

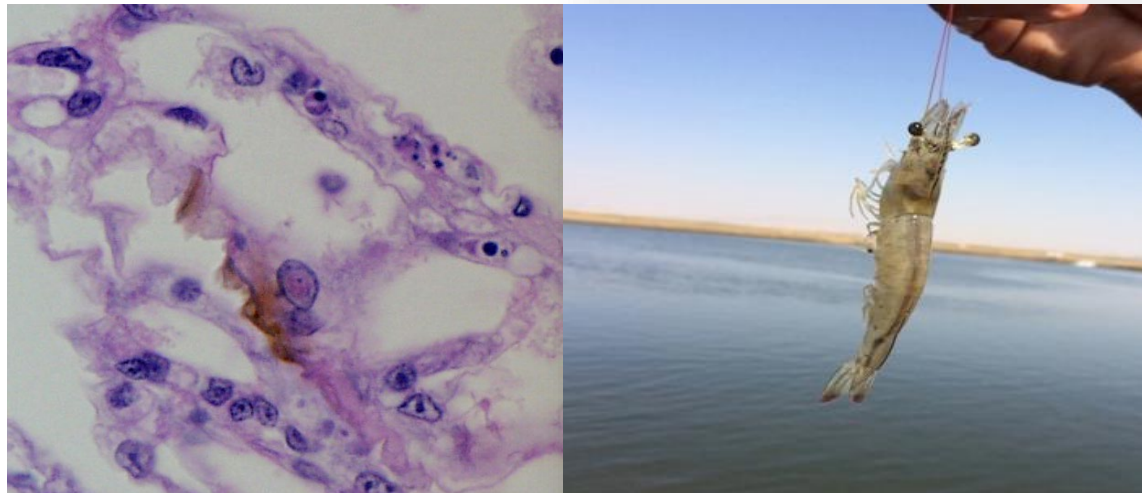
“IHHNV continues to shape shrimp industry worldwide”

Resultados preliminares de investigaciones sobre IHHNV en la industria camaronera de Ecuador

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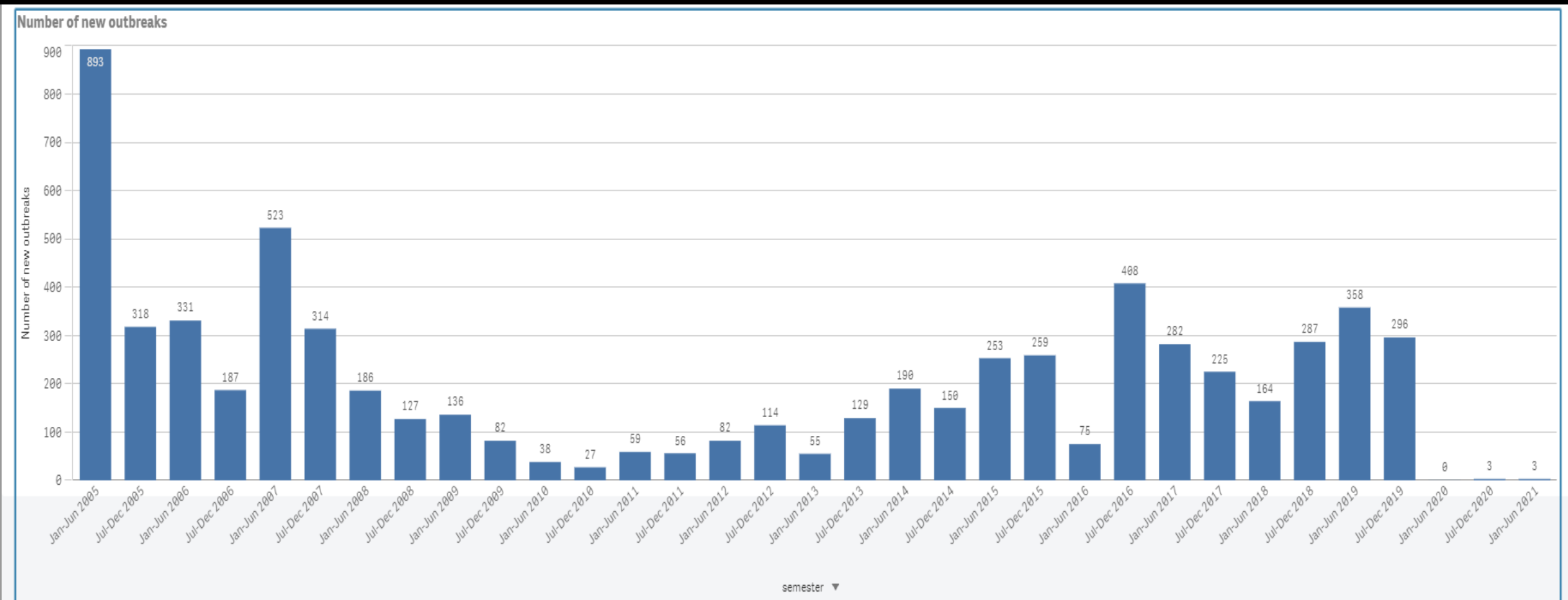


Background

- Infection with infectious hypodermal and haematopoietic necrosis virus (IHHNV). Decapod penstyldensovirus 1.
- Decapod Penstylhamaparvovirus 1 (PstHPV-1) (2020)
- The first case of IHHNV was reported in Hawaii in 1981
- IHHNV had caused mass mortalities in blue shrimp (*Penaeus stylirostris*)
- IHHNV has also been identified as the cause of 'runt deformity syndrome' (RDS) in *P. vannamei*



Events of IHNV 2005-2021 (p) OIE. WAHIS 2021



Background

Reporting of
IHHNV (events
by country and
year (WAHIS,
OIE 2021)

