

# Aquaculture Pathology Laboratory University of Arizona, USA



## Launch of the Regional Aquatic Animal Health Laboratory Network for Africa (RAAHLN-AF)

5 – 7 December 2023 Pretoria, South Africa

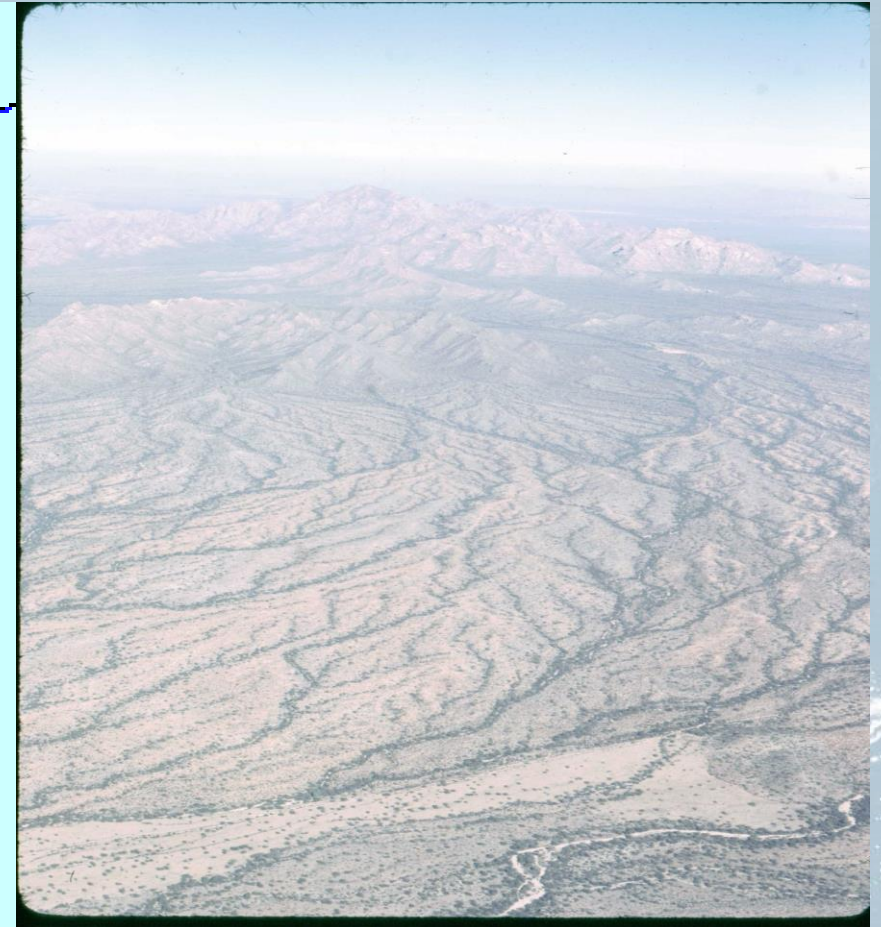


# Aquaculture Pathology Laboratory

## University of Arizona, USA



**Aquaculture  
Pathology Laboratory  
is located in Tucson,  
Arizona- A part of  
Sonoran Desert**



### Two Campuses, One Lab

- UA-Main Campus



- West Campus Agriculture Center



**WOAH Reference Laboratory of Crustacean Diseases, USDA-APHIS Approved  
& ISO 170225, ISO 17043 accredited**

# Aquaculture Pathology Laboratory

## University of Arizona, USA



### MISSIONS:

#### UA Main Campus Laboratory:

- ***Disease diagnostic services to shrimp industry and academia worldwide***
  - **Three Units: Histopathology, Molecular Diagnostics & Microbiology**
- **Educational & Training Services:**
  - ***Shrimp Pathology Short Course annually & In country training worldwide.***
  - ***International collaboration via WOAHL Twinning Projects & other projects***
- **Conducting Proficiency Tests-Harmonizing shrimp disease diagnostics worldwide**
- **Basic & applied research involving pathogen discovery, developing diagnostic tools & therapeutics**

#### WCAC- Live Animals Research Facility:

- **Disease challenge study, testing therapeutics, feed & feed additives.**
- **Disease challenge studies involving warm water fish**

# Aquaculture Pathology Laboratory

## University of Arizona, USA

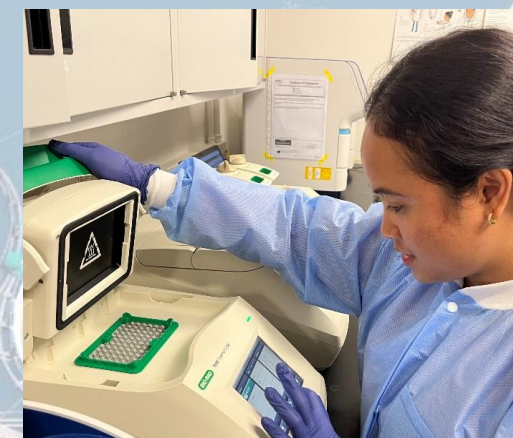
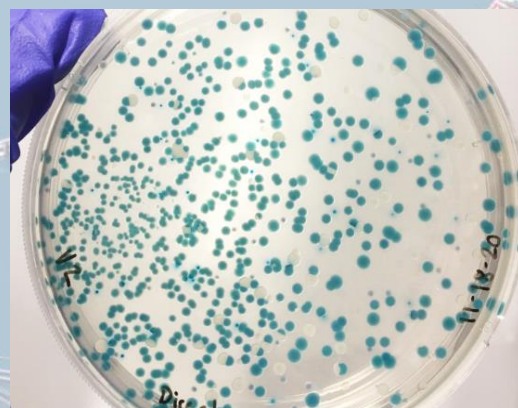
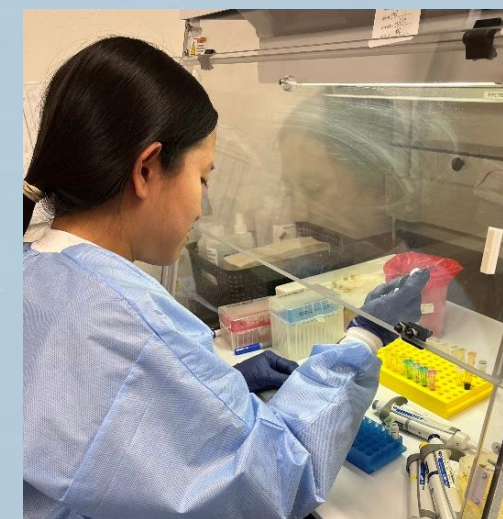


