



**MONTANA
STATE UNIVERSITY**
Institutional Animal Care & Use Committee
Xenopus and Zebrafish Larvae Policy

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 A4](#), the PHS Policy applies to the offspring of egg-laying vertebrates only after hatching. Zebrafish larvae, for example, typically hatch 3 days post-fertilization.

Zebrafish (*Danio rerio*)

General Guidelines (Adapted from NIH Guidelines):

Current OLAW interpretation of PHS policy considers aquatic species as "live, vertebrate animals" at hatching. Although this is an imprecise stage for zebrafish it can be approximated at 72 hours post fertilization. For purposes of accountability all stages of development greater than three days of age should be described in an approved MSU IACUC Animal Use Protocol. An estimate of the number of larval zebrafish from day 4-7 days post fertilization (dpf) should be included.

Since these early stages (4-7 dpf) do not feel pain or distress, it is preferable that their numbers be separated from zebrafish ≥ 8 dpf. This number can be listed as Column C in the Pain and Distress Category as a separate number from zebrafish ≥ 8 dpf.

The pain and distress categorization of the ≥ 8 dpf fish should be determined by the investigator based on the specific procedures described in the protocol. The number of animals used may need to be provided as an estimate, particularly with these young larvae, considering their size and normal housing conditions.

African Clawed Frogs (*Xenopus spp.*)

For xenopus, the beginning of the hatching process for most begins at about 2 dpf (50 hours post fertilization). The hatching process is completed over the next 48 hours and they begin to feed (4 dpf). This corresponds approximately with Nieuwkoop and Faber stage 45. For purposes of accountability all stages of development starting at 4 days of age should be described in an approved MSU IACUC Animal Use Protocol.

Since the brain is not fully developed until stage 53 (approximately day 24) it is likely that they cannot perceive pain. Larvae prior to this stage can be listed as Column C in the Pain and Distress Category.

IACUC Approval Date: 09/19/2018

Review Date: 09/19/2018

Issue Date: 09/21/2018

The pain and distress categorization of the \geq stage 53 xenopus should be determined by the investigator based on the specific procedures described in the protocol. The number of animals used may need to be provided as an estimate, particularly with these young larvae, considering their size and normal housing conditions.

References

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 - b. <https://journals.plos.org/plosone/article?id=10.1371/journal.pone.0101606>