



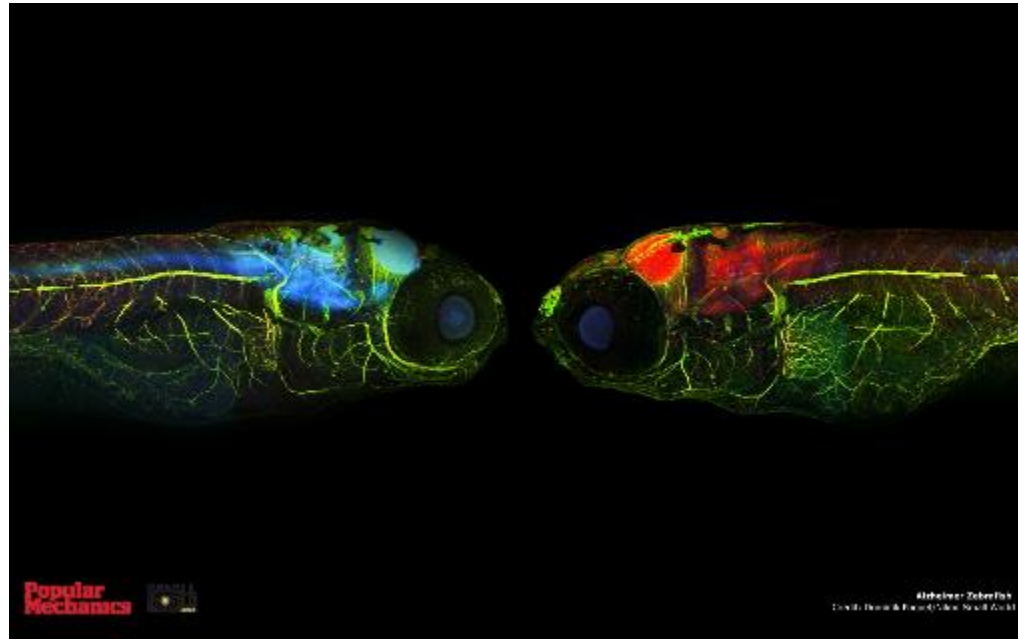
OLAW CONVERSATIONS

Reducing Administrative Burden in Zebrafish Programs and
Grant to Protocol Congruence Review

Thursday, September 16, 2021



Flexibilities and Strategies to Reduce Administrative Burden for Researchers Using Zebrafish



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Disclaimer: OLAW NOT-OD-21-118 closed on August 9, 2021. Only comments provided through the RFI Survey System by the closing date will be considered for incorporating into final guidance.

OLAW's Mission

“The Office of Laboratory Animal Welfare (OLAW) provides guidance and interpretation of the Public Health Service (PHS) Policy on Humane Care and Use of Laboratory Animals (Policy), supports educational programs and monitors compliance with the Policy by Assured institutions and PHS funding components to ensure the humane care and use of animals in PHS-supported research, testing, and training, thereby contributing to the quality of PHS-supported activities.”



Aquatic Animal Care Services

The mission of Aquatic Animal Care Services is to support researchers using aquatic and semi-aquatic animal models, primarily fish, to study vertebrate genomics and vertebrate development by employing expert husbandry techniques, by supplying efficient and timely services, and by fostering a helpful, cooperative environment. The most widely used fish model at the University of Oregon is the zebrafish (*Danio rerio*).

Fraud and Ethics
Reporting

[Make a report](#)





PHS Policy

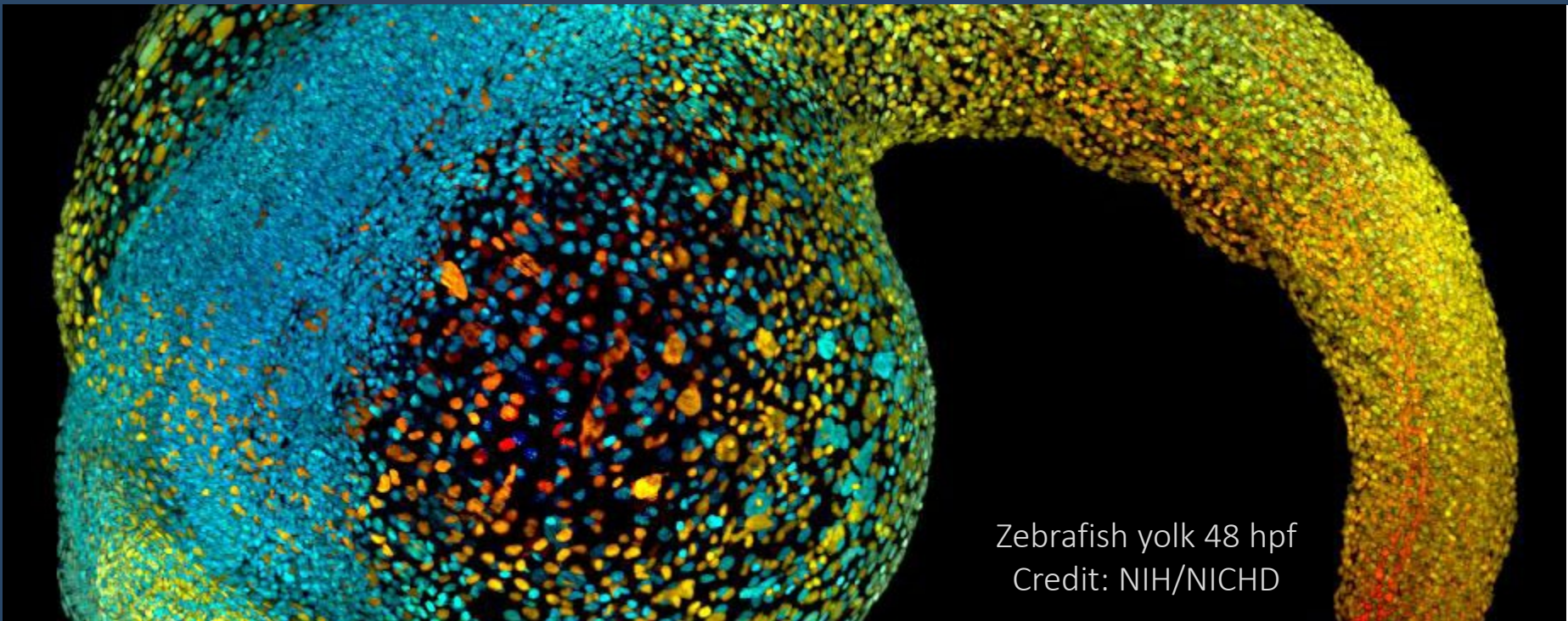
Definition of an *Animal* (III.A):