Pathology

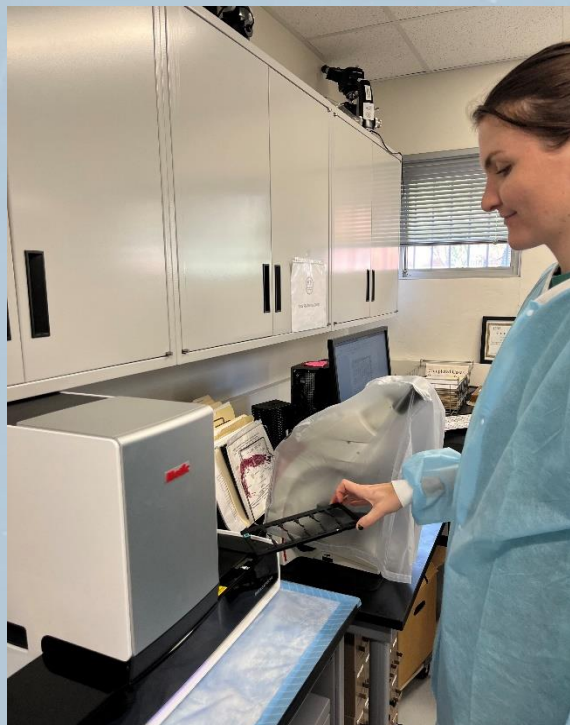
Microbiology

Molecular Diagnostics

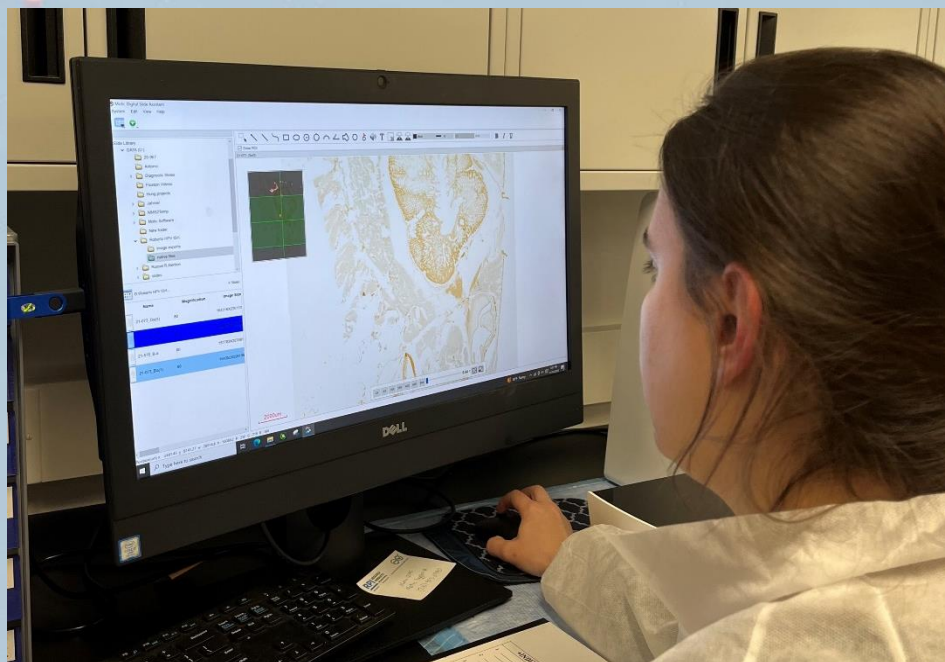
## Different Units of Shrimp Disease Diagnostics



## Developing A Digital Pathology Workflow



**Whole Slide  
Imaging**



**Annotation and  
Digital Image Analysis**



**Leveraging of Pathology  
Expertise for Teaching and  
Diagnostics**





## Acute hepatopancreatic necrosis disease

**Dr Arun Dhar**

📍 UNITED STATES OF AMERICA

**Address**

Aquaculture Pathology Laboratory, School of Animal  
and Comparative Biomedical Sciences  
University of Arizona, 1117 E Lowell St, Building 90,  
85721 Tucson

**Contact details**

+1-520 621 87.27  
[adhar@arizona.edu](mailto:adhar@arizona.edu)

[See the annual report](#) →

## Infection with *Hepatobacter penaei* (necrotising hepatopancreatitis)

**Dr Arun Dhar**

📍 UNITED STATES OF AMERICA

**Address**

Aquaculture Pathology Laboratory, School of Animal  
and Comparative Biomedical Sciences  
University of Arizona, 1117 E Lowell St, Building 90,  
85721 Tucson

**Contact details**

+1-520 621 87.27  
[adhar@arizona.edu](mailto:adhar@arizona.edu)

## Infection with infectious hypodermal and haematopoietic necrosis virus

**Dr Arun Dhar**

📍 UNITED STATES OF AMERICA

**Address**

Aquaculture Pathology Laboratory, School of Animal  
and Comparative Biomedical Sciences  
University of Arizona, 1117 E Lowell St, Building 90,  
85721 Tucson

**Contact details**

+1-520 621 87.27  
[adhar@arizona.edu](mailto:adhar@arizona.edu)

[See the annual report](#) →

## Infection with Taura syndrome virus

**Dr Arun Dhar**

📍 UNITED STATES OF AMERICA

**Address**

Aquaculture Pathology Laboratory, School of Animal  
and Comparative Biomedical Sciences  
University of Arizona, 1117 E Lowell St, Building 90,  
85721 Tucson

**Contact details**

+1-520 621 87.27  
[adhar@arizona.edu](mailto:adhar@arizona.edu)

[See the annual report](#) →

## Infection with white spot syndrome virus

**Dr Arun Dhar**

📍 UNITED STATES OF AMERICA

**Address**

Aquaculture Pathology Laboratory, School of Animal  
and Comparative Biomedical Sciences  
University of Arizona, 1117 E Lowell St, Building 90,  
85721 Tucson

**Contact details**

+1-520 621 87.27  
[adhar@arizona.edu](mailto:adhar@arizona.edu)

[See the annual report](#) →



APL served ~30-35  
countries/ year in  
2017-2023.

Live and frozen  
shrimp, feed and  
feed ingredients  
are screened for  
shrimp pathogens.



## Tissue/ Feed Sampling & Extraction of Nucleic Acid

\*  
PCR running

\*  
Sampling & Extraction



Sampling

Extraction

- Perform sampling and extraction in different rooms
- Two different sampling hoods



