Promotion of sustainable, export oriented, shrimp (*Penaeus monodon*) culture by disease prevention compliance to food safety regulations

Department of Zoology and Environmental Management

Faculty of Science

University of Kelaniya

Higher Education for the Twenty First Century (HETC) Project-QIG Window 3

Ministry of Higher Education

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Objectives of the present study

- **To investigate what are the bio security measures and better management practices (BMPs) followed by Sri Lankan hatchery and grow-out managements in their black tiger shrimp, *Penaeus monodon*, production systems presently.**

- **To identify critical bio security measures and BMPs that should be adopted to prevent the entry and spread of virulent pathogens in each system.**

- **To investigate whether there are areas in Sri Lankan coastal waters from where WSV & MBV free matured black tiger shrimp, *Penaeus monodon* could be collected.**

- **To find out whether improved nutrition (by addition of DHA & EPA in correct proportion to the brood stock diet) could increase the number of successful spawning that could be obtained from WSV and MBV negative female brood stocks.**

- **To produce "SPF" post larvae using selected, WSV and MBV negative brood stocks of black tiger shrimp while employing critical, bio-security measures and BMPs strictly.**

- **To rear "SPF" post larvae in grow-out ponds under critical, bio-security measures and BMPs to prevent occurrence of WSV, MBV disease and vibriosis in juvenile shrimp during the grow-out production cycle.**

- **To investigate whether marketable size black tiger shrimp that are harvested are free of human pathogenic bacteria such as *Salmonella* sp & *Vibrio parahaemolyticus* and free of antibiotic residues.**

Experiment 1

Regular monitoring and controlling *Vibrio* - a critical bio-security measure for Sri Lankan shrimp (*Penaeus manodon*) hatcheries

Introduction

Questionnaire survey carried out initially revealed that

Inadequacy of adopting certain BMPs and bio-security measures contribute for increased pathogenic *Vibrio* populations in water

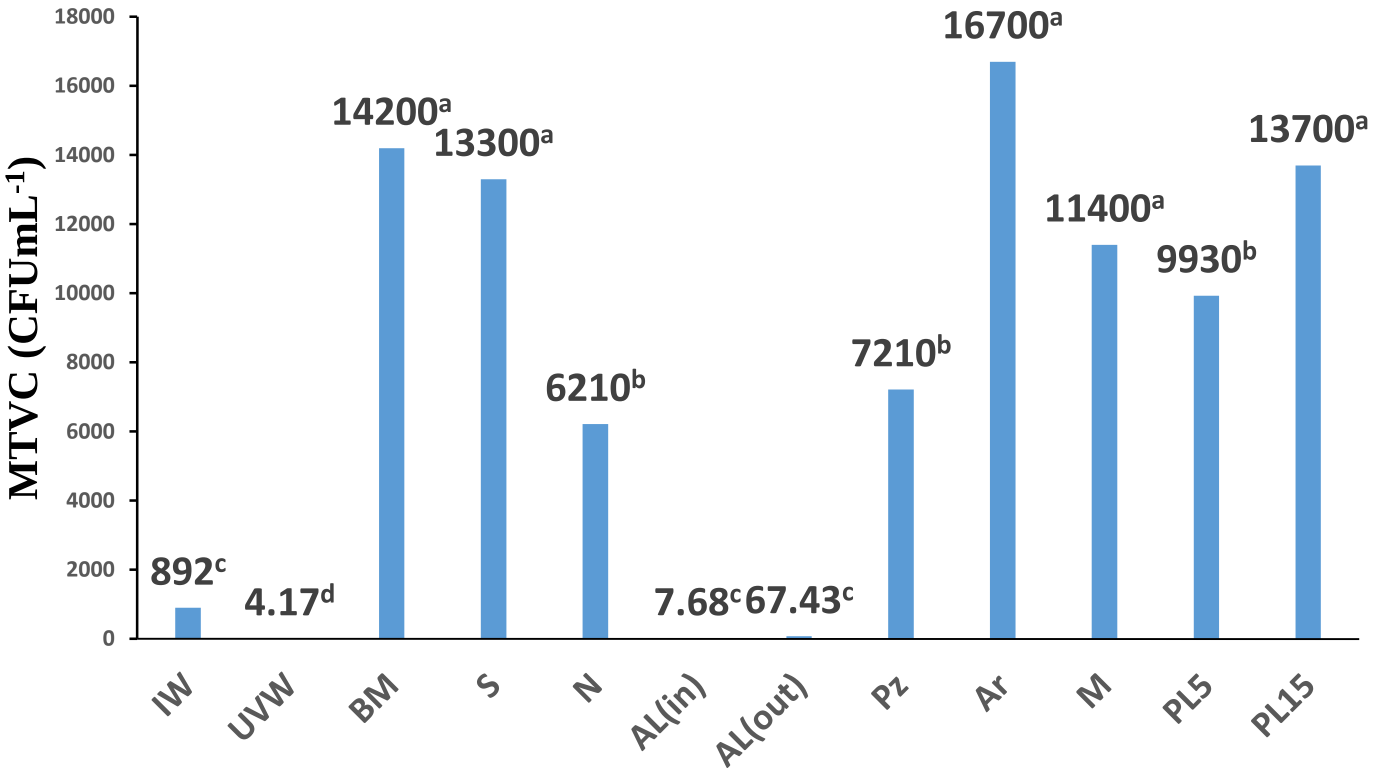
leading to high mortalities of *Penaeus monodon* larvae reared in commercial hatcheries of Northwestern Province, Sri Lanka