“Any **live, vertebrate animal** used or **intended for use** in research, research training, experimentation, or biological testing or for related purposes.”

*AWRs do not cover fish

IACUC Review of Research (IV.C.)

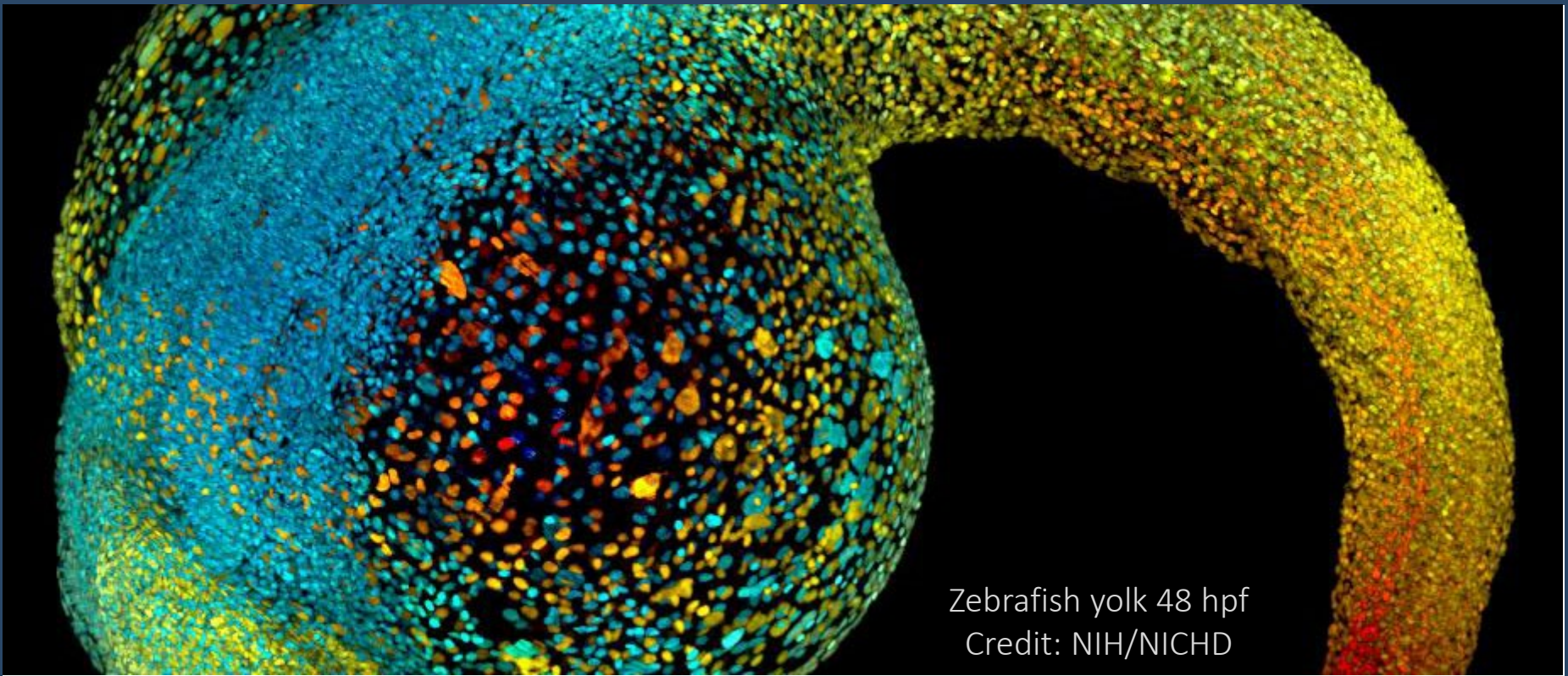
- Avoid or minimize pain and distress
- Appropriate sedation, analgesia, or anesthesia
- Humane endpoints and euthanize (AVMA)
- Comfortable housing that contributes to their health
- Medical care
- Trained personnel



