful sample vitrification. Most test will be performed without sperm samples. We will first test material integrity and stability when 3-D printed materials transition from room-temperature to liquid nitrogen (brittleness), which exerts considerable material stresses in the used plastics. Next we will test extender, and extender-cryoprotectant mixes for their capability to form a film in the loop, to maintain the film during vitrification, and the level/quality of vitrification of these solutions when injected into liquid nitrogen. The composition of solutions will be adapted for maximal vitrification. Once materials and solutions have been tested, we will test survival of cells in the cryoprotectant by collecting and pooling samples from 5 males and incubating/equilibrating them in cryoprotectant for 5, 10, 20, 30, 40, 50, and 60 minutes on ice. We will also analyze the optimal dilution of cells in the cryoprotectant solution. Cells will be frozen with slow-cooling rate methods and thawed for analysis. Cell survival and functionality will be measured after each period with Nanodrop cell density, CASA, and in vitro fertilization assays. Because pooling of samples and adjustment of cell densities yields several samples per male animal numbers will not exceed: 10 variations of cryoprotective solution x 5 cell densities x 7 incubation time frames x 5 males = 1750 males). Once the optimal incubation time, cryoprotectant composition, and cell concentration have been determined, we will attempt vitrifying cells with the optimized procedure and 3-D printed loops. We will analyze the transparency (vitrification) of samples and the integrity of the vitrified sample film in the loop immediately after freezing. Cells will be thawed and analyzed with Nanodrop, CASA and IVF to determine vitrification/survival success. We anticipate that the thawing procedure will have to be adjusted to the time frame of the vitrification procedure (accelerated). We may have to try several thawing methods. We anticipate using 50 animals x 3 thawing methods x 3 vitrification methods ~ 500 animals for successful vitrification and thawing of samples. **2250 Animals** (Note, this is an absolute maximum, because males can be re-used for harvesting sperm samples after a resting period of a few weeks; thus the same male can be used 2-4 times before euthanasia due to age, or accidental death (rare) during anesthesia).

5) We will test smart-device adaptors that attach and align phone/tablets to microscope oculars for microscopy/photography/movie recordings. We will obtain an app developed and provided by our collaborators at LSU, record short-sequences (50-60 frames) of activated sperm, and exchange sperm motility and density data with a server at LSU. Functionality, practicality, and reproducibility of data acquisition, calculation, and evaluation will be assessed and refined in collaboration with our partners at LSU. No additional animals will be used for these tests. We intend to use samples that we freeze or thaw for routine QC (test samples), line propagation, line distribution, or using samples listed in 3) and 4) for this app.

In combination with the MEGC and SeLMA/MAMC we hope to provide laboratories with low cost, efficient, standardized, and reliable sample quality control capabilities, before they use the PCPC for cryopreservation of samples in their laboratory. Streamlined QC capability at a fraction of the cost (compared to obtaining professional grade cryopreservation equipment) will empower laboratories to bank their own fish lines more effectively and efficiently, thereby reducing maintenance costs and the number of animals that need to be maintained in their facilities to propagate precious research lines. At the same time, it will also allow for standardization of cryopreservation methods across the zebrafish and other aquatic research communities, which will aid to improve genetic line preservation in aquatic biomedical laboratories and fisheries.

Total: 3450 Animals

Male/Female Conditioning

--- PLACEHOLDER for FUTURE FEATURE ---

Additional Procedure Details

Prolonged Restraint

Prolonged Restraint

Will any of the procedures listed above involve prolonged animal restraint?

No

Blood or Fluid or Tissue Collection

Blood or Fluid or Tissue Collection

Will any of the procedures listed above involve the collection of fluid from a live animal or the collection of tissues from a live animal?

Fluid can be blood, bile, spinal fluid, etc.

Do not include information about animal identification methods.

Yes

DETAILS

Provide Specimen Name

Zebrafish *Danio rerio* sperm cells/milt

Provide Collection Site

room 109

Provide Specimen Volume or Weight

1-4 μ l

Provide Number of Samples

estimated 30,000

How will specimen be discarded?

Cryopreservation storage, thawing and subsequent thawing for in vitro fertilization: no disposal

Cryopreservation storage, thawing and subsequent thawing for CASA etc QC: Samples will be cleaned off devices with running tap water, soap and bleach. In sink/to sewer disposal.

Substance Administration

Substance Administration

Will you be administering any substances to animals?

"Substances" here does not include agents used for anesthesia, analgesia, or euthanasia. Nor does it include hazardous agents. Questions about these are asked in other areas of the eAPM system.

No

Use of Controlled Substances

Use of Controlled Substances

Will controlled substances be used for anesthesia, analgesia, restraint, animal management, testing, or euthanasia?

