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Organization of the
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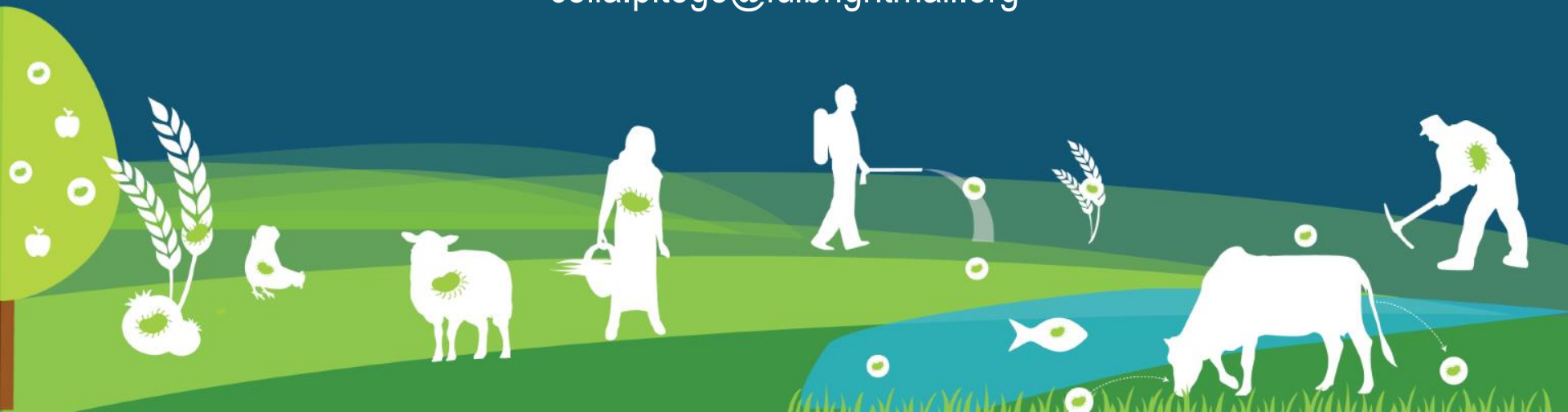
FMM/RAS/298: Strengthening capacities, policies and national action plans on
prudent and responsible use of antimicrobials in fisheries Final Workshop
in cooperation with AVA Singapore and INFOFISH

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Best Practices in Shrimp Aquaculture

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WORKING GROUP 2 MEMBERS - Putra Jaya, Malaysia



GROUP 2

PRODUCTION CYCLE STAGE

Broodstock

SPECIFIC HAZARD

on/through let former know about advantages and disadvantages

SPF/SPR/SPT STOCKS

Live Feed

Supply/Source

Pathogens carrier

Alternative to live feed

Home grown / domestic

HEALTH STATUS SPF

PREVENTATIVE MEASURES

CA APPROVED SOURCE / ACCREDITATION OF SUPPLIER

Health Certificate

ENSURE HEALTH STATUS OF PL

COA / SQA

CA Approved & Registered

Genetic inventory

ENVIRONMENTAL CHANGES

LATER & QUALITY (LAWSONIA) RESISTANT PLANT USE

awareness campaign through IEC materials

use of chemical drug

FEED QUALITY Food Quality assurance from

Validation of preventive measures

AUDIT

SAMPLING & ANALYSIS

Quarantine measures

PL quality criteria

Farm Standard (GAP, BAP)

BIOSECURITY MEASURES

Disinfected Water

Disease Occurrence

GAP handling Artemia

GOOD quality PL / Packaging

TECHNICAL ASSISTANCE

Small Scale Farm Biosecurity

Farmer Organization (Cluster of farmer) (For small scale)

HUSBANDRY/FARM MANAGEMENT

Prudent use of Antibiotic

GAP

Let the hatchery know responsible use of am.

Vehicle

Vectors

Farmer should have production goal/target

second breeding season

Indicator of good production - survival rate - uniformity in size - appearance

water discharge

water disposal

inform cluster members

information material about proper disposal of water & dead larvae

HATCHERY

NO TESTING

Disease Risk

PL Quality

NURSERY GROW-OUT

Problem in timely disease recognition

Strains with high genetic diversity being cultured

Sampling Procedures taken care of appropriate sample

technical assistance

training Diseases recognition card/manual & poster / floor

Additional Sources of Information:

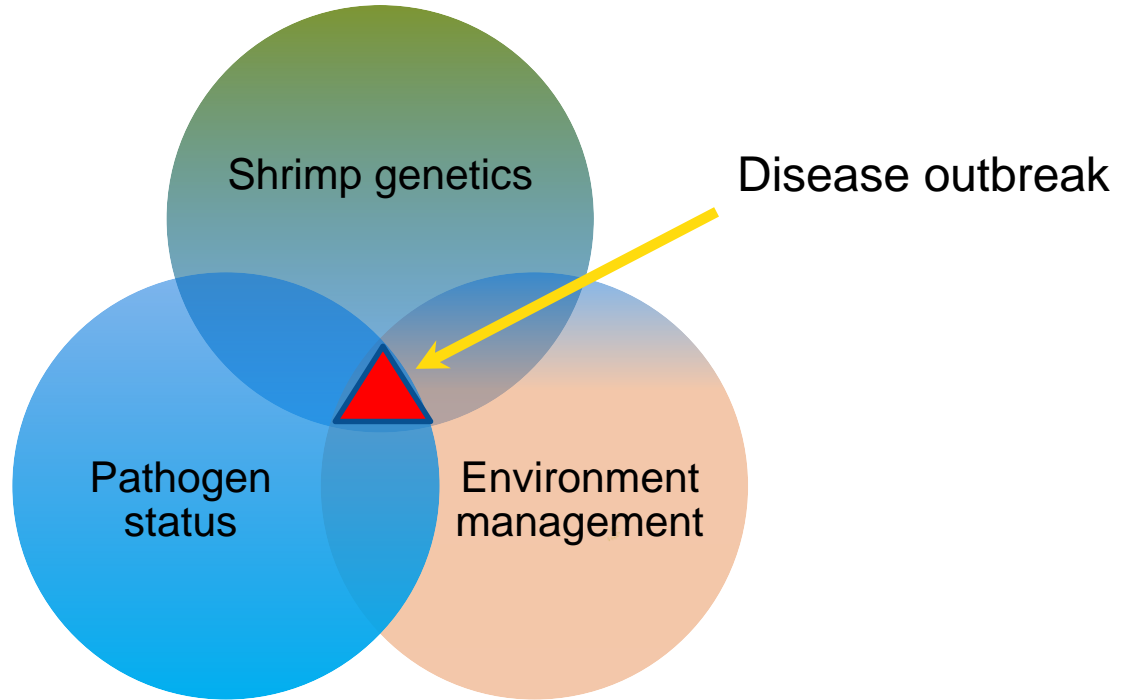
- Shrimp production matrix (tabulated guide in Putra Jaya for WG 2)
- Collated information from Dr Victoria Alday
- Manuals and publications

All phases of shrimp culture can aim for:

**GOOD BIOSECURITY = NO DISEASE = NO ANTIBIOTIC USE =
HEALTHY HARVEST = GOOD PROFIT**



Modified Snieszko circle



Definition of Biosecurity

(from FAO Monodon Manual section 2.5 page 15)

“...sets of practices that will reduce the probability of a pathogen introduction and its subsequent spread from one place to another...”. Biosecurity protocols are intended to maintain the “security” of a facility with respect to certain disease-causing organisms that may not already be present in a particular system. Biosecurity encompasses policy, regulatory and programme frameworks (including instruments and activities) in response to managing risks associated with diseases.

(from Omar's PJ presentation)

“Strategic and integrated approach that encompasses the policy and regulatory frameworks (including instruments and activities) that analyse and manage risks in the sectors of food safety, animal life and health, and plant life and health, including associated environmental risks.”

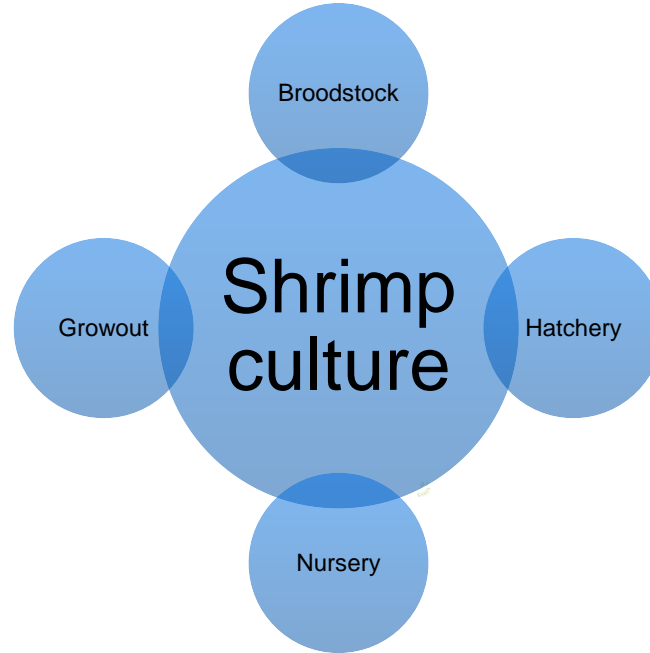
Main risk pathways for pests and diseases to spread on to, within and from a farm



Biosecurity needs in the life of shrimp

- There are critical points in the production process that can permit biological hazards.
- Spotting these needs and providing proper biosecurity throughout the shrimps's life stages is vital and difficult.

