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**UNIVERSITY
of HAWAII
SYSTEM**

Office of Research Compliance

10 July 2018

Dr. Axel Wolff
Deputy Director, Division of Compliance Oversight
Office of Laboratory Animal Welfare
RKL1, Suite 1050 (MSC 9782)
6705 Rockledge Drive
Bethesda, Maryland 20892-7982

Re: University of Hawaii Animal Welfare Assurance #A3423-01
Adverse Incident Resulting in Fish Mortalities Follow-up; COMM-OLAW-2018-5-FU

Dear Dr. Wolff,

Pursuant to OLAW's letter dated 11 June 2018, the University of Hawaii's IACUC conducted an investigation into this matter; thus, provides the following information.

As you may recall from the initial report, an adverse incident was reported to OLAW on 6 June 2018; whereby, an estimated 300 Pacific Threadfin fish, *Polydactylus sexfilis* locally known as Moi died from what was believed to be largely attributed to shipping stress. The fish were to be used in a fish nutrition study. Upon receipt, the fish were estimated to be an average of 10 grams each. The fish were air shipped in oxygenated plastic bags from private research company, the Oceanic Institute (OI) to the University of Hawaii (UH), at Hilo campus Pacific Aquaculture Coastal Resource Center (PACRC) on the Island of Hawaii.

IACUC/FAC Investigation

On Friday, 7 July 2018, an IACUC subcommittee comprised of members of the Fish Advisory Committee (FAC) conducted person-to-person interviews at the PACRC facility with personnel closely associated with this matter. The subcommittee met individually with the Dean of the College of Agriculture, Forestry, and Natural Resource Management (CAFNRM), the Principal Investigator (PI) of the study, and the PACRC Director, CAFNRM to discuss the incident. There are a few disagreements regarding what occurred with the fish which led to the mortalities. However, based on the individual interviews, the following were salient points agreed upon:

- The fish were larger than the 5-gram size requested. The sizes ranged from 5-10 grams or were all approximately 10 grams, but the majority was larger than 5 grams.
- The shipping containers did not contain any type of cooling system to keep the temperature within the boxes low. Confirmed in discussion with OI on 2 July 2018.
- There was potential toxic chemical residue on the shipping bags. A type of powder that coats the plastic of the bags to avoid sticking together may have not been removed completely. This was OI's opinion based on past experience when shipping bags that are not thoroughly rinsed prior to filling with water resulted in a large number of fish mortalities. Confirmed in discussion with OI on 2 July 2018.
- Water quality parameters were only collected from one bag of fish, prior to placing them in the quarantine tanks.
- The quarantine tank biofilter was primed approximately 4 days prior.
- The quarantine tank is located across from fish holding tanks.
- Water from the shipped fish bags were discarded outside of the fish holding tents.
- Water quality parameters differed between the facility director's readings and the PI's.

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IACUC/FAC Conclusion

The FAC subcommittee agreed that shipping stress is more than likely the primary cause which led to the fish mortalities. Potential chemical residue on the shipping bags, lack of a cooling system within the shipping boxes, larger sized fish than requested, potential water quality issues including pH changes, reduced dissolved oxygen, and increased ammonia due to temperature and dead fish all may have contributed to their deaths. However, there are other issues that are of concern with how the fish once arrived, were handled. These include:

- Testing water quality parameters in more than 1 bag. While we understand the concern for the fish that were still alive; however, it is difficult to determine if the water quality was a contributing factor in many of the fish deaths.
- Quarantine in a location separate from the current fish holding area, to reduce possibility of cross contamination. Quarantining in a separate area was a requirement of the IACUC, in the review of the SOP. This was a departure from the IACUC approved SOP.
- Euthanize and necropsy moribund fish to help determine or rule out other causes of fish mortalities.
- For Biosecurity reasons, decontaminate and dispose of shipped water away from the research animal holding area.
- Utilize a calibrated meter or testing kit to check pH. Test strips are too inaccurate.
- Proper priming of the biofilter may not have influenced the survival of the fish, but as a good measure, if biofiltration is to be used, it should be done so appropriately.

We communicated with the PACRC these concerns and expect these corrective measures and recommendations to be implemented immediately, 7 July 2018.

There is also a human resources concern at the PACRC. While it is not under the purview of the IACUC/FAC, the following is recommended. It is understood that the PI feels that the husbandry and care of the fish under his protocol should be done by hired technicians provided by the PACRC, however, it is still the responsibility of the PI to make sure that any research animals they are using are cared for appropriately. This includes husbandry and care if necessary. The PACRC Director should create policies that are used by the whole Center. Input from PIs utilizing the facility can help assist with creating these policies. This would prevent future confusion with regards to facility use, including any limitations that the facility may have. Open dialogue between the director and PIs should also occur to best determine that the desired research can be facilitated at the PACRC.

Disposition of Surviving Fish

The surviving fish have grown to approximately 29 grams and were transferred into six holding tanks. The nutrition research is currently on hold until the experimental feed is produced.

The adverse incident will be discussed by the IACUC at the 19 July 2018 business meeting.

Please contact me at (b) (6) or riveravg@hawaii.edu with any questions.