No

Food or Fluid Regulation

Food or Fluid Regulation

Will food or fluid regulation be used in this study?

Yes

What is the justification for the use of food and/or water regulation?

Provide Justification

Animals will be conditioned by being isolated from females at least two weeks before experimentation (up to 4 weeks), and maintained in 1-gallon tanks on the recirculating water system in rm 118 at a density of 9 fish per tank (9 fish per gallon; 2.5 fish / L). During isolation, fish will receive an extra feeding of Adult Master Mix flake feed at mid-day each day. The total number of feedings will be 3 per day for these fish. The conditioning improves male performance, reduces male variability between samples and males (cell density and function). Thereby, 25-50% fewer males have to be conditioned, anesthetized and squeezed for sperm samples.

What alternative methods were considered, including positive reinforcement?

Provide Alternative Methods Considered

None - this type of conditioning (more food) should be beneficial for males

What is the nature, extent, and frequency of food and/or water regulation and how was this determination made?

Provide Details

2-4 weeks, one additional feeding per day, Males isolated from females at 2.5 fish/L

What are the potential adverse consequences of the regulation on the animal's health and well-being?

Provide Animal Health and Welfare Details

Same as all other fish at ZIRC

What methods and signs will be used to assess the animal's health and well-being and how frequently will it be assessed?

Body weights should be recorded at least weekly and more often for animals requiring greater restrictions.
 (Guide, p. 31)

Provide Details about Assessment of Health and Well-Being

Daily Observation of fish according to markers for distress, discomfort, pain or humane endpoints outlined in the Procedures/Experimental Design section

Where will written records be located?

Written records should be maintained for each animal to document daily food and fluid consumption, hydration status, and any behavioral and clinical changes used as criteria for temporary or permanent removal of an animal from a protocol.
 (Guide, p.31)

Provide Location of Records

ZIRC database

**What criteria will be used to remove the animal from the study?
 Provide Criteria Used to Remove Animals from Study**

Humane endpoints, or accidental death during anesthesia

Imaging Studies

Imaging Studies

Post-Procedural Monitoring

Post-Procedural Monitoring

Procedures Training Verification

fish: Zebrafish

PROCEDURES TRAINING VERIFICATION TOOL MATRIX

	Bauer, Justin	Black, Robin	Clark, Renee	Conway, Dylan	Core, Keely
	Hwang-Shum, Jen-Jen	Lains, David	Loucks, Evyn	Marston, Dagmara	Matthews, Jen
	Murphy, Joy	Murray, Katrina "Katy"	Nasiadka, Andrzej	Quinn, Erin	Schulze, Catherine
	Smith, Calvin	Varga, Zoltan	Westerfield, Monte	Williams, Evan	Wolfe, Jacklyn

Electrosedation

Animal Species Use

fish: Zebrafish

Experimental Design and Procedures Summary

Procedures Summary

For the current aim (see highlighted subtab above), explain the experimental design and provide a clear, concise and sequential description of the procedures involving the use of animals.

This narrative should explain the overall design and include things like experimental animal groups, group sizes, and group use.

IMPORTANT: Provide a clear and concise sequential description of the procedures (especially those that are listed in the procedures tab) involving the use of animals that is easily understood by all members of the IACUC. Details of any of the procedures should be described in the "Procedures" tab. This description should allow the IACUC to understand the experimental use of an animal from its entry into the experiment to the endpoint of the study.

NOTE: You may attach diagrams, flowcharts, etc., by using the paperclip icon at the top or bottom of this page.

Provide Experimental Design (see IMPORTANT help text above):

Smith Root Inc. developed a new Zebrafish Sedation System (ZSS) prototype that allows more parameters to be adjusted and stored than previously, including 3 user settings. The new prototype is safer and technologically more advanced than the previous one we tested under protocol 12-06RRA2. The new prototype was successfully tested in 2017 at the Stowers Medical Center. ZIRC has obtained one of the ZSS prototypes and would like to test it for humane electrosedation and euthanasia of zebrafish.