Research carried out…

- As a critical bio security measure, the possible sources of *Vibrio* contamination were investigated by determining the total *Vibrio* count (from randomly selected 20 shrimp hatcheries)
- *Vibrio* count in rearing water and mortality of different larval stages were studied over two production cycles to see whether there is a relationship between *Vibrio* count and mortality of different larval stages

- ***Vibrio*** **colonies with different morphological appearance were isolated and identified using API - 20E test strips**
- **The virulency of those identified species of *Vibrio*, on mysis larvae and on twelve days old post larvae (Pl_{12}) was investigated by challenge experiments**
- **Antibiotic sensitivity tests were performed for each species of *Vibrio***

Results



IW- Incoming water, UVW- UV treated water, BM- Maturation, S- spawning tanks
N- Nauplii tanks. PZ – Protozoea tanks, My- Mysis tanks, PL- Post larvae.
AL-I, indoor algae tanks ,Algae-out, out door algae tanks Ar- *Artemia* hatching tanks

Figure 1.Mean total *Vibrio* count(CFUmL^{-1}) in water of different culture tanks in randomly selected twenty shrimp hatcheries

Conclusions

- **There was a positive relationship between total *Vibrio* count in rearing water and mortality of larvae**
- **When the total *Vibrio* count reached 1.7×10^4 $CFUmL^{-1}$ heavy larval mortalities occurred.**
- **The major sources of pathogenic *Vibrio* contamination in shrimp hatcheries are**
  - **Brood stocks of shrimp**
  - ***Artemia* nauplii**

- **The major pathogenic *Vibrio* (cause vibriosis) isolated from larval rearing tanks were**
- ***V. fluvialis,***
- ***V. alginolyticus,***
- ***V. vulnificus,***
- ***V. hrvayei and***
- ***V. parahaemolyticus***

Monitoring and & controlling *Vibrio* populations is a critical biosecurity measure

Experiment 2

Prevention/control of vibriosis in shrimp larvae using a locally produced probiotic/bioremediater

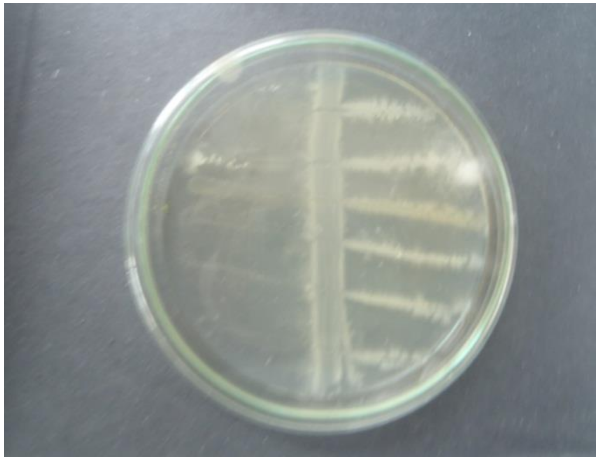
Introduction

- **Previous study revealed that in Sri Lankan shrimp hatcheries 8% to 48% mortality occurs from fertilized eggs to post larva due to vibriosis**

- **Brood stocks and *Artemia* nauplii (non disinfected) seemed to be the major sources of *Vibrio* contamination**

Objectives

- **To investigate whether WSV, MBV and *Vibrio* free good quality Pl could be produced**
  - **employing critical bio security measures and**
  - **using a locally produced probiotic/bioremediater that contain, *Bacillus subtillis* as better management practice**



After 48 hours

Plate 1. Inhibitory activity of *Bacillus subtillis* against 5 *Vibrio* species, by the cross-streak method.