Country	2014	2015	2016	2017	2018	2019	2020	2021 (p)
Australia	2	1		1	2	1	2	
Belize	1	1						
Brazil		4	4	4	4	4		
Cambodia				1				
Canada						2	1	
China (People's Rep. of)			2	2	2	2		
Chinese Taipei	2	2	2	2	1			
Colombia								1
Costa Rica	1	2	2	2	2	2		
Ecuador	3	4	4	4	4	2		
El Salvador	2			4	4	4		
Fiji		1	1					
Guatemala	1	2	2	2				
Honduras	2	2	2	2				
India	4	4	4	4	2			
Indonesia			2	2	2	1		
Madagascar				4	4	4	2	
Malaysia	1	1						
Mexico	2	2	2	2	2	2		
Nicaragua	2	2	2	2	2	2		
Panama	2	2	2	2	2	1		
Papua New Guinea	2	2	2	2	2	1		
Peru	2	2	3	3	3	3		
Philippines	2	4	4	4	4	4		
Thailand	1	2	2	2	1	2		
United Kingdom						1	1	2
United States of America						2	1	

OIE Summary of assessment of infection with IHNV

	Listing criteria						Conclusion
	1	2	3	4a	4b	4c	
IHNV	+	+	+	NA	+	-	The disease meets the criteria for listing

- Criterion No. 1** International spread of the pathogenic agent (via aquatic animals, aquatic animal products, vectors or fomites) is likely.
- Criterion No. 2** At least one country may demonstrate country or zone freedom from the disease in susceptible aquatic animals, based on provisions of Chapter 1.4.
- Criterion No. 3** A precise case definition is available and a reliable means of detection and diagnosis exists
- Criterion No. 4b** The disease has been shown to affect the health of cultured aquatic animals at the level of a country or a zone resulting in significant consequences e.g. production losses, morbidity or mortality at a zone or country level.

Current status of IHNV infectivity

- What is happening with IHNV in Ecuador?
- Is IHNV infectious?
- Are the PCR results due to insertions of IHNV within the shrimp genome?
- Does it cause mortality in endemic and exotic shrimp species?
- Does it cause RDS in *Penaeus vannamei* nowadays?

The University of Arizona, USA and Camara Nacional de Acuicultura, Ecuador

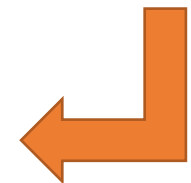
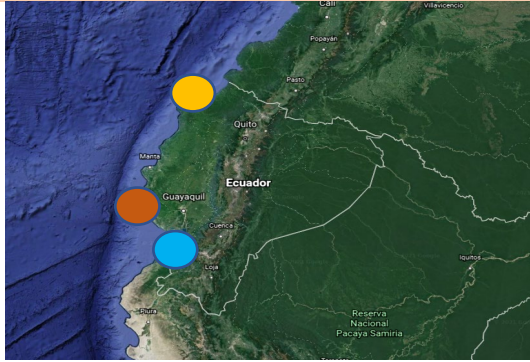
Objective 1: Determine the effect of IHHNV in shrimp farming in Ecuador

- a) Sampling
- b) Detection of IHHNV 309/389 and Real-time PCR
- c) Detection of IHHNV lesions by H&E

Objective 2: Determine the pathogenicity of the Ecuadorian IHHNV strain in three different SPF shrimp species.

- *Penaeus vannamei*
- *Penaeus monodon*
- *Penaeus stylirostris*

Shrimp sampling



- Presumed IHHNV-infected tissue
- Davidson fixed shrimp
- 95% ethanol preserved shrimp