- A) Previous testing suggested that males are more susceptible to electric pulses than females. Fish size and age might play little or no role in zebrafish response to the same electrosedation settings. However, about 35% more males died as a consequence of electrosedation at the same ZSS settings than females.
- B) Our pilot tests suggested that WIK wild types are a little more robust and tolerate slightly higher Voltages, or show better survival rates to electrosedation than AB wild types at the same ZSS settings

Thus, minor strain-specific differences exist, and males are significantly more susceptible to electrical pulses than females. We would like to conduct the following 3 tests:

Test 1 200 AB fish (100 males and females) will be electrosedated to fine-tune male and female electrosedation settings. Successfully sedated animals will be weighed on a laboratory scale, and measured under a dissecting scope (standard length, girth and/or height at the level of the pectoral fin). Fish will be placed into a recovery tank and their recovery will be documented by timing the following events. Partial recovery: 1) Recovery of opercular movements, 2) Time of first reflexive/involuntary movements, 3) Recovery of equilibrium/righting, and full recovery: 4) voluntary movements/active swimming. Experiments are scored as failure, if animals are not sedated for a full minute (the time it takes to take measurements) or if they do not regain consciousness after 10 minutes of recovery (11 minutes total). Typical full-recovery times average 2-4 minutes. Every fish will be also observed for any signs of distress, discomfort or pain, as outlined in the humane endpoints of the procedures section. After recovery, animals will be pooled and maintained in a 1-gallon tank (maximum 20 fish) and observed the same day and for 3-4 additional days (once daily) for any signs of discomfort, distress, or pain. Fish are returned to their original tank for future experimentation (at least 2 weeks resting period); or they are euthanized by hypothermal shock, and processed for incineration or histopathological analysis. The settings, dimensions, weight and age of the fish will be statistically evaluated along with behavioral recovery observations and histopathological results. **200 Animals**

Test 2 We propose to test 3 different wild-type strains to test whether strain-specific modifications are needed to develop a more generally safe and reliable application. The settings in the table of the procedure section have been developed for the AB strain. We will test with 100 WIK, 100 SAT, and 100 TU (50 of each sex), whether the above settings can be applied to these strains also or whether modifications are required. We will assess animal response in the same way as outlined in Test 1) with regard to recovery, distress, discomfort, pain, and humane endpoints. **300 Animals**

Test 3) We will test whether electrosedation is useful to streamline routine procedures at ZIRC. We will follow the process outlined in 1), and the procedures sections except that fish will be used for routine procedures such as for harvesting sperm or eggs for *in vitro* fertilization or for cryopreservation. Specifically, we want to evaluate whether any experimental advantages (such as shorter and more predictable sedation times) can be gained over MS-222 anesthesia. For this reason, we will forego the detailed analysis and observation of recovery steps as in tests 1) and 2), and will only score overall animal survival/mortality. We will also test whether gametes are affected by the electric pulse by determining and comparing sperm motility (computer assisted sperm analysis) and fertilization rates with electrosedated and MS-222 anesthetized fish. MS-222 data will be obtained from our routine operations and we request 100 animals only for the electrosedation part described here. **100 Animals**

Total: 600 Animals

Estimated time frame 1-3 years

Animal Biosafety Level

Animal Biosafety Level

For the current aim (see highlighted subtab above), will the risks associated with the experiments exceed Animal Biosafety Level 1 (ABSL-1)?

Where biologic agents are used, the Centers for Disease Control and Prevention (CDC) and National Institutes of Health (NIH) publication biosafety in Microbiological and biomedical Laboratories (BMBL; DHHS 2009) and the USDA standards (USDA 2002) should be consulted for appropriate facility design and safety procedures. These design and safety features are based on the level of risk posed by the agents used. Special facilities and safety equipment may be needed to protect the animal care and investigative staff, other occupants of the facility, the public, animals, and the environment from exposure to hazardous biologic, chemical, and physical agents used in animal experimentation. (Guide, p. 19)

No, ABSL-1 is the risk level associated with the animal use in these experiments.

Humane Endpoints

Humane Endpoints

For the current aim (see highlighted subtab above), what are the humane endpoints?

Humane Endpoints adapted from information online.

Select Animal Type(s) Used in this Aim. Check all that apply.

Fish

Types of Humane Endpoints (Check all that apply.)

Clinical or Behavioral

Aggression

Eye Bulging (Exophthalmos)

Improper Buoyancy

Twirling

Swimming in circles

--- PLACEHOLDER for FUTURE FEATURE ---

Placeholder

Final Disposition and Euthanasia

Not applicable

Euthanasia / Final Disposition

For this aim, what will be the final disposition of the animals? Check all that apply.

Euthanasia

METHODS of EUTHANASIA and CONFIRMATION of DEATH

Select Animal Type(s) Used in this Aim. Check all that apply.

Fish

What method(s) of euthanasia will be used? Check all that apply.

IACUC-Approved Methods

(zebrafish, adult) Immersion in solution of buffered tricaine methanesulfonate (MS-222), 0.03% (w/v)

Finfish should be left in the anesthetic solution for a minimum of 10 minutes after cessation of opercular movement.