Minimizing the risk of cross contamination

## PCR workstation



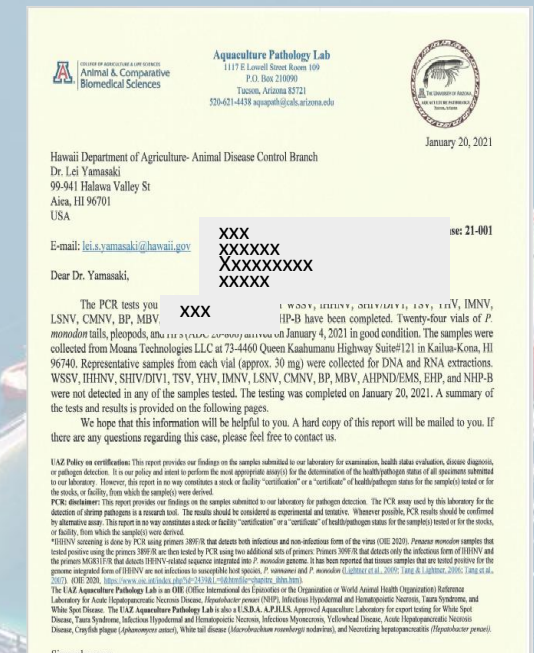
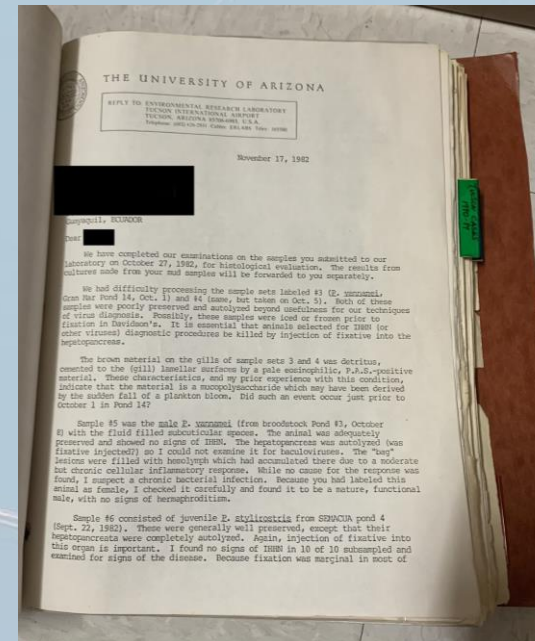
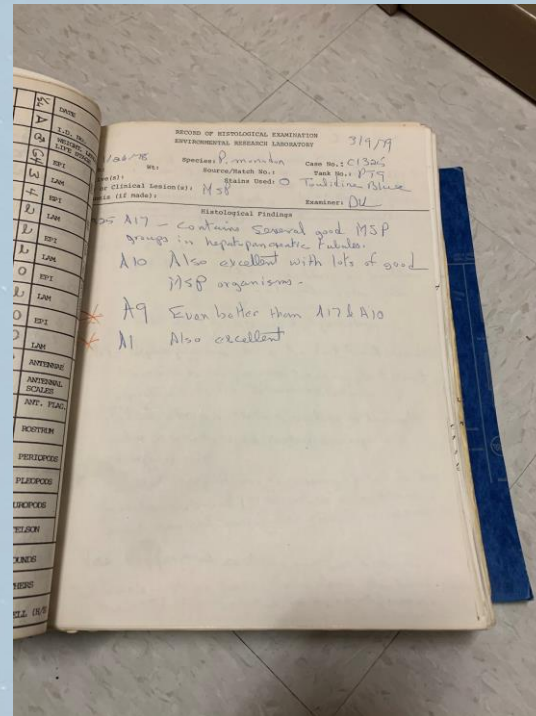
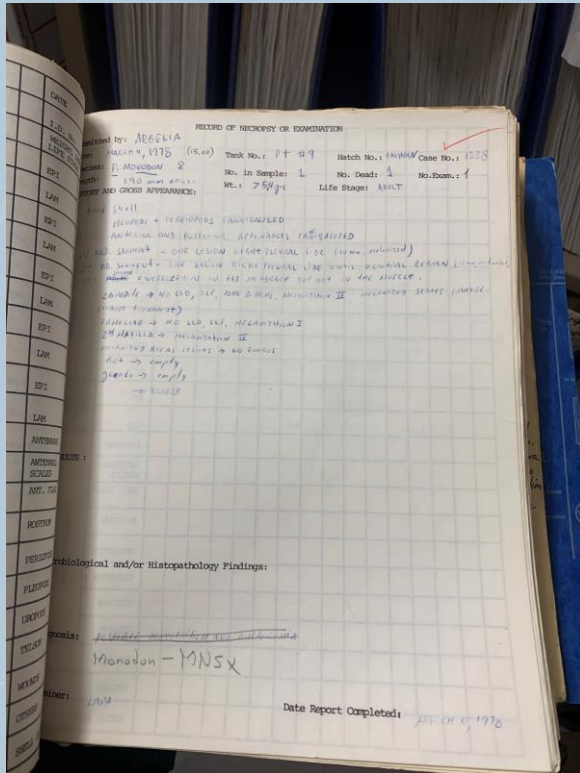
Like the sampling and extraction hoods, the PCR workstations are equipped with all the consumables needed to prevent contamination in the workstation.

## Archiving Shrimp Disease Diagnostic Case Reports






## Archiving Shrimp Disease Diagnostic Case Reports




## Archiving Shrimp Disease Diagnostic Case Reports



COLLEGE OF AGRICULTURE & LIFE SCIENCES  
Animal & Comparative  
Biomedical Sciences

Aquaculture Pathology Lab  
1117 E Lowell Street Room 109  
P.O. Box 210090  
Tucson, Arizona 85721  
Phone: 520-621-4438  
E-mail: aquapath@cal.arizona.edu



THE UNIVERSITY OF ARIZONA  
COLLEGE OF AGRICULTURE & LIFE SCIENCES  
ANIMAL & COMPARATIVE BIOMEDICAL SCIENCES


**FINAL REPORT**

Confidential, Disseminate on a Need-To-Know Basis Only

<p><b>Owner:</b> XXXXXX</p> <p><b>Animal Location:</b> XXXXXX</p> <p><b>Report Recipient:</b> XXXXXX</p>	<p><b>Case number:</b> XXXXXX</p> <p><b>Date Collected:</b> XXXXXX</p> <p><b>Collected By:</b> XXXXXX</p> <p>NAN: N/A</p> <p>Purpose: International Export Destination</p> <p>Reference Number: N/A</p> <p>Country Origin/Destination: XXXXXX</p> <p><b>Date Received:</b> XXXXXX <b>Date Completed:</b> XXXXXX</p>
--	---

NOTE: Condition of the sample(s) was good unless otherwise noted. Pooling info: 2 vials with 15 pleopods/HP sections were each submitted.

Page 1 of 2 Date Generated: 6/6/2023

Arizona's First University - Since 1885 

Case No: XXXXXX

**Table 1: Summary of Results for PCR Testing of Systemic Pathogens**

Pool ID	Identification no. (pond, tank, etc.)	Lot/Batch No.	Species	Age	Specimen Type	Sex	WSSV	IHHNV	DIV1	TSV	YHV	IMNV
XXXXXX	XXXX	XXXX	<i>L. vannamei</i>	41.2 g	Pleopods	N/A*	ND†	ND	ND	ND	ND	ND

NOTE: \*N/A - Not Available †ND - Not Detected Pooling info: 2 vials with 15 pleopods/HP sections were each submitted.

**Table 2: Summary of Results for PCR Testing of Enteric Pathogens**

Pool ID	Identification no. (pond, tank, etc.)	Lot/Batch No.	Species	Age	Specimen Type	Sex	BP	AHPND/EMS	EHP	NHP-B
XXXXXX	XXXX	XXXX	<i>L. vannamei</i>	41.2 g	Hepatopancreas	N/A*	ND†	ND	ND	ND

NOTE: \*N/A - Not Available †ND - Not Detected Pooling info: 2 vials with 15 pleopods/HP sections were each submitted.

**Protocols used:**

WSSV: Real-time PCR described by Durand and Lightner (*J. Fish Dis.*, 2002, 25: 381-389).  
 IHHNV: Real-time PCR described by Tang & Lightner (*Dis. Aquat. Org.*, 2001 44: 79-85).  
 SHIV/DIV1: Real-time PCR protocol described by Qui et al. (*J. Invertebr. Pathol.* 2020, 173, 107367).  
 TSV: Real-time RT-PCR described by Tang et al. (*J. Virol. Methods*, 2004, 109-114).  
 YHV: Real-time PCR method described by Aranguren et al. (*Dis. Aquat. Org.* 2012, 98:185-192).  
 IMNV: Real-time RT-PCR modified from Andrade et al. (*Aquaculture*, 2007, 264: 9-15).  
 BP: OIE 2019 Chapter 2.2.11. Tetrahedral Baculovirus (*Baculovirus penaei*)-Manual of Diagnostic test for aquatic animals.  
 AHPND/EMS: Real-time PCR described by Han et al. (*Aquaculture*, 2015, 442: 12-15).  
 EHP: Real-time PCR described by Liu et al., 2018 (*J. Invertebr. Pathol.* 151: 191-196).  
 NHP-B: Real-time PCR described by Aranguren et al. (*Aquaculture*, 2010, 307: 187-192).

**UAZ Policy on certification:** This report provides our findings on the samples submitted to our laboratory for examination, health status evaluation, disease diagnosis, or pathogen detection. It is our policy and intent to perform the most appropriate assay(s) for the determination of the health/pathogen status of all specimens submitted to our laboratory.

