

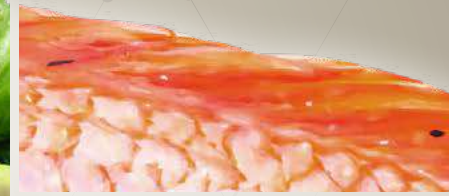
# ANNUAL REPORT 2021

## RESEARCH AND DEVELOPMENT PROGRAM ON FISH HEALTH IN AQUACULTURE

P21300040170502



NATIONAL FISH HEALTH RESEARCH DIVISION (NaFish)  
FISHERIES RESEARCH INSTITUTE (FRI)  
DEPARTMENT OF FISHERIES (DOF)







# **ANNUAL REPORT 2021**

## **RESEARCH AND DEVELOPMENT PROGRAM ON FISH HEALTH IN AQUACULTURE**

**P21300040170502**

**@Pusat Penyelidikan Kesihatan Ikan Kebangsaan (NaFisH),**  
Institut Penyelidikan Perikanan (FRI) Malaysia 2022

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Annual Report **NaFisH** 2021

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# DIRECTOR WORDS

The year 2021 will be forever remembered due to global economic crisis caused by Covid-19. Operation of NaFisH in 2021 was challenging, due to reinstatement of Movement Control Order (MCO) restrictions from 13 January until 31 May 2021 covering various phases, total lockdown in June 2021 and national recovery plan which ended in November 2021. Due to MCO restrictions, our earlier plan to organize the 11<sup>th</sup> Symposium on Diseases in Asian Aquaculture (DAA11) was postponed to 2022. Despite all the challenges, NaFisH team and partners managed to complete 16 fish health research projects in 2021, including two field trials conducted on two sites. Under RMK-12, two MOU projects aimed for potential digitalization plans in fish health management were signed between NaFisH and Universiti Utara Malaysia, as well as NaFisH and Biogenes Technology Sdn Bhd.

I am pleased to highlight that NaFisH managed to utilize 99.99% of RM1.6 million R&D funding for 2021. Additionally, we continued to excel when two of our innovations were awarded at international level. BP2 was awarded as a winner of the Commonwealth Secretary-General's Innovation for Sustainable Development while KRIPeK received silver medal during the 5th International Innovation, Design & Articulation.

Lastly, I would like to thank everyone at NaFisH and our collaborators for pushing us forward despite the challenges of the Covid-19 pandemic.

**Dr. Kua Beng Chu**

Director National Fish Health Research Division (NaFisH)



# PROJECT PROFILE

## FINANCIAL PROJECTIONS

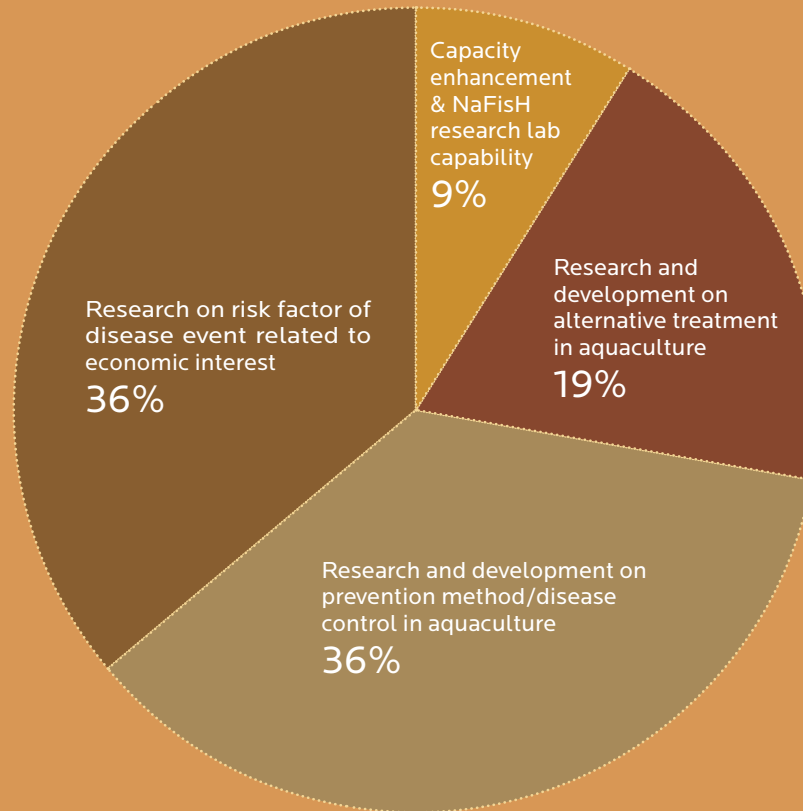


CEILING COST  
RMK12

**RM20M**



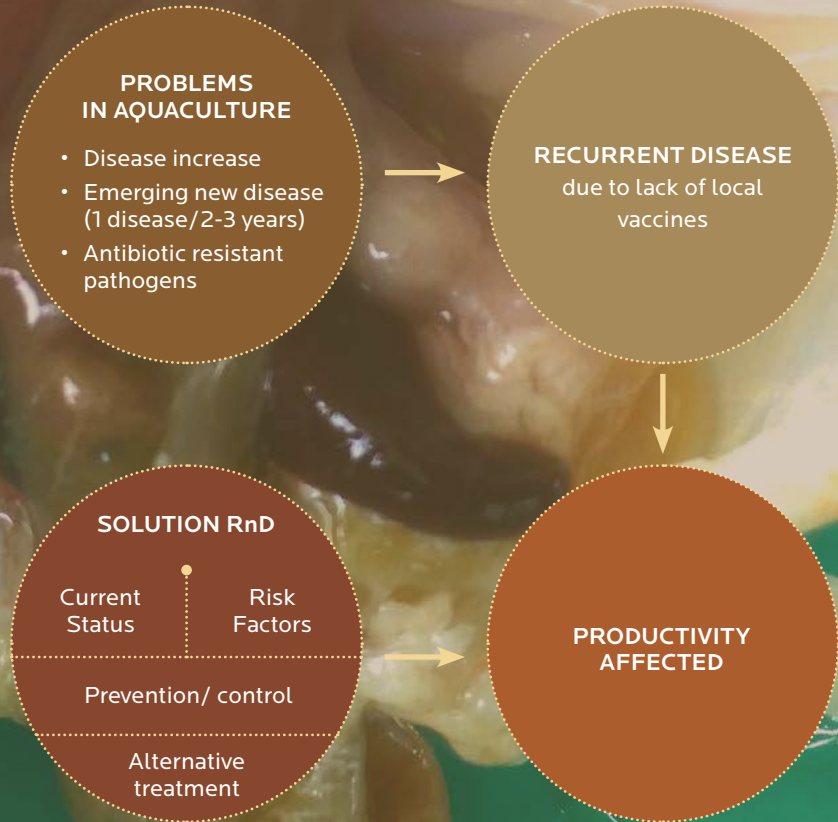
**RM1.6M**  
2021



## CATEGORY

**NON-PHYSICAL - RESEARCH**

## BRIEF DESCRIPTION OF PROJECT



## TARGET GROUP



- AQUACULTURE FARMERS/ ENTREPRENEUR
- DEVELOPMENT AGENCY/ GOVERNMENT
- SCIENTIST

## LOCATION

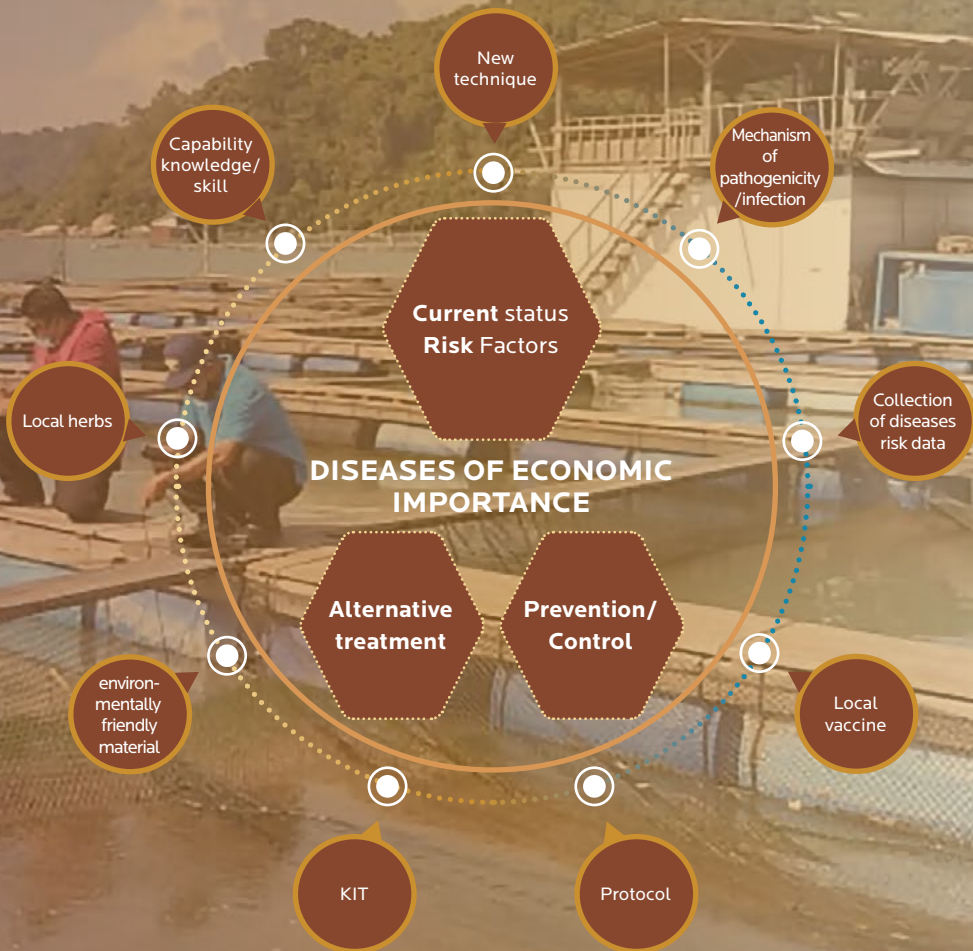


- TERENGGANU,
- PAHANG
- KEDAH
- PERAK
- PENANG
- JOHOR
- SARAWAK
- NEGERI SEMBILAN

# METHODOLOGY/IMPLEMENTATION MODEL

## OBJECTIVES

1. Verify the **current status** of economically important diseases in aquaculture
2. Method development in control & prevention of disease identified
3. Development of methodology in alternative treatment for disease in aquaculture