(AVMA Guidelines for the Euthanasia of Animals (2013), p. 72)

(zebrafish, all developmental stages) Rapid Chilling (Hypothermic Shock)

Transfer from acclimatized temperatures to water associated with a 2° to 4°C ice slurry must occur rapidly with as little transfer of warmer water as possible.

(AVMA Guidelines for the Euthanasia of Animals (2013), p. 73)

Other Method(s)

Accidental death by electrosedation (infrequent, usually males)

How will you confirm animal death? Check all that apply.

Other

Fish can be euthanized or maintained alive for future electrosedation testing, but are never returned to the breeding colony or transferred to other protocols/tests. I.e. the same animal may be electrosedated 2-3 times over a period of 6-9 months. A minimal resting period between electrosedation tests of 2 weeks is observed.

Antibody Production

Antibody Production

Will you produce antibodies as part of this Aim?

No

--- PLACEHOLDER for FUTURE FEATURES --- (Use of Visual Recordings, Photography, or Audio Recordings)

Surgery

Surgery

Will you be performing surgery as part of this Aim?
No

Unexpected Outcomes

Unexpected Outcomes

Will this Aim introduce novel experimental variables that may affect animal health and welfare?
Yes

What is the plan for monitoring the health and welfare of animals exposed to novel experimental variables?

Provide Plan for Monitoring Animal Health and Welfare

Animals will be observed during the recovery period as outlined above and their recovery steps are timed and statistically evaluated with their body condition factor. Animals will be monitored as outlined above for pain distress and discomfort or humane endpoints. Animals will be monitored during recovery, after experimentation, at the end of the day of experimentation and then daily once for 3-4 days. If humane endpoints or unexpected outcomes are observed fish will be euthanized by IACUC approved methods (rapid chilling).

What method of communication will you use to report animal welfare concerns to the IACUC when the initial characterization of a genetically modified animal (GMA) reveals a condition that negatively affects animal well-being? (Check all that apply.)

Email

Animal number calculation for experimental part Electro sedation

fish: Zebrafish			
	Min	Max	Procedure or Experimental Group
	(200)	Test 1
+	(300)	Test 2
+	(100)	Test 3
	0	600	fish: Zebrafish

Procedures, Pre-Defined and Manually Entered

Not applicable

Description of Procedures

We propose to test the following ZSS settings for male and female fish:

Sex	DC Pulse duration	Pulse Voltage differential	Pulse Frequency	Pulse Duty cycle
AB Male	Until loss of equilibrium, but no longer than 7 seconds	8 V (1/2-sized chamber) 15 V (full-sized chamber)	30 Hz	40%
AB Female	Until loss of equilibrium, but no longer than 10 seconds	10-11 V (1/2-sized chamber) 20 V (full-sized chamber)	30 Hz	40%

Procedure: Fish are netted from 20-gallon tanks into 1-gallon housing tank for transport, or are taken in their 1-gallon tank from the Main Fish facility (room 118) to room 109. The chamber of the ZSS electroseparator is filled with fish system water, a fish is placed into the ZSS electroseparator chamber, and a transparent lid is placed over the chamber. An electric pulse is applied (table) with the green start button, and as soon as the fish is sedated, the same button is pushed again. During the pulse (after the first push) a count-down timer measures the time it takes to immobilize the fish. With the second push, the electric pulse is stopped and a clock times the seconds and minutes of the experimentation and recovery period. For our purposes, electroseparation is considered successful if the fish loses equilibrium and is immobilized for up to 1 minute. During this time, we perform the following measurements as a stand-in for other experiments (and to better understand the electroseparation process).

Immediately after sedation, the fish is removed from the chamber with a perforated spoon and excess water is drained by gently rolling the fish on a stack of paper towels. The fish is transferred with the spoon to a laboratory scale and its mass is determined. The fish is then transferred (spoon) under a dissection stereo microscope and its length, height, or girth is determined. Once these measurements have been taken (usually 50-60 seconds after the start of sedation), the fish is returned to a recovery tank and is observed during its entire recovery. The sequence of recovery is: 1) recovery of opercular movements, followed by 2) reflexive/involuntary movements, 3) recovery of equilibrium/righting, and 4) full recovery/voluntary movements/active swimming, usually within 2-4 minutes, which is similar to recovery from MS-222 anesthesia. The minutes and seconds when these recovery stages occur are recorded in an excel spreadsheet, along with the body dimensions, and these values are used to determine body condition factor, timing of recovery steps, in relationship to the applied electrical pulse. Fish remain in a recovery chamber until the entire group has been tested. This way, any unusual outcomes after recovery can be observed. Fish are then moved back to the main fish facility (r, 118), and placed on the recirculation system with appropriate labeling. The fish are observed once more after experimentation has finished (during clean-up), and again in the evening of the tests. They are then observed once daily for 3-5 days, and either returned to their original tank or euthanized according to approved methods for histopathological testing. Fish are only returned to their original tanks if they are not used for any other procedure than future electroseparation, and dedicated stocks are used to eliminate biosafety concerns by accidentally mixing fish stocks.

