



DEPARTMENT OF HEALTH & HUMAN SERVICES

PUBLIC HEALTH SERVICE  
NATIONAL INSTITUTES OF HEALTH

FOR US POSTAL SERVICE DELIVERY:

Office of Laboratory Animal Welfare  
6700B Rockledge Drive, Suite 2500, MSC 6910  
Bethesda, Maryland 20892-6910  
Home Page: <http://grants.nih.gov/grants/olaw/olaw.htm>

FOR EXPRESS MAIL:

Office of Laboratory Animal Welfare  
6700B Rockledge Drive, Suite 2500  
Bethesda, Maryland 20817  
Telephone: (301) 496-7163  
Facsimile: (301) 402-7065

December 16, 2019

Re: Animal Welfare Assurance  
A3356-01 [OLAW Case L]

Joseph R. Gladden, Ph.D.  
Interim Vice President for Research  
and Sponsored Programs  
University of Mississippi  
(b) (4) Barr Hall - P.O. Bo907x 1848  
University, MS 38677

Dear Dr. Gladden,

The Office of Laboratory Animal Welfare (OLAW) acknowledges receipt of your December 13, 2019 letter reporting an adverse event affecting fish at the University of Mississippi, following up on an initial telephone report on November 13, 2019. According to the information provided, OLAW understands that 12 of 24 puffer fish died due to a problem with water quality. Specifically, the ammonia level spiked due to a filter being overwhelmed which caused the nitrifying bacteria level to drop.

The corrective actions consisted of contacting the Attending Veterinarian (AV), Principal Investigator, and caretaker. Ammonia levels were lowered by changing out water, adding chemicals, and reestablishing the nitrifying bacteria. Unfortunately, all but one of the fish died or were euthanized. To prevent a recurrence, a second filter will be installed, new bacteria stocks will be obtained, ammonia buffers will be stocked, and ammonia levels will be carefully monitored. The AV and animal care staff will monitor the water quality logs. The study is supported by NSF grant #1638289.

Based on its assessment of this explanation, OLAW understands that measures have been implemented to prevent recurrence of this problem. OLAW concurs with the actions taken by the institution to comply with the PHS Policy on Humane Care and Use of Laboratory Animals. Please contact OLAW if the proposed corrective actions are found to be unsuccessful. Thank you for keeping OLAW apprised on this matter.

Sincerely,

(b) (6)

Axel Wolff, M.S., D.V.M.  
Deputy Director  
Office of Laboratory Animal Welfare

cc: IACUC Chair  
NSF Animal Welfare Officer



December 13, 2019

Dr. Axel V. Wolff, M.S., D.V.M.  
Director  
Division of Compliance Oversight  
Office of Laboratory Animal Welfare  
National Institutes of Health  
Rockledge 1, Suite 360, MSC 7982  
6705 Rockledge Drive  
Bethesda, MD 20892-7982

University of Mississippi Animal Welfare Assurance#: A3356-01

Dear Dr. Wolff,

As the University's Institutional Official, I am providing this final report regarding a recent animal welfare incident. A preliminary incident report was given to your office by Mandy King, our IACUC chair and Director for Research Integrity and Compliance, and Harry Fyke, our Attending Veterinarian, via telephone. The research in this case was funded by NSF (award # 1638289). The sequence of events is detailed below for your records, review, and comment.

#### Incident

On Saturday, November 2, 2019 the ACS (animal care supervisor) observed that approximately 12 of 24 pufferfish (*Canthigaster* spp.) had died since the previous check 24 hours earlier.

