

Algae in abundance: au natural, or a violation of *Xenopus* husbandry standards?

Xenopus laevis, the African Clawed Frog, is well known to many laboratory animal facilities. This extremely aquatic and unique animal has no tongue, no externally visible ears, claws on some of its toes, a lateral line system analogous to that seen in fish, and in the wild is found in warm, stagnant ponds that are typically covered with algae. It is this last characteristic that became problematic for the Great Eastern University IACUC.

Dr. Simon Eaton had three 25-gallon tanks, each with two female *X. laevis* frogs which he kept in an 80°F, temperature-controlled room near his office. Because Eaton was the only *Xenopus* user on campus, and because the number of frogs was small, the IACUC allowed

Eaton to care for his own animals. The lab staff fed the animals daily and manually changed 10% of the dechlorinated water weekly. If any problems were noticed, the lab staff would immediately contact the vivarium. The vivarium's animal care and veterinary staff checked the frogs twice weekly.

During a routine semi-annual inspection by the IACUC, the inspectors reported that they could barely observe the frogs due to algae overgrowth in the tanks. This was cited as a potential significant violation of husbandry standards. When questioned, Eaton explained that his new technician wanted to keep the tanks as natural as possible for the frogs, and because they normally lived in stagnant, algae covered

ponds, he agreed to letting the tanks become covered with algae. Eaton added that he had initially discussed this with the animal facility personnel, and they accepted his rationale. There were no health problems with the animals, so he did not see why the IACUC should be concerned.

Should the IACUC be concerned or was Eaton's lab acting within the standards of care for *X. laevis*? □

Jerald Silverman ✉

University of Massachusetts Medical School,
Worcester, MA, USA.

✉e-mail: Jerald.Silverman@umassmed.edu

Published online: 23 July 2020
<https://doi.org/10.1038/s41684-020-0595-0>

The clarity of SOPs

While *The Guide* states that “the success and adequacy of the [aquatic] system depend on its ability to match the laboratory habitat to the natural history of the species”¹, the system also needs to be conducive to the specific research activities being performed. In Dr. Eaton's case, the presence/absence of algae became a research variable.

The specificity required for aquatic husbandry standards is significantly less than what is available for other species; specifically: “The type of life support system used depends on several factors including the natural habitat of the species, age/size of the species, number of animals maintained, availability and characteristics of the water required, and the type of research”¹. This does not, however, imply that research-driven husbandry practices for aquatics should always be described in an IACUC-approved protocol.

Great Eastern University (GEU)'s IACUC established a mechanism to review and approve “PI-husbandry” for these animals. It was at this time that Eaton and the Attending Veterinarian (AV) should have established standard operation procedures (SOPs) for husbandry and AV management of the animals and the room. These SOPs would specify, at an institutional level, the

criteria for evaluating and maintaining, for example, water quality, animal health, and sanitization. Any modifications to the SOPs should be reviewed and approved by the AV prior to implementation. Furthermore, any deviations from the institutionally accepted husbandry standards would need to be detailed and justified in the IACUC-approved protocol.

The concern for Eaton and GEU arose when IACUC members became concerned that the health and welfare of the animals were jeopardized due to assumed poor husbandry practices. Adequate visualization of animals is a general necessity; however, there are other ways IACUCs can determine that there are satisfactory husbandry practices and animal health oversight in place. For example, along with the established husbandry SOPs, Eaton, his lab members, and veterinary staff perform biweekly inspections, and must maintain room sheets detailing variables such as animal health checks, water quality assessments, and room temperature. In addition, viewing animal health (or treatment) reports for Eaton's animals would show how frequently (or not) the veterinary staff identified any health or welfare concerns.

The remaining question is whether Eaton consulted with the AV before making

the change in husbandry practices and, if not, would the AV have considered this a departure from the institutionally accepted husbandry standards, requiring IACUC review. GEU should have a well-described process for oversight of “PI-husbandry” areas, which includes, for example, the extent of veterinary oversight, SOPs for the care of the animals, and clear criteria for when modifications require veterinary and/or IACUC review and approval. Since it appears that the health and welfare of Eaton's animals is not in question, GEU's IACUC should be concerned if a formalized process for oversight of “PI-husbandry” areas has not been established, if the IACUC is not aware of it, and/or if the PI has not been properly educated and/or informed of the requirements as a “PI-husbandry” lab. □

Lauren Danridge ✉

University of Michigan Animal Care & Use Office
(ACUO), Ann Arbor, MI, USA.

✉e-mail: danridlm@med.umich.edu

Published online: 23 July 2020
<https://doi.org/10.1038/s41684-020-0596-z>

References

1. Institute for Laboratory Animal Research. *Guide for the Care and Use of Laboratory Animals* 8th ed. (National Academies Press, Washington, DC, 2011).