The ZSS is cleaned before and after experimentation (or before changing test groups/fish stocks) by spraying the chamber, lid, and electrode plates (removable) with 70% EtOH twice, - wiping the parts clean with paper towels after the first spraying and letting them air dry after the second. Electrodes are made of bleach-resistant metal; plastic and metal parts are also treated with 1% bleach for 1 hour between different fish stocks. The apparatus is always rinsed with fresh system water before tests begin again to remove traces of EtOH or bleach.

--- PLACEHOLDER for FUTURE FEATURE ---

Additional Procedure Details

Prolonged Restraint

Prolonged Restraint

Will any of the procedures listed above involve prolonged animal restraint?

No

Blood or Fluid or Tissue Collection

Blood or Fluid or Tissue Collection

Will any of the procedures listed above involve the collection of fluid from a live animal or the collection of tissues from a live animal?

Fluid can be blood, bile, spinal fluid, etc.

Do not include information about animal identification methods.

No

Substance Administration

Substance Administration

Will you be administering any substances to animals?

"Substances" here does not include agents used for anesthesia, analgesia, or euthanasia. Nor does it include hazardous agents. Questions about these are asked in other areas of the eAPM system.

No

Use of Controlled Substances

Use of Controlled Substances

Will controlled substances be used for anesthesia, analgesia, restraint, animal management, testing, or euthanasia?

No

Food or Fluid Regulation

Food or Fluid Regulation

Will food or fluid regulation be used in this study?

No

Imaging Studies

Imaging Studies

Post-Procedural Monitoring

Post-Procedural Monitoring

Procedures Training Verification

fish: Zebrafish

PROCEDURES TRAINING VERIFICATION TOOL MATRIX

	Bauer, Justin	Black, Robin	Clark, Renee	Conway, Dylan	Core, Keely
	Hwang-Shum, Jen-Jen	Lains, David	Loucks, Eryn	Marston, Dagmara	Matthews, Jen
	Murphy, Joy	Murray, Katrina "Katy"	Nasiadka, Andrzej	Quinn, Erin	Schulze, Catherine
	Smith, Calvin	Varga, Zoltan	Westerfield, Monte	Williams, Evan	Wolfe, Jacklyn

Additional Procedure Training Details

Additional General Training Details

Are there any additional General Training details for the IACUC to consider?

No

Anesthesia/Analgesia

Anesthesia

Procedure	Species	Drug	Concentration	Dose	Route	Frequency
-----------	---------	------	---------------	------	-------	-----------

Additional Anesthesia Information

Do you need to request an anesthetic not available via the menu?

If you have selected a procedure via the pull-down menu and the anesthetic you would like to use is not available in the "Drug" pull-down menu, describe your need here.

No

Did you enter any procedures manually that require anesthesia?

Select "Yes" if you manually entered any procedures that require anesthesia on the "Procedures" tab.

Yes

Fin Clip (Survival) and Testes dissection (non-survival)

- MS-222 (Tricaine)
- 168 mg/L
- Immersion bath
- Once, before fin clip (168 mg/L; survival procedure)
- Once, before testes dissection (MS-222 overdose 200-300 mg; euthanasia, non-survival procedure)

Analgesia

Procedure	Species	Drug	Concentration	Dose	Route	Frequency
-----------	---------	------	---------------	------	-------	-----------

Additional Analgesia Information

Do you need to request an analgesic not available via the menu?

If you have selected a procedure via the pull-down menu and the analgesic you would like to use is not available in the "Drug" pull-down menu, describe your need here.

No

Did you enter any procedures manually that require analgesia?

Select "Yes" if you manually entered any procedures that require analgesia on the "Procedures" tab.

Yes

Before fin clips, or before squeezing adults to obtain gametes (survival procedures). A low dose of MS-222 has been shown to have a sedative effect and increases survival chances when fish are revived after anesthesia.

- MS-222 (Tricaine)
- 30-50 mg/L

- Immersion bath
- Once, before fin clip or squeezing (168 mg/L; survival procedure)

General Training

Principal Investigator Qualifications

General Training Assurance

Will you as principal investigator assure that personnel listed in this project will be allowed adequate time to attend training sessions?