Zebrafish yolk 48 hpf
Credit: NIH/NICHD

U.S. Government Principles

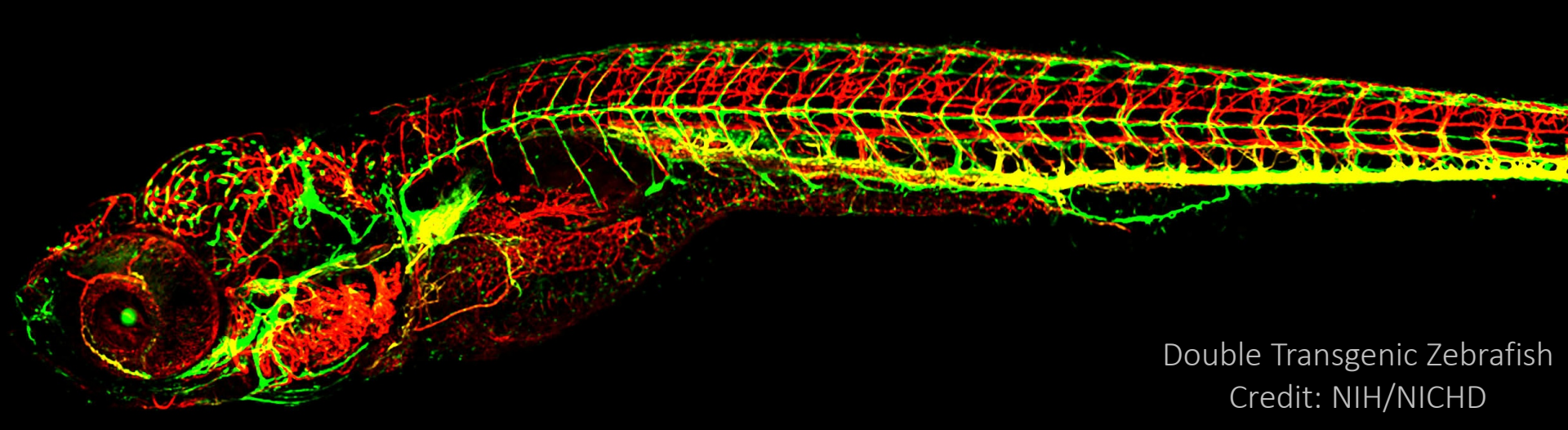
- V. “Procedures with animals that may cause more than momentary or slight pain or distress ...sedation, analgesia, or anesthesia...”
- VI. “...otherwise suffer severe or chronic pain or distress that cannot be relieved should be painlessly killed”
- IX. “Where exceptions are required ... the decisions should not rest with the investigators ... but should be made ... by an appropriate review group...”



Zebrafish yolk 48 hpf
Credit: NIH/NICHD

U.S. Government Principles

- IV. Proper use of animals, including the **avoidance or minimization of discomfort, distress, and pain** when consistent with sound scientific practices, is imperative. **Unless the contrary is established, investigators should consider that procedures that cause pain or distress in human beings may cause pain and distress in other animals.**



Double Transgenic Zebrafish
Credit: NIH/NICHD

Applicability to Embryonated Eggs

OLAW FAQ A.4. Does the PHS Policy apply to live embryonated eggs?

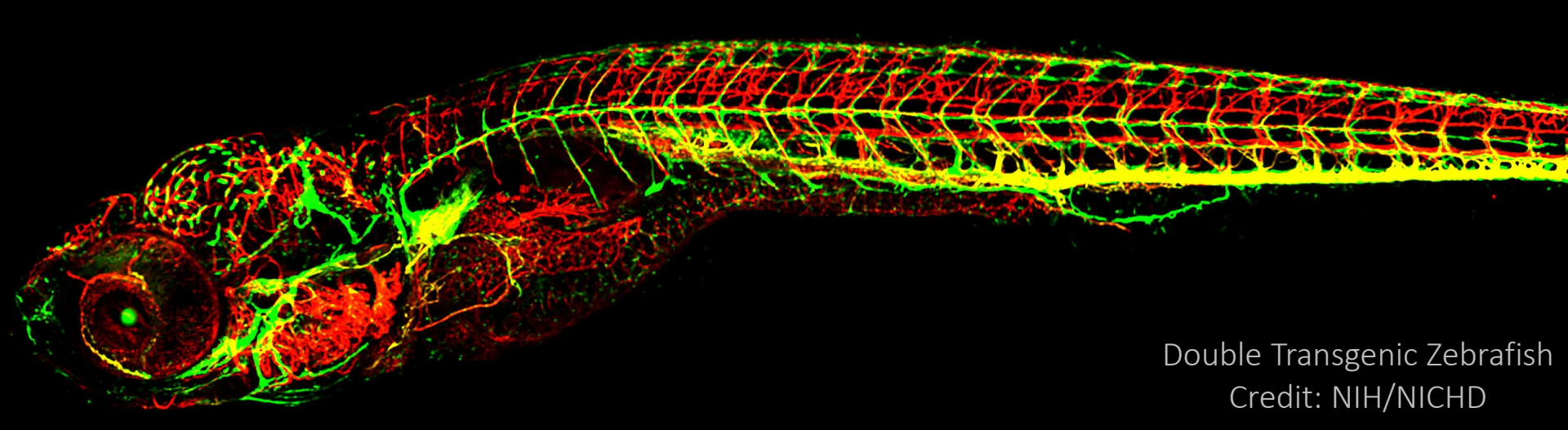
“Although avian and other egg-laying vertebrate species develop backbones prior to hatching, OLAW interprets the PHS Policy as [applicable to their offspring only after hatching](#). The egg-laying adult animal is covered by the Policy. OLAW expects Assured institutions to have policies and procedures in place that address the care or euthanasia of animals that hatch unexpectedly.”

Important Concepts:

Guidance is and will continue to be based on **developmental stage** (e.g., immediately after hatching) **vs single timepoint** (e.g., 3 dpf) = FLEXIBILITY

Previous and current guidance uses “hatching” – **alternate terminology** for zebrafish?

