Skretting

Stoping Post-Larva as a Disease Vector, specifically for White Spot, WSSV

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Agenda

1. What is White spot, WSSV
2. The Theories:
   - SPF: Shrimp Patogen Free
   - APE: All Pathogen Exposed
3. Thermall Treatment to eliminate White spot from Post-Larva
4. The use of Raceways/Nursery as a Biological Filter
5. Conclusion

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What is White Spot, WSSV

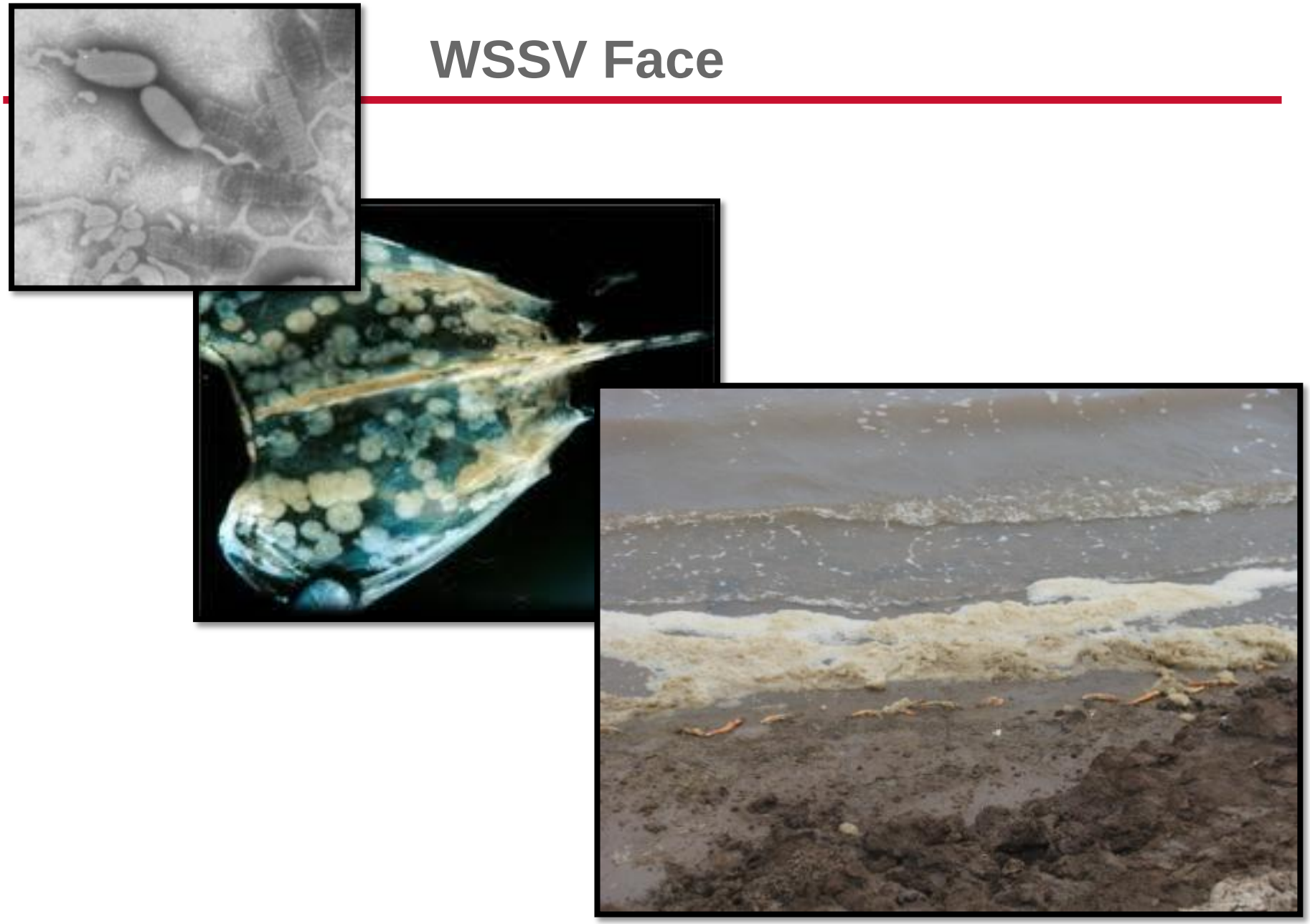
1. Genus Whispovirus within the Nimaviridae family. Virions of WSSV are ovoid or ellipsoid to bacilliform in shape, have a regular symmetry, and measure 120-150nm in diameter and 270-290nm in length. Most notable is the thread or flagella like extension at the end of the virion.

2. Survival outside the host: It is viable outside the host at least 30 days at 30 degrees Celsius in sea water under laboratory condition, and viable in ponds for 5-7 days.

3. Hosts: extremely wide range of hosts. The virus can infect a large range of crustaceans including marine, brackish or fresh water penaids, crabs and crayfish.

4. Susceptible stages of the host: All life stages from egg to brood stock.

WSSV Face



Background:

- White spot first appeared in Asia in 1992-1993.

White Spot symptoms:

- Erratic swimming, slow lethargic shrimp.
- Reduced feeding.
- Followed by dead shrimp on the sides of the ponds, these shrimp have a red color and white spots on the cephalothorax.
- In the next 3 to 10 days after confirmation of the disease the pond will experience very high mortality.
- It is important to point out that WSSV can live in water up to 120 days in an experimental form. In the wild it does not resist more than 5-7 days, time enough to propagate the disease. (Maeda and Cols. 1998)

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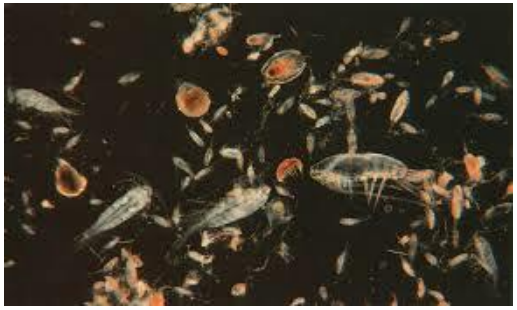
WSSV Vectors; Where does it come from:



WSSV

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WSSV VECTORS

Vol. 63: 107–111, 2005 | DISEASES OF AQUATIC ORGANISMS Dis Aquat Org | Published February 28

Polychaete worms — a vector for white spot syndrome virus (WSSV)

2.2.5. Persistent infection with lifelong carriers

Persistent infection occurs commonly and lifelong infection has been shown (9). Viral loads during persistent infection can be extremely low and potentially undetectable by any available diagnostic test.