**PCR disclaimer:** This report provides our findings on the samples submitted to our laboratory for pathogen detection.

The UAZ Aquaculture Pathology Lab is an OIE (Office International des Epizooties or the Organization or World Animal Health Organization) Reference Laboratory for Acute Hepatopancreatic Necrosis Disease, *Hepatobacter penaei* (NHP), Infectious Hypodermal and Hematopoietic Necrosis, Taura Syndrome, and White Spot Disease. The UAZ Aquaculture Pathology Lab is also a U.S.D.A. APHIS. Approved Aquaculture Laboratory for export testing for White Spot Disease, Taura Syndrome, Infectious Hypodermal and Hematopoietic Necrosis, Infectious Myonecrosis, Yellowhead Disease, Acute Hepatopancreatic Necrosis Disease, Crayfish plague (*Aphanomyces astaci*), White tail disease (*Microbrachium rosenbergii nodavirus*), and Necrotizing hepatopancreatitis (*Hepatobacter penaei*).

**Tests conducted by:**

*Rika Nakamura*


Rika Nakamura  
Research Technician

**Results authorized by:**

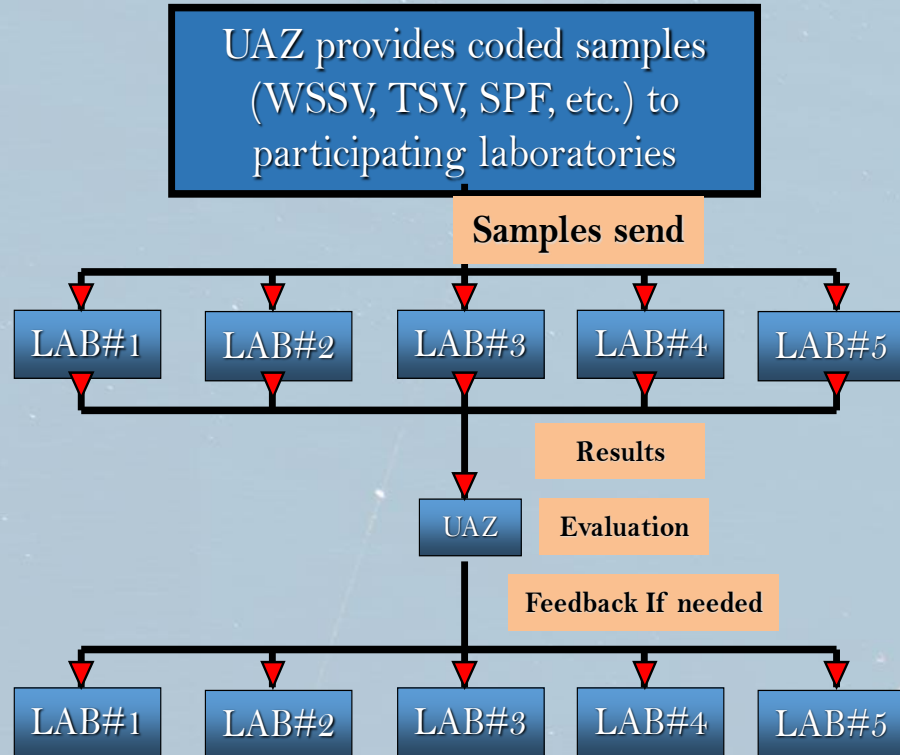
*Arun K. Dhar*

Dr. Arun K. Dhar  
Director  
Aquaculture Pathology Laboratory  
Office: 520-621-8727

Page 2 of 2 Date Generated: 6/6/2023

Arizona's First University - Since 1885 

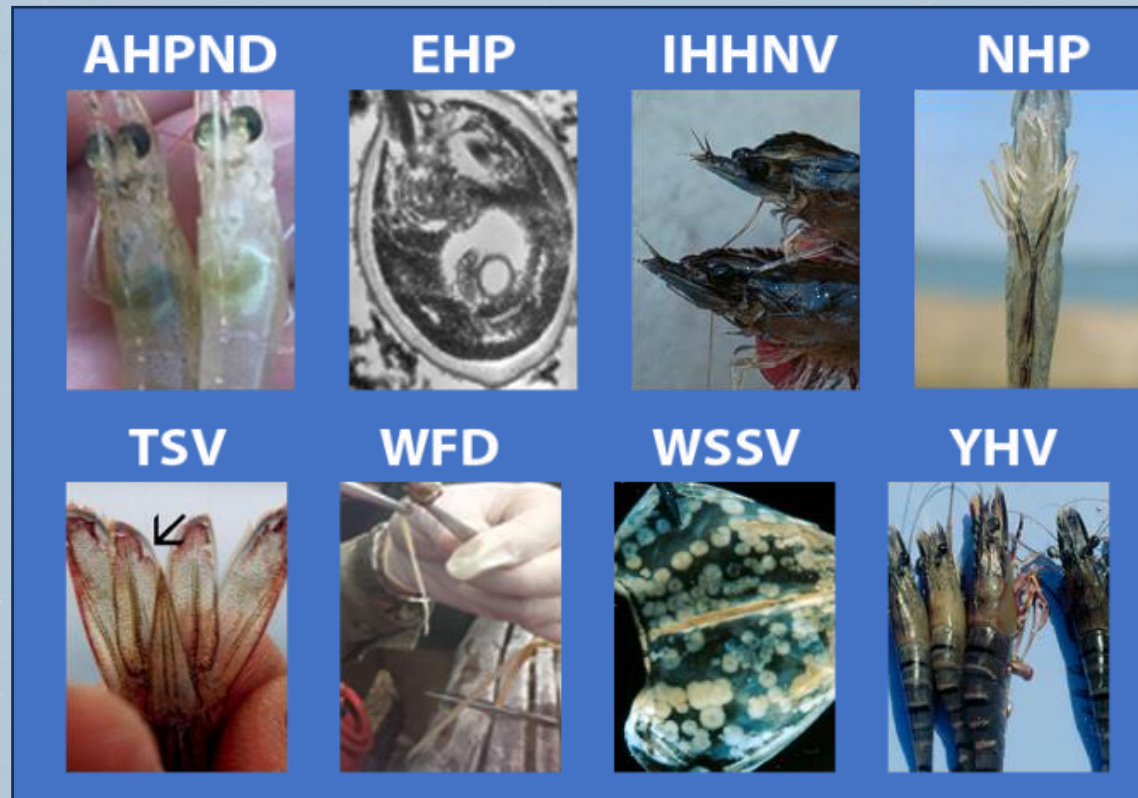
# Aquaculture Pathology Laboratory University of Arizona, USA



- Conduct Proficiency Test (Ring Test) to harmonize shrimp disease diagnostics twice a year (in February & August).
- Disease diagnostic laboratories from many countries around the world participate.

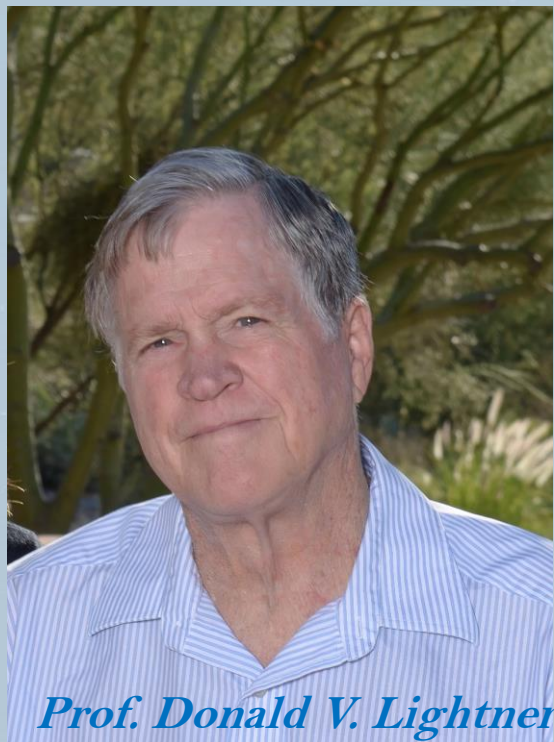
## University of Arizona, USA

- Generate, archive and distribute biological reference products and other reagents for shrimp disease diagnosis worldwide.