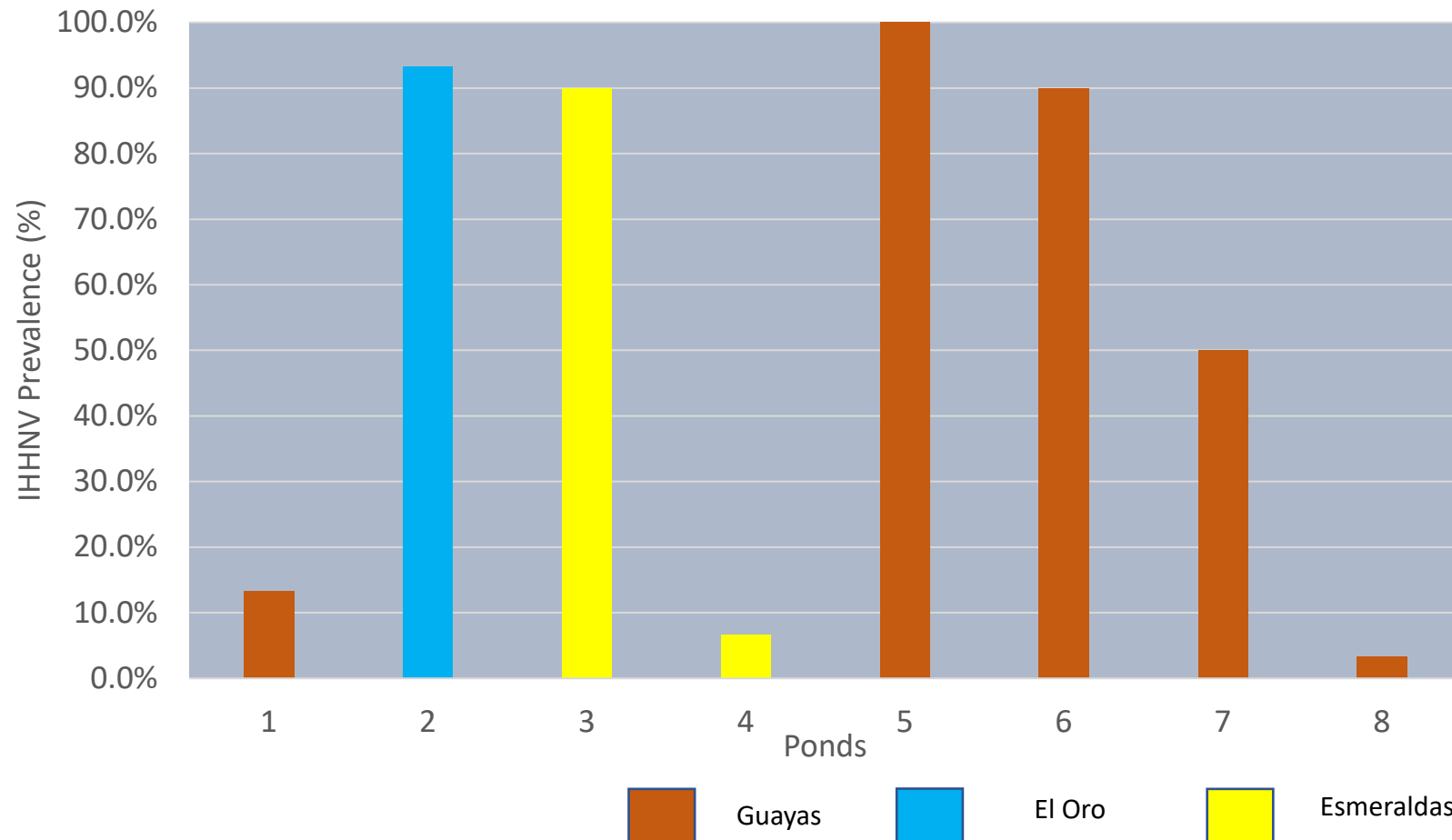


Methodology

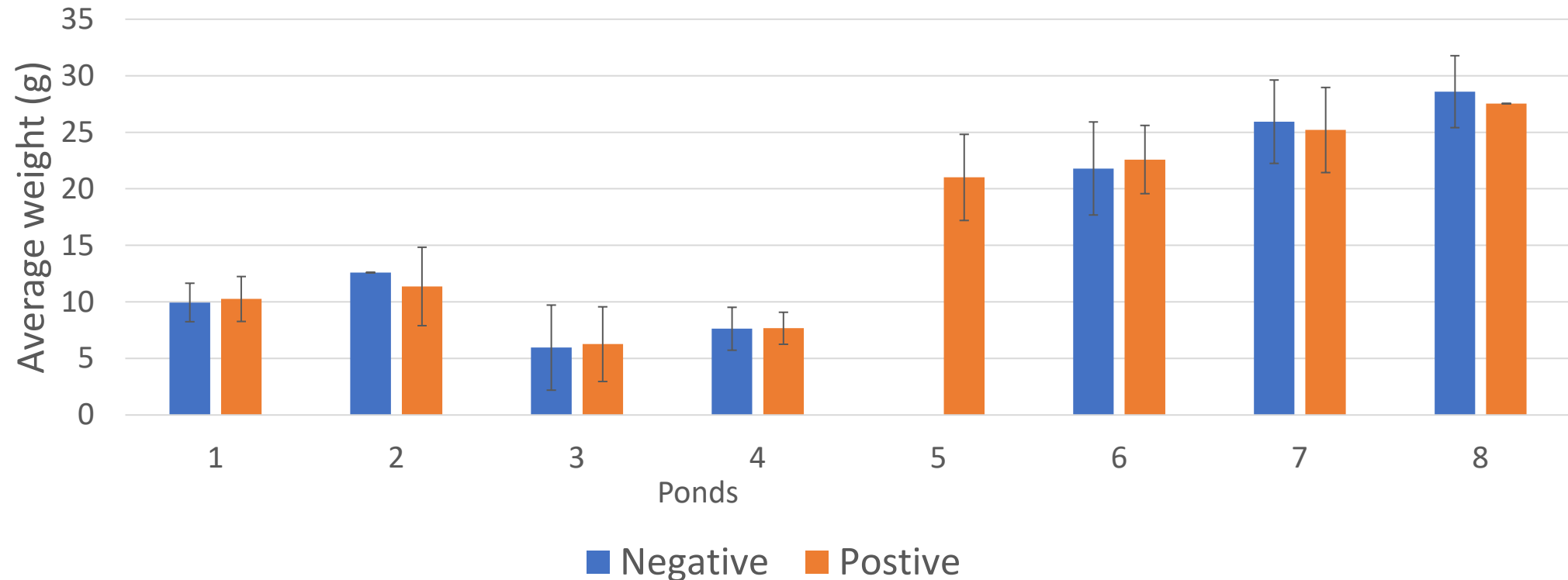
Grow-out ponds with history of IHNV /ponds with positive result of IHNV were selected

Region	Number of shrimp	Weight (g) mean	SD	CV(%)
Guayas	30	10.0	1.7	17.2
El oro	30	11.5	3.4	29.3
Esmeraldas	30	6.2	3.3	52.7
Esmeraldas	30	7.6	1.9	24.3
Guayas	30	21.0	3.8	18.1
Guayas	30	22.5	3.1	13.6
Guayas	30	25.6	3.7	14.4
Guayas	30	28.6	3.1	11.0