# RESEARCH ACTIVITIES YEAR 2021

SCOPE OF PROJECT	LOCATION	RESEARCH COMPONENT
1. Field study of economically important diseases and Risk Factors	FRI BM/NaFiSH (Penang)	• <b>Fresh Water Fish Diseases</b> (TiLV, Edwardsiellosis) in Pahang, Perak & Kedah
	FRI BM/NaFiSH (Penang)	• <b>Marine Fish Diseases</b> (VNN, Iridovirus, parasite capsalid & bacteria) in Kedah, Penang & Terengganu
	FRI BM/NaFiSH (Penang)	• <b>Marine Shrimp Diseases</b> (AHPND & EHP) in Penang
	FRI BM/NaFiSH (Penang)	• <b>Emerging &amp; Re-emerging Diseases</b> (DIV1)
2. R&D of disease diagnosis control and prevention in aquaculture	FRI TD (Terengganu)	• <b>Vaccines</b> (VNN, Vibriosis) in Terengganu dan Penang
	FRI BM/NaFiSH (Penang)	• <b>Protocol/kit</b> (Cryptocaryoniasis, EHP, AHPND, VNN & TiLV) in Penang
3. R&D in alternative treatment in aquaculture	FRI TD (Terengganu)	• Screening of Antimicrobial Activities from Potential Herbs in Terengganu and Penang
	FRI BM/NaFiSH (Penang)	• Alternative Treatment/prevention of Diseases in Terengganu and Penang
4. Capacity building of Research Laboratory at NaFiSH	FRI BM/NaFiSH (Penang)	• Capacity Building of Research Laboratory



# RESEARCH & DEVELOPMENT FLOW



**RESEARCH AND DEVELOPMENT PROGRAM ON FISH HEALTH IN AQUACULTURE (P21300040170502)**



Physical removal by substrate  
Break & Protect 2  
(BP<sub>2</sub>)

**1**

Field study of economically important diseases and Risk Factors



Feed-Based Vaccine Against Streptococcus

**2**

R&D of disease diagnosis control and prevention in aquaculture



**3**

R&D in alternative treatment in aquaculture

**4**

Capacity building of Research Laboratory at NaFisH



# SCOPE 1: EPIDEMIOLOGY

# 1

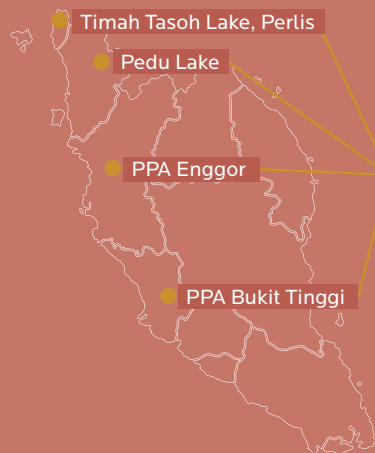
## EPIDEMIOLOGY OF TiLV IN FRESHWATER FISH

Afzan Muntaziana Mohd Pazai, Rimatulhana Ramly, Shahidan Hashim, Fahmi Sudirwan and Azila Abdullah

### OBJECTIVES

1. Prevalence of TiLV in wild black tilapia and tinfoil barb
2. TiLV status in Tilapia & tinfoil barb broodstocks
3. TiLV status in wild fish (2021)

### SAMPLING SITES



Timah Tasoh Dam,  
Perlis



Pedu Cage Culture



PPA Enggor

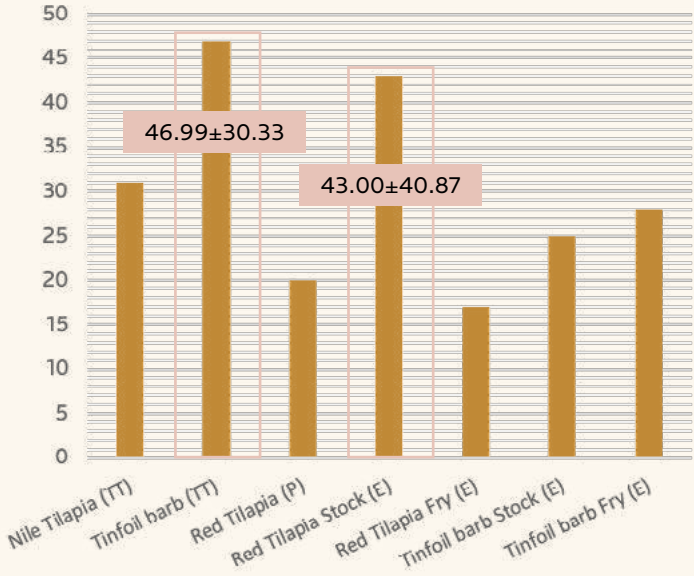


PPA Bukit Tinggi

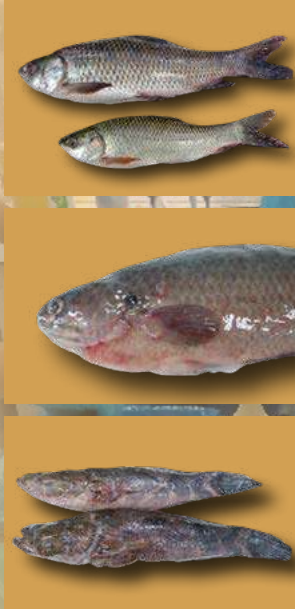
Status & output -  
PPA Bukit Tinggi  
is STILL negative

**STATUS & OUTPUT- TT: TIMAH TASOH LAKE, P: PEDU & E: PPA ENGGOR WERE POSITIVE**

Mean Prevalence of TiLV detected in fish at various stages & sampling sites during study period of 2018-2020



**STATUS & OUTPUT - WILD, CAPTURED FISH & MOLLUSC: NEGATIVE**



# 2

## EPIDEMIOLOGY OF *EDWARDSIELLA ICTALURI* IN CAGE CULTURED *PANGASIVUS SPP* IN PAHANG RIVER

Rimatulhana Ramly, Amirah Syahidah Nordin and Nur Nazifah Mansor

### OBJECTIVES

1. To determine the disease status of *Pangasius spp* due to *E. ictaluri*
2. To determine the risk factors of *E. ictaluri* towards *Pangasius spp*.

- Edwardsiellosis is one of bacterial diseases that infected freshwater fish, mainly catfish.
- *E. ictaluri* is one of the bacterial species which cause Bacillary Necrosis of Pangasianodon (BNP) (Crumlish et al. 2002).
- *E. ictaluri* also known by “Enteric Septicaemia of Catfish” (ESC) is responsible to the massive losses in catfish industry across the world (Crumlish et al., 2002).



## METHODOLOGY

### THIS STUDY WAS CONDUCTED IN 2 CULTURE CYCLES:

- 1<sup>st</sup> cycle: June 2021 - January 2022
- 2<sup>nd</sup> cycle: February 2022 - September 2022

### SAMPLING SITES IN TEMERLOH:

- Kg Bintang
- Teluk Ira

### SAMPLING SITES IN PEKAN:

- Kg Belimbing
- Kg Tanjong Pulau

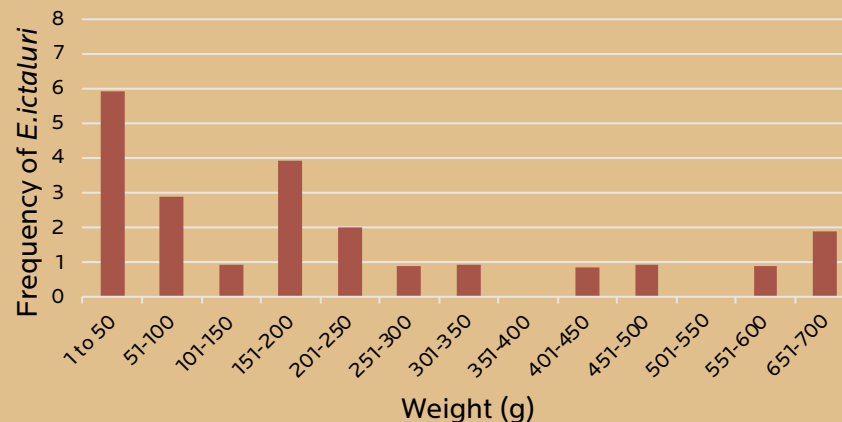
## RESULTS

*E. ictaluri* infects all sizes of *P. hypophthalmus* except at 351-400 g and 501-550 g body weight, with highest prevalence at size below 50 g. There is no *E. ictaluri* isolated from *P. hypophthalmus* cultured at Kg. Belimbing most probably due to topographical factors, the density of fish in the cage and the water current.

There is no correlation between water parameters and the presence of *E. ictaluri* in *P. hypophthalmus*, except in Kg. Tanjong Pulau, where the prevalence of *E. ictaluri* has a high correlation with temperature ( $P < 0.005$ ,  $P = 0.001$ ).



Fish Health Project Collaborations Between FRI (NaFiSH, FRITD, FRIGL) & IPTA (IIUM)



Frequency of *E. ictaluri* vs Weight (g)

# 3

## EPIDEMIOLOGY OF ACUTE HEPATOPANCREATIC NECROSIS DISEASE (AHPND) IN MARINE SHRIMPS

Padilah Bakar, Rohaiza Asmini Yahya and Kua Beng Chu

### OBJECTIVE

1. To determine the status of AHPND in white shrimps (*P. vannamei*) culture in Penang.

- Study population/zone of *P. vannamei* under superintensive tank culture
- Stocking density 300-350 pcs/m<sup>2</sup>
- Two cycles culture production (Oct. 2020 - May, 2021)



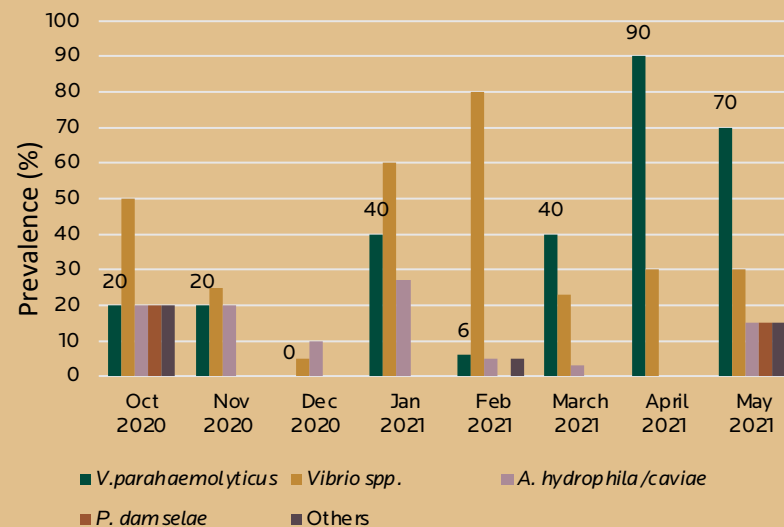
## METHODOLOGY

1. Polymerase chain reaction (PCR) for detection of *PirAB<sup>vp</sup>* toxin genes from hepatopancreas tissues (IQ2000™ AHPND/EMS)
2. Culture and isolation of bacteria (API Kit)

## RESULTS AND DISCUSSION

- 135 samples were analyzed. AHPND was not detected in *P. vannamei* from 2 cycle culture production.
- *V. parahaemolyticus* was isolated with high prevalence (20-90%), only 2 isolates carrying plasmid with toxin *PirA/B* deletion.