2.2.6. Vectors

Vectors include rotifers (31), marine molluscs, polychaete worms (25) and non-decapodal crustaceans including *Artemia salina* (2) and the copepods, as well as non-crustacean aquatic arthropods such as sea slaters (Isopoda) and Euphydradae insect larvae. All these species can accumulate high concentrations of viable WSSV, although there is no evidence of virus replication (9).

75%. Polychaetes collected from areas near shrimp farms showed a higher level of contamination. Laboratory challenge experiments confirmed the field observations, and >60% of worms exposed to WSSV inoculum were proved to be WSSV positive after a 7 d exposure. It was also confirmed that *P. monodon* broodstock could be infected with WSSV by feeding on WSSV contaminated polychaete worms. Though the present study indicates only a low level infectivity in wild polychaetes, laboratory experiments clearly indicated the possibility of WSSV transfer from the live feed to shrimp broodstock, suggesting that polychaete worms could play a role in the epizootiology of WSSV.

KEY WORDS: Broodstock · Live feed · *Penaeus monodon* · Polychaete worms · Vector · White spot syndrome virus · WSSV

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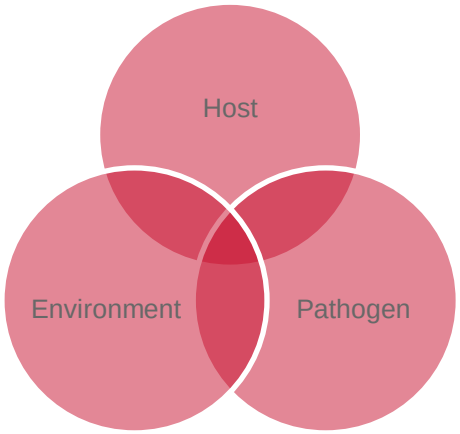
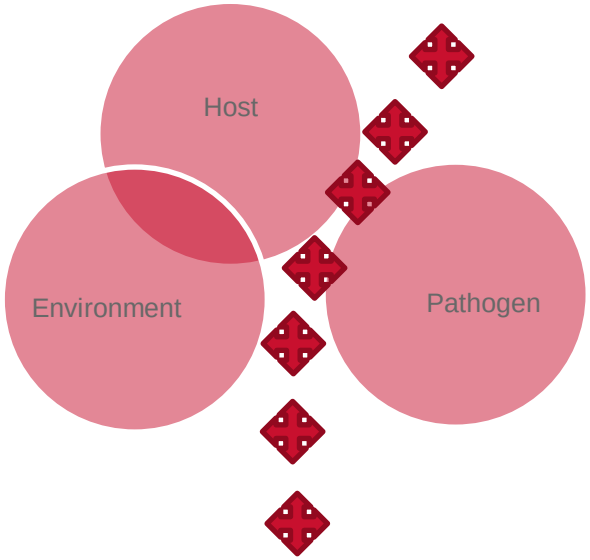
The Theories for broodstock production:

Pathogen + Host + Environment = Disease



APE: All Pathogen Exposed

Exclude pathogen = No Disease



SPF: Shrimp Pathogen Free

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SPF: Shrimp Pathogen Free

SPF Shrimp: Asking the right questions, clearing the myths & mysteries

By Shaun M. Moss, Ph.D.
Oceanic Institute

newly constructed Nucleus ...ding Center at Oceanic ...itute. The USMSFP established world's first and most ad... ...ced breeding genetic selection ...gram for marine shrimp.

The first population of specific pathogen free (SPF) Pacific white shrimp (*Litopenaeus vannamei*) was developed in 1989 when Dr. Don Lightner and his colleagues at the University of Arizona imported about 15,000 postlarvae from a commercial hatchery in Sinaloa, Mexico.

These shrimp underwent histological evaluation and did not appear to be infected with any known pathogen. About 10,000 of these "candidate" SPF shrimp were shipped to the Oceanic Institute in Hawaii where they were raised to broodstock, mated and spawned to produce SPF offspring.

Since then, SPF shrimp have played an important role in the U.S. shrimp farming industry. Despite that recognition, the SPF concept is not clearly understood by many stakeholders in the industry—that SPF shrimp are free of specified pathogens.

Three essential criteria are required for a pathogen to be included on an SPF list. These are: 1) the pathogen must be reliably diagnosed, 2) it must be physically excluded from a facility, and 3) it must pose a significant threat to the industry.

Although there is no internationally recognized SPF list used by the global shrimp farming industry to date, the current working list of specific pathogens for SPF penaeid shrimp in the United States includes eight viruses, one prokaryote, and certain classes of parasitic protozoa (see Table 1 on P4).

It is important to note that this list is dynamic and will be revised and expanded as new pathogens are identified and more accurate disease diagnostic tools become available.

- First SPF population developed in the US in 1989.
- Post-Larva from a commercial hatchery in Sinaloa, Mexico.
- 3 criteria's for a pathogen to be added to the SPF list:

1) The pathogen has to be able to be detected.
2) In has to be able to be eliminated form a hatchery or commercial set up.
3) It must represent a risk to the industry.