V1:*V. alinolyticus* (type:1), V2: *V. vulnificus*, V3:*V. parahaemolyticus*
V4:*V. alginolyticus* (type:2), V5:*V. fluvialis*, V:*V harveyi*

Conclusions

- **Good quality post larvae could be produced using a suitable probiotic/bioremediater, *Bacillus subtillis* (a BMP) to control pathogenic *Vibrio* sp in culture water, instead of using broad spectrum antibiotics**
- **Vertical transmission of pathogenic *Vibrio* could be prevented by disinfection of eggs and nauplli using correct concentrations of disinfectants with correct exposure time**

- **Horizontal transmission of pathogenic *Vibrio* could be prevented by disinfecting *Artemia* nauplii before feeding mysis and post larvae**
- **Antibiotics also could control mortality of larvae but some Pl were positive for *Vibrio* and quality and survival of post larvae were low compared to the post larvae reared with the probiotic/bioremediater treatment**

Experiment: 3

Isolation and identification of *Vibrio* species that cause white feces disease in cultured *Penaeus monodon* (black tiger shrimp) with methods to prevent/control

Introduction

- **Since 2010, a significant part of the harvested cultured shrimp (*Penaeus monodon*) was rejected by processors due to loose shell condition.**

- **Some shrimp farmers harvested the shrimp for local market before completing the production cycle**

Objectives

- **To investigate the occurrence of WFS in grow-out ponds**
- **To isolate and identify the causative pathogenic *Vibrio* species**
- **To find out the combined effect of**

1. proper disinfection of culture water (a bio security measure) and BMPs

2. zero water exchange and

3. the use of locally produced probiotic/bioremediater containing *Bacillus subtilis*

Results

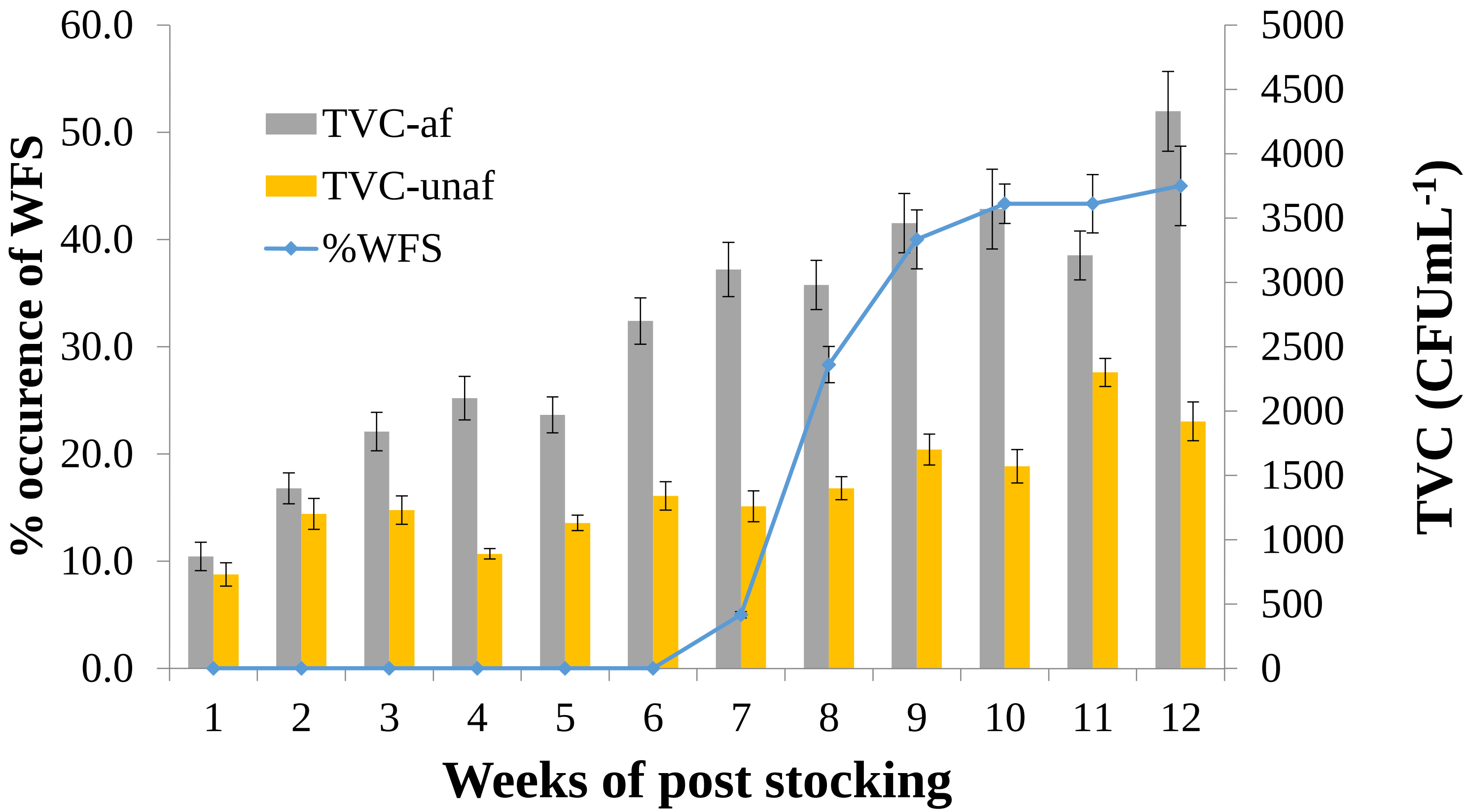
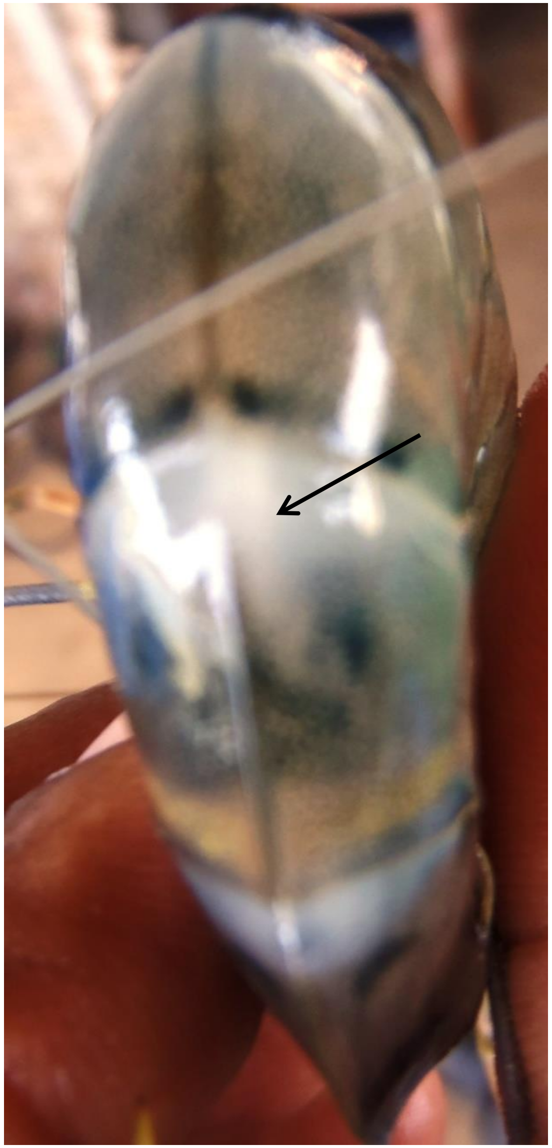