Illustrate the production cycle and the different components of each (sample below)

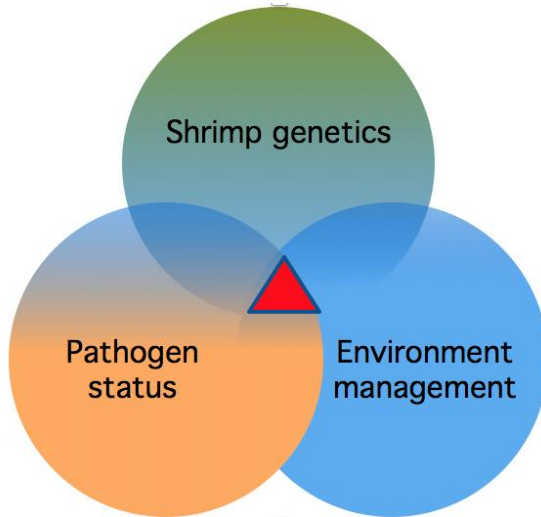


Each of these components would require:

1. Clean water
2. Clean rearing facilities
3. Clean feed
4. Hygienic protocols
5. **Dry out and break cycle**



Site Selection and Environment



- ❖ Site selection
 - Far from incursions
 - Clean water
- ❖ Facility design
 - Modular
 - Reservoir
 - Effluent containment
- ❖ Hygiene
 - Siphon out dead shrimp
 - Waste management of treated water



❖ Choose right shrimp genetics

- tolerant shrimp for low biosecurity
- SPF & high performers for high biosecurity systems

❖ Keep pathogens out!

- Dry out facilities regularly
- Disinfect water
- Feeds
- Prevent air & droplets contamination

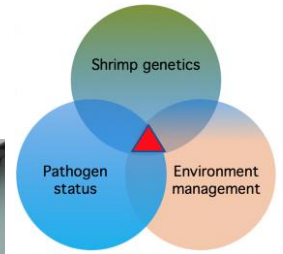
❖ Limit visitors (vehicles, humans, birds, other carriers)

❖ Install hand-wash stations, foot baths, and wheel washes or tyre baths

❖ Put up warning signs

❖ Change footwear/ wear boots

BROODSTOCK



Broodstock

Biosecurity in shrimp aquaculture starts with clean broodstock that supply eggs and nauplii for hatcheries.

- Certification of broodstock health status is supported by disease surveillance records, audit records of rearing facilities, and on-growing protocols, including feeds and feeding.
- Specific pathogen free (SPF) broodstock are guaranteed free of specific diseases through periodic testing of shrimp and their rearing facilities

A female broodstock (gravid female) exhibits an ovary filled with mature eggs that runs from the head down to the tail (white structure as illustrated (from Bell and Lightner 1988))



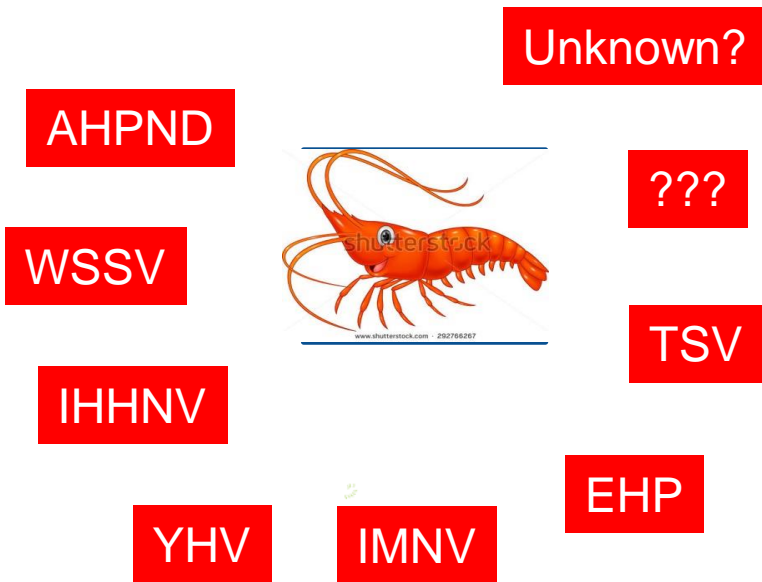
Sources and Kinds of Broodstock

1. Non-SPF and locally-sourced
 - a. Wild-caught
 - b. Home-grown from grow-out ponds
2. Imported specific pathogen free (SPF) stocks
 - a. Direct importation from Hawaii and the US
 - b. Multiplication centers within countries

Regardless of source, new broodstock should be **quarantined**

Newly-acquired broodstock should be kept separate or quarantined upon arrival and should not be mixed with existing stocks in the facility until their health status is ascertained.