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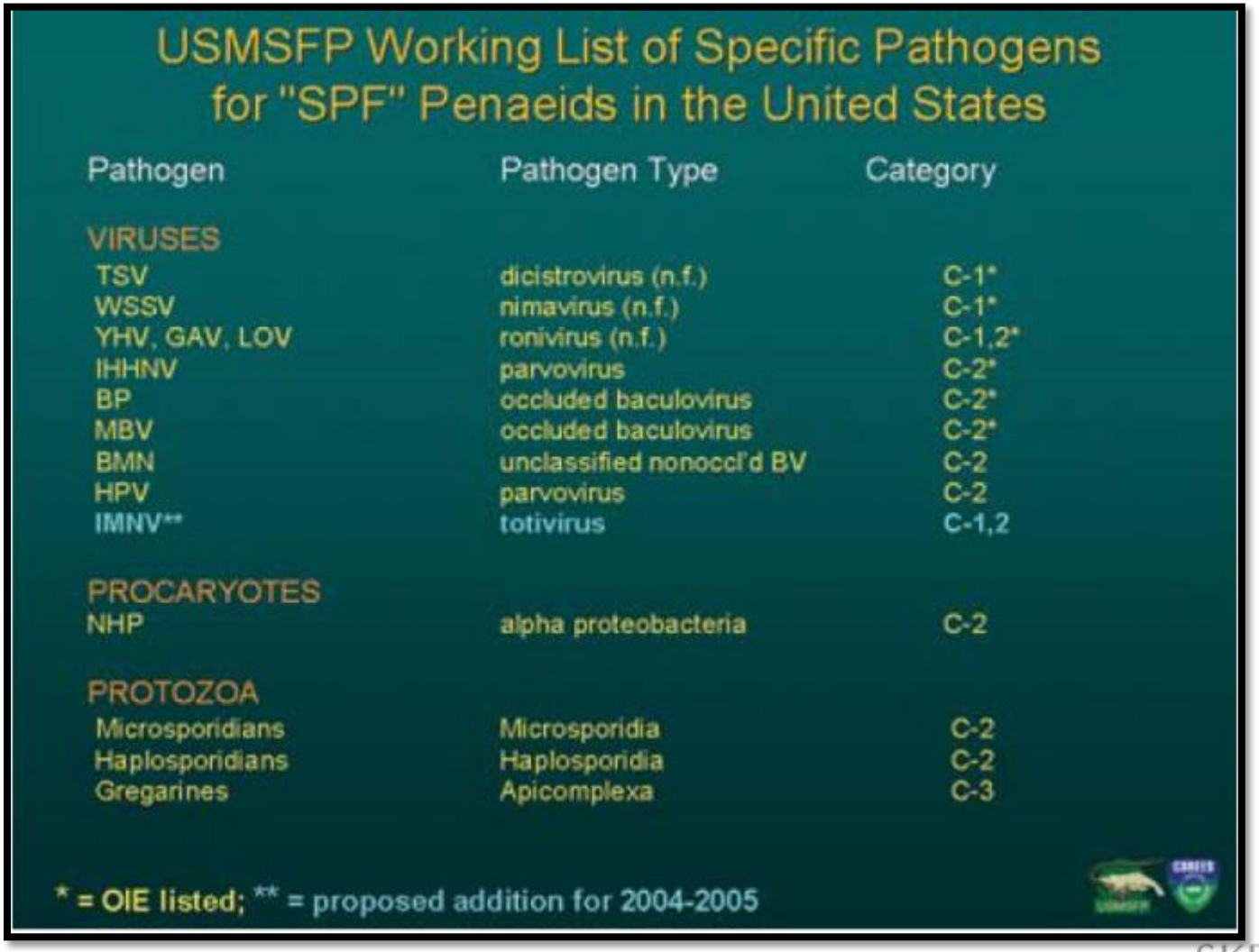
SPF Working List



USMSFP Working List of Specific Pathogens for "SPF" Penaeids in the United States

Pathogen	Pathogen Type	Category
VIRUSES		
TSV	dicistrovirus (n.f.)	C-1*
WSSV	nimavirus (n.f.)	C-1*
YHV, GAV, LOV	ronivirus (n.f.)	C-1,2*
IHHNV	parvovirus	C-2*
BP	occluded baculovirus	C-2*
MBV	occluded baculovirus	C-2*
BMN	unclassified nonoccl'd BV	C-2
HPV	parvovirus	C-2
IMNV**	totivirus	C-1,2
PROCARYOTES		
NHP	alpha proteobacteria	C-2
PROTOZOA		
Microsporidians	Microsporidia	C-2
Haplosporidians	Haplosporidia	C-2
Gregarines	Apicomplexa	C-3

* = OIE listed; ** = proposed addition for 2004-2005

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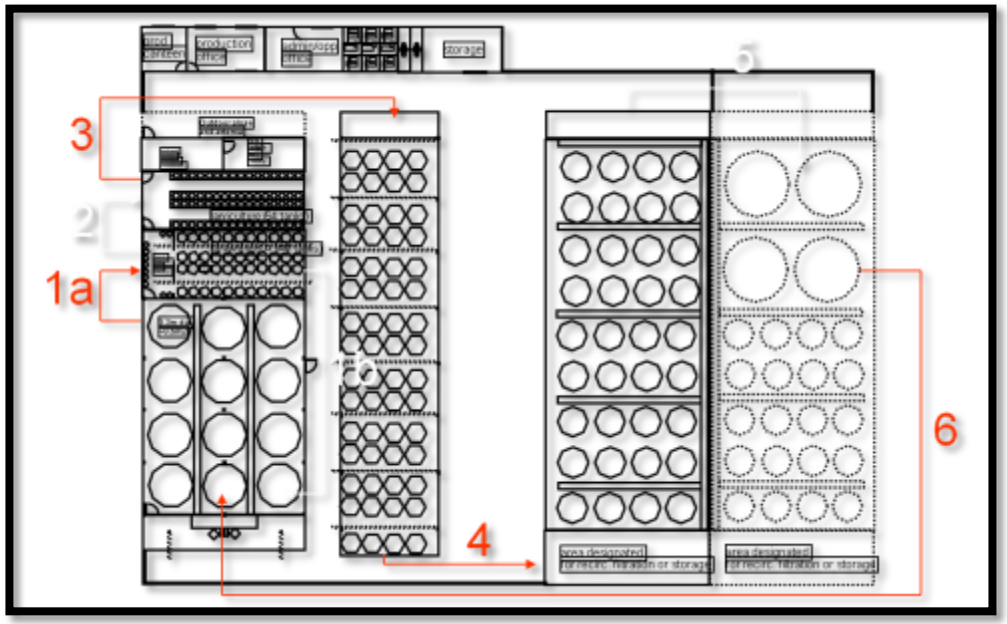
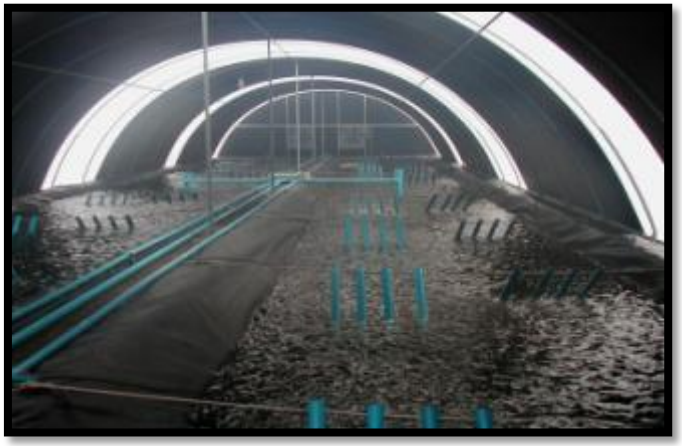
Genetic Nucleus, for SPF broodstock production:

Water disinfection,
Vector control
Feed Control
Strict Biosecurity





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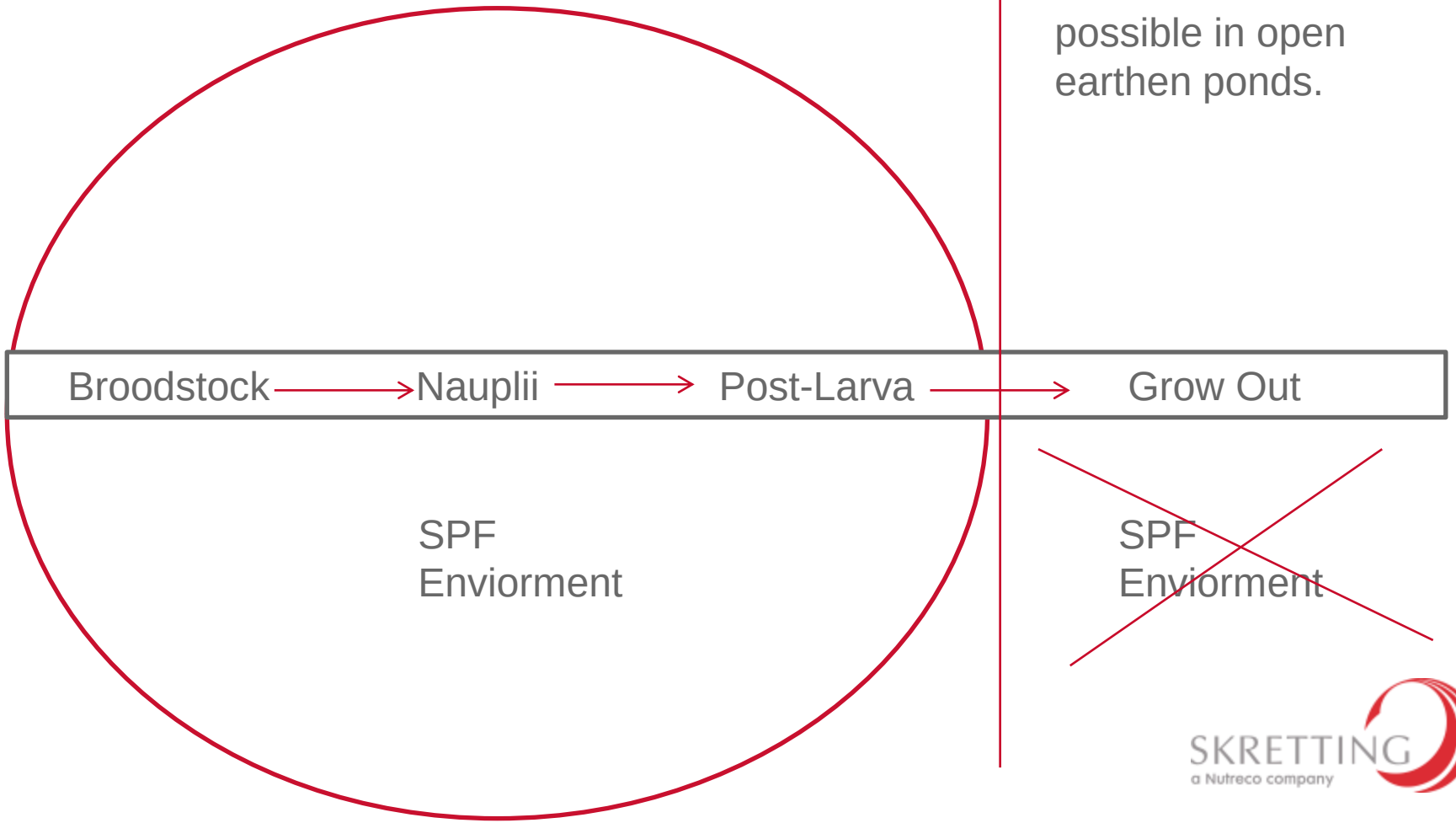
SPF Post-Larva

Disinfection and control is possible in the hatchery environment

Little control of vectors or full disinfection not possible in open earthen ponds.



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LEVELS OF BIOSECURITY

- NBC all phases
- Maturation Departments
- Algae Departments
- Larviculture Department
- Artemia Department
- Pack Out Area
- Office and Dirty area of the Hatcheries

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Critical Control Points

Vehicle Movement and Supply Distribution

- Biosecurity prior to the arrival of the hatchery.
- Biosecurity while moving around the hatchery
- Biosecurity of all operatives involved.

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People Movement

- Entrance to the hatchery are restricted to hatchery personnel on working hours.
- People working in a department will not cross over to another department without authorization.
- Hatchery gates shut at all times, vendors and visitors will be announced and the entrance will be under authorization of the hatchery manager.
- Visitors and staff will use boots at all times while in the hatchery.
- Boots and equipment will not be shared between departments.
- No crustaceans will be allowed in the hatchery.