Figure 1 The mean total *Vibrio* count (TVC) in culture water and occurrence of WFS over one production cycle from randomly selected 60 grow-out ponds (data are provided as mean ± standard error)

TVC-af : total *Vibrio* count in WFS affected ponds, TVC-unaf,: total *Vibrio* count in unaffected ponds, % WFS: percentage occurrence of WFS

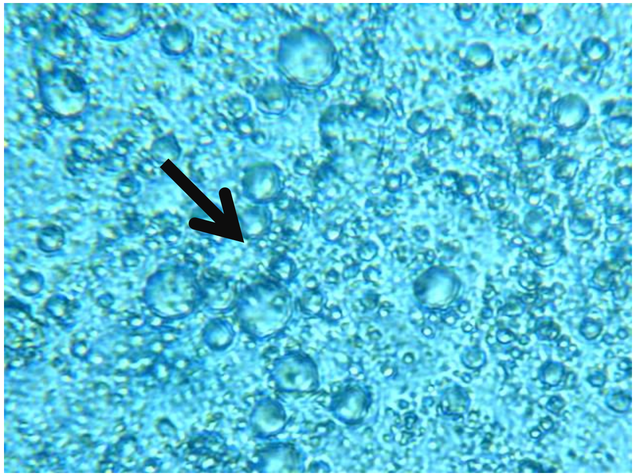


White color gut in infected shrimp

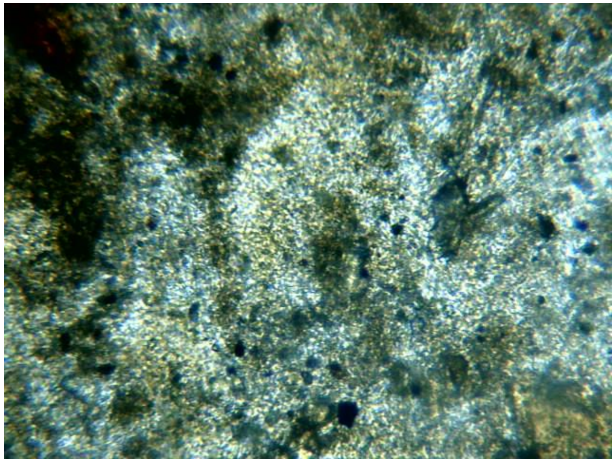
Strings of white faeces floating on the water surface