Non-SPF broodstock have **unknown** health status and may have been exposed to known and unknown pathogens



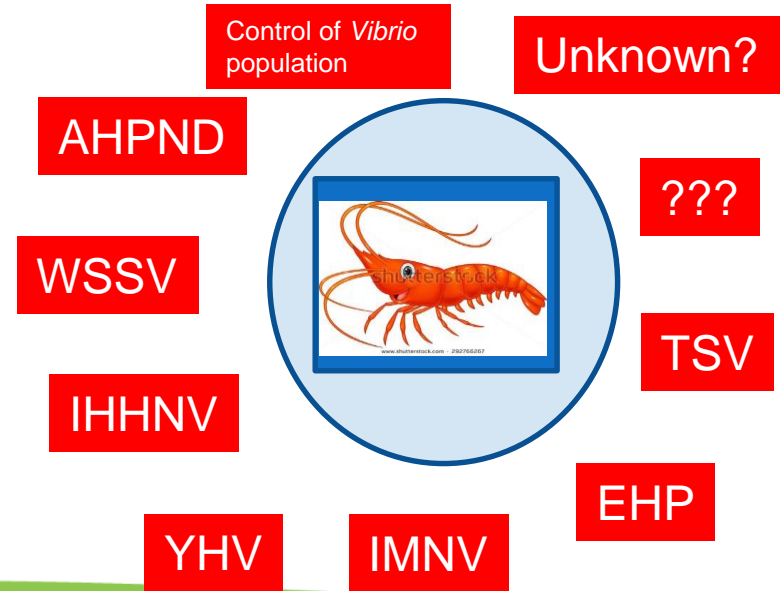
Sources and Kinds of Broodstock

1. Locally-sourced
 - a. Wild-caught
 - b. Home-grown from grow-out ponds
2. Specific Pathogen Free (SPF) stocks
 - a. Directly imported from Hawaii, Florida, Singapore and other countries
 - b. Produced in multiplication centers within countries

Quarantine of new broodstock

Newly-acquired broodstock should be kept separate or quarantined upon arrival and should not be mixed with existing stocks in the facility until their health status is ascertained.

SPF broodstock have known health status and are certified free from certain known pathogens. However, freedom from pathogens is merely through physical barriers and strict biosecurity of their closed holding systems. Thus, SPF status is temporary and dependent upon provision of similar level of biosecurity of the new facility, in case of transfer.

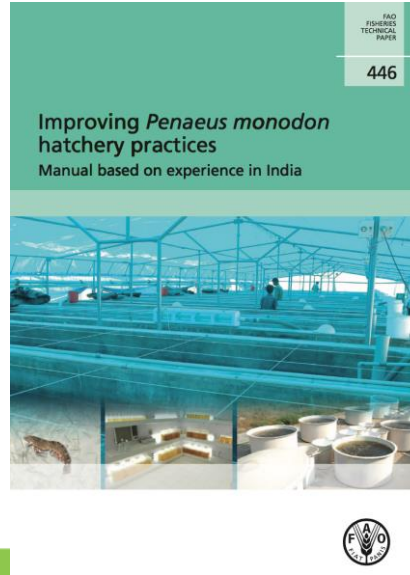


Caring for Broodstock

Requirements:

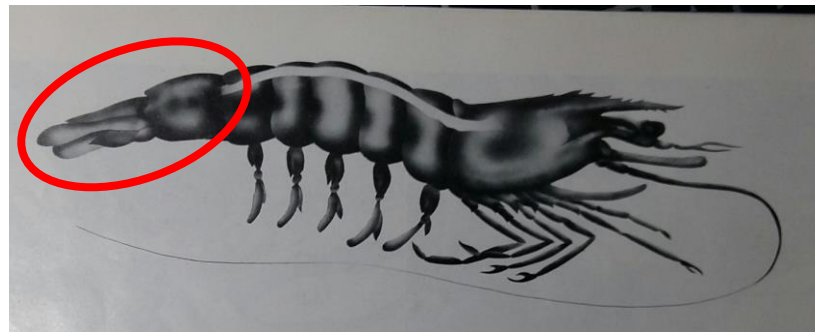
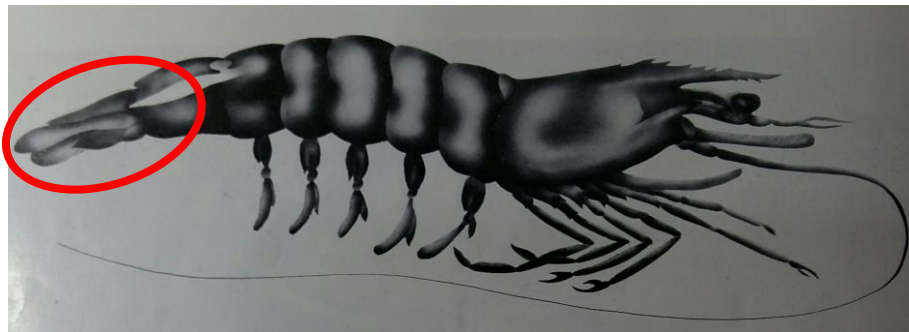
1. Clean water
2. Clean rearing facilities
3. Clean feed
4. Hygienic protocols