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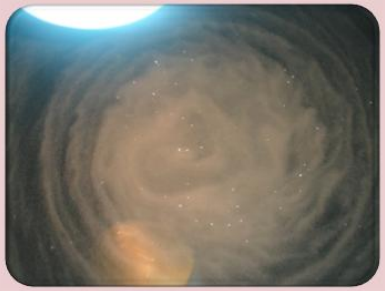
Quality Assurance of Feed and Supplies

- Purchasing pathogen free feed and supplies.
- Monitoring the feeds purchased and checking their background.
- Sending Feeds to a PCR Lab to check for WSSV<IHNNV<TSV<YHV<GAV



High Nauplii Production

- Same quality and amount of Naupli per female
- Standarization
- Reduced Vibrio quantity

Reduced Disease Risk

- Eliminating fresh feeds as vectors
- Constant and Stable Nutrient Delivery
- 2 year shelf life
- No more freezers

Clean Water

- Reduced Patogens
- Reduced Water Exchange
- High Biosecurity

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Water Treatment

- Disinfection of the primary water intake by chlorination.
- Disinfection of the water by ozone in the secondary water line.
- Disinfection of the water by chlorination in the third and final water line.
- Filtration of the water up to 1 micron
- Ultraviolet light for Algae department.
- Use of biofiltration to reduce water consumption and reduce the risk of pathogen inclusion.

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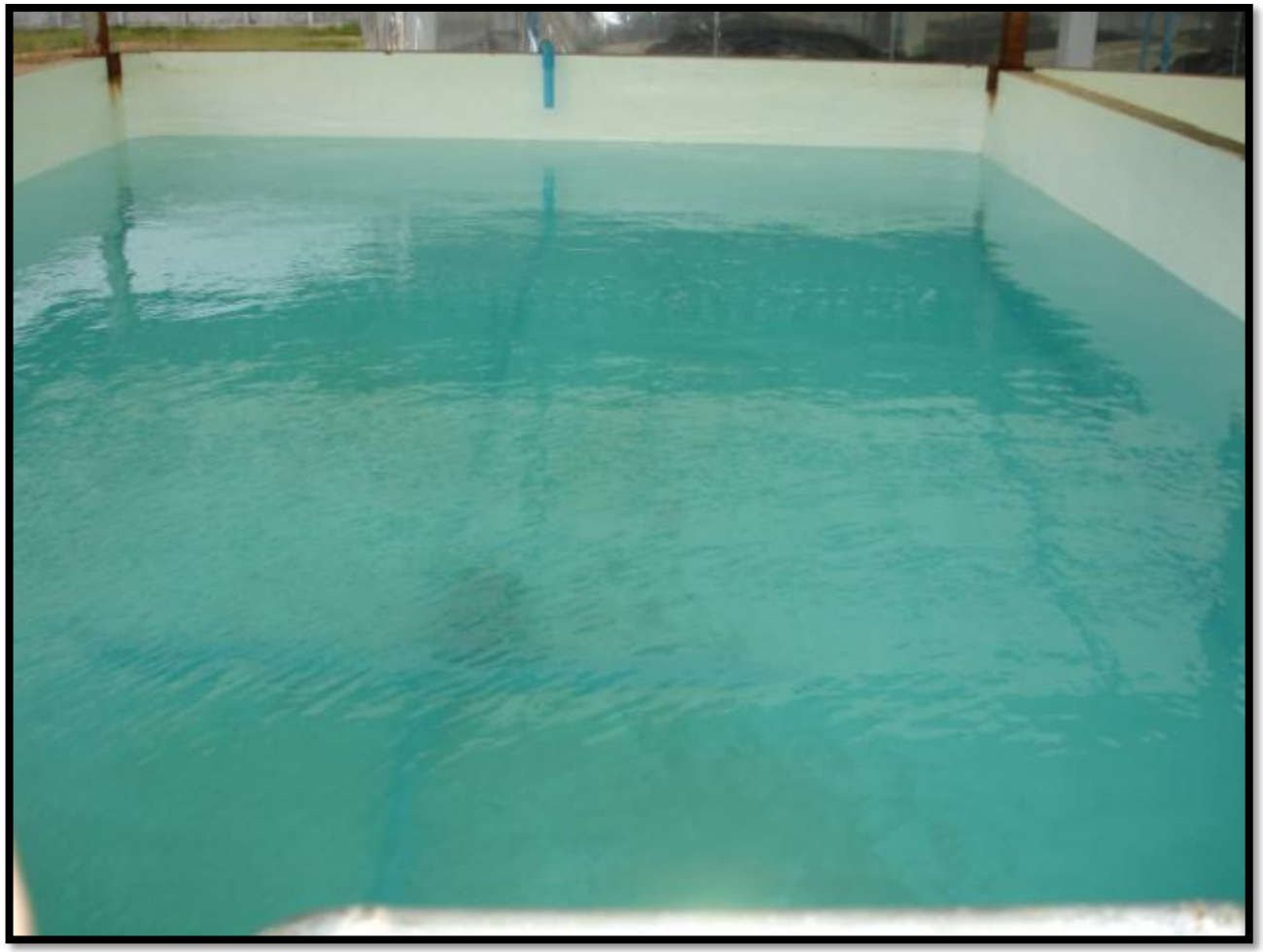
Water in the Hatchery should look like this:



- Free of Vibrio spp.
- Free of WSSV.
- Free of Pathogens.
- Free of Algae.
- Free of animals, fish and crustaceans, etc.
- To do this you need mechanical filtration, biological filtration, chlorine, ozone, and or U.V.