## *A hallmark contribution in educational and training service of Prof. Donald V. Lightner: Shrimp Pathology Short Course*



*Prof. Donald V. Lightner  
1945-2021*





## *Shrimp Pathology Short Course in the University of Arizona*



Launch of the Regional Aquatic  
Animal Health Laboratory Network for Africa (RAAHLN-AF)

5 – 7 December 2023 Pretoria, South Africa



## Virtual & In-county Training in Peru-2021

**Curso virtual**

# Métodos de diagnóstico para enfermedades de Langostinos

Subproyecto PNIPA ACU002



## Conducting in-country training on shrimp disease diagnostics



Bangladesh- November 2017



Indonesia 2018



Saudi Arabia 2018



Honduras, 2019



Mexico, 2018

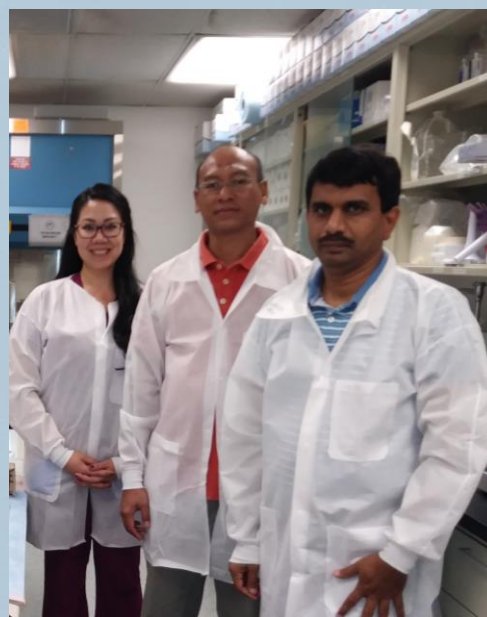


India 2023

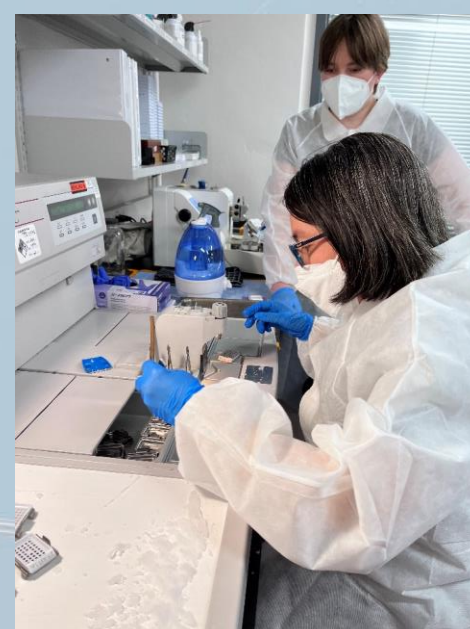
## WOAH-Member country scientists undergoing training in the Aquaculture Pathology Laboratory-University of Arizona



**Mexico**



**Indonesia**

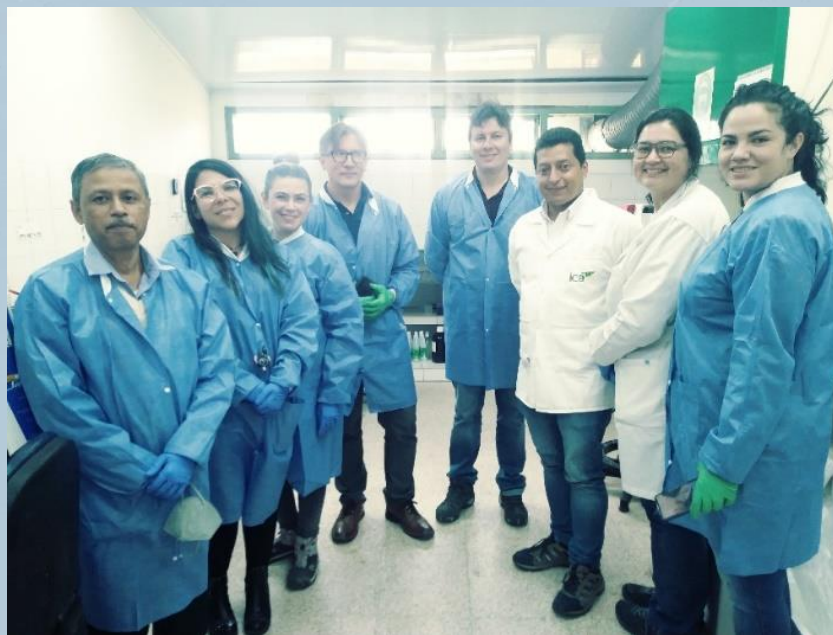


**Vietnam**



**Colombia**

## WOAH-Twinning Project in Indonesia-2018, Colombia-2023 & Saudi Arabia-2019



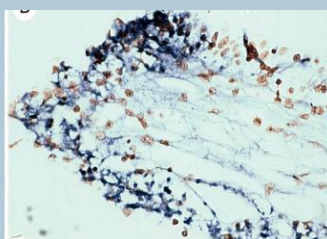
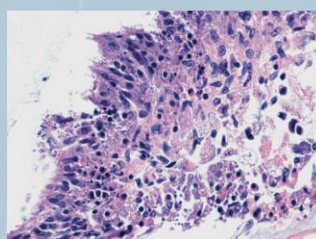
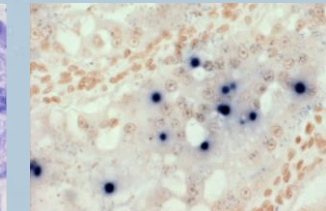
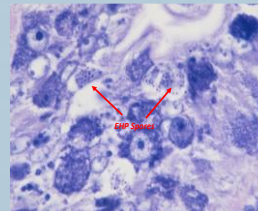
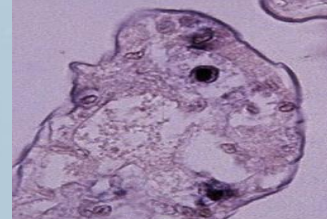
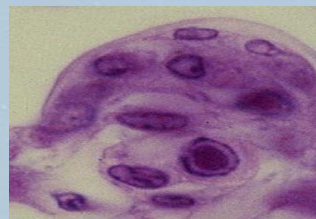
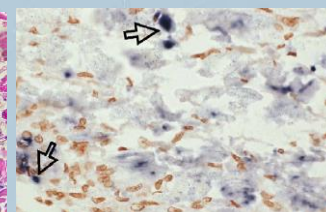
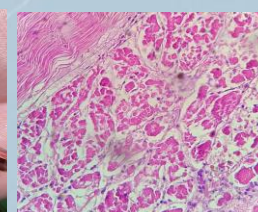
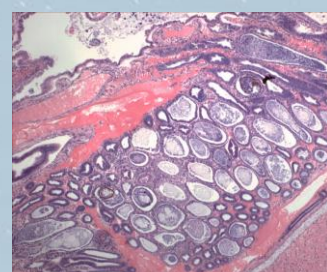
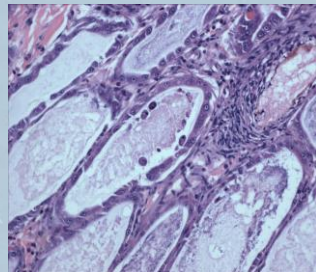
**Indonesia-2018**