Yes

Required General AUP Training

ALL: Animal Occupational Health Awareness (>=85%)

L1-A-1 Overview and Purpose

L1-A-2 Organizational Structure

L1-A-3 Nature of Research, Testing and Teaching Programs

L1-A-4 Key Institutional Representatives

L1-A-5 Accreditation History

L1-A-Introductions

L1-B-1 Animal Welfare Act

L1-B-2 U.S. Government Principles

L1-B-3 U.S. Public Health Service (NIH) Policy

L1-B-4 Other Regulations and Regulatory Agencies

L1-B-Federal Regulations and Policies

L1-C-01 Institutional Animal Care and Use Committee (IACUC)

L1-C-02 Animal Care Services (ACS)

L1-C-03 University Policy on the Use of Animals

L1-C-04 Code of Ethics

L1-C-05 PHS Assurance of Compliance

L1-C-06 Concern Policy

L1-C-07 Reportable Events Policy

L1-C-08 Occupational Health and Safety Program

L1-C-09 Transportation of Live Vertebrate Animals

L1-C-10 Euthanasia Policy

L1-C-11 Anesthesia Policy

L1-C-University of Oregon Policies

L1-D-1 Animal Use Protocol

L1-D-2 Protocol Amendment Policy

L1-D-3 Peer Review of Research Services

L1-D-4 Cooperative Research Policy

L1-D-5 Designated Member Review

L1-D-6 Guidelines for Pain, Distress and Discomfort in Zebrafish

L1-D-Protocol Related Policies

L2-ZIRC 1 Facility Safety and Security

L2-ZIRC 2 Zebrafish Processing

L2-ZIRC 3 Zebrafish Health

L2-ZIRC 4 General Zebrafish Procedures

L2-ZIRC 5 Feeding

L2-ZIRC 6 Facility Maintenance

L2-ZIRC 7 Aquaculture

L2-ZIRC 8 Zebrafish Distribution

L2-ZIRC Aquaculture System Shut Down and Start Up

L2-ZIRC Aquarium and Plastic Tank Cleaning Procedure

L2-ZIRC Aretmia Decapsulation
L2-ZIRC Bacterial Plating for UV Sterilizer Monitoring
L2-ZIRC Brine Shrimp (Artemia) Hatching Procedure and Feeding
L2-ZIRC Daily Monitoring of Fish Morbidity and Mortality
L2-ZIRC Embryo Media
L2-ZIRC Embryo Surface Sterilization
L2-ZIRC Euthanasia by Hypothermal Shock
L2-ZIRC Euthanasia of Fish for PCR or Fixation
L2-ZIRC Feeding Procedures
L2-ZIRC Fin Clipping and Fin Clip Processing
L2-ZIRC Fish Acquisition/Receiving
L2-ZIRC Fish Maintenance (strain identification and propogation)
L2-ZIRC Fixing Zebrafish for Histopathology
L2-ZIRC General Breeding
L2-ZIRC Molecular Genotyping of Mutant and Transgenic Lines
L2-ZIRC Nursery
L2-ZIRC Operation in Time of Crisis
L2-ZIRC Paramecia Feeding
L2-ZIRC Paramecia Maintenance
L2-ZIRC Powdered Food Preparation
L2-ZIRC Prospore Autoclave Sterilazation Monitoring
L2-ZIRC Providing Fish & Materials
L2-ZIRC Quarantine Room and General Procedures
L2-ZIRC RODAC Procedure
L2-ZIRC Sentinel Fish Program
L2-ZIRC Sperm Cryopreservation
L2-ZIRC Sperm Thawing Procedure
L2-ZIRC Washroom Procedure
Level 1
Level 2
Physician's Clearance
ZIRC Level 2

Additional General Training Details

Are there any additional General Training details for the IACUC to consider?

No

Protocol/Forms Links

Links to other Protocols or Forms

Not applicable

Additional details for linked protocols and forms

Not applicable

Alternatives

Rationale for Animal Use

Not applicable

Search Data

Search Conducted	Searched From	Searched To	Database	Hit Count	Search Keywords
22-Feb-2018				3	Zebrafish, Genetic, Resource, Stock, Center

Search Data

Should additional databases be added?

No

Rationale for Animal Use and Consideration of Alternatives

In accordance with the information provided above, please provide a written narrative description that gives the rationale for the use of animals, the choice of species, and the methods and sources used to determine that alternatives were not available or appropriate for this study. The narrative should also address each of the 3 R's defined above.

Provide Narrative:

A database search was not conducted as alternatives do not actually exist. As a resource center, ZIRC serves a relatively unique (replicated only twice worldwide) and very specific function that supports the assurance statements of all zebrafish researchers in general. Resource Centers exist for other species, however, ZIRC is supported by NIH/ORIP to function as one for zebrafish specifically. Two other zebrafish Resource Centers exist, with which ZIRC cooperates to support assurance statements: 1. EZRC - the European Zebrafish Resource Center and 2. CZRC, the China Zebrafish Resource Center.