https://www.zfin.org/zf_info/zfbook/stages/index.html

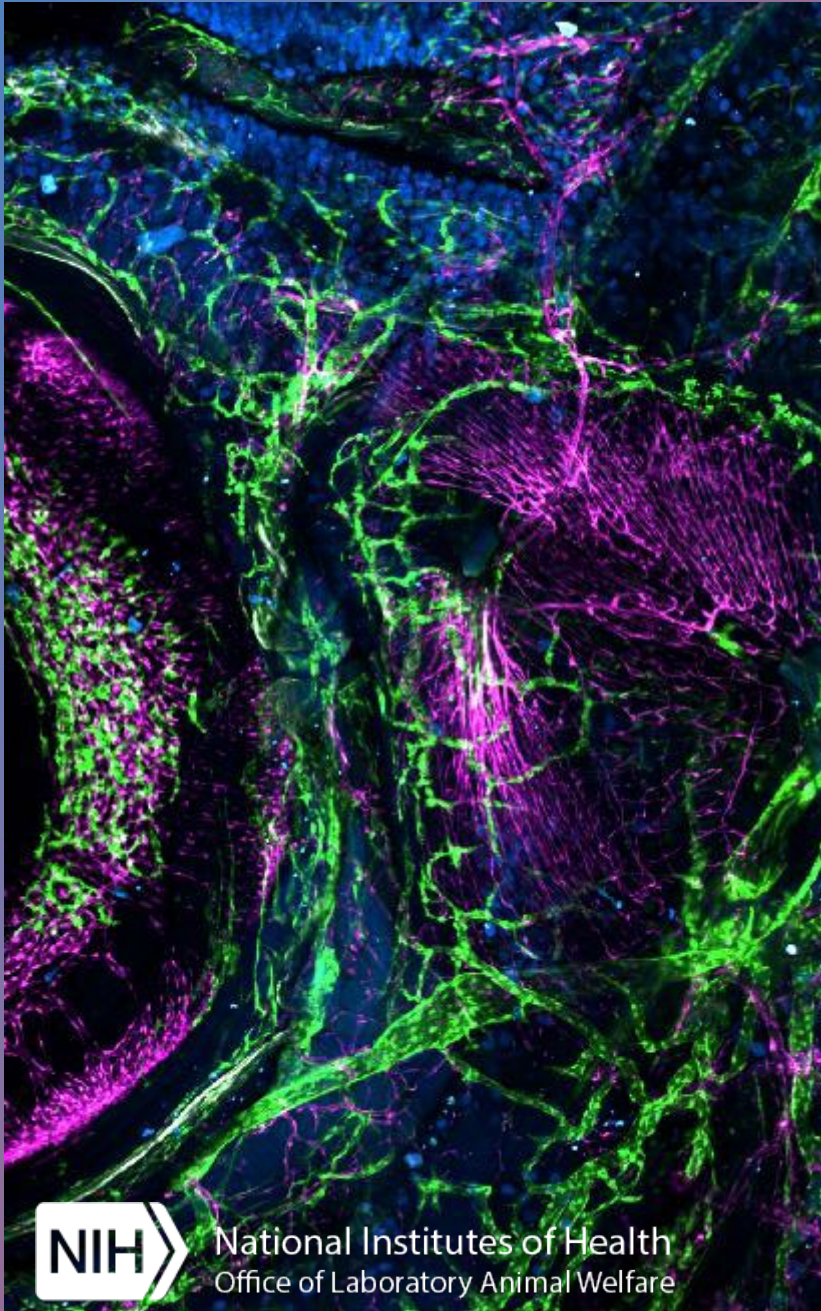


Double Transgenic Zebrafish
Credit: NIH/NICHD

Applicability to Embryonated Eggs

OLAW FAQ A.5. Does the PHS Policy apply to larval forms of amphibians and fish?

“Yes, larval forms of fish and amphibians have vertebrae and are covered by the PHS Policy. As noted in FAQ A.4., the PHS Policy applies to the [offspring of egg-laying vertebrates only after hatching](#). Zebrafish larvae, for example, typically hatch 3 days post-fertilization.”



National Institutes of Health
Office of Laboratory Animal Welfare

21st Century Cures Act 2034 (d) Animal Care and Use in Research

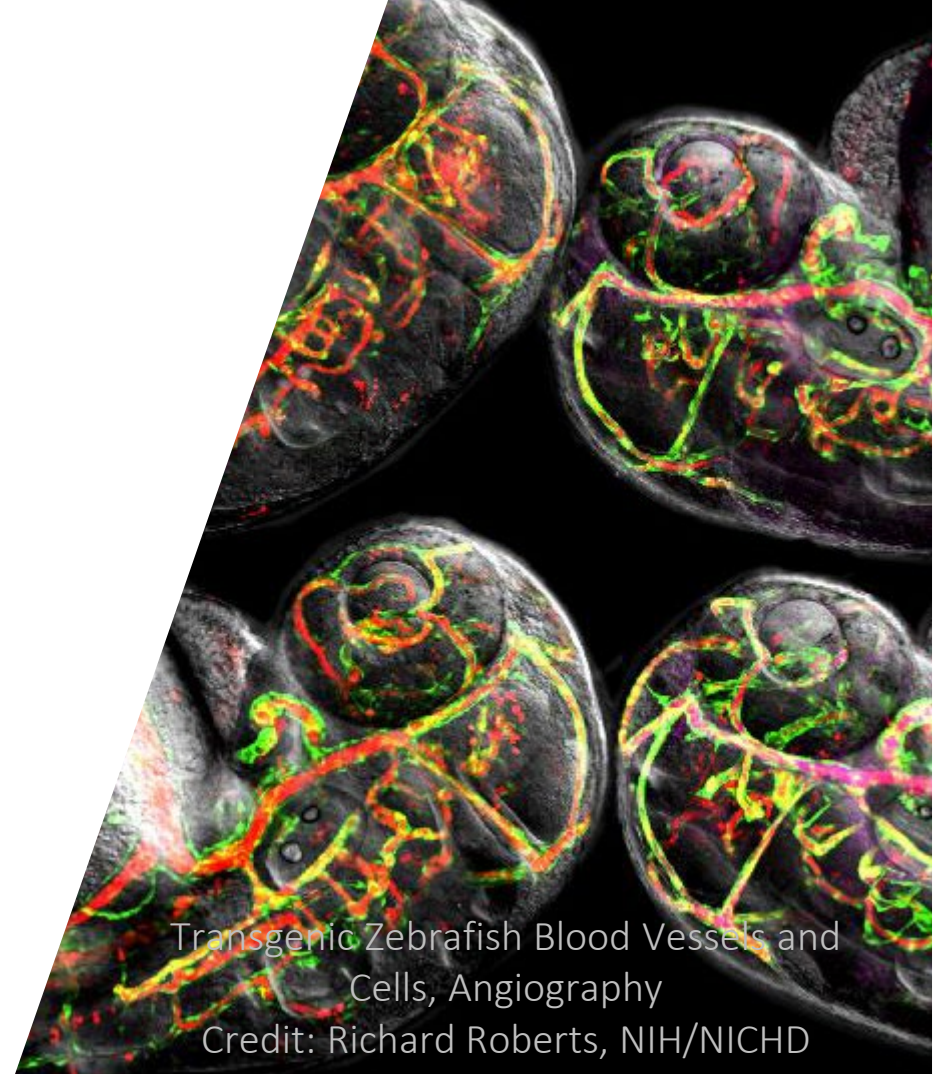
“Complete a review of applicable regulations and policies for the care and use of laboratory animals and make revisions, as appropriate, to **reduce administrative burden on investigators while maintaining the integrity and credibility of research findings and protection of research animals.**”