Hepatopancreas and gut from WFD infected shrimp

Hepatopancreas and gut from uninfected shrimp



Lipid droplets in the gut contents of the WFD infected shrimp



Gut contents of the uninfected shrimp

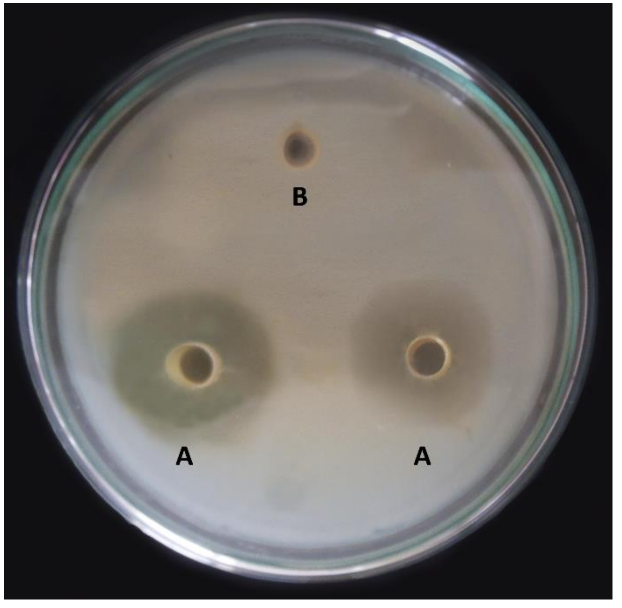


Plate: X

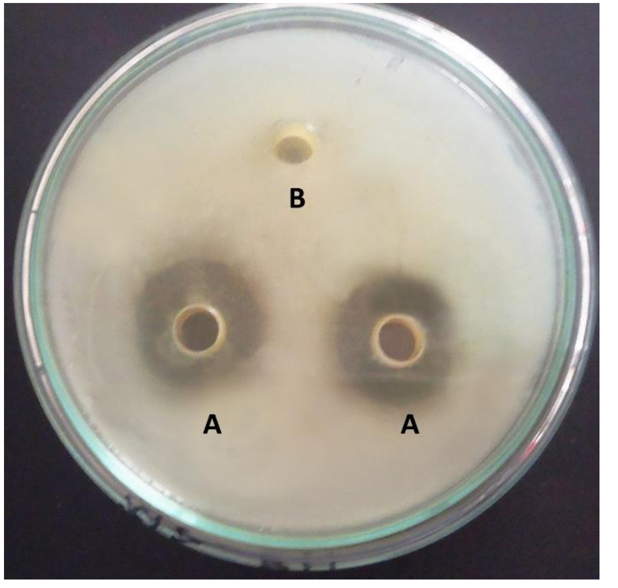


Plate: Y

Plate X Inhibitory zones (A) produced by cell free extract of *Bacillus subtilis* on a lawn of *V. alginolyticus*

Plate Y Inhibitory zones (A) produced by cell free extract of *Bacillus subtilis* on a lawn of *V. fluvialis*

Conclusions

- **The *Vibrio* species that cause "WFD" in cultured black tiger shrimp in Northwestern province, are *V. alginolyticus* and V. *fluviails***

- **Out of two *Vibrio* species that contribute for the development of "WFD", <u>the major pathogen is *V. alginolyticus*</u>**

- **Disinfection of culture water**
- **Zero water exchange**
- **Regular application of *Bacillus subtillis* to culture water as a bioremediotor**
- **and incorporation of *Bacillus subtilius* to feed as a probiotic**

could prevent the occurrence of white feces disease in cultured shrimp in Northwestern province, Sri Lanka

Experiment: 04

Different isolates of *Bacillus subtilis* from gastrointestinal tract of wild caught black tiger shrimp, *Penaeus monodon* to improve a locally produced probiotic/bioaugmenter for controlling pathogenic *Vibrio* in Sri Lankan shrimp culture systems

Our previous studies have confirmed that

- **A locally produced probiotic/bioaugmenter (containing locally isolated strain of *Bacillus subtilis*) could contribute significantly in**

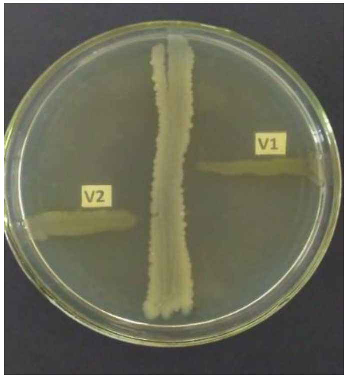
  production of healthy post larvae of *P. monodon* and