Refer to illustrations from *P. monodon* (FAO Fisheries Technical Paper 446) and *P. vannamei* (FAO Fisheries Technical Paper 450) manuals



Broodstock

A female broodstock releases fecal material during spawning. The feces contains gut-associated bacteria, many of which are opportunistic vibrios, as well as gut-infecting parasites and viruses. A management practice or device that effectively prevents the release of the spawner's fecal material into the spawning water would drastically prevent opportunistic bacterial infection in larvae.

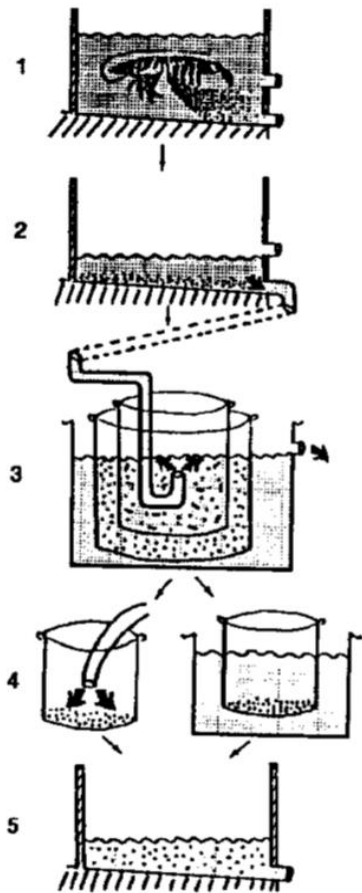


It is important to collect and wash eggs with care to prevent bacterial build-up on their surfaces. Alternatively, nauplii should be collected using their phototactic response taking very little of their hatching water into the new larval rearing tank (photos from Bell and Lightner 1988)

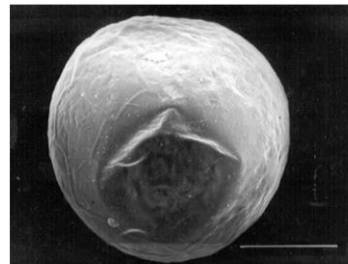


Steps in shrimp egg-washing

It is also important to collect eggs and wash them with care to prevent bacterial build-up on their surfaces (biofilm formation). Egg washing technique was found very effective in preventing Baculovirus midgut gland necrosis infection in *Penaeus japonicus* hatcheries, eradicating the disease almost completely. This technique will also prevent gut-associated infectious bacteria.



Sources of *Vibrio harveyi* in *Penaeus monodon* hatcheries: Spawners release fecal material in spawning water; adherence resulted to **biofilm** formation on egg surface



Penaeus monodon egg with bacterial plaques on the surface



The bacterial plaques are composed of actively dividing cells similar to *Vibrio harveyi*

Alternatively, nauplii could be collected using their phototactic response taking very little of their hatching water into the new larval rearing tank (drawing from Momoyama and Sano (1992))



Take Home Messages Broodstock and Hatchery

1. Broodstock source is important
2. The health status of the broodstock should be certified by the competent authority of the exporting country.
3. Purchase from accredited sources with good track record only
4. Always separate new stocks from old stocks; quarantine
5. Provide hygienic and biosecure facilities for broodstock
6. Some diseases originate from female spawners necessitating re-evaluation of spawning techniques and hatchery management
7. Husbandry techniques in hatcheries could influence the associated bacterial flora of postlarvae
8. Keep up-to-date records