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HEALTH ASSURANCE

WHAT	HOW	FREQUENCY	WHO
Broodstock Importation	Documentation with each shipment testifying free of disease	Every shipment	Facility Manager
Broodstock Transfer (in Country)	Virus PCR test 10 broodstock for every 500 broodstock sent. Have to be free of primary viruses (IHHNV,TSV,WSSV and YHV)	Every shipment	Facility Manager
Nii Movement	Checking broodstock every month	Once per month	Maturation Manager
Post-Larvae	PCR detection (WSSV,IHHNV,YHV y TSV)	Send at Post-Larvae 5-8	Distribution Manager

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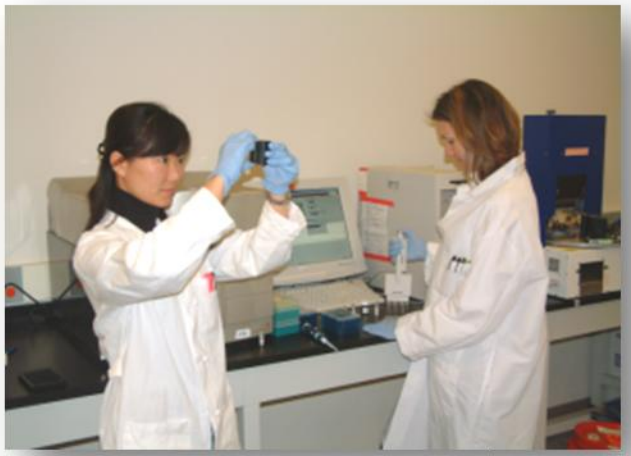
Three Step Monitoring Plan

1. Level 1: Daily visual observation of animals and environment. (Mortality in tanks, lesions on brood stock ,etc.)
2. Level 2: Daily microscopic examination, squash mounts of samples, and bacteriology.
3. PCR diagnostics of samples.



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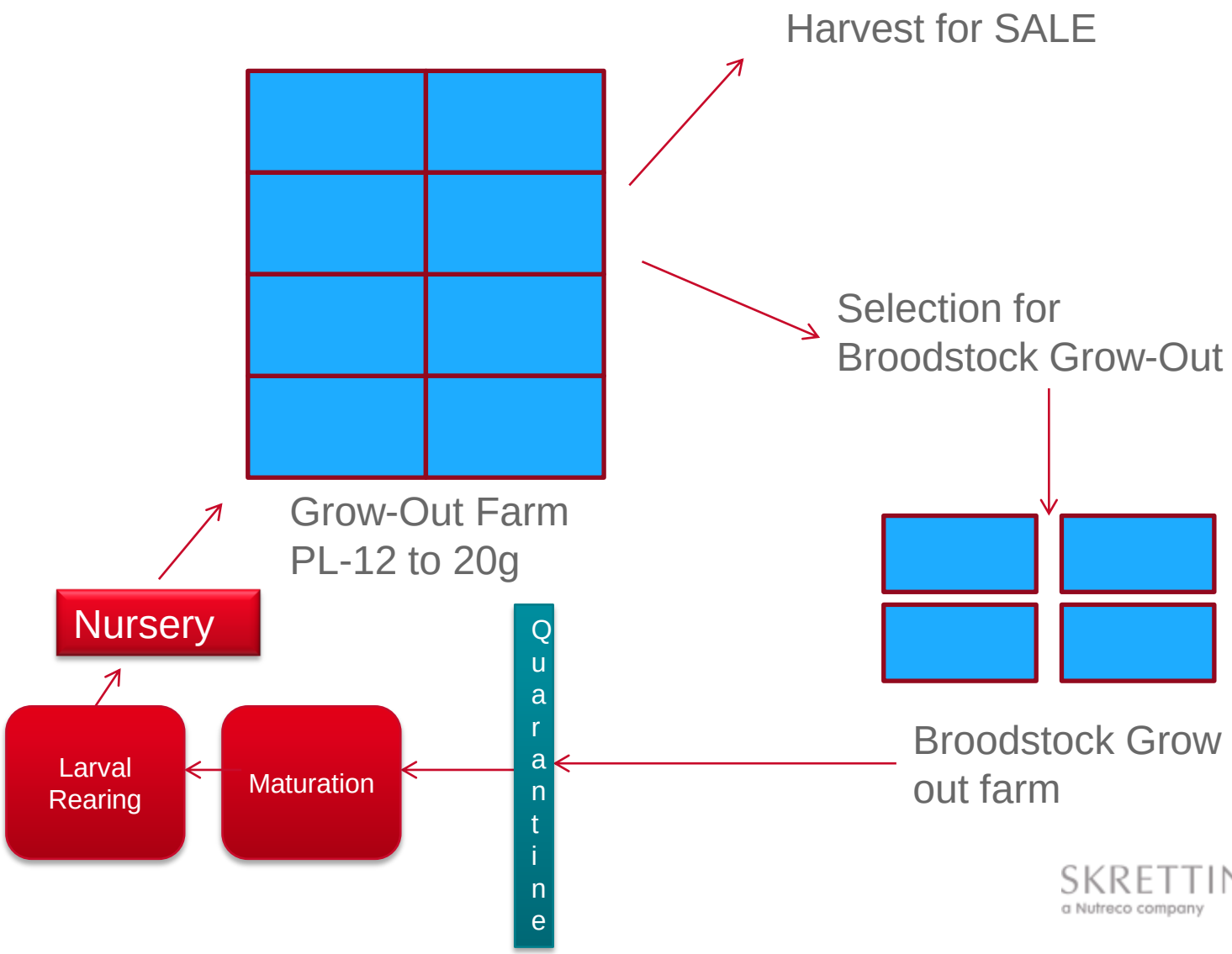
APE: All Pathogen Exposed (Ecuador)



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White Spot can be eliminated by:

- The virus can be eliminated fiscally or chemically.
- Exposing the virus at 55 degrees Celsius for 90 minutes or at 50 degrees or 70 degrees Celsius for 50 minutes.
- Use low PH exposures, exposing WSSV to a PH=1 for 10 minutes or at PH=3 for 1 hr.
- High Alkalinities can also eliminate WSSV, PH=12 for 10 minutes at 25 degrees Celsius.
- U.V. Light at an intensity of 9*105/cm2 for 1 hr.
- Ozone at 100ppm
- Benzalkonium Chloride 50% at a concentración of 75ppm for 10 min.
- Povidine Iodine at 100ppm concentration for 10 min.
- HTH Chlorine at 65%, concentration of 5ppm for 3 hours or 10 ppm for 1.5 hrs.

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Thermal Treatment to Eliminate White Spot from Post-Larva

1. Post-Larva is a vector for white spot in the grow out ponds.
2. White spot can be eliminated from Post-larva threw clean Genetic brood stock, SPF.
3. White spot can be eliminated from Post-Larva threw maintaining the water temperature for 7 days at 32 (+1) degrees Celsius.

Thermal treatment can be done in any stage:

We recommend doing it from:

Mysis I to PL-4 in the hatcheries.