1. The ZIRC serves other research groups to *replace* other vertebrate model organisms with zebrafish for some of their research goals. In addition, because many lines are maintained in a centralized, efficiently operating facility, ZIRC also helps to *reduce* the overall number of zebrafish maintained globally for research purposes. Cryopreservation research at ZIRC is also aimed at *reducing* the number of animals that have to be maintained as live stocks at ZIRC or elsewhere. Thus, ZIRC activities are aimed to support two of the "R's" in the Assurance Statement XI.A (Alternatives): Replace and Reduce. ZIRC's husbandry activities and publications are in addition aimed to *refine* and optimize maintenance standards and therefore support the third "R" also.

2. Several regional Stock Centers (Taiwan, China, Japan, Germany) support the operations of ZIRC, because we will be able to focus better on the specific research programs of NIH and US based researchers. Importantly, shipping live fish to countries with complicated local regulations will become less frequent. Because Stock Centers are better equipped to exchange and work with cryopreserved samples and generate live animals by thawing and in vitro fertilization, we will exchange frozen samples between Stock Centers in bulk, facilitating the regional distribution of live fish strains and minimizing potential discomfort or waiting times of animals in transit. In the long run, we aim to mirror the (cryopreserved) genetic stock inventories between ZIRC, EZRC, and CZRC to further reduce the numbers of animals that need to be maintained alive worldwide. The cooperation between Stock Centers thus refines animal handling and husbandry (shipping) and reduces overall animal numbers.

Standard Justification for Animal Use and Choice of Species

I am using zebrafish on this protocol.

**Standard Justification for Zebrafish
Zebrafish Justification**

The zebrafish has become widely accepted throughout the world as a particularly useful preparation to analyze how vertebrate development is regulated at the cellular, genetic, and molecular levels. There are a number of reasons for this assessment: (1) the fish are easy to maintain in large numbers and readily reproduce under laboratory conditions; (2) adult fish can be subjected to mutagenesis and mutations can be screened in the first generation by analyzing

haploid embryos; (3) the zebrafish embryo has few cells relative to other vertebrates, thus making it a "simple" model for more complex vertebrates such as ourselves; (4) the embryos are optically clear and develop very rapidly and externally (not inside the mother or an eggshell) so that the events involved in the differentiation of tissue, such as the nervous system, can be readily observed; (5) direct access to the developing embryos make it possible to introduce foreign genetic material and to perform cell labeling and other experimental manipulations; and (6) the zebrafish is a small animal so that large numbers, required for genetics, can be kept and studied.

Assurance Regarding Unnecessary Duplication of Previous Experiments

As principal investigator, I certify the experiments in this project do not unnecessarily duplicate previous experiments.

Replacement

Not applicable

Reduction

Not applicable

Refinement

Not applicable

Safety & Hazards

Not applicable

Use of Animal Biological Products or Animal Cell Lines

Are animal derived biologicals or cell lines used on animals under on this protocol?

No

Not applicable

Use of Infectious Agents

Are infectious agents used on animals under this protocol?

No

Not applicable

Use of Recombinant or Synthetic Nucleic Acid Molecules (rsNA)

Is recombinant/synthetic DNA used on animals under this protocol?

No

Not applicable

Use of Genetically Engineered Animals / Transgenic Animals

Will genetically engineered animals be used or created under this protocol?

Yes

What activates or silences the genetic modification(s)? Check all that apply.

Virus

ZIRC does not generate any transgenic organisms, however, they are bred in our facility for identification and propagation purposes, before they are redistributed to other research labs.

A portion of ZIRC transgenic lines are so called "*la*" alleles. They contain an inactivated retroviral vector which, once integrated in the zebrafish genome, cannot replicate. The inserted vector is used to disrupt genes and their normal function (i.e. it creates a mutant line). Most *la* lines/samples have multiple insertion loci (sometimes several dozens), which are all listed as distinct alleles on the ZIRC ordering pages.

Other

ZIRC does not generate any transgenic organisms, however, they are bred in our facility for identification and propagation purposes, before they are redistributed to other research labs.