**Colombia-2023**

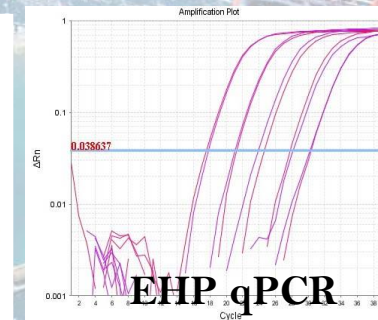
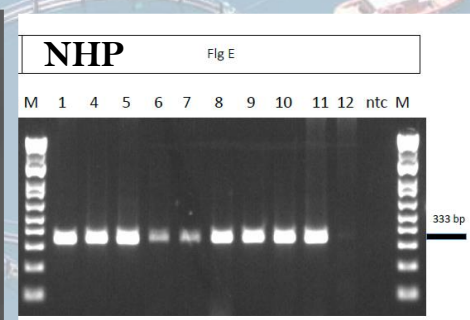
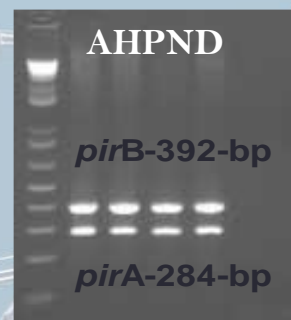
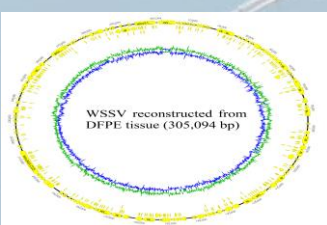
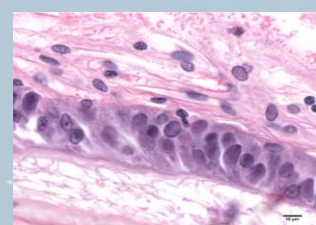
**Saudi Arabia-2019  
(restarting)**

- ✓ Determining Etiology
- ✓ Developing Bioassay

- ✓ Histopathology
- ✓ *In situ* Hybridization

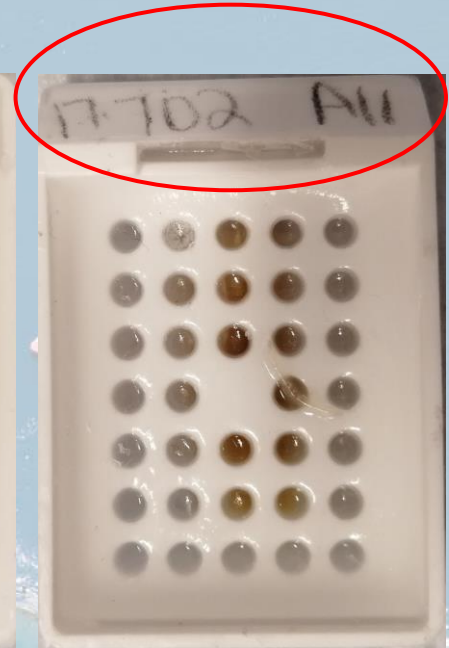
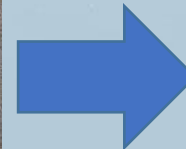


- Molecular detection**
- ✓ PCR
  - ✓ Real-time PCR

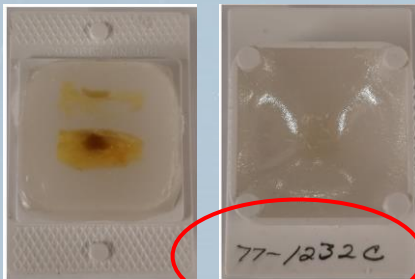


APL has a collection of >110,000 histology  
tissue blocks dating back to mid 70's

Block used in Cruz-Flores et al., (2020) to  
reconstruct the WSSV genome



Each block provides us with a snapshot of  
the genetic information of the pathogen and  
the host from the past!

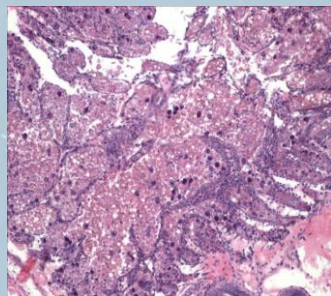




# Expediting pathogen discovery workflow by combining histopathology & genomic tools



**Histological processing  
(3-4 days)**



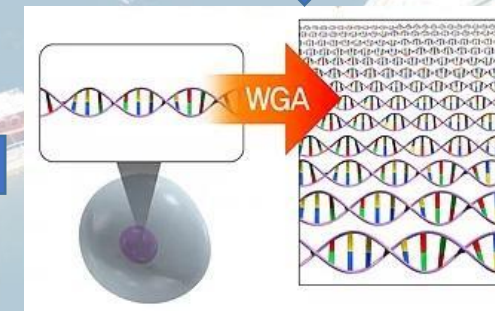
**Identification of lesions  
by H&E (2 days)**



**Laser Capture  
Microdissection of the  
lesions (1 day)**



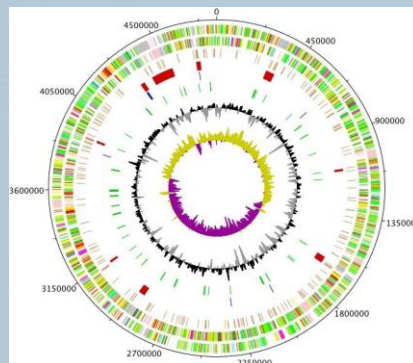
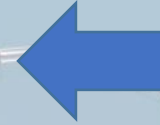
**Nucleic acid extraction  
(1 day)**



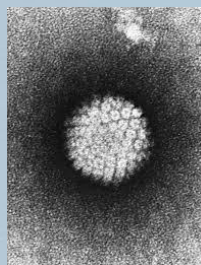
**Whole Genome  
Amplification (1 day)**