IHHNV prevalence



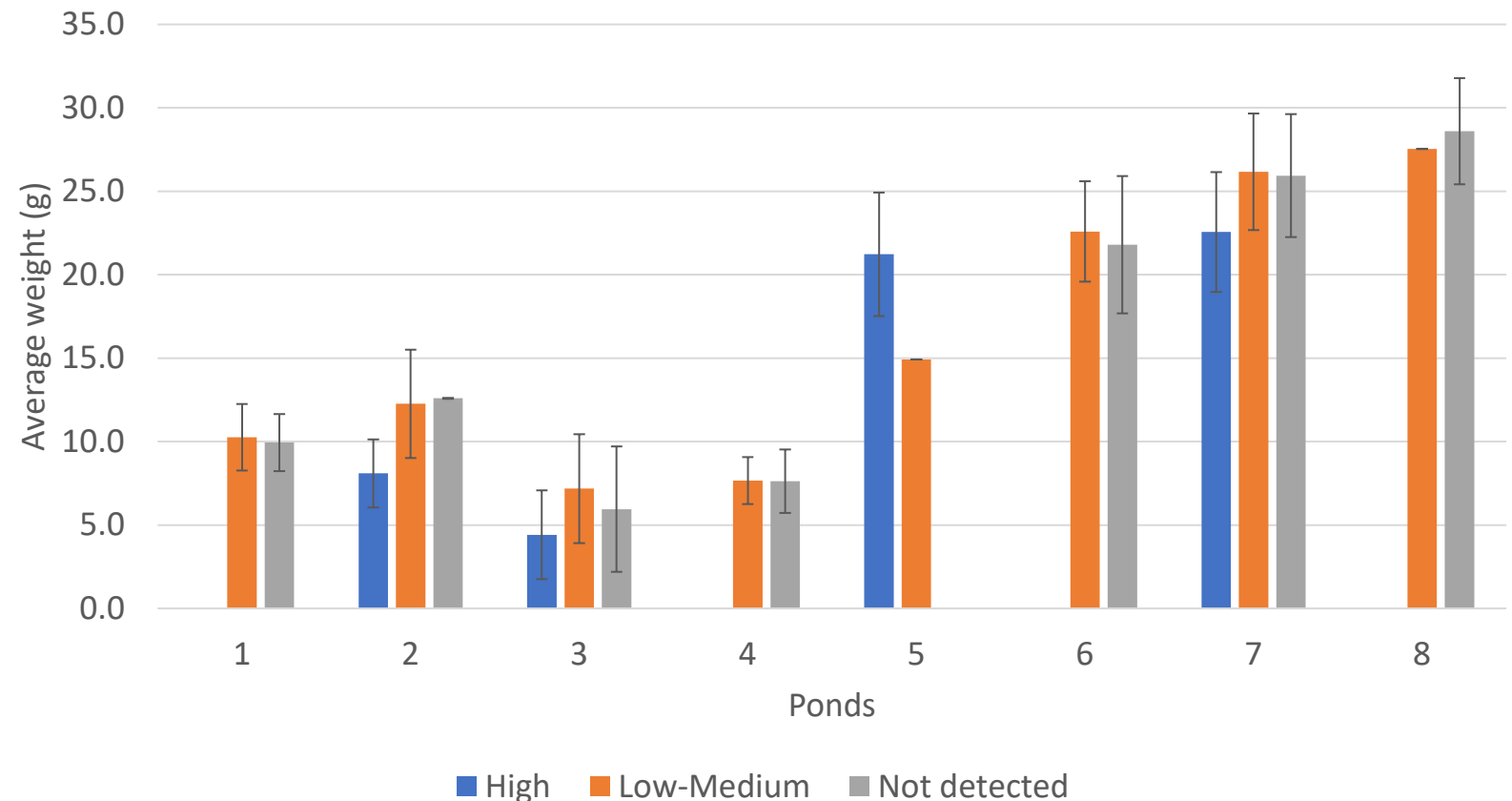
Average weight (g) vs. shrimp samples IHNV positive and not detected



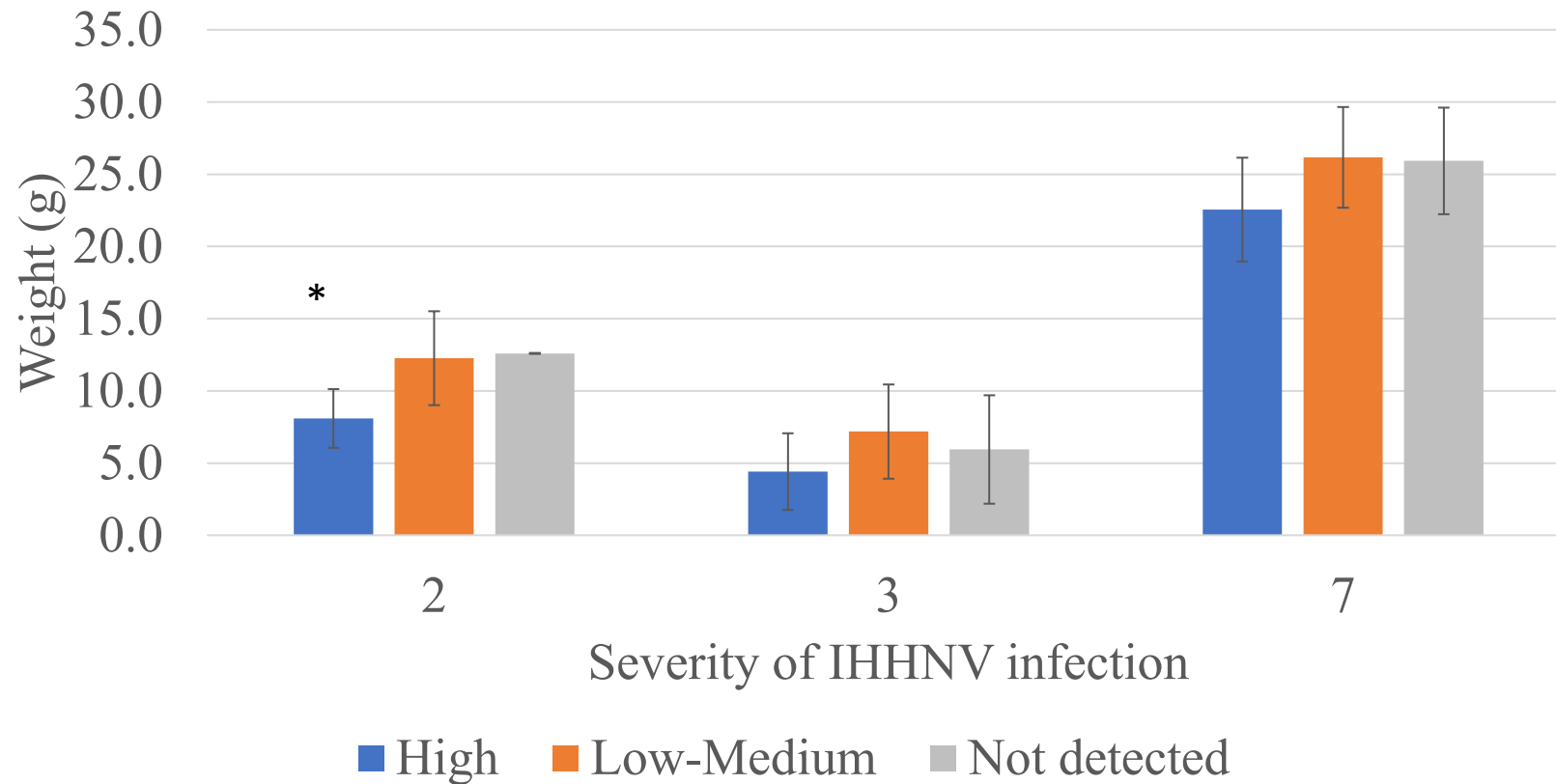
Association of IHNV copy number vs. weight