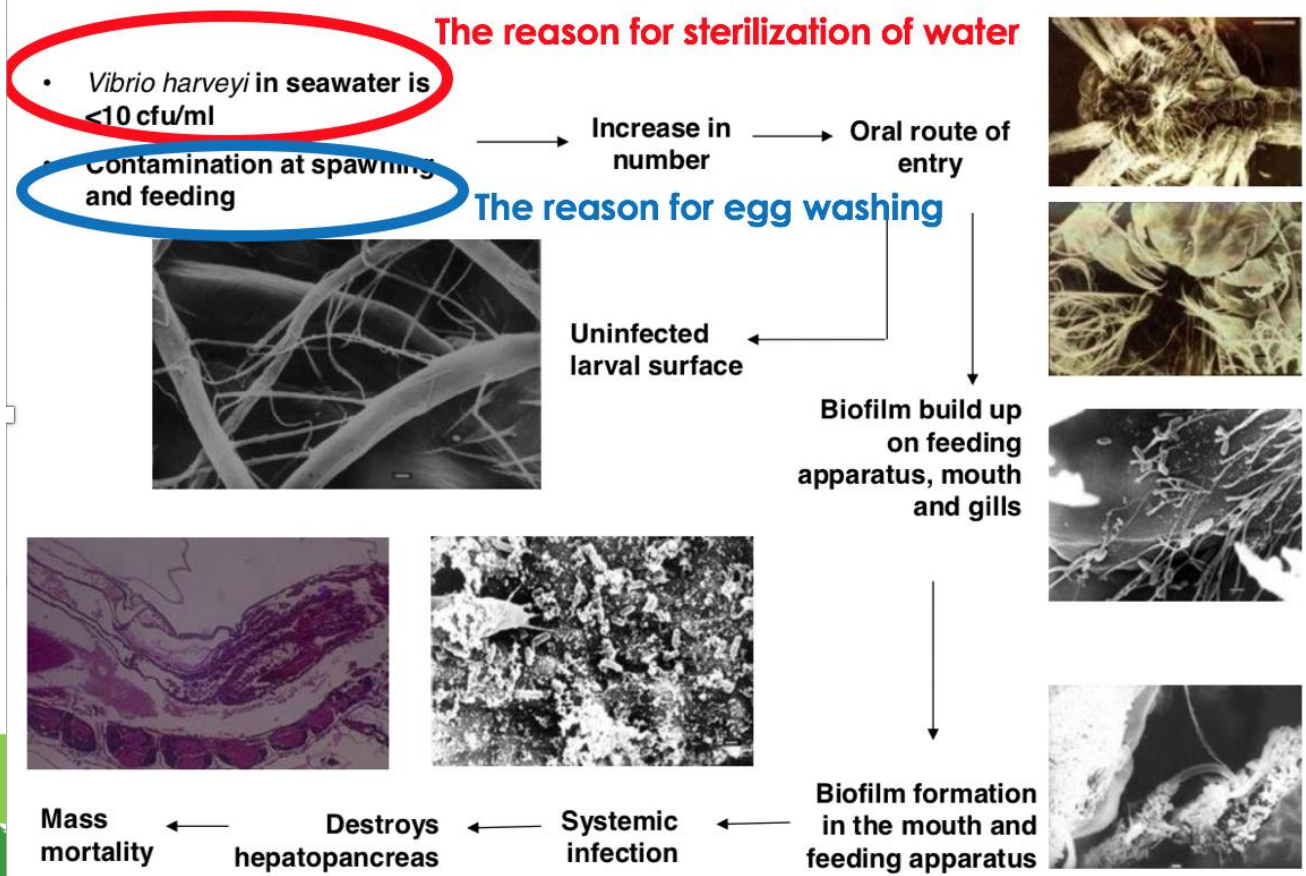
Hatchery: avoid prophylactic use of antibiotics

Comparison of luminescent bacterial load of hatchery-reared and wild-caught postlarvae

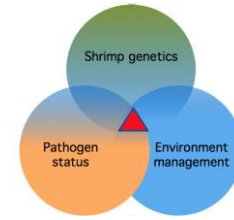
Stage	Number of Batches Examined	Batches Negative for Luminescent Bacteria (%)	Range of Associated Luminescent Bacteria
Hatchery-reared postlarvae = 272 batches			
PL 12	97	59 (61)	$5.0 \times 10^0 - 1.3 \times 10^5$
PL 13	36	12 (33.3)	$2.5 \times 10^0 - 8.9 \times 10^4$
PL 14	25	11 (44)	$5.0 \times 10^0 - 2.5 \times 10^4$
PL 15	37	11 (30)	$7.0 \times 10^1 - 1.7 \times 10^4$
PL 16	18	6 (33.3)	$5.0 \times 10^0 - 9.9 \times 10^4$
PL 17	28	9 (32)	$5.0 \times 10^0 - 3.0 \times 10^5$
PL 18	31	7 (23)	$2.0 \times 10^2 - 4.0 \times 10^4$
Wild-caught PLs	31	18 (58)	$5.0 \times 10^0 - 3.5 \times 10^2$

Hatchery: monitor bacterial population in rearing water using TCBS

Fate of *Penaeus monodon* larvae during *Vibrio harveyi* infection



Hatchery rearing water management



Incoming water disinfection:

- Ozone or
- Sand filter (washing and disinfection of sand every 6 months with 200ppm calcium hypochlorite)

- Calcium hypochlorite 30ppm

- Validation of water treatment: microbiology (TCBS no green colonies) (PHOTOS)