Performance standard only half done

It is easy to understand why the IACUC would be concerned when observing the *Xenopus* in an algae-covered tank; after all, most IACUC members are accustomed to seeing furry animals in clean cages as most institutions probably utilize warm-blooded animals more than cold-blooded ones. Yet this scenario is important because *Xenopus* are probably the most widely used amphibian in animal research today.

The Guide for the Care and Use of Laboratory Animals does not contain many frog-specific recommendations for the care of these animals. This could leave the investigators and the IACUC at a bit of a loss when it comes to evaluating the animals during a semi-annual inspection. But it is important to realize that even

though the *Guide* may have few specific recommendations, many of the general recommendations of the *Guide* apply. We would argue that the most important recommendation would be the use of performance standards.

The *Guide* identifies a performance standard as “a standard or guideline that, while describing a desired outcome, provides flexibility in achieving this outcome by granting discretion to those responsible for managing the animal care and use program, the researcher, and the IACUC.”

In this scenario, one could argue that, given a paucity of regulatory standards for the housing of *Xenopus*, a performance standard should be the guiding principal when the evaluation of the care of *Xenopus* is performed. It appears that the

implementation of this standard is what has broken down.

The Principle Investigator (PI), Dr. Eaton, and his staff have been caring for the *Xenopus* as allowed by the IACUC. The PI is of the understanding that all is fine because the frogs’ housing is as ‘natural’ as possible, it was discussed with animal facility personnel, and there appeared to be no health problems for the animals. The fact that the animals are healthy is of prime importance. The IACUC, however, appears to be unaware of the specifics and therein lies the problem.

The Attending Veterinarian (AV), as opposed to simply “animal facility personnel,” should have discussed the husbandry and care of the animals with Eaton to determine what would be appropriate. As part of the IACUC protocol, the PI should provide a Standard Operating Procedure (SOP) of the husbandry schedule, which would include documentation of items such as water changes, water testing, temperature, feeding, etc. In that way, the IACUC would have known that there may be algae in the tanks and that its presence in the tanks does not equal inadequate care of the frogs. The AV could explain the procedures as to how the animals will be maintained by the researchers to the IACUC during the protocol review process. If the AV had little experience with *Xenopus*, and the IACUC was concerned, they could invite a biologist who has experience with these animals to advise them.

The ultimate indicator of adequate care for this species is going to be the health of the animals. In this scenario, it appears that the animals are healthy so the answer to the question is yes, the IACUC should have some concerns but those concerns should not necessarily be regarding the outcome (as the animals are healthy) but the process used by which that outcome was achieved (husbandry and care procedures).

Alison D. Pohl[✉] and Ron G. Wallace
UConn Health, Farmington, CT, USA.
[✉]e-mail: pohl@uchc.edu

Published online: 23 July 2020
<https://doi.org/10.1038/s41684-020-0598-x>

References

1. Institute for Laboratory Animal Research. *Guide for the Care and Use of Laboratory Animals* 8th ed. (National Academies Press, Washington, DC, 2011).

A WORD FROM OLAW

In response to the issues posed in this scenario, the National Institutes of Health - Office of Laboratory Animal Welfare (NIH-OLAW) provides the following clarification:

In this scenario, IACUC inspectors report concerns to the committee about algae overgrowth in tanks in a satellite *Xenopus* frog room. Because there are no apparent health problems with the frogs housed in the more natural habitat, the principal investigator is not concerned and does not find it outside the standards of care.

The PHS Policy requires the IACUC, as an agent of the institution, to inspect at least once every six months all of the institution’s animal facilities including satellite facilities using the *Guide for the Care and Use of Laboratory Animals* as a basis for evaluation¹. The expectations of both the PHS Policy and the *Guide* is for institutions to establish standards for IACUC and veterinary oversight of all animal species whether housed in central or satellite facilities^{2–4}. The *Guide* states that “establishing standard operating procedures can assist an institution in complying with regulations, policies, and principles as well as with day-to-day operations and management².” While it is the IACUC’s responsibility to review husbandry standards that directly impact animal welfare, it may engage the expertise of others to assure the standards are appropriate for the species. The IACUC may also allow standards that deviate from commonly acceptable practices

if justified for scientific reasons⁵. Likewise, the veterinarian may allow deviations for veterinary care or husbandry reasons. Standard operating procedures or written performance standards are clearly needed for Great Eastern University’s frog room that address water turbidity and ensure daily observations, adequate oxygenation, and limited biofilm accumulation while providing a more natural habitat². □

Patricia Brown[✉]

Director, OLAW, OER, OD, NIH, HHS, Bethesda, MD, USA.