PL 23 to PL 30 in the nurseries or 7 days before harvesting and sending the PL to grow out.

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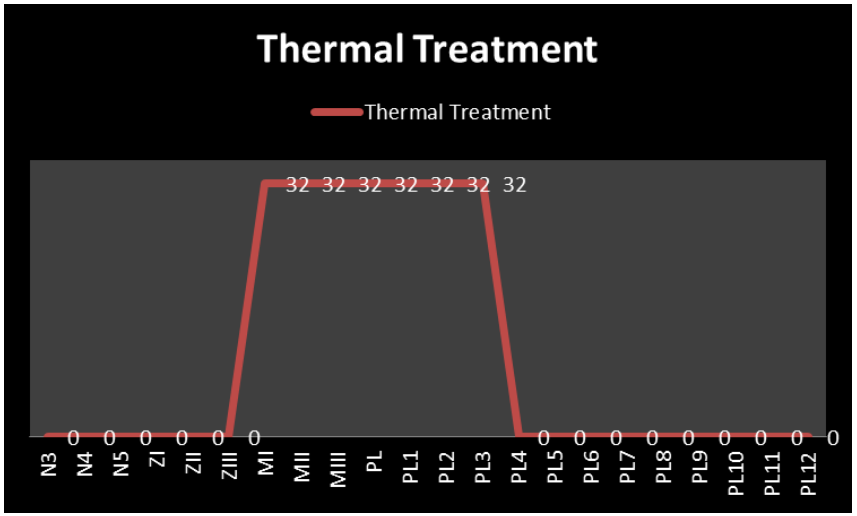
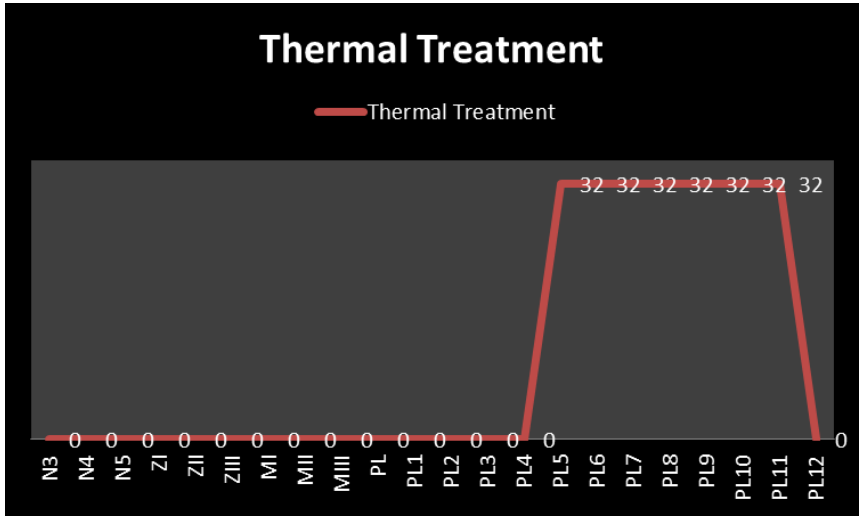
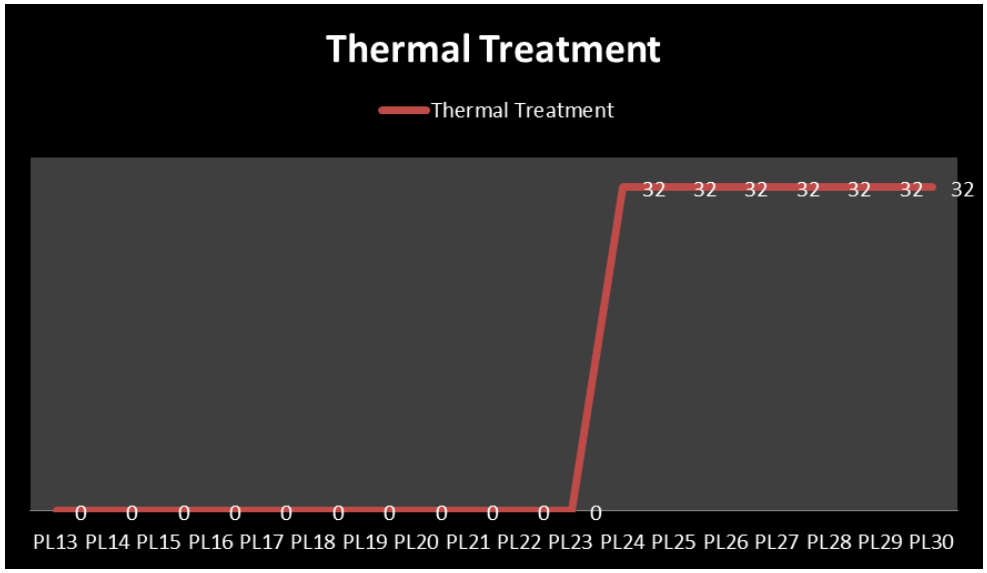
Thermal Treatment at Diferent stages







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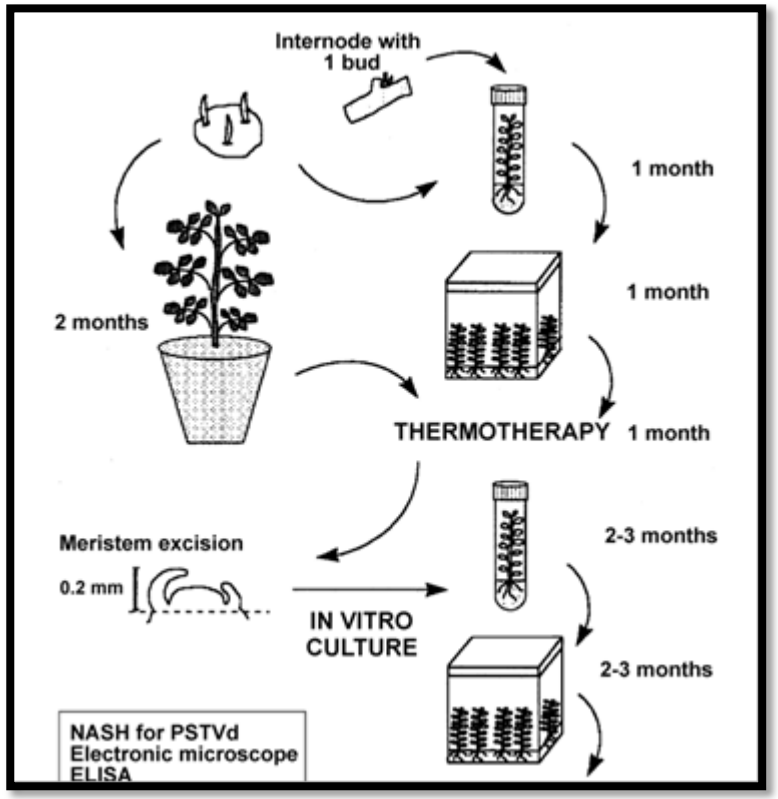
Post-Larvae, The science behind it;