**Future Plan:** Determine AHPND status from collaboration project with FRIGP & FRITD and case diagnosis



# 4

## PARASITE MONOGENEAN CAPSALID INFESTATION IN MARINE FARMED FISH

Rohaiza Asmini Yahya, Padilah Bakar, Khairul Azri Aziz, Nur Ashikin Arbi, Nur Samihah Mazlan & Kua Beng Chu

### INTRODUCTION

1. Diagnostic cases carried out in 2019 and 2020 have identified four species namely, red snapper, seabass, golden pompano and grouper were infested with monogenean capsalid.
2. The parasite localized at fins, eyes and body.
3. Clinical signs of capsalid infestations include scale drop, haemorrhage on the body and eyes, followed with secondary infections and subsequently, mortality.
4. *Benedenia* sp. and *Neobenedenia* sp. are the two species that have been identified.

### OBJECTIVE

To determine the current status of capsalid monogenean infestation for control measures of ectoparasites in marine fish culture at Pulau Jerejak, Pulau Pinang.



## RESULTS & DISCUSSION

A total of 108 red snappers were sampled in November and December, 2021, with an average weight of  $18.64 \pm 3.31$  g and  $26.81 \pm 6.04$  g, respectively. Results showed prevalence of 36.7% and 97.4% in November and December, 2021, respectively. High capsalid infestations was identified in cages and the study will be continued in 2022.



# 5

## EPIDEMIOLOGY OF DECAPOD IRIDESCENT VIRUS 1 (DIV1) FROM NORTHERN REGION OF MALAYSIA

Padilah Bakar and Kua Beng Chu

### OBJECTIVE

1. To determine the status of DIV1 in shrimp culture

### METHODOLOGY

- *P. vannamei*, *M. rosenbergii*, *P. monodon*, crayfish *C. quadricarinatus* and *P. clarkii* are susceptible species.
- Nested PCR for SHIV (Qiu et al. 2017) was used in 2020-2021.
- Real time PCR (qPCR) for DIV1 was used in 2022



## RESULT & DISCUSSION

DIV1 was not detected in crayfish *C. quadricarinatus*, *P. clarkii*, fresh water prawn (*M. rosenbergii*) and white shrimps (*P. vannamei*)

**Future Plan:** Screening of DIV1 status in marine shrimp culture (collaboration with FRIGP, FRITD and FRIPS)

Date of sampling	Shrimp spp.	Location	No. of sample	PCR DIV1
1. 8 Apr. 2021	<i>C. quadricarinatus</i>	Penang	30	-ve
2. July 2021	<i>P. clarkii</i>	Penang	30	-ve
3. Aug. 2021	<i>P. vannamei</i>	Penang	30	-ve
4. Sept. 2021	<i>M. rosenbergii</i>	Kedah	30	-ve
5. Nov. 2021	<i>M. rosenbergii</i>	Perak	30	-ve



Crayfish *C. quadricarinatus*



*P. clarkii*



*M. rosenbergii*



*P. vannamei*

# 6

## ENTEROCYTOZON HEPATOPENAEI (EHP) OF WHITE SHRIMP IN HIGH DENSITY CULTURE

Rohaiza Asmini Yahya, Padilah Bakar, Nur Ashikin Arbi & Kua Beng Chu



### INTRODUCTION

- The parasitic disease microsporidia *Enterocytozoon hepatopenaei* (EHP) affects shrimp growth and produces a variety of shrimp sizes but does not cause high mortality.
- The hepatopancreas is the primary organ affected by EHP infection.



### OBJECTIVE

To determine the status of EHP infection in white shrimp in one cycle culture production of high-density culture.



### RESULTS AND DISCUSSION

- A total of 416 hepatopancreas samples of white shrimps were analyzed using PCR for detection of spore wone protein (SWP) of EHP parasite.
- Prevalence of EHP at 10-70% was found in the post larval stages (PL1-16) while 5-61% in the juvenile.
- The results showed that the post larvae were infected with EHP at an early stage. Farmers were advised to perform a preliminary screening before admission of seeds into the pond culture, proper disinfection for all equipments and water treatment before use.

## SCOPE 2: R&D OF DISEASE DIAGNOSIS, CONTROL AND PREVENTION

# 7

## DEVELOPMENT OF LOCAL VNN VACCINE

### PHASE 1: DETERMINATION OF VACCINE CANDIDATE

Azila Abdullah and Hazreen Nita MK

#### OBJECTIVES

1. To develop recombinant VNN vaccine from local isolate
2. To develop bioencapsulation procedure ← We are here
3. To develop vaccine strategies for VNN

#### OVERALL METHODOLOGY

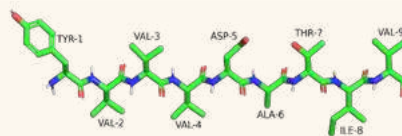
Characterization of Local Betanodavirus

In Silico Studies, Molecular Docking & Molecular Dynamic Study

Construction Of Recombinant Plasmid & Colony Screening

Expression Of Targeted Proteins In Prokaryotic System

#### OUTPUT: CANDIDATE VACCINE



Recombinant

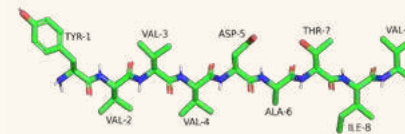
Plasmid

Transformation



#### ACHIEVEMENTS/OUTPUT:

#### VACCINE CANDIDATE OUTPUT: PATENTING PROCESS



2E : EASY & EFFECTIVE

#### SHIELD STOCK

FISH VACCINE TO BEGIN

#### STRATEGIES

Bioencapsulated Fry Broodstocks Grower



Fish Health Project Collaborations Between FRI (NaFish, FRITD, FRIGL) & IPTA (IIUM & UMK)

# 8

## DEVELOPMENT OF LOCAL VNN VACCINE

### PHASE 2: PROTECTION OF SEABASS LARVAE AGAINST VNN USING BIOENCAPSULATED LIVE FEED VACCINE

Azila Abdullah, Nur Fatin Afifah Osman Manah, Shaharah Idris and Hazreen Nita MK

#### OBJECTIVES

1. To establish disinfection protocol of rotifer We are here
2. To establish bioencapsulation protocol for VNN vaccine
3. To determine the protective capacity provided by the bioencapsulated recombinant VNN vaccine against Viral Nervous Necrosis (VNN)



#### EXPERIMENTAL DESIGN



The study was continued to determine effective disinfection rate for rotifer

1 <sup>st</sup> experiment	Treatments SirehMAX™ OTC EraBaju	Concentrations 10 ppm 10 ppm 3 ppm	No. of rotifers 300 ind/mL	Sampling time - 1, 2, 3, 4, 5, 24, 48 hours
2 <sup>nd</sup> experiment	SirehMAX™	Concentrations 10 ppm 20 ppm 30 ppm	No. of rotifers 500 ind/mL	Sampling time - 1 hour
3 <sup>rd</sup> experiment	SirehMAX™	Concentrations 10 ppm 20 ppm 30 ppm	No. of rotifers 200, 300, 400, 500 ind/mL	Sampling time - 1 & 2 hours
4 <sup>th</sup> experiment	SirehMAX™	Concentrations 10 ppm 20 ppm 30 ppm	No. of rotifers 300 ind/mL	Sampling time - 1, 2, 3, 4, 5 hours
5 <sup>th</sup> experiment	SirehMAX™	Concentrations 10 ppm 20 ppm 30 ppm	No. of rotifers 400 ind/mL	Sampling time - 1, 2, 3, 4, 5 hours

Streaking  
- confirmation  
of existence  
bacteria

Survival rate  
Rotifers  
counting

Fish Health Project Collaborations Between FRI (NaFish, FRITD, FRIGL) & IPTA (IIUM & UMK)

## OBJECTIVES

1. To determine NNV whole genome of local isolate
2. To identify the possibility of reassortment in local NNV strain

## CURRENT STATUS

- New reassortant strain have been found involving the SJNNV and RGNNV (Toffolo et al., 2007).
- Due to the segmented nature of NNV genome, fish nodavirus can undergo genetic shift resulting in the generation of reassortant viruses.
- Notably, the occurrence of the reassortant strains RGNNV/SJNNV and SJNNV/RGNNV has been reported only in the Mediterranean Sea (Toffan et al., 2017).
- Based on the RNA 1 partial sequence and RNA 2 sequence obtained, we can assume that the local isolate is RGNNV and not reassortant strain.
- But further studies are being planned to confirm this statement

## METHODOLOGY

Optimised Component	1X (μl)
2X Mytaq One Step mix	12.5
10μM Forward primer	0.5
10μM Reverse primer	0.5
Rnase inhibitor	0.5
RT-enzyme	0.25
DEPC water	7.75
Master mix per tube	23
sample	2
<b>Total per tube</b>	<b>25</b>

### 1. Primer Design

RPSJRGNNV  
F1R1  
F2R2

### 2. RNA Extraction

Trizol  
• AVA  
• BE  
• KL22

### 3. Optimization and RT-PCR

- MyTaq™ One Step RT-PCR kit (Bioline)
- DreamTaq DNA polymerase (Thermo Fisher)