#### Initial Response

Upon discovering the fish deaths on the afternoon of November 2<sup>nd</sup>, the animal care supervisor alerted the AV (attending veterinarian) and communicated with the person responsible for the daily fish care. The PI (principal investigator) was notified by their personnel. The AV checked the fish on the morning of Sunday, November 3, 2019 and did not see any signs of disease in the remaining fish. After a discussion between all parties, it was determined that a spike in the ammonia due to the nitrifying bacteria in the filter system shutting down was the cause of the deaths. The system was supported by a single bio-wheel filter unit (rated to handle the water volume in the system). Nonetheless, that system became overwhelmed. Steps were taken to lower the ammonia levels by partial water changes, the addition of chemicals to the water, and reestablishing the nitrifying bacteria. Despite these efforts, all but 1 of the remaining pufferfish died or were euthanized due to morbidity over the next few days.

#### IACUC Response and Corrective Action

The IACUC office was notified of the occurrence on Monday, November 11<sup>th</sup> by both the PI and the AV. On November 14<sup>th</sup> the PI was asked for a detailed report of the incident which was submitted promptly.

The PI outlined the following plan to try to prevent this issue before any more fish were obtained:

1. A second bio-wheel filter will be installed and new stocks of nitrifying bacteria obtained in order to control the system in a redundant manner. Thus, even if one bio filter slows down the overall system should be fine for many hour with the redundancy.
2. Ammonia buffers will be kept on-hand to drive an ammonia spike down immediately.
3. A much more robust ammonia testing procedure will be implemented that should show elevations well in advance of the lethal concentrations that were seen during this incident.

#### Conclusion

The IACUC concurred with the PI's corrective action plan. The Attending Veterinarian and Laboratory Animal Care staff will regularly review future water quality recordkeeping logs maintained by the research team to ensure increased diligence to husbandry.

Sincerely,

(b) (6)

Joseph R. Gladden, Ph.D.  
Vice Chancellor for Research and Sponsored Programs  
Institutional Official

**Wolff, Axel (NIH/OD) [E]**

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**From:** OLAW Division of Compliance Oversight (NIH/OD)  
**Sent:** Monday, December 16, 2019 10:10 AM  
**To:** Mandy L. King  
**Cc:** OLAW Division of Compliance Oversight (NIH/OD)  
**Subject:** RE: Final report from University of Mississippi A3356-01

Thank you for this report, Ms. King. We will send a response soon.

Axel Wolff, M.S., D.V.M.  
Deputy Director, OLAW

**From:** Mandy L. King <mlking9@olemiss.edu>  
**Sent:** Monday, December 16, 2019 9:59 AM  
**To:** OLAW Division of Compliance Oversight (NIH/OD) <olawdco@od.nih.gov>  
**Cc:** iacuc@olemiss.edu; HARRY CARTER FYKE <hfyke@olemiss.edu>  
**Subject:** Final report from University of Mississippi A3356-01

On behalf of University of Mississippi's Institutional Official, Dr. Gladden, please find the attached final report regarding a recent incident.

We are happy to answer any additional questions you might have and look forward to your response.

Regards,

**Mandy King**  
Director of Research Integrity and Compliance and IACUC Chair  
Office of Research and Sponsored Programs  
The University of Mississippi  
(b) (4) Barr Hall  
University, MS 38677-1848  
USA  
O: (b) (6) | F: (b) (6)  
[mlking9@olemiss.edu](mailto:mlking9@olemiss.edu) | [www.olemiss.edu](http://www.olemiss.edu)



## Initial Report of Noncompliance

By: Carol

Date: 11/13/19

Time: 12:00

Name of Person reporting: Mandy Wang

Telephone #: (b) (6)

Fax #:

Email:

Name of Institution: U of Mississippi - Oxford

Assurance number: A3356

Did incident involve PHS funded activity? NSF

Funding component: \_\_\_\_\_

Was funding component contacted (if necessary): \_\_\_\_\_

What happened?

New fish arrived + died due to poor water quality

Species involved: Puffer Fish

Personnel involved:

Dates and times:

Animal deaths: 18

Projected plan and schedule for correction/prevention (if known): \_\_\_\_\_

Establish helpful bacteria in water (denitrifying)

Projected submission to OLAW of final report from Institutional Official:

OFFICE USE ONLY

Case # \_\_\_\_\_