- Addition of probiotics to disinfected water prior stocking to mature the water

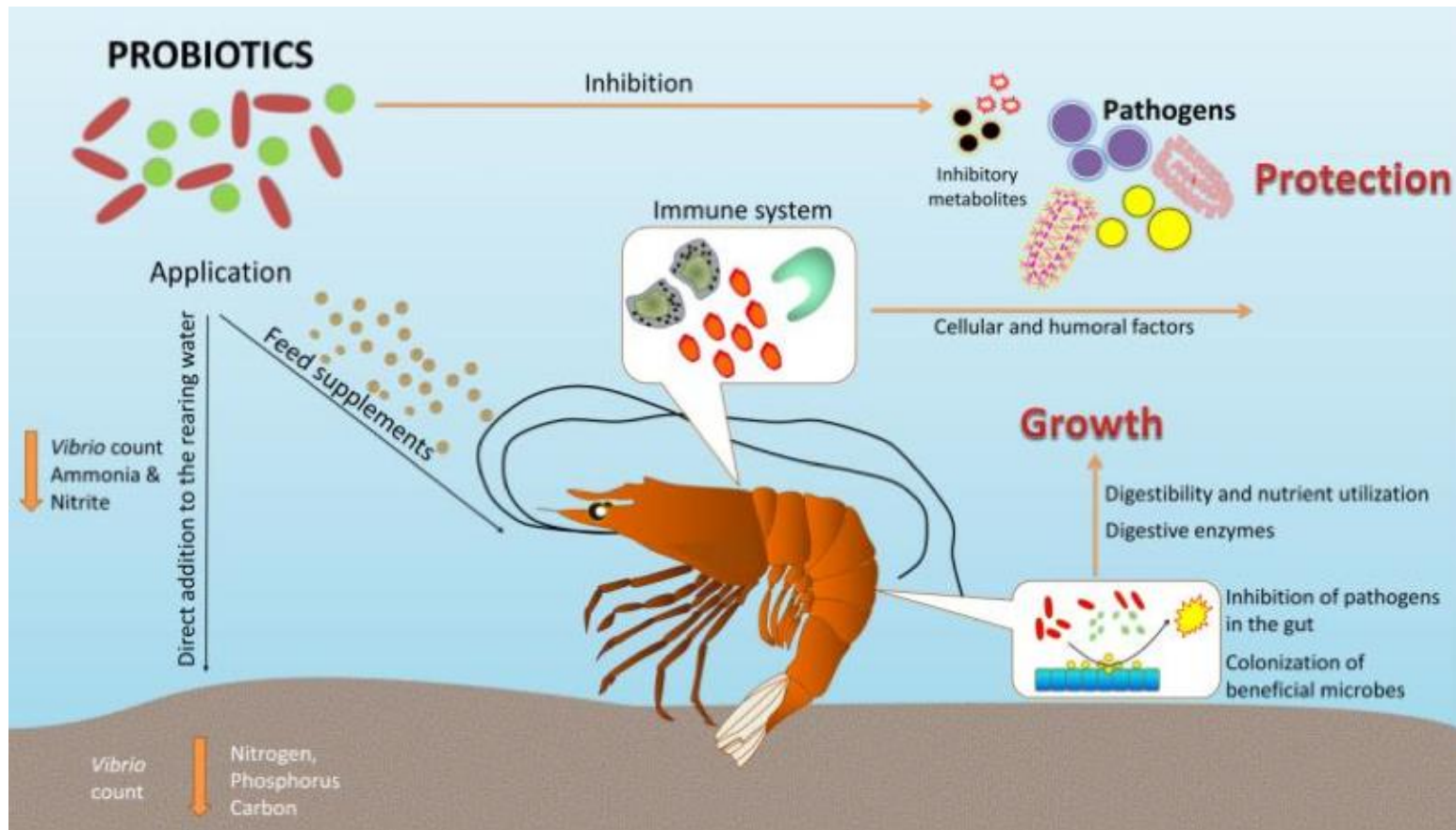
An Example of Microbiological Index (MI)

- Marine Agar plate (M): for total bacterial count
- TCBS Agar plate (T): for total *Vibrio* count
 - Yellow colony (TY): generally for less harmful *Vibrio* species
 - Green colony (TG); generally for harmful *Vibrio* species