**NGS (3 weeks)**

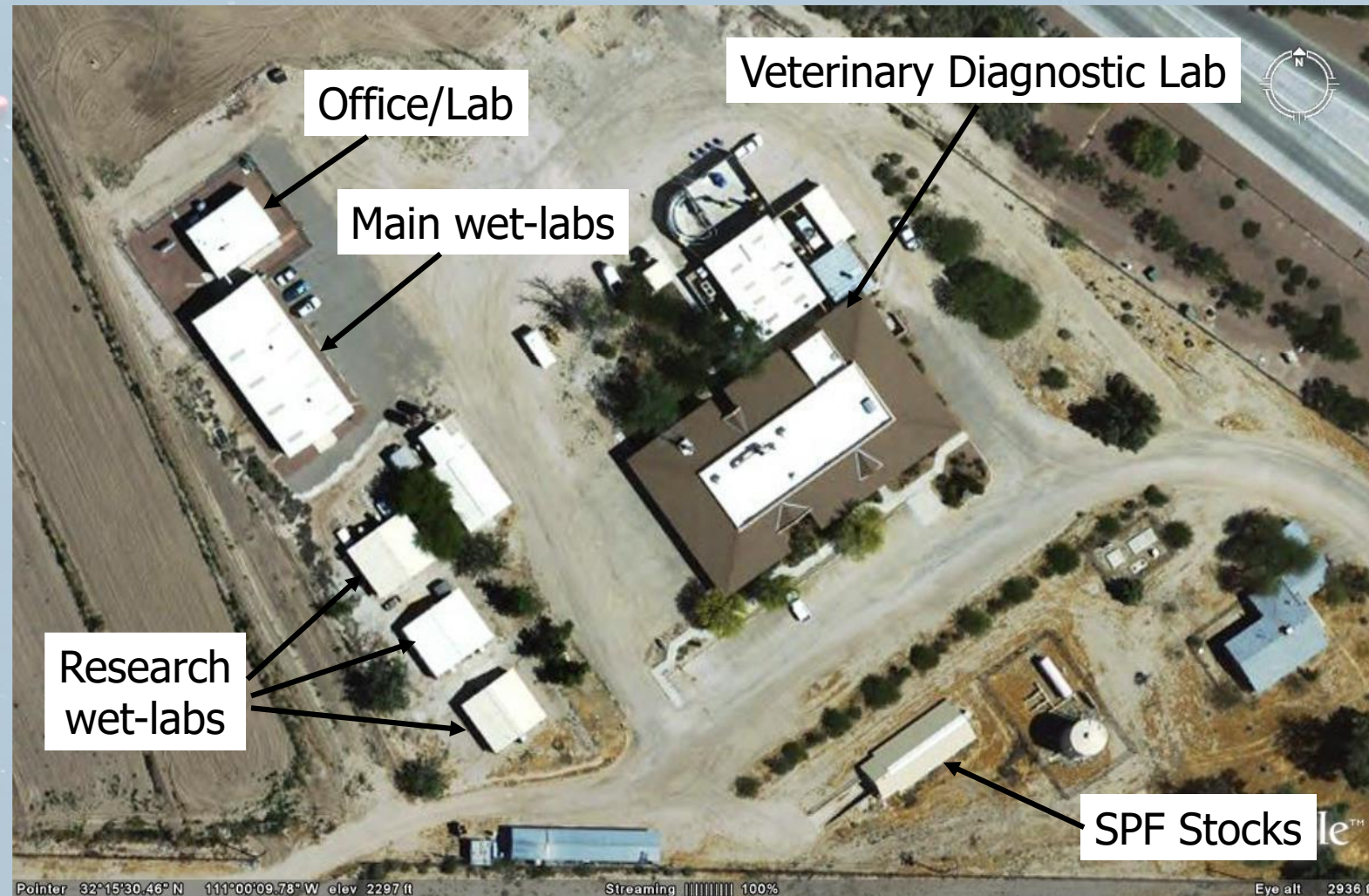


**Bioinformatics (2 weeks)**



**NEW PATHOGEN**

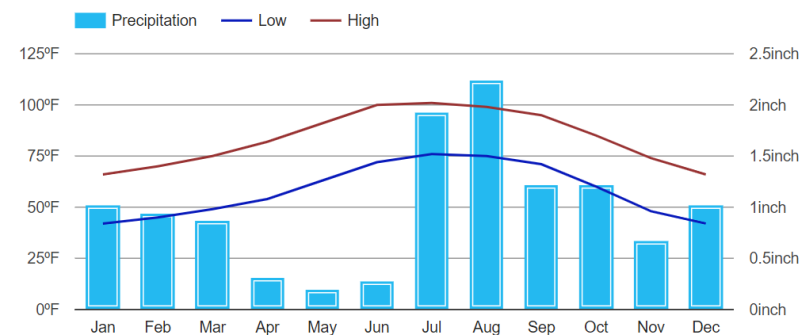
## Aerial view of live animal facility of West Campus Agriculture Center



- The live animal facility of West Campus Agriculture Center is over 200 miles (320 km) from the nearest body of salt water.
- The EPA lists Southern Arizona as the region with the highest average UV index in the United States (source: EPA).
- UV radiation has proven to inactivate both bacteria and viruses.



Tucson Climate Graph - Arizona Climate Chart



**Specific Pathogen Free  
live animals are  
maintained in a  
completely separated  
building and monitored  
regularly for disease-free  
status.**



Different size tanks and systems are used for experimental challenge





## Our Partners

- **Shrimp Farmers**
- **Scientific Colleagues**
- **Pharmaceutical Companies**
- **Agrochemical Corporations**
- **Filtration Designers**
- **Shrimp Breeding Facilities**
- **Food Distribution Firms**

## Challenges of a WOAHA Reference Laboratory

- Maintaining ISO accreditation
  - Very costly and time consuming
  - Requires maintaining significant technical expertise and operational knowhow
- Maintaining biological resources
  - Expensive and very challenging especially when the pathogen culture cannot be archived in freezer (e.g. EHP)
- Trainings of Ref. Lab staff
  - Detailed, long-term planning is required to ensure that the quality of the training and diagnostic/research work do not suffer.

## Challenges of a WOAHA Reference Laboratory

- Annual Reporting
  - Requires detailed information to be kept about every case
  - Lab staff must be trained to maintain databases of information to ensure accurate and timely reporting
- Conducting disease diagnostic training
  - While valuable for both trainers and trainees, teaching necessitates pulling staff from diagnostic and research activities
- Accommodating needs of other laboratories/ researchers at a free of charge



- Pathogen discovery
- Determining etiology
- Developing diagnostic tools

SCIENTIFIC REPORTS  
natureresearch

OPEN Genome reconstruction of white spot syndrome virus (WSSV) from archival Davidson's-fixed paraffin embedded shrimp (*Penaeus vannamei*) tissue

Roberto Cruz-Flores, Hung N. Mai, Siddhartha Kanrar, Luis Fernando Aranguren Caro & Arun K. Dhar<sup>1,2</sup>

scientific reports

OPEN The emerging pathogen *Enterocytozoon hepatopenaei* drives a degenerative cyclic pattern in the hepatopancreas microbiome of the shrimp (*Penaeus vannamei*)

Jesús Antonio López-Carvallo<sup>1</sup>, Roberto Cruz-Flores<sup>1,2</sup> & Arun K. Dhar<sup>2,3</sup>

OPEN Rapid, CRISPR-Based, Field-Deployable Detection Of White Spot Syndrome Virus In Shrimp

Timothy J. Sullivan<sup>1,2</sup>, Arun K. Dhar<sup>2</sup>, Roberto Cruz-Flores<sup>2</sup> & Andrea G. Bodnar<sup>2</sup>

viruses

Article

## Detection and Phylogenetic Analyses of Taura Syndrome Virus from Archived Davidson's-Fixed Paraffin-Embedded Shrimp Tissue

Lauren Marie Ochoa, Roberto Cruz-Flores and Arun K. Dhar \*

MDPI

Journal of Invertebrate Pathology 200 (2023) 107960

Contents lists available at ScienceDirect

Journal of Invertebrate Pathology

journal homepage: [www.elsevier.com/locate/jip](http://www.elsevier.com/locate/jip)

Detection of a novel microsporidium with intranuclear localization in farmed *Penaeus vannamei* from Latin America

Arun K. Dhar<sup>a</sup>, Roberto Cruz-Flores<sup>a,b</sup>, Hung N. Mai<sup>a</sup>, Luis Fernando Aranguren Caro<sup>a</sup>, Pablo Intriago<sup>c</sup>, Xavier Romero<sup>c</sup>

viruses

MDPI

Article

## Identification of a Novel Solinvivirus with Nuclear Localization Associated with Mass Mortalities in Cultured Whiteleg Shrimp (*Penaeus vannamei*)

Roberto Cruz-Flores<sup>1,2,4</sup>, Thales P.D. Andrade<sup>2,3,4</sup>, Hung N. Mai<sup>2</sup>, Rod Russel R. Alenton<sup>2</sup> and Arun K. Dhar<sup>2,4</sup>