Three categories:

- Not detected
- Low-medium : >1
 $\leq 1 \times 10^2$ copies/ng DNA
- High: $>1 \times 10^2$ copies/ng
DNA



Association of IHHNV copy number vs. weight



Objective 2: Determine the pathogenicity of the Ecuadorian IHNV strain in three different shrimp species.

Penaeus monodon



Penaeus vannamei



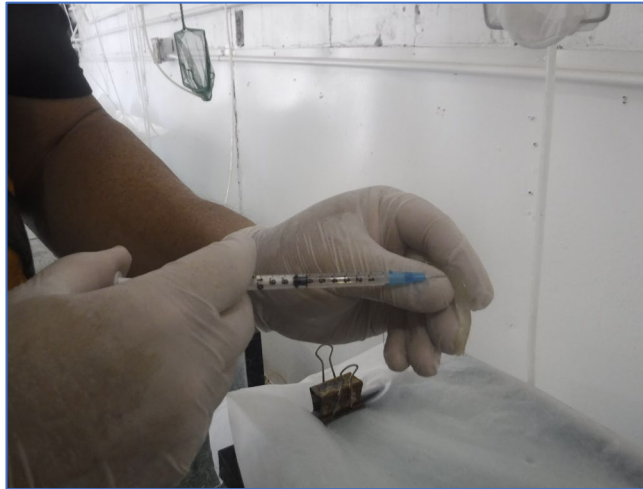
Penaeus stylirostris



IHHNV Challenge test



IHHNV from
Ecuador: farms
positive for
IHHNV and
negative for
WSSV (21-270, 21-
271)



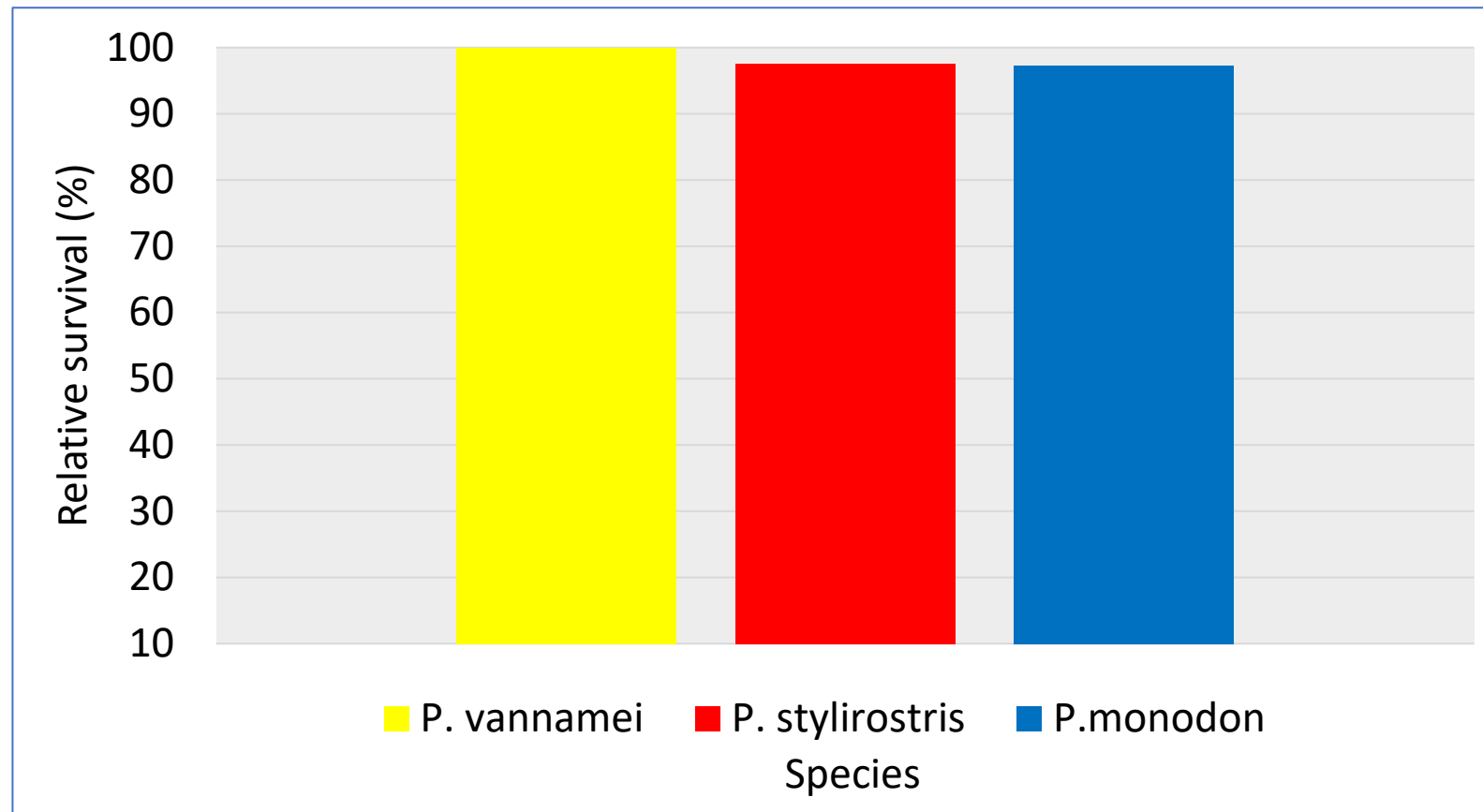
Average weight: 1.0 g $\pm$ 0.5
Temperature: 27-28.5 °C
pH: 7.5-8.1
Salinity: 30 ppt
Ammonia 0.0-0.5 ppm
Nitrite: 0.0-0.25 ppm