- **in production of healthy marketable size shrimp in grow-out ponds (Hettiarachchi et al., 2013; Hettiarachchi et al., 2014)**

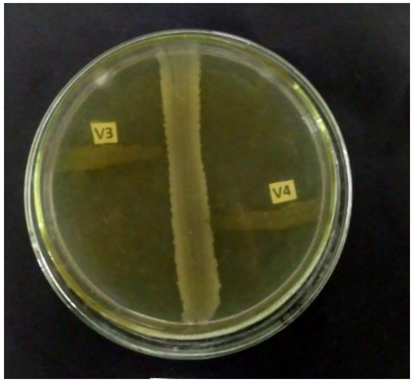
Objectives

- **To isolate and identify different strains of *Bacillus subtilis* from the gastrointestinal tract of wild *P. monodon* collected from estuaries of Northwestern province**

- **To investigate the antagonistic properties of isolated different strains of *Bacillus subtilis* on pathogenic *Vibrio* species (that were isolated from cultured shrimp)**



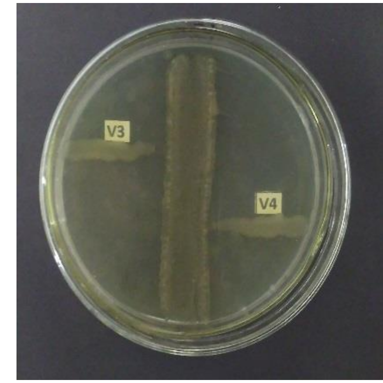
B12



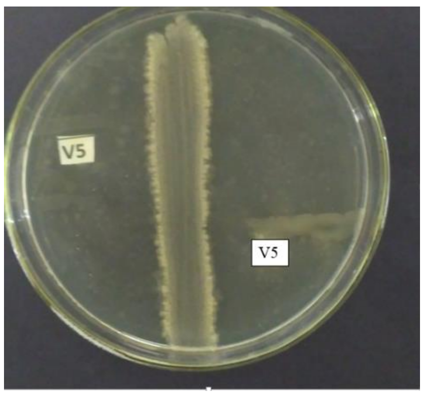
B12

B12

B13



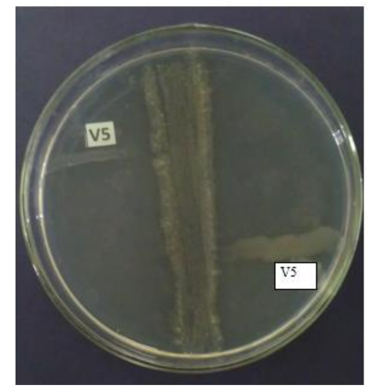
B13



B13

B15

B15



B15

Inhibition activity of 3 selected strains of *Bacillus subtilis* against pathogenic *Vibrio* species (cross streak method; Monthon, et al., 2008)

V1: *V. alginolyticus* (type-1) V2: *V. alginolyticus* (type-2), V3: *V. parahaemolyticus* (type-1) V4: *V. Parahaemolyticus* (type-2) , V5: *V. harveyi*

Conclusions

- **Eight strains of *Bacillus subtilis* were isolated from gastrointestinal tract of wild *P. monodon* collected from different estuaries located in the North Western Province**
- **Out of those eight strains of *Bacillus subtilis*, isolates B12, B13 and B15 showed the**
  - **Widest range of salt tolerance**
  - **Widest range of pH tolerance**
  - **Highest antagonistic activity against known pathogenic *Vibrio* species (that had been isolated from cultured *P. monodon*)**

Conclusions

- **Those three strains of *Bacillus subtilis* could be used to improve the locally produced probiotic/bioaugmenter for shrimp culture in Sri Lanka**

Expreiment:05

Reproductive performances of brood stocks of black tiger shrimp, *Penaeus monodon* when docosa hexaenoic acid (DHA) and eicosa pentaenoic acid (EPA) are added to the maturation diet