Virology 555 (2021) 117-121

Contents lists available at ScienceDirect

Virology

journal homepage: [www.elsevier.com/locate/virology](http://www.elsevier.com/locate/virology)

Complete genome reconstruction and genetic analysis of Taura syndrome virus of shrimp from archival Davidson's-fixed paraffin embedded tissue

Roberto Cruz-Flores<sup>a</sup>, Hung N. Mai, Arun K. Dhar

Aquaculture Pathology Laboratory, School of Animal and Comparative Biomedical Sciences, Building 90, The University of Arizona, Tucson, AZ, USA

Aquaculture 554 (2022) 730159

Contents lists available at ScienceDirect

Aquaculture

journal homepage: [www.elsevier.com/locate/aquaculture](http://www.elsevier.com/locate/aquaculture)

Novel infectious myonecrosis virus (IMNV) variant is associated with recent disease outbreaks in *Penaeus vannamei* shrimp in Brazil

Thales P.D. Andrade<sup>a</sup>, Roberto Cruz-Flores<sup>b,c</sup>, Hung N. Mai<sup>b</sup>, Arun K. Dhar<sup>b,4</sup>

RESEARCH ARTICLE

## Experimental reproduction of White Feces Syndrome in whiteleg shrimp, *Penaeus vannamei*

Luis Fernando Aranguren Caro<sup>a</sup>, Hung N. Mai, Roberto Cruz-Flores<sup>a</sup>, Frances Lauren Agcalao Marcos, Rod Russel R. Alenton, Arun K. Dhar

Journal of Virological Methods 323 (2024) 114840

Contents lists available at ScienceDirect

Journal of Virological Methods

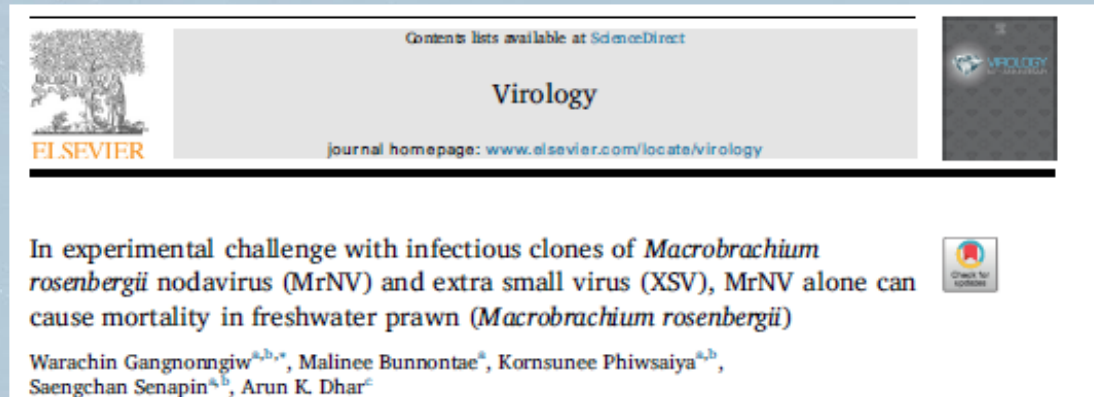
journal homepage: [www.elsevier.com/locate/jvromet](http://www.elsevier.com/locate/jvromet)

Comparison of Polymerase Chain Reaction (PCR) assay performance in detecting *Decapod penstylhamaparvovirus 1* in penaeid shrimp

Arun K. Dhar<sup>a,1</sup>, Roberto Cruz-Flores<sup>a,1</sup>, Hung N. Mai<sup>a</sup>, Janet Warg<sup>b</sup>



- **Developing oral delivery of therapeutics**
- Prevention alone is not enough in reducing losses due to infectious diseases in shrimp aquaculture.
- Need to develop therapeutics with commercial viability.





PNAS Nexus, 2023, 00, 1–9

<https://doi.org/10.1093/pnasnexus/pgad278>

Advance access publication 23 August 2023

Research Report

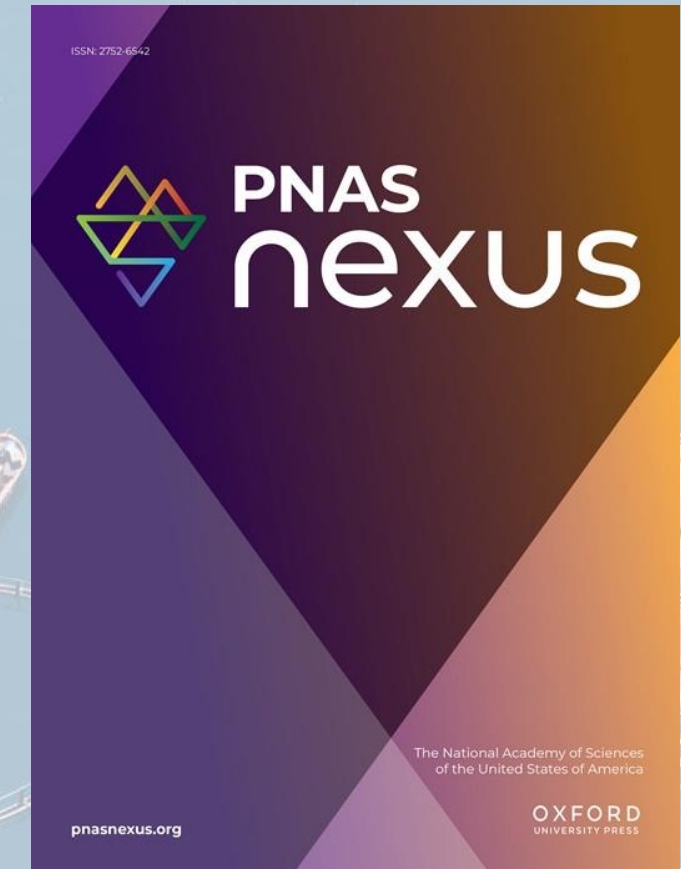
## Engineering a replication-incompetent viral vector for the delivery of therapeutic RNA in crustaceans

Rod Russel R. Alenton , Hung N. Mai and Arun K. Dhar 

Aquaculture Pathology Laboratory, School of Animal and Comparative Biomedical Sciences, The University of Arizona, Tucson, AZ 85721, USA

\*To whom correspondence should be addressed: Email: [adhar@arizona.edu](mailto:adhar@arizona.edu)

Edited By: Richard Stanton



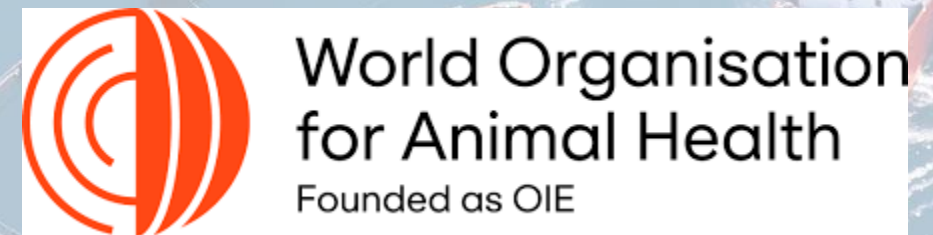
## FUNDING ACKNOWLEDGEMENT



United States Department of Agriculture  
National Institute of Food and Agriculture



**GOVERNMENTAL  
AGENCIES & SHRIMP  
INDUSTRY WORLDWIDE**





**The laboratory is self-funded**

# Aquaculture Pathology Laboratory University of Arizona, USA



*Thank You*