IHHNV Challenge test set up

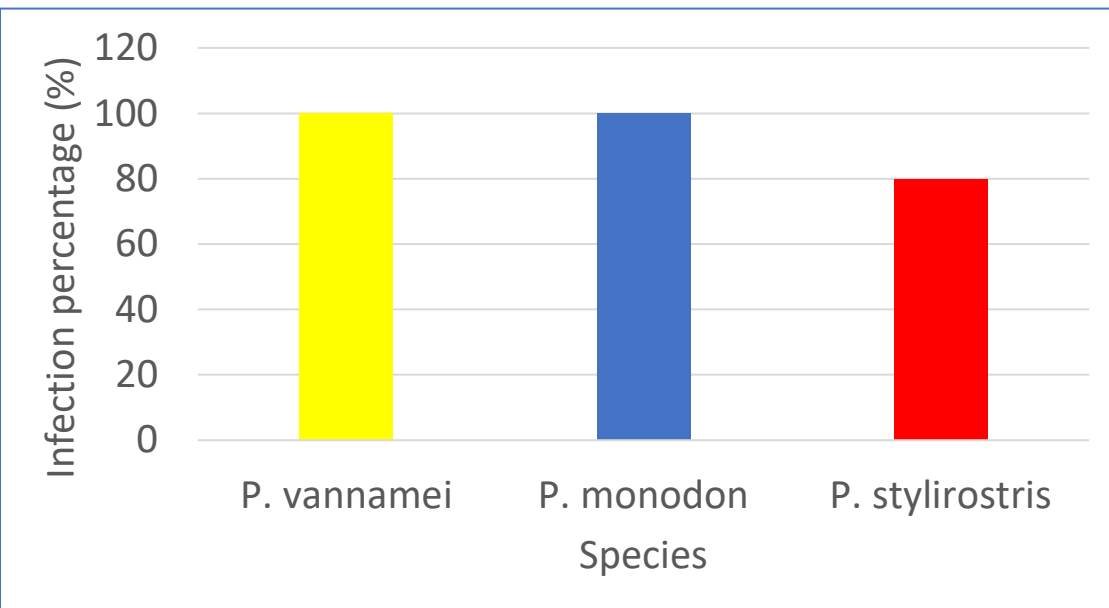
Tank Number	Tank Definition	Number Stocked	Species
1	Negative Control	20	P. vannamei
2	Negative Control	20	P. monodon
3	Negative Control	20	P. stylirostris
4	IHHNV Challenge	20	P. vannamei
5	IHHNV Challenge	20	P. vannamei
6	IHHNV Challenge	20	P. vannamei
7	IHHNV Challenge	20	P. monodon
8	IHHNV Challenge	20	P. monodon
9	IHHNV Challenge	20	P. monodon
10	IHHNV Challenge	20	P. stylirostris
11	IHHNV Challenge	20	P. stylirostris
12	IHHNV Challenge	20	P. stylirostris

Results: Survival of shrimp upon an IHNV Challenge at 30 days post infection

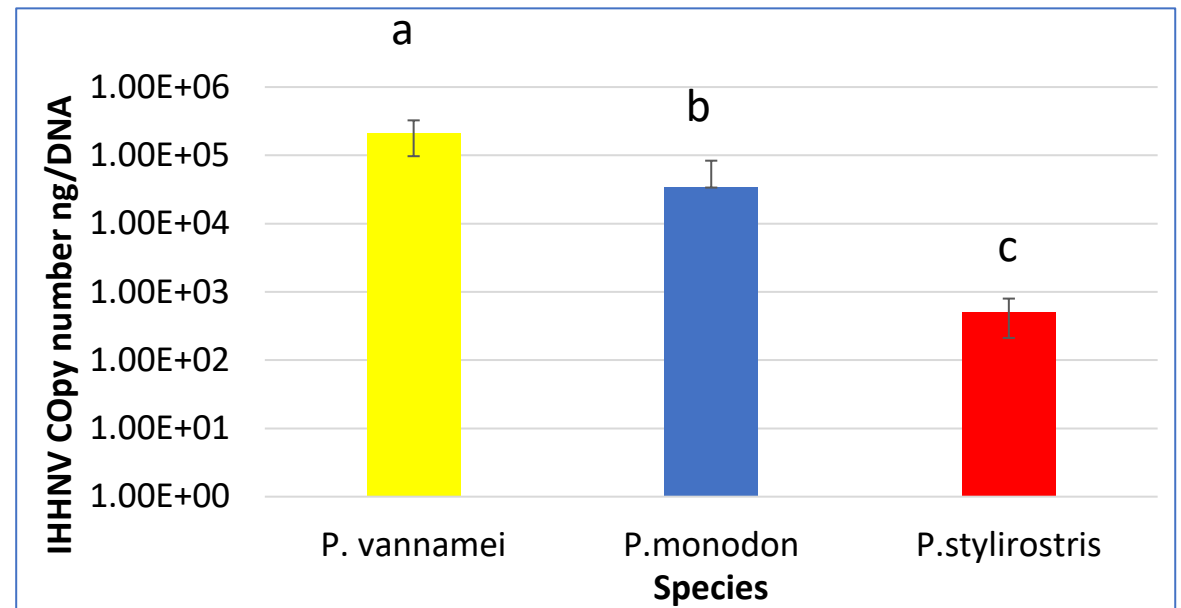


Results

Percentage of infection (%)