OLAW Committed to...

Review existing guidance,

Clarify the requirements, and

Seek public comments on updated guidance.



Transgenic Zebrafish Blood Vessels and Cells, Angiography
Credit: Richard Roberts, NIH/NICHD

NOT-OD-21-118: Request for Information (RFI) on Flexibilities to Reduce Administrative Burden While Continuing to Apply the PHS Policy to Zebrafish Immediately After Hatching

Opened: May 7, 2021

Closed: August 9, 2021



National Institutes of Health
Office of Laboratory Animal Welfare



Zebrafish Skeleton, Scales,
Lymphatic System
Credit: NIH/NICHD

“The NIH is seeking input on the flexibilities available to [reduce administrative burden](#) with research involving zebrafish larvae [while continuing to apply the PHS Policy to zebrafish immediately after hatching.](#)”

NOT-OD-21-118

Closed August 9, 2021

Justifications – PHS Policy

PHS Policy definition of an *animal*:
“live, vertebrate animal...intended for use”

- UO tracks embryos

Zebrafish develop anatomical structures characteristic of vertebrates prior to hatching.



Zebrafish Scales
Credit: NIH/NICHD

Justifications – Lack of Data



Lack of consensus based on scientific evidence to indicate they do not feel pain immediately after hatching.



U.S. Government Principle IV states: “...avoidance or minimization of discomfort, distress... is imperative. Unless the contrary is established, investigators should consider that procedures that cause pain or distress in human beings may cause pain or distress in other animals.”



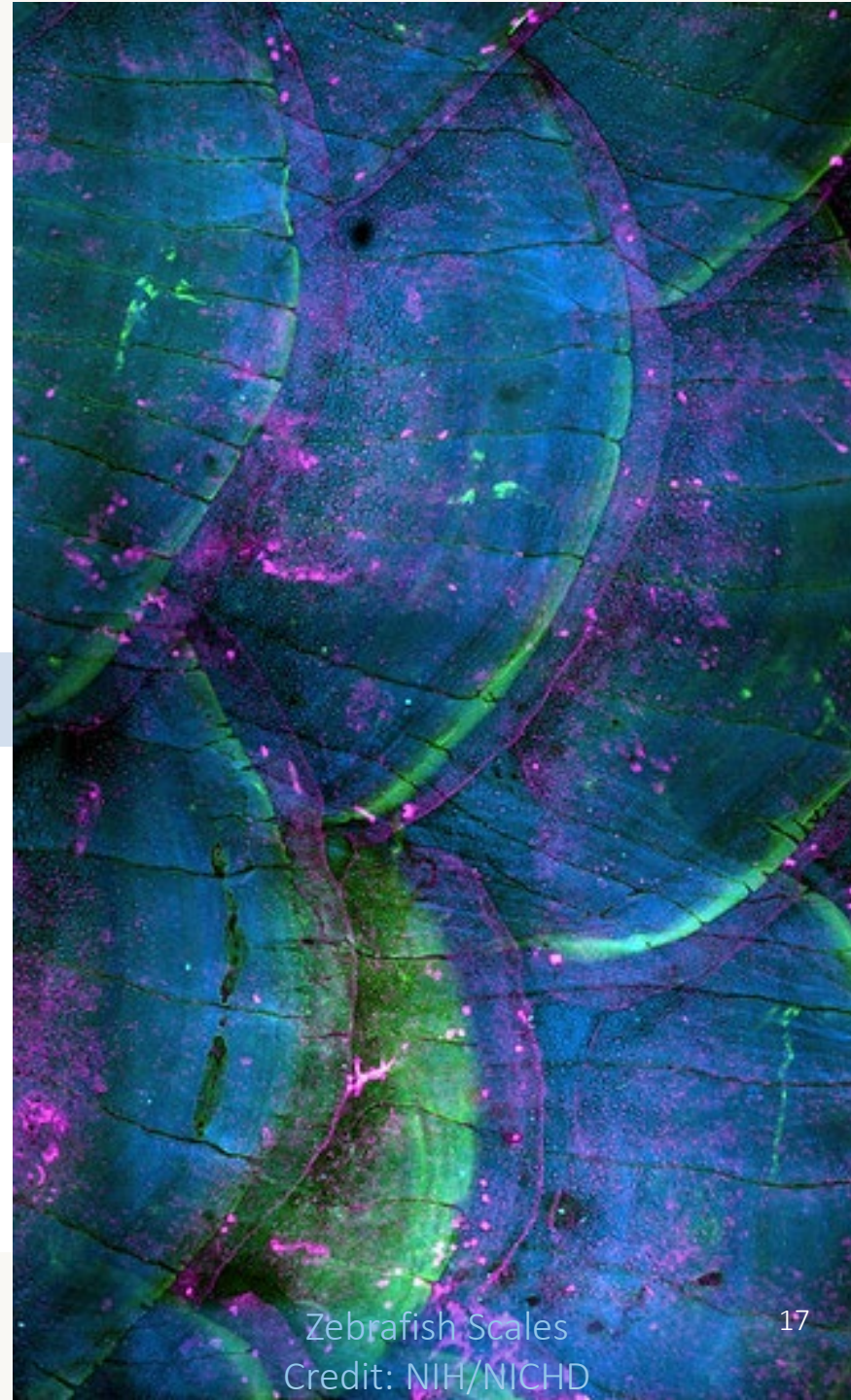
Precautionary principle



Justifications - 5 dpf Larvae Behave Like Adults to Some Noxious Stimuli

At 5 dpf, zebrafish larvae respond as adults do to noxious stimuli, indicating that protections should be in place *by this time*.