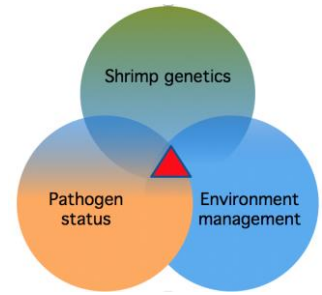
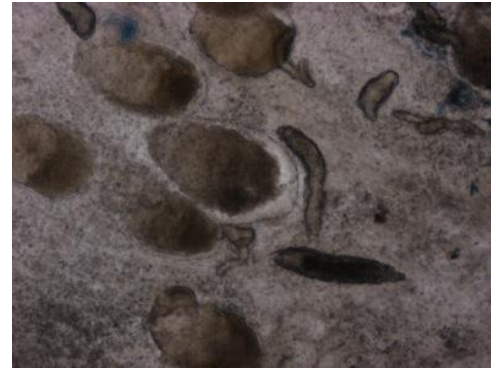
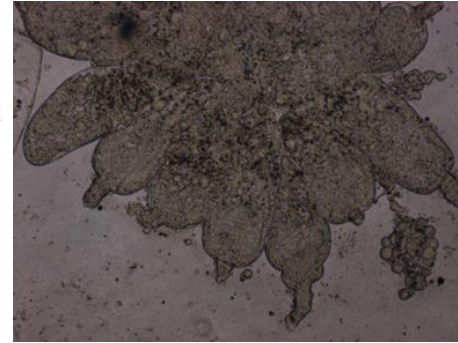
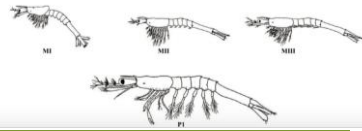
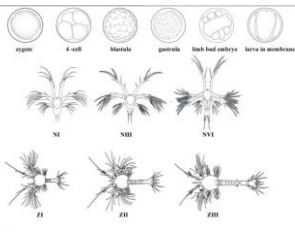
Good Water Quality Index from microbiology

1. $M = > 20 \times \text{Total } Vibrio (T)$
2. $M = \text{usually } > 10^5$ High = Dissolved oxygen 6 – 8 mg/L
3. $T < 10^3$, and $TG > TY$ Low = Dissolved oxygen < 2 to 3 mg/L
4. $TG < 10^3$, $< 5 \times 10^2$ is better
5. Higher DO can increase shrimp tolerance to *Vibrio*

NOTE: The MI for each farm may be different.



Hatchery management



FEEDS

- ❖ Clean pure cultures of algae or diatoms
- ❖ Disinfected brine shrimp, *Artemia*

PL quality and health assessment

- No melanization, no tubular constrictions, no deformities
- Confirm PL health by PCR for WSSV, EHP, AHPND (incubation of PLs in general bacteriological media for 24h prior to PCR)
- Stress test: 32ppt to 0ppt with >90% survival
- Microbiology (TCBS no green colonies)



Best practices for shrimp grow-out

- Quality of postlarvae = various criteria applied in different countries (Refer to FAO Technical Paper 450)
- **Good quality seeds for all** = a biosecurity threat somewhere, is a biosecurity threat everywhere. Small-scale farmers should have access to good quality postlarvae from SPF broodstock.

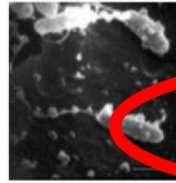
QUALITY OF Environment

- Thorough drying of pond bottom and pond preparation
- Aeration for water circulation
- Others



Fate of *Penaeus monodon* juveniles to sub-adults after infection with *Vibrio harveyi*

Examine closely the strategies for creating “microbially mature” water and application of probiotics.

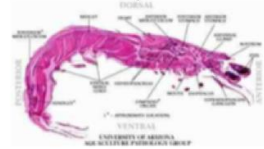


Vibrio harveyi in the environment
<10 cfu/ml

Increase in number

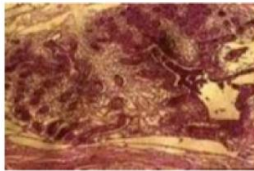
The reason for sterilization of water

The strategy may have been wrong.



Build up in the hepatopancreas

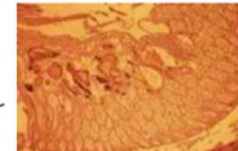
Acute Infection



Massive inflammatory response

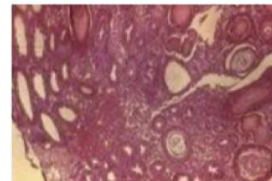
Mortality

Chronic Infection



Focal necrosis in the hepatopancreas

Recovery;
slow growth

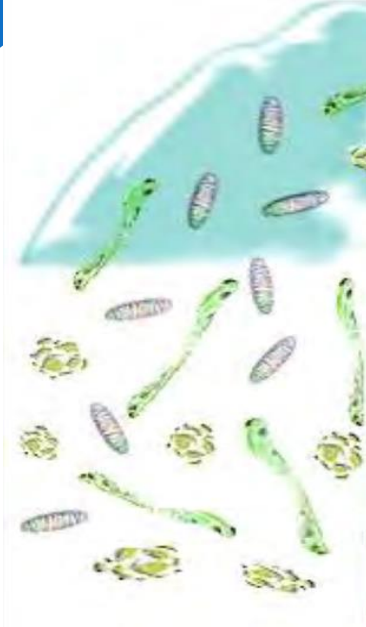


Severe melanization, fibrosis,
granuloma formation

Mortality



DISEASE BARRIERS



Good shrimp strains and genetic improvement

Good nutrition and immunostimulation

Minimize risk factors

Reduce infectious pressure

Vigilant surveillance and diagnosis

Prudent use of antibiotics



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