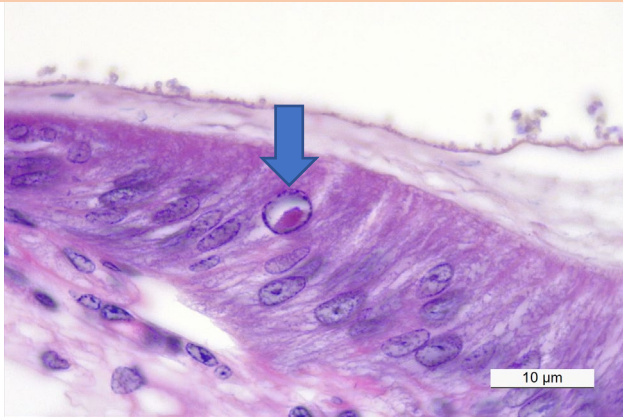


IHHNV copy number / ng DNA

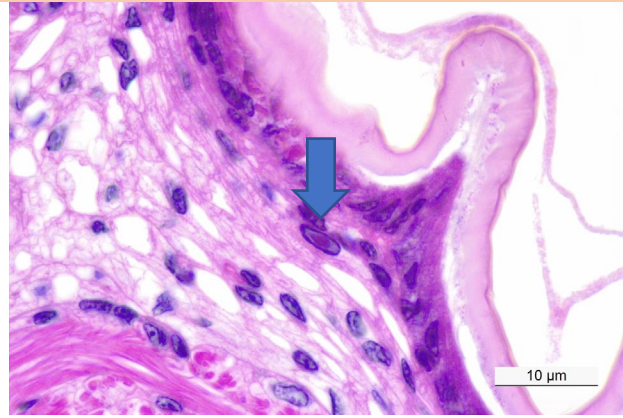


IHHNV histological lesions after 30 days post infection

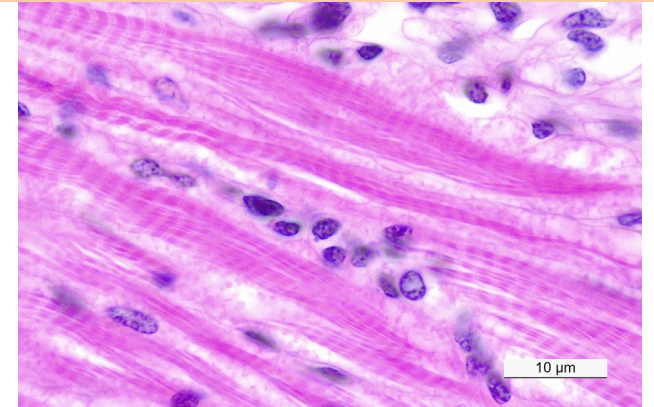
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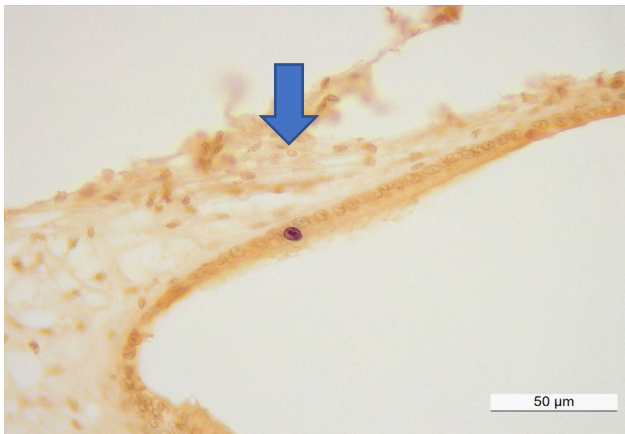
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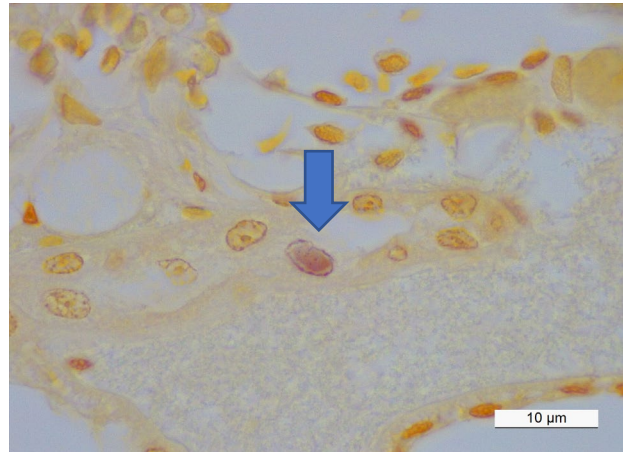
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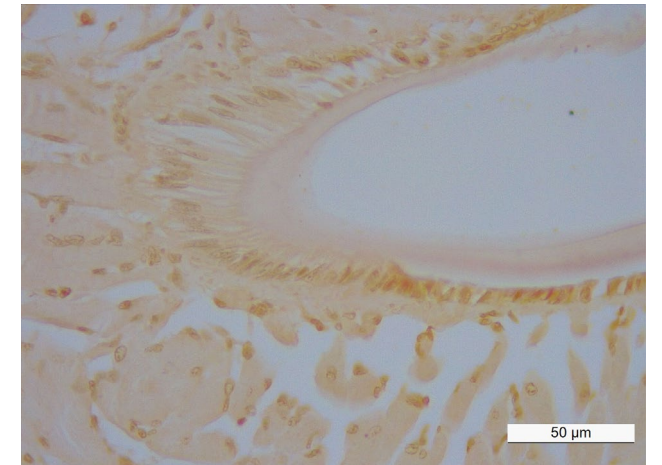
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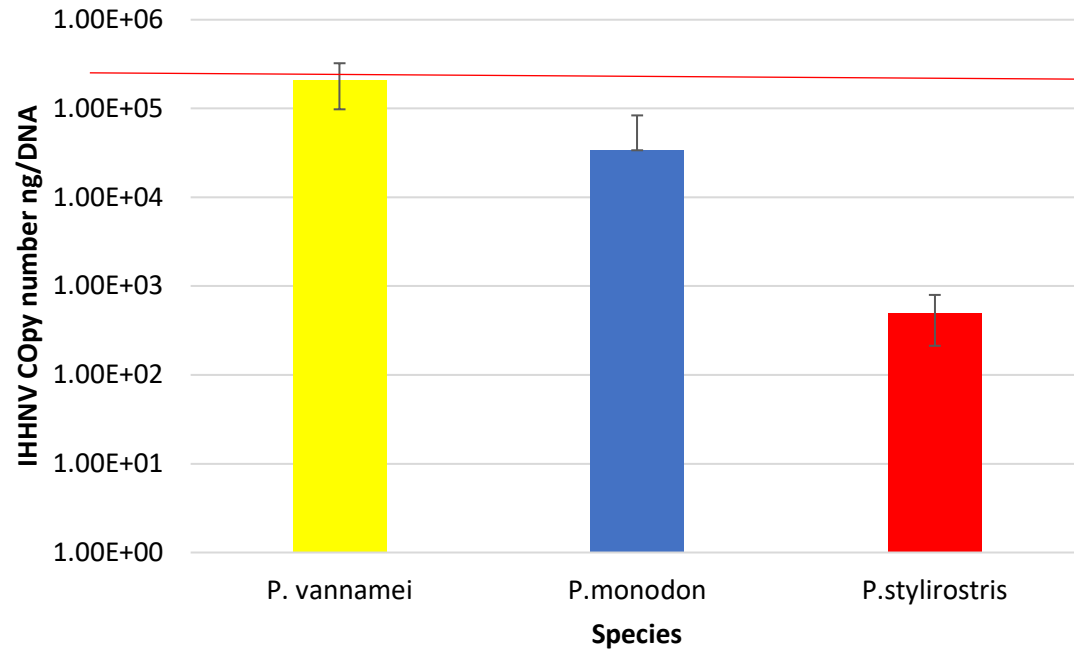
Penaeus vannamei