### 4. DNA Purification

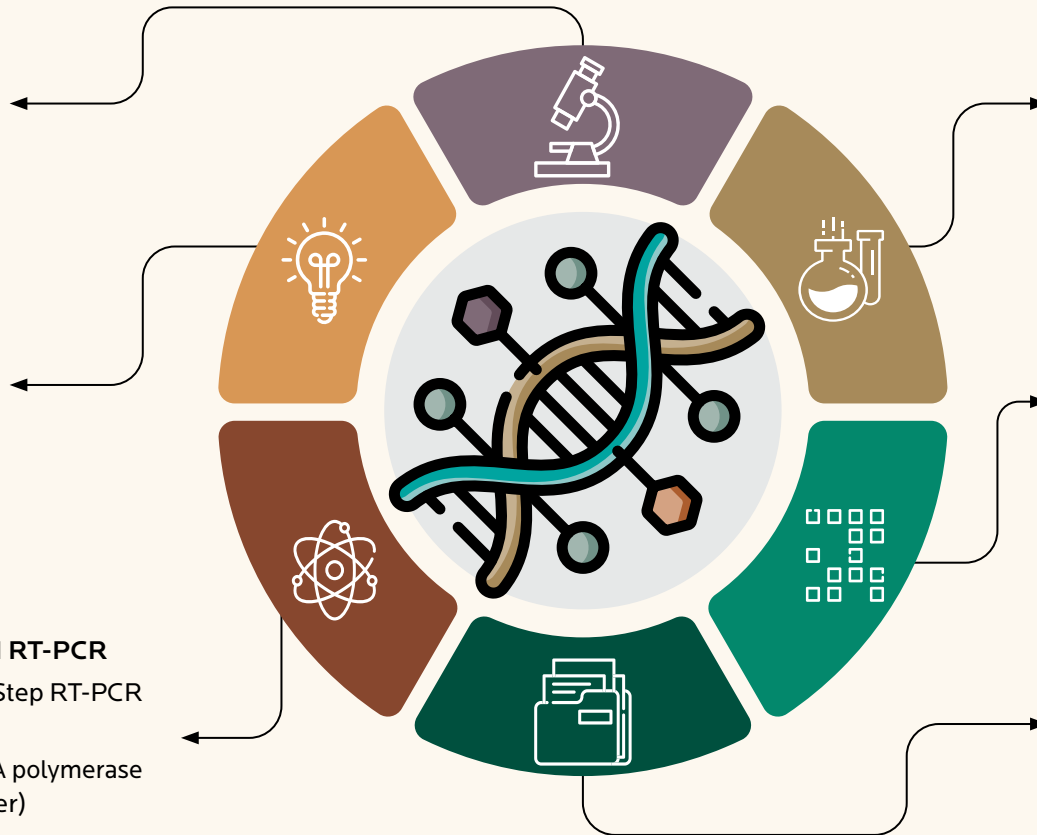
Macherey Nagel™  
NucleoSpin™ Gel and  
PCR Clean-up Kit  
• PCR clean up  
• Gel Extraction

### 5. DNA Sequencing

Apical (1<sup>st</sup> Base)  
• Single pass DNA sequencing  
• Difficult Template Sequencing (GC rich)

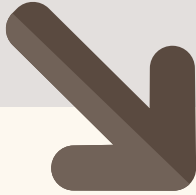
### 6. Sequencing Analysis

MEGA Software  
• DNA alignment  
• Blast



## VIRAL INFECTION

Genus: Betanodavirus  
Species: RGNNV Lorem  
Strain/isolates virulence?



## VIRAL NERVOUS NECROSIS DISEASE



## PATHOGENICITY

Risk factors in Malaysia situation?

## PROBLEMS

100% mortality in fry & juvenile  
Vertical transmission



## OBJECTIVES

To determine:

- Stress parameter in relation to temperature fluctuation
- Effect of temperature fluctuation on VNN induction

## DISCUSSION & CONCLUSION

### EXPERIMENT 1

- All fish showed dark body coloration and lethargic during 7 days experimental period especially on the 2nd day of experiment.
- Corticols level recorded highest at day 2, and slowly decreasing until it reached certain levels in all the 3 groups.
- RBC was also increased in all the group on the 3rd day of experiment. These results showed that this fish can tolerate to the stress rapidly and effectively.
- Tempertaure fluctuation helped in induce stress and thus increase the rate of VNN infection in seabass.

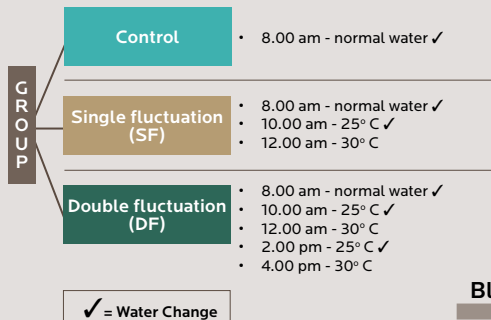
## DISCUSSION & CONCLUSION

### EXPERIMENT 2

- Fish challenge with KL22 had the highest cumulative mortality.
- All the sick and dead fish were tested positive by PCR that confirmed the clinical signs and mortalities caused by betanodavirus infection. The partial virus coat protein sequence at 248 bp (Nishizawa et al., 1994)
- No vacuolation observed in all strains due to the acute infection.
- **Conclusion :** Temperature fluctuation was confirmed as a main predisposing factor during acute betanodavirus infection and KL22 is the most virulent isolate in Asian seabass.

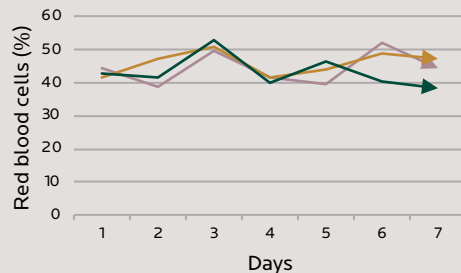
## METHODOLOGY

### EXPERIMENT 1



Blood

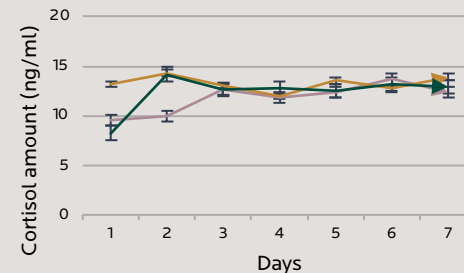
### RED BLOOD CELLS



Serum

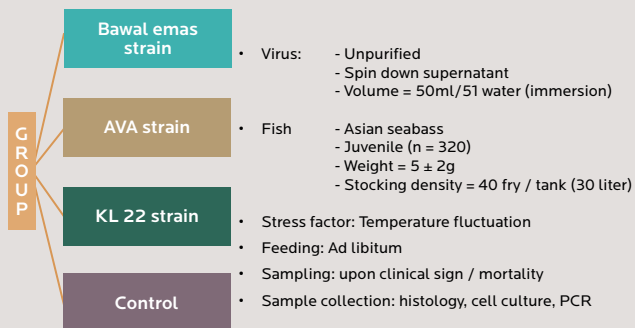
## RESULTS

### CORTISOL AMOUNT AGAINST DAY



## METHODOLOGY

### EXPERIMENT 2

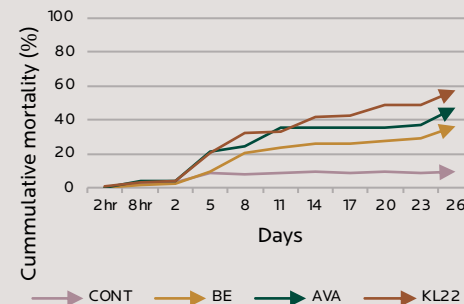


### TCID<sub>50</sub> of the strains:

- KL22 - 10<sup>4</sup> TCID<sub>50</sub>/mL
- BE - 10<sup>7</sup> TCID<sub>50</sub>/mL
- AVA - 10<sup>6</sup> TCID<sub>50</sub>/mL

## RESULTS

### CUMULATIVE MORTALITY AGAINST DAY



# 11

## DEVELOPMENT OF INACTIVATED VIBRIO VACCINE FOR GROUPER BROODSTOCKS

Sufian Mustafa, Shaharah Mohd Idris and Azila Abdullah

### OBJECTIVE

1. To evaluate passive transfer of maternal antibodies and their existence in eggs, larvae and fry of tiger grouper.

### METHODOLOGY

- Administration of inactivated *V. alginolyticus* vaccine followed by two booster doses at two weeks interval



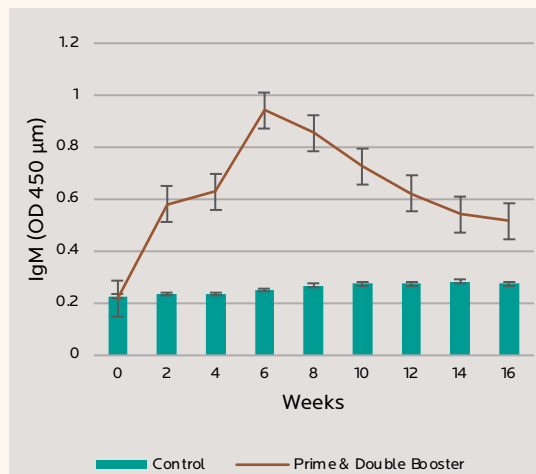
Fish Health Project Collaborations Between FRI (NaFish, FRITD, FRIGL) & IPTA (IIUM & UMK)

## RESULTS

1. Vaccination strategy could evoke a maximum level of IgM antibody in tiger grouper broodstock.
2. The protection provided against *V. alginolyticus* was 14 weeks.
3. Antibody & T cell memory were vertically transferred to the eggs and larvae & making them able to produce self-antibody against *V. alginolyticus* as early as 21 day after hatching.
4. This characteristic is very crucial since the critical period of vibriosis was at larvae rearing stage; after egg yolk completely absorbed until 60 days old.

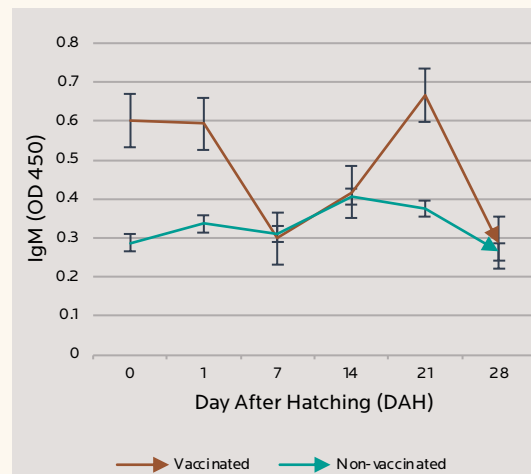
**Output: 1 publication:** Understanding the active vibriosis vaccination to broodstock could evoke passive transfer of maternal antibodies to their eggs, larvae and fries of tiger grouper.

Comparison of IgM antibody level of tiger grouper (*E. fuscoguttatus*) between vaccinated and non-vaccinated groups



**Fig 1.** Level of IgM antibody in blood serum and protective level (COV) against *V. alginolyticus* double vaccinated to the tiger grouper broodstock.

Level of specific antibody IgM in eggs and larvae of tiger grouper (*E. fuscoguttatus*) from vaccinated and unvaccinated broodstocks



**Fig 2.** IgM antibody levels in egg, larval and fry of tiger grouper obtain from matured tiger grouper brooder which introduced inactivated *V. alginolyticus* vaccine.