Objectives

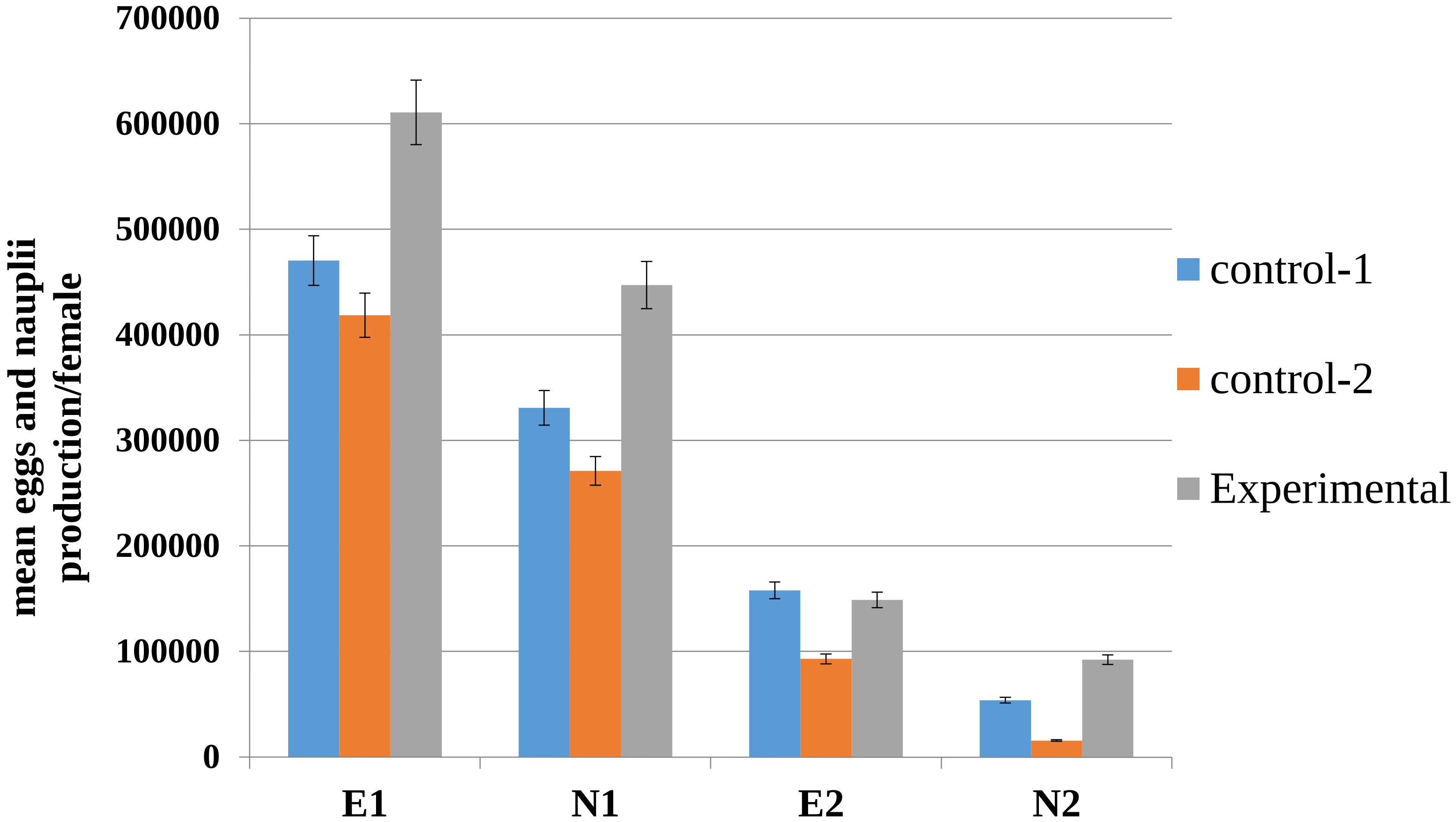
To find out whether improved nutrition, by addition of docosa hexaenoic acid (DHA) & eicosa pentaenoic acid (EPA) to the maturation diet, could improve reproductive performances of black tiger shrimp, *Penaeus monodon*

CD1

CD2

ED

CD1: Control diet -1 (normal feed), CD2 : Formulated feed for Control diet -2, ED: Formulated feed for experimental diet



E1 - eggs production during 1st month, N1 – nauplii production during 1st month, E2 - eggs production during 2nd month, N2 – nauplii production during 2nd month, vertical bars indicate SE of the mean

Figure 03. Mean total eggs and healthy nauplii production per female *Penaeus monodon* fed on experimental and control diets at 1st (during first 3-4 spawns) and 2nd (during 5-8 spawns) months of maturation period

Conclusions

- **Substitution of 50% of the normal feed of brood shrimp (squid flesh & beef liver) with the experimental feed containing additional amount of DHA & EPA could significantly increase**
  - **maturation rate**
  - **spawning rate**
  - **fertilization rate**
  - **hatching rate and**
  - **production of healthy nuaplii**

- **The brood stock that received DHA and EPA could produce healthy active nauplii for an extended period compared to the shrimp in control groups**

Presently working on

Identification of disease-resistant stock of black tiger shrimp, *Penaeus monodon* in Sri Lankan coastal sea