Penaeus monodon

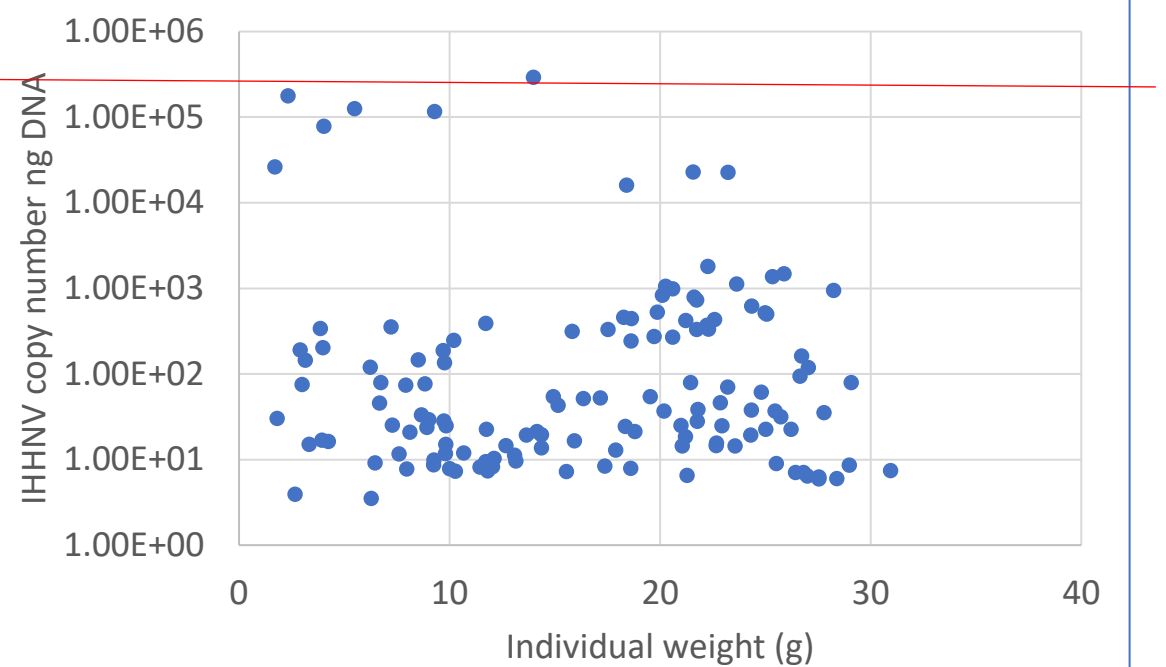
Penaeus stylirostris

IHHNV copy number in a challenge test vs. copy number in grow out ponds

Experimental challenge



Grow out ponds (*P. vannamei*)



**Phylogenetic analysis of
IHHNV isolates from Ecuador
and other geographical
regions based on the whole
genome, Evolutionary
analysis by Maximum
Likelihood method**



How can we put this information in international context /Final remarks

- IHHNV strain from Ecuador does not cause any mortality in SPF species *P. vannamei* & *P. stylirostris*.
- IHHNV does not cause any mortality in the exotic species *P. monodon*
- Runt deformity syndrome (RDS) was not observed in *P. vannamei* under the experimental challenge carried out.
- IHHNV can cause infections in the penaeids shrimp *P. vannamei*, *P. monodon* & *P. stylirostris*. This has been confirmed by Real-time PCR, End point PCR, histopathology and *in-situ* hybridization.

How can we put this information in international context /Final remarks

- The tissular lesions caused by IHNV are mild even in shrimp with a very high copy number. (*P. vannamei*).
- Level of IHNV infection on shrimp weight in a grow-out pond needs to be further investigated.