Initiating oversight beyond hatching, such as free-feeding or swimming, independent of the consideration for the potential to feel pain or distress, could negatively impact animal welfare.



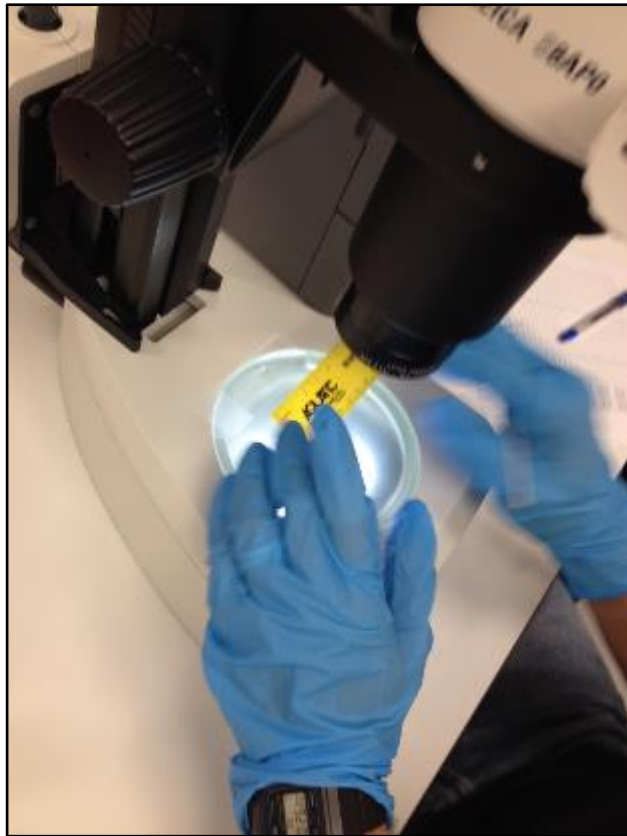
Zebrafish Scales
Credit: NIH/NICHD

Burden
Reduced:

OLAW Flexibility: Use Standard or Core Protocols

*Protocol
Creation and
Review*

UO Implementation to Reduce Burden:
Creating a protocol and, later, its amendments



Maintain a standard library of **experimental procedures** that can be used “as-is” or modified per project then submitted for approval.

- Genome manipulation via CRISPR/Cas9 system
- Genome manipulation via *Tol-2* system
- Fin Amputation
- Intraperitoneal Injection

UO Implementation to Reduce Burden:
Creating a protocol and, later, its amendments

Maintain IACUC-approved **SOPs for population management, anesthesia, euthanasia, veterinary notifications, documentation, etc.** Only departures from the institution's IACUC-approved standard need explanation.

Quarantine

- Handling and Acclimation

Population Management

- Genotyping from fin clips, progeny phenotype, etc.

Anesthesia and Analgesia

- MS-222, Eugenol, gradual cooling, etc.

Euthanasia

- Rapid chilling, MS-222, etc.



Burden
Reduced:

*Protocol
Creation and
Review*

OLAW Flexibility: Use Standard or Core Protocols

UO Implementation to Reduce Burden:
Creating a protocol and, later, its amendments

Maintain IACUC-approved SOPs for **standard environment, housing, and management**, then let researchers using those standards simply choose “Y” on the protocol form. Only departures from the institution’s IACUC-approved standard need explanation.

UO standard species-specific...

- Water quality parameters
- Feed types and feeding frequency
- Illumination and photoperiod
- Noise/sound ranges
- Temperature ranges
- Tank/cage change schedule
- Tank/cage type(s)
- Environmental enrichment



Animal tracking

UO Implementation to Reduce Burden:

Using only *approximate* animal numbers

- Allow estimates for zebrafish numbers, both in protocol review and post-approval reporting.
- Use template (“boilerplate”) language for zebrafish species 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...”



Burden
Reduced:

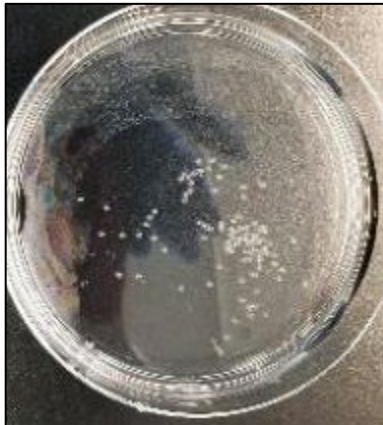
OLAW Flexibility: Track *Approximate* Animal Numbers

PHS Policy IV.D.1.A., *Guide* (p. 87)

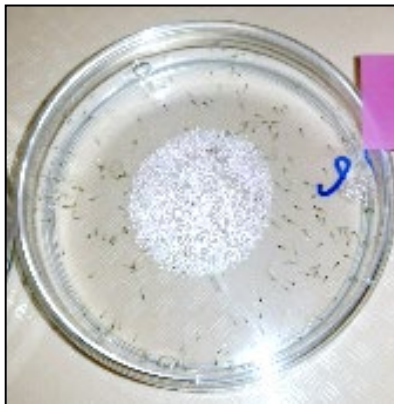
Animal tracking

UO Implementation to Reduce Burden:

Using only *approximate* animal numbers



(100 embryos)



(Overcrowded)

Life Stage at 28.5 °C	Equipment Type	Approximate Volume	Maximum Stocking Density	Comments
Embryo	Petri dish, 100 mm x 20 mm	100 ml	100	
Use for fish that will be reared or fish that will be studied after hatching.				
Embryo	Petri dish, 100 mm x 20 mm	100 ml	not specified	
For 0 hours post fertilization (hpf) to 60 hpf at 28.5 °C, use for fish that will be screened. At 60 hpf, fish must be either humanely euthanized or rehoused at 100 fish per dish or lower density.				
Early larval	Petri dish, 100 mm x 20 mm	100 ml	100	
Use for fish that will be reared in the AqACS Zebrafish Facility Nursery .				
Early larval	Beaker	100 ml	100	
Use for fish from weaker strains that will be reared in the AqACS Zebrafish Facility Nursery .				