Sincerely,



Victoria G. Rivera, MPA
Director, Office of Research Compliance
Institutional Official, Animal Welfare Program

c: Manuel G. Himenes, Jr., Chair, UH IACUC

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DEPARTMENT OF HEALTH & HUMAN SERVICES

PUBLIC HEALTH SERVICE
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FOR US POSTAL SERVICE DELIVERY:

Office of Laboratory Animal Welfare
Rockledge One, Suite 360
6705 Rockledge Drive - MSC 7982
Bethesda, Maryland 20892-7982
Home Page: <http://grants.nih.gov/grants/olaw/olaw.htm>

FOR EXPRESS MAIL:

Office of Laboratory Animal Welfare
Rockledge One, Suite 360
6705 Rockledge Drive
Bethesda, Maryland 20817
Telephone: (301) 496-7163
Facsimile: (301) 402-7065

June 11, 2018

Re: Animal Welfare Assurance
#A3423-01 (OLAW Case 2B)

Ms. Victoria G. Rivera
Interim Director for Research Compliance
Institutional Official for Animal Welfare Program
University of Hawaii-Manoa
2425 Campus Road, (b) (4)
Honolulu, HI 96822

Dear Ms. Rivera,

The Office of Laboratory Animal Welfare (OLAW) acknowledges receipt of your June 6, 2018 letter reporting an adverse event involving fish at the University of Hawaii. According to the information provided, OLAW understands that approximately 300 fish died during or shortly after transit from a private research company to the University. The deaths were attributed to shipping stress.

The incident will be reviewed by the Institutional Animal Care and Use Committee to determine if noncompliance had occurred or if there were specific problems surrounding the shipment.

In order for OLAW to have a complete understanding of this matter, please provide any additional information regarding the IACUC's findings, the disposition of the surviving fish, as well as any preventive measures taken. Please provide this information by **July 13, 2018**.

Sincerely,

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

cc: IACUC Chair

(b) (6)



**UNIVERSITY
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6 June 2018

Dr. Axel Wolff
Director, Division of Compliance Oversight
Office of Laboratory Animal Welfare
RKL1, Suite 1050 (MSC 9782)
6705 Rockledge Drive
Bethesda, Maryland 20892-7982

Re: University of Hawaii Animal Welfare Assurance #A3423-01
Adverse Incident Resulting in Fish Mortalities; COMM-OLAW-2018-5

Dear Dr. Wolff,

I am writing to OLAW to report an adverse incident which resulted in the death of an estimated 300 Pacific Threadfin fish, *Polydactylus sexfilis* locally known as Moi. The fish were estimated to be average of 10 grams each. The fish were air shipped in oxygenated plastic bags from private research company, the Oceanic Institute (OI) to the University of Hawaii (UH), at Hilo campus Pacific Aquaculture Coastal Resource Center (PACRC) on the Island of Hawaii. The OI is located on the island of Oahu; therefore, at this time, the travel distance between the islands travel is not considered to be a determining factor in the cause of the fish mortalities. Furthermore, the mortalities are not attributed to the ongoing volcanic activities occurring on the island of Hawaii.

The fish were to be used a fish nutrition study. Funding for the study originated from the Center for Tropical and Subtropical Aquaculture (CTSA), U.S. Department of Agriculture. PHS, VA, or NSF funding was not being used to support the study.

Timeline and Response

On Friday, 25 May 2018, a UH Hilo Principal Investigator reported that on 24 May 2018 an adverse incident occurred whereby a large shipment of Moi numbering 504 fish was air shipped from OI to the PACRC. The fish were shipped in 28 large plastic bags at a density of 18 fish per bag. Upon receipt of the shipment, 170 of the fish were found dead. The surviving fish were transported to the PACRC and placed in quarantine. The PI observed the following morning that 61 fish had perished overnight. After 2 days of acclimation and quarantine, 269 Moi survived and were reported exhibiting normal swimming behavior but were considered too stressed to serve as research subjects at this point.

The mortalities of the fish have been largely attributed to shipping stress; however, the IACUC will inquire whether there may have been departures from the IACUC approved animal use protocol and PACRC facility standard operating procedures concerning the receipt of newly acquired fish, shipping methods, acclimation and quarantine, etc.

IACUC

The adverse incident will be discussed by the IACUC at the 21 June 2018 business meeting. The University's Fish Advisory Committee, an IACUC subcommittee comprised of aquatic species subject matter experts will likely conduct the inquiry, then report back to the IACUC its findings.

Please contact me at [redacted] or niverawg@hawaii.edu with any questions.

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Dr. Axel Wolff

COMM-OLAW-2018-1

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Sincerely,

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Victoria G. Rivera, MPA
Interim Director, Office of Research Compliance
Institutional Official, Animal Welfare Program

c: Manuel G. Himenes, Jr., Chair, UH IACUC

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Morse, Brent (NIH/OD) [E]

From: OLAW Division of Compliance Oversight (NIH/OD)
Sent: Thursday, June 07, 2018 3:15 PM
To: (b) (6) OLAW Division of Compliance Oversight (NIH/OD)
Cc: Victoria Rivera
Subject: RE: University of Hawaii Reports Adverse Incident

Thank you for this report (b) (6) I will forward it to Dr. Wolff for his review.

Sincerely, Brent Morse

Brent C. Morse, DVM, DACLAM
Acting Director
Division of Compliance Oversight
Office of Laboratory Animal Welfare
National Institutes of Health

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From: (b) (6)@hawaii.edu]
Sent: Thursday, June 07, 2018 1:25 PM
To: OLAW Division of Compliance Oversight (NIH/OD) <olawdco@od.nih.gov>
Cc: Victoria Rivera <riveravg@hawaii.edu>
Subject: University of Hawaii Reports Adverse Incident

Good Afternoon Dr. Wolff and OLAW Compliance Staff:

On behalf of Ms. Victoria Rivera, Interim Director for Research Compliance, Institutional Official for the UH Animal Welfare Program, please find the attached report.

Sincerely,

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