**Table 1.** Levels of antibody (IgM) on egg and larvae of tiger groupers (*Epinephelus fuscoguttatus*) from vaccinated and Non-vaccinated broodstock

	0	1	7	14	21	28
Vaccinated	0.61±0.06 <sup>a</sup>	0.60±0.09 <sup>a</sup>	0.31±0.05 <sup>a</sup>	0.42±0.08 <sup>a</sup>	0.67±0.06 <sup>a</sup>	0.30±0.07 <sup>a</sup>
Non-Vaccinated	0.29±0.04 <sup>b</sup>	0.33±0.04 <sup>b</sup>	0.31±0.01 <sup>a</sup>	0.41±0.02 <sup>a</sup>	0.38±0.03 <sup>b</sup>	0.23±0.01 <sup>a</sup>

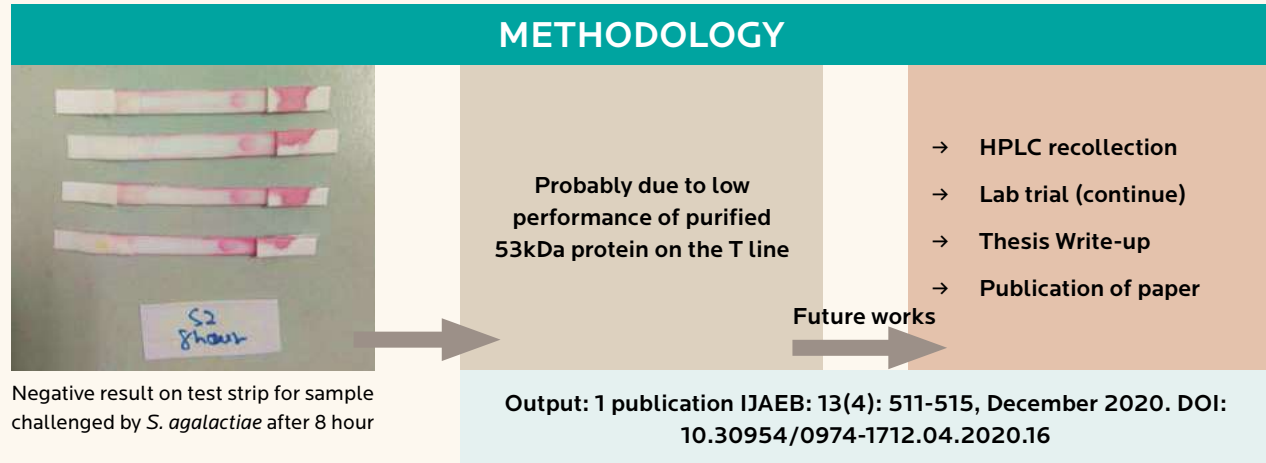
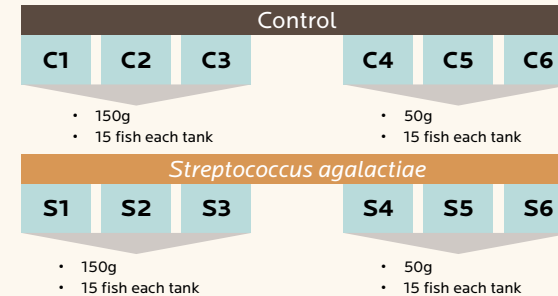
Muhamad Faizal bin Mohd, Nur Nazifah Mansur and Rimatulhana Ramly

## OBJECTIVES

1. To profile and explore molecular changes of fish body mucus after subsequent challenge to streptococcosis causative agent (*Streptococcus agalactiae*). **DONE**
2. To prepare and assemble a rapid detection kit towards the detection of *S. agalactiae*. **DONE**
3. To evaluate the effectiveness of developed rapid detection kit against streptococcosis. **ONGOING**

## LAB TRIAL

(*Streptococcus agalactiae*)

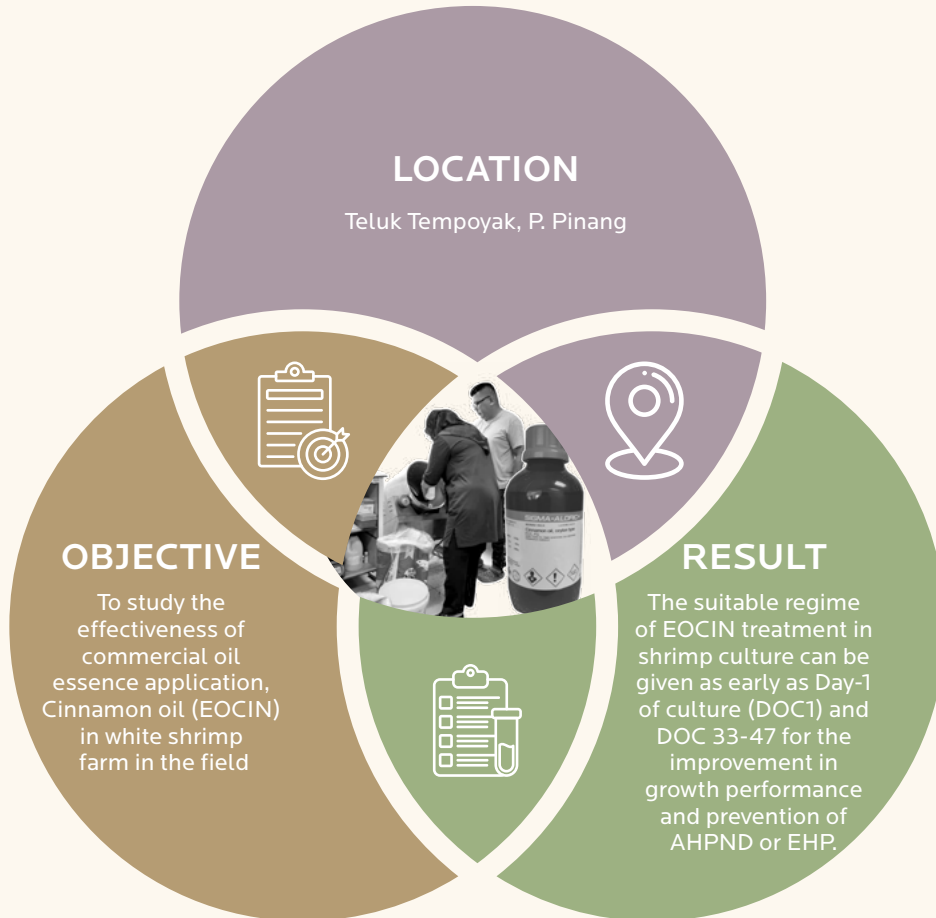




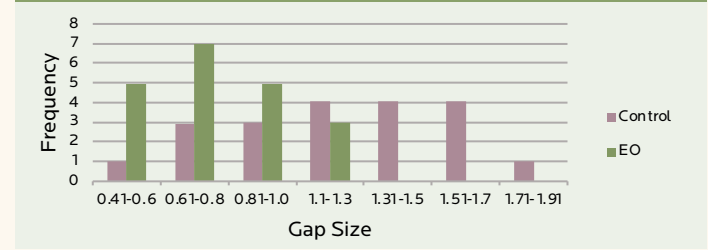
**SCOPE 3:  
R&D IN ALTERNATIVE  
TREATMENT IN  
AQUACULTURE**

## STUDY ON IMPROVING THE HEALTH MANAGEMENT OF WHITE SHRIMP IN SUPERINTENSIVE FARMING

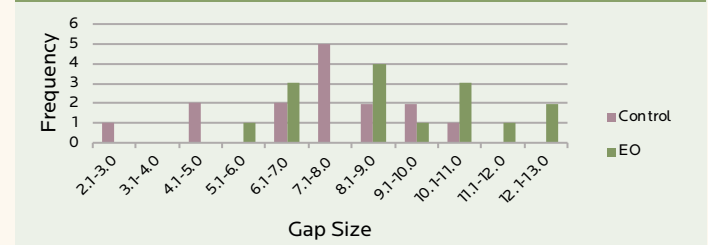
Rohaiza Asmini Yahya, Nur Ashikin Arbi, Nur Samihah Mazlan, Wan Muhamad Hazim Wan Muhammad Sajiri & Kua Beng Chu



**PL 6**



**DOC 47**



# 14

## GUT MICROBIOME OF HYBRID GROUPER, *EPINEPHELUS SP.* AFTER ORAL DIET OF COMMERCIAL CINNAMON OIL

Rohaiza Asmini Yahya, Nur Ashikin Arbi, Nur Samihah Mazlan, Wan Muhamad Hazim Wan Muhammad Sajiri & Kua Beng Chu



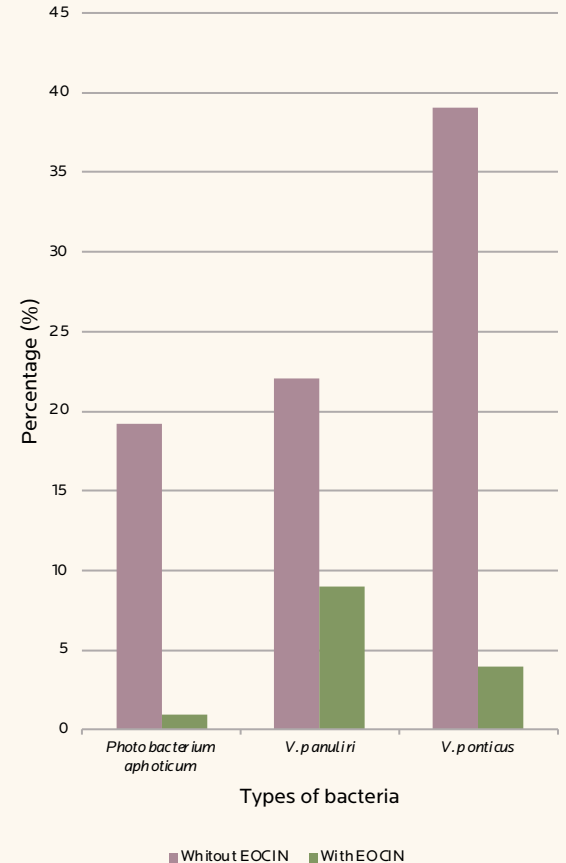
The microbial community in the gastrointestinal (GI) consists of mainly bacteria and yeast that play a very important role in determining the growth, digestion, nutrition, disease resistance and immunity of the host. Manipulation of the GI microbial community can help promote host health against disease.



The main goal of the study was to explore whether the intake of commercial cinnamon oil (EOCIN) by grouper would affect the intestinal microbiota profile.



- Five guts from each individual hybrid grouper received EOCIN continuously for 14 days and grouper receiving pellets without EOCIN were removed for microbiome analysis.
- Results showed significant differences in the GI microbiome of bacteria in hybrid grouper mainly from the Vibrionaceae group, compared to hybrid grouper without EOCIN. A low percentage (1-10%) of the three species *Photobacterium aphoticum*, *Vibrio panuliri* and *V. ponticus* in hybrid grouper intestines received EOCIN compared to 25-40% in fish without EOCIN.
- In conclusion, continuous intake of EOCIN can modulate the intestinal microbiota in hybrid grouper and provide a scientific basis for developing effective strategies in grouper health management.



## OBJECTIVE

- To find the optimum extraction method for antimicrobial activity of herbs.