Some transgenes have a dominant effect and can therefore be maintained by outcrossing to wild-type AB fish. For example, all heterozygous carriers of GFP encoding transgenes will express green fluorescence at appropriate times of their embryonic development or during adulthood under the control of a promoter DNA sequence. Conversely, there are transgenes that have no effect on their own, typically they need to be crossed to another transgenic line in order to effect expression. For example, the Gal4 protein is generated under the control of an endogenous zebrafish gene regulatory element. On its own it has no effect, because the yeast Gal4 protein has no function in vertebrates. However, when the line is crossed to a UAS-reporter transgene carrier, Gal4 proteins can bind to the UAS sequence and thereby activate reporter gene expression. Reporter genes can be enzymes that catalyze a color reaction in the fish/embryo, or fluorescent proteins that can be detected by fluorescence microscopy.

Another type of transgenes has been created with a *tol2* transposon vector, which facilitates (site-specific) integration of cloned promoter and/or reporter elements. These transgenes are also inactive once they integrated in the zebrafish genome and can not excise/relocate (transpose) again.

There are 3 general types of transgenes in the collection: 1) enhancer traps (allele postfix "Et" - e.g. smb576Et) where the transgene inserted in the gene's enhancer/regulatory elements (246 records); 2) gene traps, (allele postfix "Gt" - e.g. j1098cGt), where the transgene inserted in the gene's coding sequence or introns/exons (532 records); and "regular" transgenic lines (allele postfix "Tg" - e.g. hi1640Tg) where the transgene inserted in randomly in the genome (5521 records); this last type can contain endogenous regulatory sequences with reporters, or (the majority now) virus inserts that disrupt gene functions.

A database of transgenic lines handled at ZIRC can be found at <http://zebrafish.org/fish/lineAll.php>.

Provide the likely phenotypic result(s) of the modification(s):

No Problems for the Animal

Are there any special care or special monitoring requirements for the animals?

No

Not applicable

Use of Chemical Hazards

Will chemical hazards be used with animals named on this protocol and are those chemical

hazards listed in the table above?

No

Not applicable

Use of Radiological Hazards

Will radiological hazards be used with animals named on this protocol and are those radiological hazards listed in the table above?

No

Not applicable

Other Hazards and Agents

Are there any additional hazards and agents that apply to this protocol you have listed in the table above?

Human or Non-Human Primate (NHP) Biological Products and Cell Lines

Does this protocol use tissues, samples, or cell lines derived from human or non-human primate sources?

Waste and Animal Disposal Procedures

Describe disposal procedures for carcasses, recombinant/synthetic materials, contaminated items, and other hazardous materials.

IMPORTANT: Describe disposal procedures for items related to radiological work under the "Use of Radiological Hazards" group.

Provide Description:

All adult animal carcasses (post-euthanasia) are collected in zip-lock bags and maintained in a freezer until collected by a commercial vendor for incineration. Embryos are dissolved in bleach over night (500 mg/L), which neutralizes pathogens and also destroys any DNA.

Additional Safety Considerations

Are there any additional safety considerations not listed in the previous sections?

No

Field Studies

Will any part of this project be conducted in the field?

Assurances

INVESTIGATOR AGREEMENTS and ASSURANCES

1. I agree to abide by the University of Oregon policies for the care and use of animals; the provisions of the NIH Guide to the Care and Use of Laboratory Animals; and all federal, state, and local laws and regulations governing the use of animals in research. I understand that emergency veterinary care will be administered to animals showing evidence of pain or illness, in addition to routine veterinary care as prescribed for individual species in the Standard Operating Procedures.
2. I declare that all experiments involving live animals will be performed under my supervision or that of another qualified biomedical scientist listed on this protocol.
3. I certify that all personnel having direct animal contact, including myself, have been trained in humane and scientifically acceptable procedures in animal handling, administration of anesthetics, analgesics, and euthanasia to be used in this project. I assure that personnel will be allowed adequate time to attend training sessions.
4. I understand that personnel with live animal contact are required to participate in the Occupational Health and Safety Program.
5. I further declare that the information provided in the accompanying protocol is accurate to the best of my knowledge. Any proposed revisions to the animal care and use data will be promptly forwarded in writing to the IACUC for approval, including changes in personnel and location.
6. I am aware that any deviation from an approved protocol or violations of pertinent policies, guidelines or laws could result in immediate suspension of this project.

Amendment Information

Full Committee (FCR) or Designated Member (DMR) Review Amendment

Please select all that apply.

Details for amendment types can be found on the UO IACUC Protocol Amendment Policy .

Veterinarian Verification and Consultation (VVC) Amendment

Please select all that apply.

Details for amendment types can be found on the UO IACUC Protocol Amendment Policy .

Administrative Changes (AR) Amendment

Please select all that apply.

Details for amendment types can be found on the UO IACUC Protocol Amendment Policy .

Please provide a detailed separate justification for all changes listed above and if applicable, describe how the change(s) relates to the objective of the research

Please review the original protocol and confirm that all Principal Investigator assurances apply to proposed change(s).