Using Microsatellite DNA marker

- **Both 317 bp microsatellite DNA marker and 457 bp RAPD- SCAR marker were employed to identify WSV susceptible and disease resistant stocks of wild *P. monodon* in Sri Lankan coastal sea.**
- **After amplification by polymerase chain reaction, they provided a highly statistically significant DNA fingerprint of 317 bp (Scar marker-1) and 457 bp (Scare marker-2) only in disease resistant populations but not in disease susceptible shrimp populations**

This is the first identification of the presence of WSV resistant gene in wild black tiger shrimp (*P. monodon*) stocks in coastal sea of Sri Lanka using 317 bp and 457 bp microsatellites RAPD-SCAR DNA markers

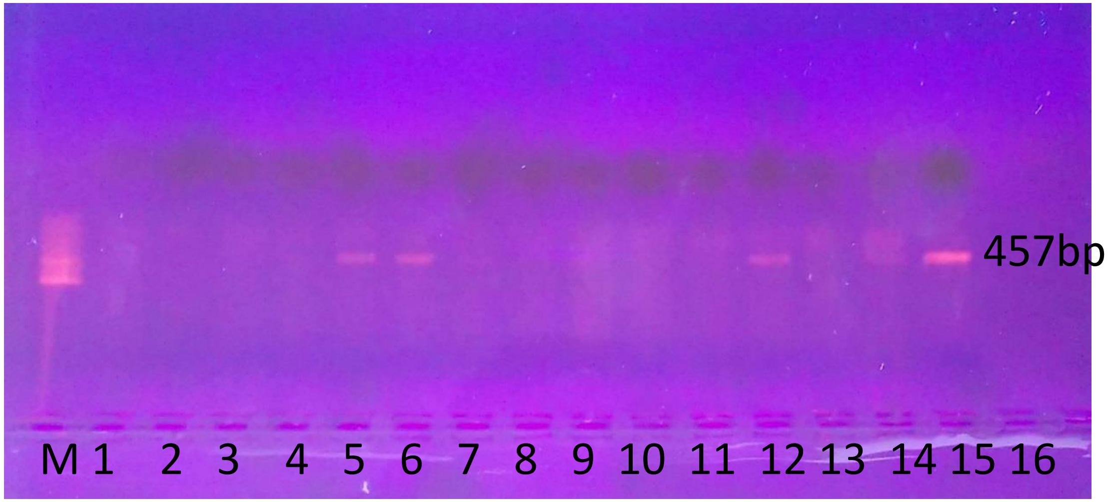


Figure 1. Scar primer -1, after PCR amplification generated 457bp DNA bands in WSV resistant *P. monodon* which was absent in WSV susceptible shrimp

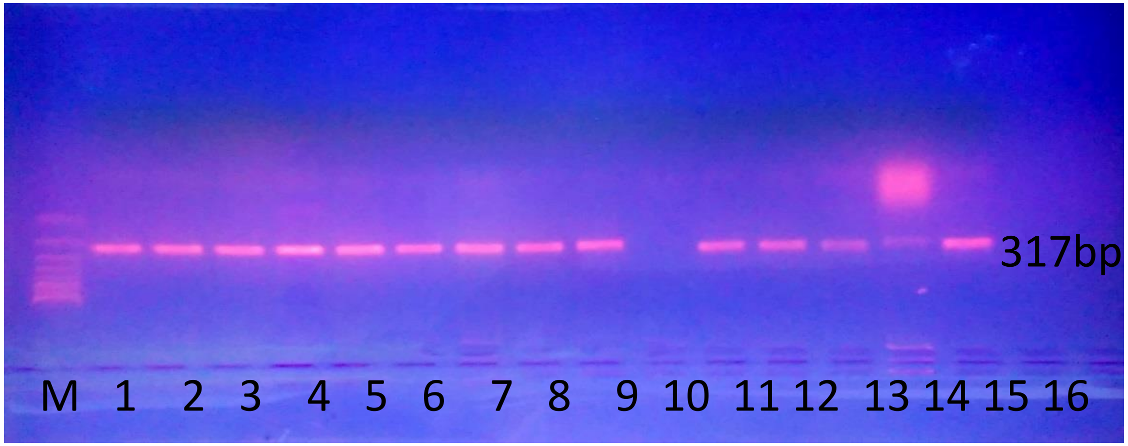


Figure2. Scar primer -2, after PCR amplification produced 317bp in WSV resistant *P. monodon* which was absent in WSV susceptible shrimp.

Acknowledgement

- To HETC QIG Window 3 Grants, Ministry of Higher Education
- Faculty of Science, University of Kelaniya
- Supervisors of the Research Project
- King Aqua services Pvt Ltd
- Microbtek Lab Holdings, Dalugama, Kelaniya

Thank you