## PLANT SAMPLE



Gelenggang



Senduduk



Mahkota Dewa



Dukung Anak



Semambu

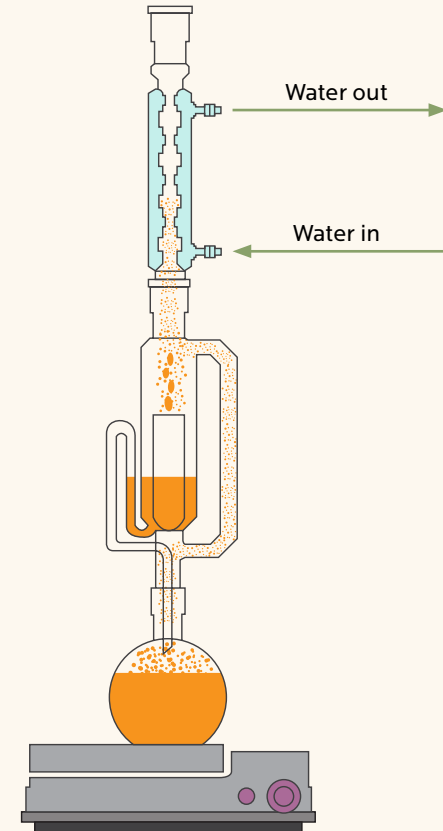


Mengkudu

- V. vulnificus*
- V. alginolyticus*
- A. hydrophila*
- S. agalactiae*
- E. tarda*



DISC DIFFUSION METHOD



A. SOXHLET EXTRACTION

Figure 1: Soxhlet Extraction Method using ethanol as solvent

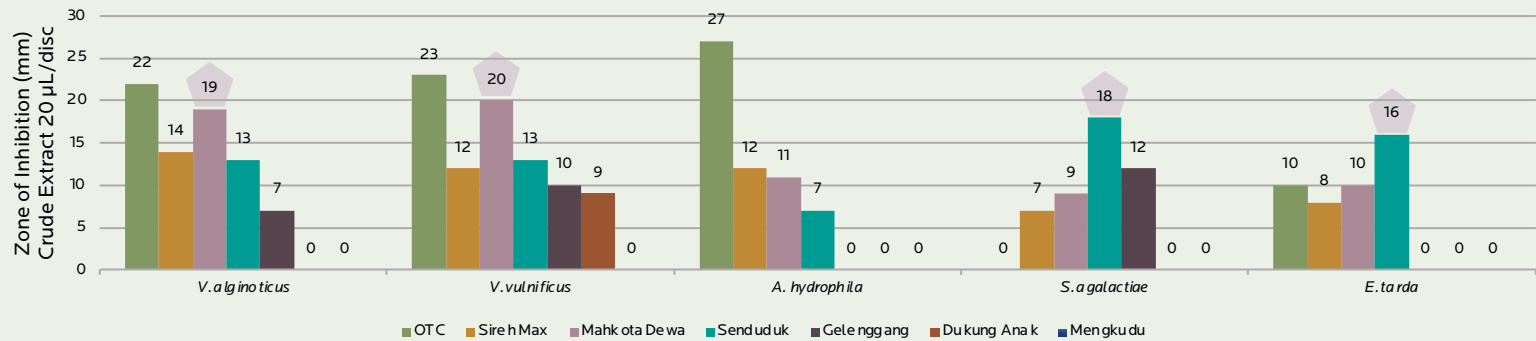
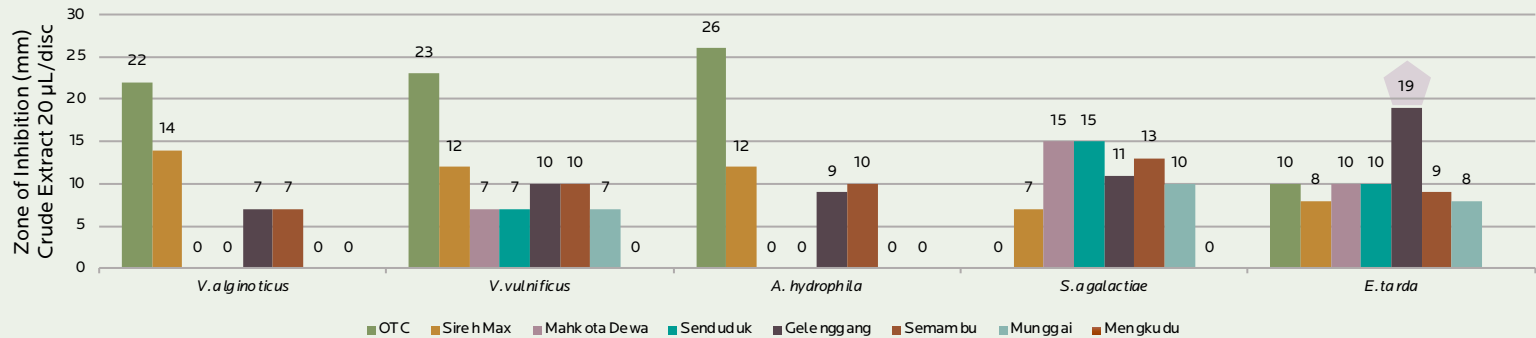


Figure 2: Maceration Extraction Method using ethanol as solvent



**OUTPUT:**

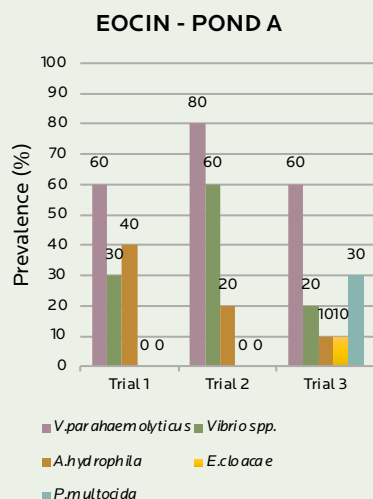
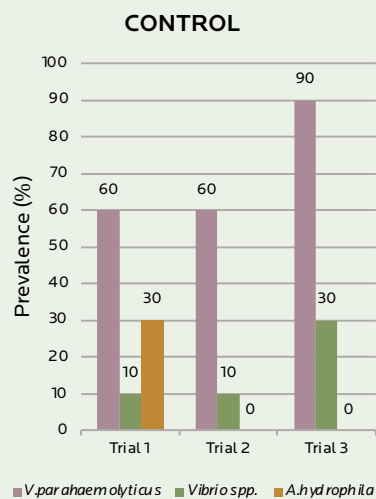
- Mahkota dewa, Senduduk and Gelenggang exhibiting antimicrobial activities
- indicates potential for alternative medication for bacterial fish pathogen

# FIELD STUDY: EFFECT OF EOCIN (CINNAMON OIL) ADDITIVE ON BACTERIAL PREVALENCE AND AHPND IN WHITE SHRIMPS (*P. VANNAMEI*) SUPERINTENSIVE TANK CULTURE

Padilah Bakar, Rohaiza Asmini Yahya and Kua Beng Chu

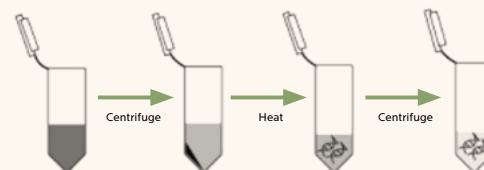
## OBJECTIVES

- To determine effect of EOCIN in feed pellet on bacteria and AHPND in white shrimps.
- Stocking density (300 pcs/m<sup>3</sup>) - shrimps 300,000 pcs/tank (600 m<sup>3</sup>) from DOC-14 until harvest (DOC 78).
- EOCIN treatment (3 replicates), control (2) at 1.5% everyday for 2 weeks followed by once a week until harvest.

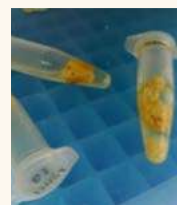


## METHODOLOGY

### CTAB DTAB EXTRACTION METHOD



### A. PCR



### B. Bacteria analysis



## RESULTS AND DISCUSSION

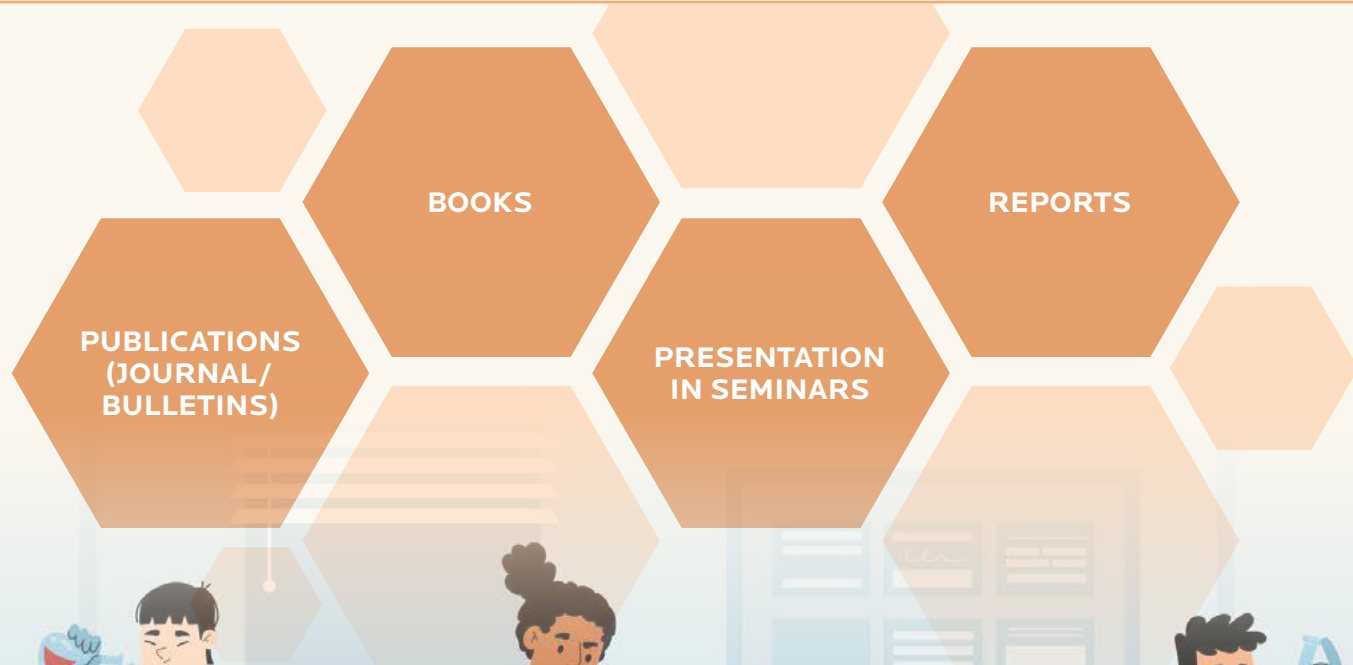
- V. parahaemolyticus* prevalence was high (50-80%) in EOCIN treated white shrimps and control (60-90%).
- AHPND (*PirA/B* toxin genes) was NOT detected from both groups
- Plasmid with toxin 1 deletion was found in EOCIN treated group of *P. vannamei* (n=1/70) & *V. parahaemolyticus* isolated from them (n=8/70).