Space Recommendations and Housing Density Standards



(30 vs. 50 larvae)

Use Guidance on Flexibilities for Conducting Semiannual Inspections of Animal Facilities, (NOT-OD-21-164, August 2, 2021)

“1. The PHS Policy Footnote 8 and 9 C.F.R. § 2.31(c) (3) of the AWRs allow IACUCs to determine the best means of evaluating the institution’s facilities. For areas housing non-Animal Welfare Act (AWA)-regulated species, the IACUC may use as few as one qualified individual or ad hoc consultant, who need not be an IACUC member or institutional employee, to conduct the facility inspections. Qualified individuals should have training and a working knowledge of the PHS Policy, Guide, and the AWRs to appropriately evaluate the facilities and identify deficiencies and animal welfare issues.”



**Burden
Reduced:**

UO Implementation to Reduce Burden:
Animal care and use flexibilities

*Daily husbandry
and care;
recordkeeping*



- ✓ Use centralized training
- ✓ Use centralized staff and services
- ✓ Use centralized facilities

Implemented with NIH Cost and
Rate Setting Manual

[https://grants.nih.gov/grants/policy/
air/rate_setting_manual_2000.pdf](https://grants.nih.gov/grants/policy/air/rate_setting_manual_2000.pdf)

Training

Use centralized training resources.



About 100 embryos

Onboarding new
personnel

- Understanding layout of facilities
- Incident safety

Basic husbandry
and vet care

- Handling/netting zebrafish
- Understanding biosecurity and fomites
- Breeding fish
- Caring for embryos and larvae
- Observing fish for signs of pain, discomfort, distress, and disease

Burden
Reduced:

Transportation

UO Implementation to Reduce Burden: Animal care and use flexibilities



Use centralized staff and services.

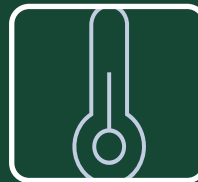


Veterinarian review and approval



Shipping

- Logistics, packaging, tracking



Receiving and Quarantine

- Acclimating



Burden
Reduced:

OLAW Flexibility: Use DMR v. FCR

Protocol Review

Use designated member review (DMR) unless full committee review (FCR) is requested

Resources on DMR & FC

- PHS Policy IV.C.2.
- NOT-OD-09-035
- OLAW FAQ D.19.



Burden
Reduced:

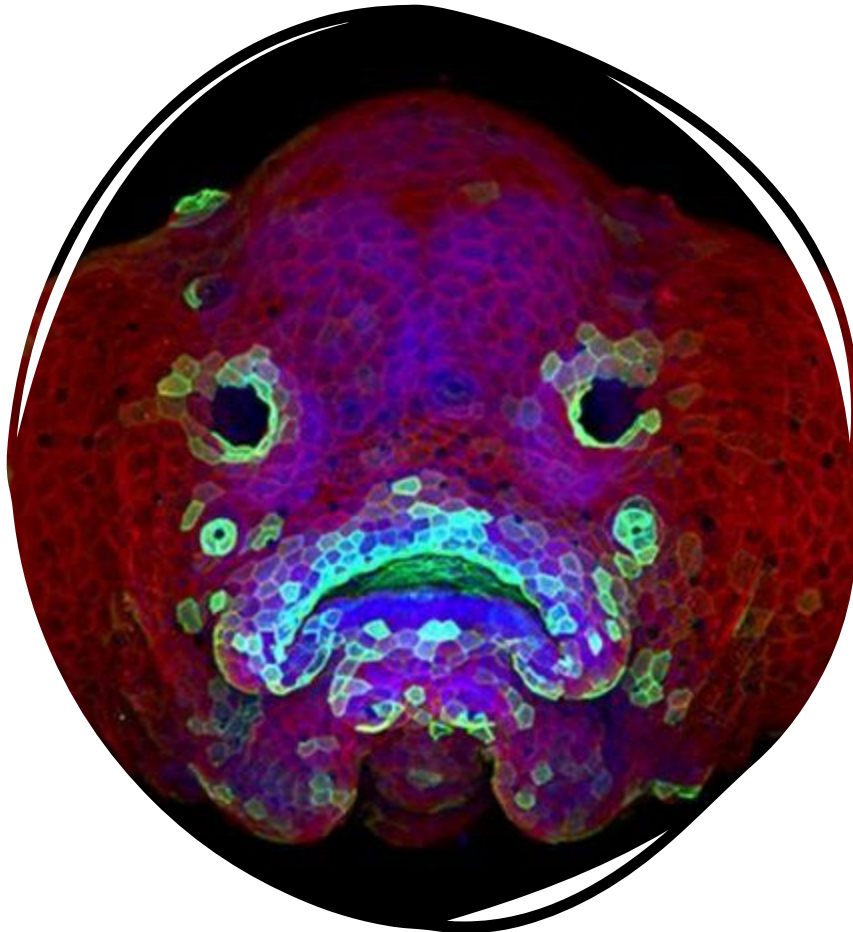
Protocol Review

OLAW Flexibility: Include a Zebrafish Expert

Include a zebrafish researcher / animal care technician on IACUC or as an ad hoc consultant.