[✉]e-mail: brownp@od.nih.gov

Published online: 23 July 2020
<https://doi.org/10.1038/s41684-020-0600-7>

References

1. Office of Laboratory Animal Welfare, National Institutes of Health. *Public Health Service Policy on Humane Care and Use of Laboratory Animals*. (US Department of Health and Human Services, Bethesda, Maryland, USA, 2015). <https://olaw.nih.gov/policies-laws/phs-policy.htm>
2. Institute for Laboratory Animal Research. *Guide for the Care and Use of Laboratory Animals*. 8th edn. (National Academies Press, Washington, D.C., 2011).
3. National Institutes of Health. *Public Health Service Policy on Humane Care and Use of Laboratory Animals - Frequently Asked Questions. Animal Use and Management, Question F.16*. (US Department of Health and Human Services, Bethesda, MD, USA, revised 2017). <https://olaw.nih.gov/guidance/faqs#f16> (accessed June 15, 2020).
4. National Institutes of Health. *Public Health Service Policy on Humane Care and Use of Laboratory Animals - Frequently Asked Questions. Institutional Responsibilities, Question G.10*. (US Department of Health and Human Services, Bethesda, MD, USA, revised 2017). <https://olaw.nih.gov/guidance/faqs#g10> (accessed June 15, 2020).
5. Brown, Patricia & Gipson, Chester *Lab Animal* 41, 41–43 (2012).



Animal preferences vs regulatory standards of care

This scenario seems to be exploring a potential conflict between U.S. Government Principle VII¹, which states that “the living conditions of animals should be appropriate for their species and contribute to their health and comfort,” with the cardinal rule of animal research² that all animals should be observed daily for signs of illness, injury, or abnormal behavior. This case asks the question: do we as animal caretakers and welfare personnel provide the most natural habitat for a research animal at the expense of established and regulated standards of care? What is an acceptable way forward in such scenarios?

In the scenario described, are these animals currently involved in a study protocol approved by the university’s IACUC? From simple calculations, Dr. Eaton has six female animals on site. This number seems very small for any approved research protocol. Additionally, are they providing too much space for each animal and in that way also indirectly affecting observation of the animals? According to *the Guide* and the Blue book³, *X. laevis* adults may be housed at stocking densities in the range of one frog per 2–3 L to four frogs per 5–10 L. In this scenario they are stocking at one female frog to about 47.3L of water. This amount of space per animal coupled with the algal cover may directly contribute to the deficiencies in animal observation.

In the wild, *X. laevis* are secretive, private animals and are commonly found in murky water, which provide a visual barrier to predators⁴. Most universities and research centers that use these animals acknowledge this behavioral characteristic and suggest the use of visual barriers, hides, tanks with darkened or opaque sides, and artificial and/or natural plants as appropriate. However, from my reading, not many references suggest letting tanks get significantly covered with algae. As a matter of fact, *The Guide* acknowledges that algal growth is common in aquatic systems, but it recommends limiting algal growth to allow viewing of the animals in their enclosure.

Xenopus sp. are covered under the Public Health Service Policy, which follows and adheres to *The Guide*². Therefore, the inspectors were correct in highlighting the algal overgrowth in the tanks as a barrier to the proper viewing of the animals. In my opinion the scientist and his staff did not show ill-intent but an honest attempt to provide a naturalistic habitat for the animals in their care. They sought the relevant advice and were vigilant in their care of the animals. I do not think it constitutes a significant violation of husbandry standards. I think the inspectors should note the finding in their reports and present their findings to the IACUC. The

IACUC in turn can then investigate if and how Eaton and his staff were able to meet the daily animal observation requirement in the existing housing scenario. All bodies involved—the IACUC, the veterinary staff and the laboratory staff—should then meet to discuss how conditions can be modified to balance the animals’ habitat preferences while still satisfying the regulatory standards of care. □

Jenelle Johnson ✉

School of Veterinary Medicine, The University of the West Indies (The UWI), St. Augustine, Trinidad and Tobago.

✉e-mail: Jenelle.johnson2@sta.uwi.edu

Published online: 23 July 2020

<https://doi.org/10.1038/s41684-020-0597-y>

References

1. Interagency Research Animal Committee (IRAC). U.S. Government Principles for Utilization and Care of Vertebrate Animals Used in Testing, Research, and Training. Federal Register, May 20, 1985
2. Institute for Laboratory Animal Research. *Guide for the Care and Use of Laboratory Animals*. 8th edn. (National Academies Press, Washington, DC, 2011)
3. O’Rourke D. P. & Rosenbaum M. D., Chapter 18 - *Biology and Diseases of Amphibians*, Editor(s): J. G. Fox, L. C. Anderson, G. M. Otto, K. R. Pritchett-Corning, M. T. Whary, In *American College of Laboratory Animal Medicine, Laboratory Animal Medicine*, 3rd edn, pp 931-965, (Academic Press, 2015)
4. Tinsley, R.C., Loumont, C., Kobel, H.R., *Geographical distribution and ecology*. In: Tinsley, R.C., Kobel, H.R. (Eds.), *The Biology of Xenopus* (Clarendon Press, Oxford, UK, 1996a)