## FUTURE PLAN

- Determine AHPND status from case diagnosis and collaboration study with FRIGP, FRITD & FRIPS
- Pathogenicity study of AHPND strains (*V. parahaemolyticus*)

- 
- DOCUMENTATION OUTPUT
  - COLLABORATIONS
  - TECHNICAL SERVICES
  - EVENTS
  - INNOVATION
  - CONTRIBUTION
  - RESEARCH GATE PROFILE
  - DIRECTORY

# DOCUMENTATION OUTPUT



## TECHNICAL/SCIENTIFIC ARTICLE IN JOURNALS

1. Wan Muhd HWS, Muhd Hafiz B & Kua BC. (2021). Occurrence of *Enterocytozoon hepatopenaei* (EHP) infection on *Penaeus vannamei* in one rearing cycle. Diseases of Aquatic Organisms.144:1-7. doi: 10.3354/dao03571.
2. SN Pauzi SN, Hassan MG., Yusoff N., Harun NH., Abu Bakar AB & Kua BC. (2021). A review on image processing for fish disease detection. Journal of Physics: Conference Series. 1997 012042. doi:10.1088/1742-6596/1997/1/012042
3. Mohd Syafiq MR, Azila A, Rimatulhana R, NurNazifah M, Norazsida R & Mohd Firdaus N. (2021). Current status and advances of fish vaccines in Malaysia (Manuscript Number: VETWORLD-2021-10-567)- Submitted
4. Faizal M, Nur-Nazifah M, Syafiq Ridzuan, Wan Norazlan, Azila A and Rimatulhana R. (2020). Protein Profiling of *Oreochromis* spp. Epidermal Mucus Subsequent to Challenge of Common Freshwater Bacteria (2020). International Journal of Agriculture, Environment and Biotechnology. 13(4): 511-515. DOI: 10.30954/0974-1712.04.2020.16



## DOCUMENTATION OUTPUT

### MAGAZINE/BULLETIN/NEWSLETTER

1. Haslawati B, **Kua BC** and Raja Yana Meleessa RHA. (2021). Aquatic invasive alien species in Malaysia: status and challenges in managing the conflict and complexity. FRI Newsletter 24: 3-8
2. **Kua BC** dan Eleanor DL. (2021). Managing incoming alien species through CIASA, Department of Fisheries Malaysia. FRI Newsletter 24:18-19
3. **Afzan Muntaziana MP & Azila A.** (2021). "Kajian Penyakit Tilapia Lake Virus (TiLV) pada Ikan Tilapia Merah (*Oreochromis sp.*) dan Lampam Sungai (*Barbonymus schwanenfeldii*) di Pusat Pengembangan Akuakultur (PPA) Bukit Tinggi, Pahang.". Berita Perikanan Bil. (118) Edisi September 2021. Ms 26.
4. **Rohaiza Asmini Y. & Afzan Muntaziana MP** (2021). Artikel 'NaFisH Jayakan Program Hari Bertemu Pelanggan Secara Maya', Hari Bertemu Pelanggan. Berita Perikanan Bil. 118, Sept. 2021, Ms 34.

### BOOKS

1. **Kua BC, Azila A, Rimatulhana R, Padilah B, Mohd Syafiq MR, Afzan Muntaziana MP, Rohaiza AY dan Kamisa A.** (2021). Laporan Projek Pembangunan: Penyelidikan Penyakit Ikan Akuakultur dan Pembangunan Vaksin/Kit Diagnosis RMK-11 (2016-2020). Institut Penyelidikan Perikanan, Jabatan Perikanan Malaysia, ISBN 978-967-2946-11-3, 118 pp.
2. **Rimatulhana R, Azila A, Kamisa A and Fahmi S** (2021). Kompilasi Laporan Kes Diagnosis Penyakit Ikan (2016-2020). Institut Penyelidikan Perikanan, Jabatan Perikanan Malaysia, Pulau Pinang, Malaysia ISSN, 178pp.



## PRESENTATION IN SEMINARS/ CONFERENCES







1. **Kua Beng Chu.** Country report: EHP in Malaysia. Virtual invited speaker for Online Consultation on Strategies for Hepatopancreatic Microsporidiosis caused by *Enterocytozoon hepatopenaei* (EHP), 9-10 Feb. 2021
2. **Kua Beng Chu, Rohaiza Asmini Yahya, Nur Ashikin Arbi.** KRIPeK: A Solution for Swim Bladder Disorder in Tiger Grouper (*Epinephelus fuscoguttatus*). Extended abstract for 5<sup>th</sup> International Innovation, Design and Articulation (i-IDEA™2020), online, 8 -22 March 2021
3. **Kua Beng Chu.** Country presentation: Sharing lessons from Malaysia: Active surveillance. Virtual invited speaker for course on the design and implementation of an active surveillance for Tilapia Lake Virus (TiLV) using 12-point Checklist for a multidisciplinary team (Philippines and Vietnam), 26 March – 15 April 2021
4. **Rohaiza Asmini Y, Padilah B, Imelda RR & Kua BC.** Microsporidian Parasite, *Enterocytozoon hepatopenaei* at Early Stage of Culture in Whiteleg Shrimps (*Penaeus vannamei*) of East Malaysia. Poster presentation at Webinar Penyelidikan Perikanan, Fisheries Research Institute (FRI), Gelami Lemi, Negeri Sembilan, 27-28 May 2021
5. **Padilah B, Rohaiza AY & Kua BC.** Acute hepatopancreatic necrosis disease (AHPND) in *Penaeus vannamei* culture in Malaysia. Poster presentation at Webinar Penyelidikan Perikanan, Fisheries Research Institute (FRI), Gelami Lemi, Negeri Sembilan 27-28 May 2021.
6. **Azila A.** (2021). Status of Tilapia Lake Virus (TiLV) in Malaysia. Oral presentation at FRI Seminar, 27-28 May 2021
7. **Rimatulhana R, Azila A, Munira M, Zuraidah R, Kamisa A, Afzan Muntaziana MP.** (2021). Molecular characterisation of Tilapia lake virus (TiLV) isolated from different localities in Malaysia. Abstract & poster presentation at FRI Seminar, 27-28 May 2021.
8. **Afzan Muntaziana MP, Azila A & Kua BC.** (2021). Fish Disease Cases in Malaysia Reported to the National Fish Health Research Division Center (NaFiSH) from 2003 to 2010. Abstract & poster presentation at FRI Seminar, 27-28 May 2021
9. **Azila Abdullah.** Penyakit Viral nervous necrosis (VNN): Fakta & Harapan. Program Bicara Penyakit Ikan Siri 1: VNN: sejarah, Pencegahan & pengawalan. (146 kumpulan sasar). Online Webinar pada 28.7.2021

## REPORTS

- 1. Padilah B & Rohaiza AY.** Case diagnosis 1/2021 (P. vannamei), 2 March, 2021. Report sent to shrimp farmer, Asia Aquaculture Holding, Teluk Tempoyak, Penang. File No. Prk. ML (Peny) K.106.16.JLD.6 (28)
- 2. Padilah B & Kua BC.** Case diagnosis 2/2021 (Arapaima), 25 Feb. 2021. Report sent to Veterinary Consultant, Istana Bukit Kecil, Alor Setar, Kedah. File No. Prk. ML (Peny) K.106.16.JLD.6 (29)
- 3. Afzan Muntaziana MP, Kua BC, Azila A & Rohaiza Asmini Y.** Case diagnosis 3/2021 (Tilapia hitam), 10 Feb. 2021. Report sent to Trapia Malaysia Sdn. Bhd. & Pusat Perikanan Banding, Perak. File No. Prk. ML (Peny) K.106.16.JLD.6 (30)
- 4. Rimatulhana R, Kua BC & Afzan Muntaziana MP.** Case diagnosis 4/2021, 24 May, 2021. Report sent to Pengarah Perikanan Negeri Johor. File No. Prk. ML (Peny) K.106.16. Jld 6 (31)
- 5. Padilah B & Kua BC.** Case diagnosis 5/2021 (ikan hiasan), 15 March, 2021. Report sent to Sealife Malaysia, Legoland Malaysia Resort, Gelang Patah, Johor. File No. Prk. ML (Peny) K.106.16.JLD.6 (32)
- 6. Padilah B & Kua BC.** Case diagnosis 6/2021 (ikan siakap), 12 March, 2021. Report sent to Max Akuatic, Pantai Merdeka, Kedah & CS Fishery Penang. File No. Prk. ML (Peny) K.106.16.JLD.6 (33)
- 7. Afzan Muntaziana MP & Azila A.** Case diagnosis 7/2021(Tilapia GIFT), 2 March, 2021. Report sent to a farmer, (En. Hairul Abu Bakar) Tasik Pedu, Kedah. File No. Prk. ML (Peny) K.106.16.JLD.6 (34)
- 8. Padilah B, Rimatulhana R & Kua BC.** Case diagnosis 8/2021(Puyu) A&B, 6 April, 2021. Report sent to Cemerlang Agro Enterprise, Gunung Semanggol, Daerah Kerian, Perak. File No. Prk. ML (Peny) K.106.16.JLD.6 (35)
- 9. Rimatulhana R, Kua BC & Afzan Muntaziana MP.** Case diagnosis 9/2021, 16 June, 2021. Report sent to Pengarah Perikanan Negeri Kedah. File no. Prk. ML (Peny) K.106.16.JLD.6. (41)
- 10. Padilah B, Rimatulhana R & Kua BC.** Case diagnosis 10/2021 (puyu induk & juvenil Kulim), 6 April, 2021. Report sent to Cemerlang Agro Enterprise, Gunung Semanggol, Daerah Kerian, Perak. File no. Prk. ML (Peny) K.106.16.JLD.6 (36)