OLAW Expectations



6-dpf Zebrafish Larva

Credit: Ruize & Eisenhoffer, NIH/NIGMS

1. OLAW will continue requiring full implementation of the PHS Policy to research activities involving zebrafish immediately after hatching.
2. Assured institutions must have policies and procedures in place that address the care or euthanasia of animals that hatch unexpectedly.
3. Data integrity and animal welfare must not be negatively impacted and must be consistent with the PHS Policy, U.S. Government Principles, *Guide*, and AVMA Guidelines for Euthanasia.

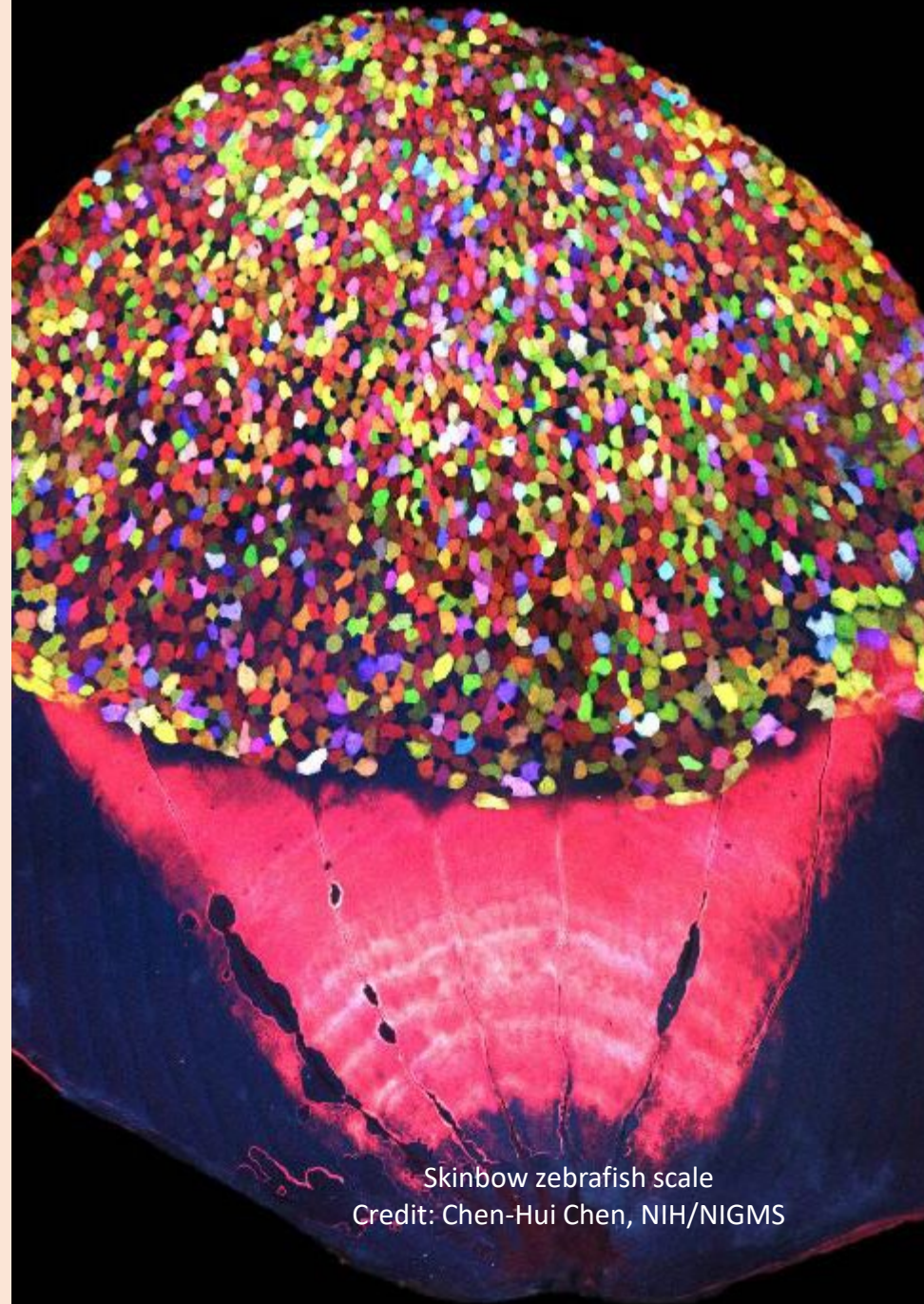
Questions?

Visit OLAW's zebrafish webpage for more information:

<https://olaw.nih.gov/policies-laws/21st-century-cures-act/Zebrafish>

UO Aquatic Animal Care Services

<https://aqacs.uoregon.edu>



Skinbow zebrafish scale
Credit: Chen-Hui Chen, NIH/NIGMS



UNIVERSITY OF
OREGON

Aquatic Animal Care Services

Resources

NOT-OD-21-118: Request for Information (RFI) on Flexibilities to Reduce Administrative Burden While Continuing to Apply the PHS Policy to Zebrafish Immediately After Hatching

NOT-OD-21-164: Guidance on Flexibilities for Conducting Semiannual Inspections of Animal Facilities

OLAW FAQ A.4. Does the PHS Policy apply to live embryonated eggs?

OLAW FAQ A.5. Does the PHS Policy apply to larval forms of amphibians and fish?

OLAW FAQ F.12. What are the institution's responsibilities in ensuring that animals are shipped safely and in reporting adverse events that occur in shipment of animals to or from the institutions?

[Zebrafish.org](http://zebrafish.org) – search for and order lines

Zebrafish Husbandry Association – zhaonline.org – Promotes the advancement of zebrafish research husbandry through continual learning.

Zebrafish International Resource Center (ZIRC) – repository for husbandry techniques and the latest research. <https://zfin.org/ZDB-LAB-991005-53>

[Zfin.org](http://zfin.org) – data, publications, news, meetings, jobs