11. **Padilah B, Rimatulhana R & Kua BC.** Case diagnosis 11/2021 (Puyu induk Bukit Merah), 6 April, 2021. Report sent to Cemerlang Agro Enterprise, Gunong Semanggol, Daerah Kerian, Perak. File No. Prk. ML (Peny) K.106.16.JLD 6 (37)
12. **Rohaiza Asmini Y, Rimatulhana Ramly & Kua BC.** Case diagnosis 12/2021 (Siakap), 14 April, 2021. Report sent to a farmer (Mr. Kwan Pok Li), Tanjung Dawai, Kedah. File No. Prk. ML (Peny) K.106.16.JLD 6 (39)
13. **Padilah B, Rimatulhana R & Kua BC.** Case diagnosis 13/2021 (Sepat), 5 April, 2021. Report sent to Cemerlang Agro Enterprise, Gunong Semanggol, Daerah Kerian, Perak. File No. Prk. ML (Peny) K.106.16.JLD 6(38)
14. **Rohaiza Asmini Y, Rimatulhana Ramly, Fahmi Sudirwan & Kua BC.** Case diagnosis 14/2021 (Kerapu), 5 July, 2021. Report sent to LS Marine, Penang. File No. Prk. ML (Peny) K.106.16.JLD 6 (45)
15. **Rohaiza Asmini Y, Padilah B & Kua BC.** Case diagnosis 15/2021 (Udang putih), 25 August, 2021. Report sent to Asia Aquaculture Holding (Mr. Teh), Penang. File No. Prk. ML (Peny) K.106.16.JLD 6 (42)
16. **Padilah B & Rohaiza AY.** Case diagnosis 16/2021 (*P. vannamei*), 28 September, 2021. Report sent to shrimp farmer, Asia Aquaculture Holding, Teluk Tempoyak, Penang. File No. Prk. ML (Peny) K.106.16.JLD.6 (43)
17. **Padilah B & Noor Hanis AH.** Case diagnosis 17/2021 (Koi), 9 Nov. 2021. Report sent to AKUATAR, FRI Batu Maung, Penang. File No. Prk. ML (Peny) K.106.16.JLD.6 (46)
18. **Padilah B & Rimatulhana R.** Case diagnosis 18/2021 (Keli), 12 Nov. 2021. Report sent to Biosecurity Division, Tunjang, Kedah, DoF and farmer (Nekmat Bin Abdul Rahim), Tobiari, Kedah. File No. Prk. ML (Peny) K.106.16.JLD 6 (48)
19. **Padilah B & Rohaiza AY.** Case diagnosis 19/2021 (*P. monodon* & *P. vannamei*), 25 Nov. 2021. Report sent to Biosecurity Division, Tunjang, Jitra kedah, DoF. file No. Prk. ML (Peny) K.106.16.JLD 6 (41)

# COLLABORATIONS IN FISH HEALTH RESEARCH

			
<p><b>2 PROJECTS</b></p>	<p><b>1 PROJECT</b></p>	<p><b>4 PROJECTS</b></p>	<p><b>1 PROJECT</b></p>
<p><b>FRI TD</b></p>	<p><b>FRI GL</b></p>		
<ul style="list-style-type: none"> <li>• Screening of local herbs as an alternative treatment for fish diseases</li> <li>• Vaccine trial for <i>Vibriosis</i> in marine fish hatchery</li> </ul>	<p>Research on survival rate of shrimp juvenile at hatchery level</p>	<ul style="list-style-type: none"> <li>• Development of immunodiagnostic kit for rapid detection of freshwater fish-borne Streptococcosis</li> <li>• Identification on molecular characterization of <i>E.ictaluri</i> from <i>P.hypohthalmus</i> cultured In Pahang river, Malaysia</li> <li>• Development of recombinant vaccine for VNN disease in marine fish</li> <li>• Assesment of VNN infection in tiger grouper (<i>E.fuscoguttatus</i>) by immunological techniques</li> </ul>	<p>Detection of protozoa white spot disease ,<i>C.irritans</i> parasite in marine fish culture Using AI (artificial intelligent) method</p>



# TECHNICAL SERVICES



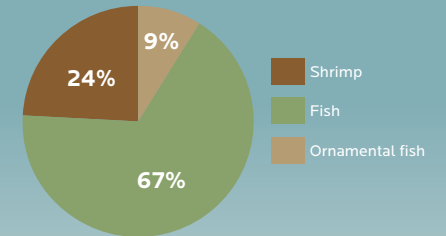
INTERNAL EXPERTISE   
EXTERNAL EXPERTISE 



9 consultations on Fish/shrimp health management



22 case investigations



## EVENTS IN 2021

'Hari Bertemu Pelanggan NaFish' on 27 Jan 2021



About 26 people joined the online program consisting of 15% from IPP, 15% farmers and 70% from private sector.

Consultations and Technical Services



Consultation to farmers during a field visit to fish farm for disease investigation and sampling of fish culture for laboratory test at Bukit Merah, Perak on 18 March, 2021

## R&D collaboration



The online ceremony announcing the winners in The commonwealth 2021 innovation competition on 29 Sep. 2021



Meeting with UUM for MOU agreement in collaboration study between NaFiSH-UUM (7 Jan. 2021)



Progress meeting on R&D of the collaboration projects between NaFiSH, FRITD, FRIGL and IIUM



# INNOVATION

## INTELLECTUAL PROPERTY

A

(pending for UI): Identification of vaccine candidate for VNN

B

UI2021005765: KRIPeK (bloating stomach treatment Kit)

C

Break and Protect 2

On-site solution for swim bladder disorder symptoms



**WINNER**  
**BREAK & PROTECT 2**



Certificate awarded to

*Kas Basyi Cha and Nur Achiqin Arbi,  
Department of Fisheries, Government of Malaysia*

Winner

Commonwealth Secretary-General's Innovation  
for Sustainable Development Awards 2021

28 September 2021

The Rt Hon Patricia Scotland GC,  
Commonwealth Secretary-General

Commonwealth Secretary-General's Innovation for Sustainable  
Development Awards (29 Sep. 2021)

## CONTRIBUTION TO DEPARTMENT AND COUNTRY

Name of Officers	Activities/Contributions	Level within Department/Country
<b>Kua Beng Chu</b>	• Alternate focal point for OIE, Aquatic Animal Health in Malaysia	DOF/Country
	• Alternate focal point for OIE, Asean Network on Aquatic animal health Centres (ANAAHC)	Country
	• Vice President for Executive committee Fish Health Section of Asian Fisheries Society 2017/2022	Country
	• OIE resource person for aquatic animal health management	Country
	• Head of Technical Committee in Parasitology Laboratory Analysis, Fisheries Department Malaysia (DOF)	DOF
	• Head of Technical Committee in Import Risk Analysis for IAS, DOF Malaysia	DOF
	• Head of Technical Committee in organizing 11th International Symposium on Diseases in Asian Aquaculture (DAA11)	FRI/DOF/Country
	• Technical Committee in organizing 58th Annual Scientific Conference of the Malaysian Society of Parasitology and Tropical Medicine	Country
	• Working Technical Expert in <i>Ad-Hoc</i> Identification of Parasites in fish and Fisheries Products	Expert Consultant/DOF/Country
	• Supervising Committee to UMT student (1 MSc)	DOF
• Supervising Committee to UUM student (1 Msc)	DOF	
<b>Azila Abdullah</b>	• Chief Editor, Malaysian Fisheries Journal (MFJ), FRI	FRI/DOF
	• Technical Working Group (TWG) in Veterinary Drug (technical working group for veterinary vaccine)	DOF/Country
	• Head of Security Officer at NaFisH	FRI/DOF
	• <i>OIE Resource person</i>	DOF/Country
	• Supervising Committee to IIUM students (Co-supervisor to 1 PhD and 3 MSc)	DOF
	• 'Jawatankuasa pemutus sebutarga'	DOF
	• 'Jawatankuasa Pengurusan aset alih kerajaan'	DOF
<b>Rimatulhana Ramly</b>	• Technical Committee in Laboratory Virology Analysis, Department of Fisheries Malaysia	DOF
	• Assessment and Registration of Commercial Vaccine	DOF/Country
	• 'Jawatankuasa Pengurusan aset alih kerajaan'	FRI
	• Technical Committee in Organizing 11th International Symposium on Diseases in Asian Aquaculture (DAA11)	FRI/Country
	• OIE Resource person	DOF/Country
	• Editorial Secretariat, <i>Malaysia Fisheries Journal</i> (MFJ), Fisheries Research Institute (FRI), Jabatan Perikanan Malaysia (DoF), Malaysia	FRI/DOF

Name of Officers	Activities/Contributions	Level within Department/Country
<b>Padilah Bakar</b>	• Editorial Secretariat, <i>Malaysian Fisheries Journal</i> (MFJ), FRI, Department of Fisheries	FRI/DOF
	• Technical Committee in Bacteriology Analysis of Fish Diseases, Department of Fisheries	DOF
	• Technical Committee in Import Risk Analysis (IRA) for IAS, Department of Fisheries Malaysia	DOF
	• 'Jawatankuasa Pemutus Sebutharga' Fisheries Research Institute Batu Maung	FRI/DOF
	• Technical Committee in Organizing 11th International Symposium on Diseases in Asian Aquaculture (DAA11)	FRI/DOF/Country
	• Technical Working Group 1 (TWG1), Awareness and education, National Action Plan of AMR, DOF Malaysia	DOF
<b>Rohaiza Asmini Yahya</b>	• Technical Committee of Laboratory Parasitology Analysis and Histopatology	FRI
	• 'Jawatankuasa Pembuka Sebutharga FRIBM'	DOF
	• 'Jawatankuasa Penerimaan Barang, NaFish'	FRI
	• Officer in-charge of 'Store Verification asset' at FRIBM	FRI
	• Technical Committee in Organizing 11th International Symposium on Diseases in Asian Aquaculture (DAA11)	FRI/DOF/Country
<b>Afzan Muntaziana Mohd. Pazai</b>	• Technical Committee in Organizing 11th International Symposium on Diseases in Asian Aquaculture (DAA11)	FRI/DOF/Country
	• Sport and Recreation Club, FRI, Pulau Pinang (KESURI). <i>Member Club No.: PPM-003-07-02071991</i>	DOF
	• 'Jawatankuasa Teknikal Sebutharga' FRIBM	FRI/DOF
	• 'Jawatankuasa Aset Tak Alih dan Aset Alih Kerajaan'	FRI/DOF
	• Development Officer 'Pegawai Pembangunan' NaFish	FRI/DOF
	• Officer in-charge of 'Store Verification asset' at FRIBM	FRI
	• Sensory Analysis Panel FRIBM	FRI
	• Coordinator Officer KRISTE.MY 2.0 System (2021-2023)	DOF

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Name of Officers	Citations	Reads	Research Interest Score	Full text read	H-index
Dr. Kua Beng Chu	73	2,055	298.1	660	12
Dr. Azila Abdullah	36	2747	191.4	751	8
Dr. Rimatulhana Ramly	20	1135	85.5	449	3
Dr. Padilah Bakar	5	665	28.5	162	3
Cik Rohaiza Asmini Yahya	0	0	0	0	0
Cik Noor Hanis Abu Halim	23	1,595	68.8	120	3
Afzan Muntaziana Mohd. Pazai	33.23	2591	64.7	41.73	5

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The background features a close-up, reddish-brown image of a fish, likely a salmon, with its scales and fins visible. A white hexagonal grid pattern is overlaid on the image, particularly prominent in the top-left and bottom-right corners.

# **ANNUAL REPORT 2021**

## **RESEARCH AND DEVELOPMENT PROGRAM ON FISH HEALTH IN AQUACULTURE**

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