Thermal therapy is the use of high temperatures to eradicate pathogenic agents such as virus and bacteria.

This methods are used commonly in agriculture, where the plants are treated at high temperatures for a certain time to eliminate viral pathogens.

As an example we took this diagram from the Training Manual for Plant Virology, CIP. Where it describes the eradication of a virus in a specific plant.

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The Science behind it;

WALAILAK JOURNAL

Article

Effects of Water Temperature on the White Spot Syndrome Virus Infection in Postlarvae *Litopenaeus Vannamei*

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Abstract

This study evaluated the effects of high water temperature (32 ± 1 °C) on the white spot syndrome virus (WSSV) infection in *Litopenaeus vannamei* postlarvae (PL_{15}). WSSV challenge was done by immersion. One group of PL_{15} was continuously maintained at 32 ± 1 °C until the end of the experiment after challenge and a control group of PL_{15} was constantly maintained at 28 ± 1 °C until the end of the experiment after challenge. Other groups were kept at 32 ± 1 °C until temperature was altered from 32 ± 1 °C to 28 ± 1 °C at 0, 1, 3, 5 and 7 days after infection. Gross signs and mortality were monitored every 12 h until the end of the experiment. WSSV infections were confirmed by nested-PCR, histopathology, immunohistochemistry and bioassay methods. Challenged shrimp were kept at 32 ± 1 °C for 0, 1, 3 and 5 days before the temperature was reduced to 28 ± 1 °C revealing that maintaining the temperature at 32 ± 1 °C for a longer period could delay clinical signs and onset of mortalities. Nevertheless, 100 % mortalities occurred in all groups and the control group within 7 days. All moribund PL_{15} were WSSV-positive by nested-PCR assay as well as histopathology, immunohistochemistry and bioassay methods. In contrast, PL_{15} constantly maintained at 32 ± 1 °C until the end of the experiment, and for 7 days after challenge before switching to 28 ± 1 °C did not show clinical signs and mortality. Surviving PL_{15} from both groups were WSSV-negative by nested-PCR assay as well as histopathology, immunohistochemistry and bioassay methods. This study clearly indicated that postlarvae maintained constantly at 32 ± 1 °C for 7 days were able to eliminate/clear WSSV infection.

Keywords: White spot syndrome virus, temperature, infection, *Litopenaeus vannamei*, postlarvae

- Study done in Walailak University, Thailand.
- Proved the effectiveness of the thermal treatment.
- White Spot was eliminated from the post-larva that was treated for 7 days at a temperature of 32 degrees Celsius +1.

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Thermal Therapy

Available online at www.sciencedirect.com
ScienceDirect
Aquaculture
ELSEVIER
Aquaculture 261 (2006) 842–849

Effect of high water temperature (33 °C) on the clinical and virological outcome of experimental infections with white spot syndrome virus (WSSV) in specific pathogen-free (SPF) *Litopenaeus vannamei*

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Abstract

White spot syndrome virus (WSSV) is the most lethal pathogen of cultured shrimp. Previous studies done with undefined WSSV titers showed that high water temperature (32–33 °C) reduced/delayed mortality of WSSV-infected shrimp. This study evaluated the effect of high water temperature on the clinical and virological outcome of a WSSV infection under standardized conditions. Groups of specific pathogen-free *Litopenaeus vannamei* were challenged either by intramuscular or oral routes with a low (30 SID_{50}) or a high (10,000 SID_{50}) virus titer. Shrimp were kept (i) continuously at 27 °C, (ii) 30 °C or (iii) 33 °C; (iv) maintained at 33 °C before challenge and 27 °C afterwards, or (v) kept at 27 °C before challenge and 33 °C afterwards. Shrimp were maintained at the respective temperatures for 120 h before challenge and 120–144 h post challenge (hpc). Gross signs and mortality were monitored every 12 h until the end of the experiment. Dead and surviving shrimp were screened for WSSV infection (VP28-positive cells) by indirect immunofluorescence (IIF). Shrimp kept continuously at 27 °C or 30 °C, or switched to 27 °C post challenge developed gross signs within 24 hpc, first mortalities at 36–60 hpc and 100% cumulative mortality between 60 and 144 hpc depending on the virus titer. All dead shrimp were WSSV-positive. In contrast, shrimp kept at 33 °C continuously or after WSSV challenge showed no signs of disease and low mortalities (0–30%) regardless of the virus titer. Dead and surviving shrimp were WSSV-negative. Further, early virus replication was studied in two groups of shrimp: one maintained at 27 °C before and after challenge and one switched from 27 °C to 33 °C after challenge with 10,000 SID_{50}. Immunohistochemistry (IHC) analysis showed that WSSV-positive cells were first displayed at 12 hpc in shrimp kept at 27 °C and by 24 hpc the infection became systemic. In contrast, shrimp kept at 33 °C did not display WSSV-positive cells at 12 or 24 hpc. This work confirms previous reports that high water temperature prevents the onset of disease and significantly reduces mortality of WSSV-inoculated shrimp regardless of the route of inoculation or virus titer used. This strategy may have practical applications to control WSSV in tropical shrimp farming countries.

Keywords: WSSV replication; VP28; Water temperature; *Litopenaeus vannamei*; SPF

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Nursery's

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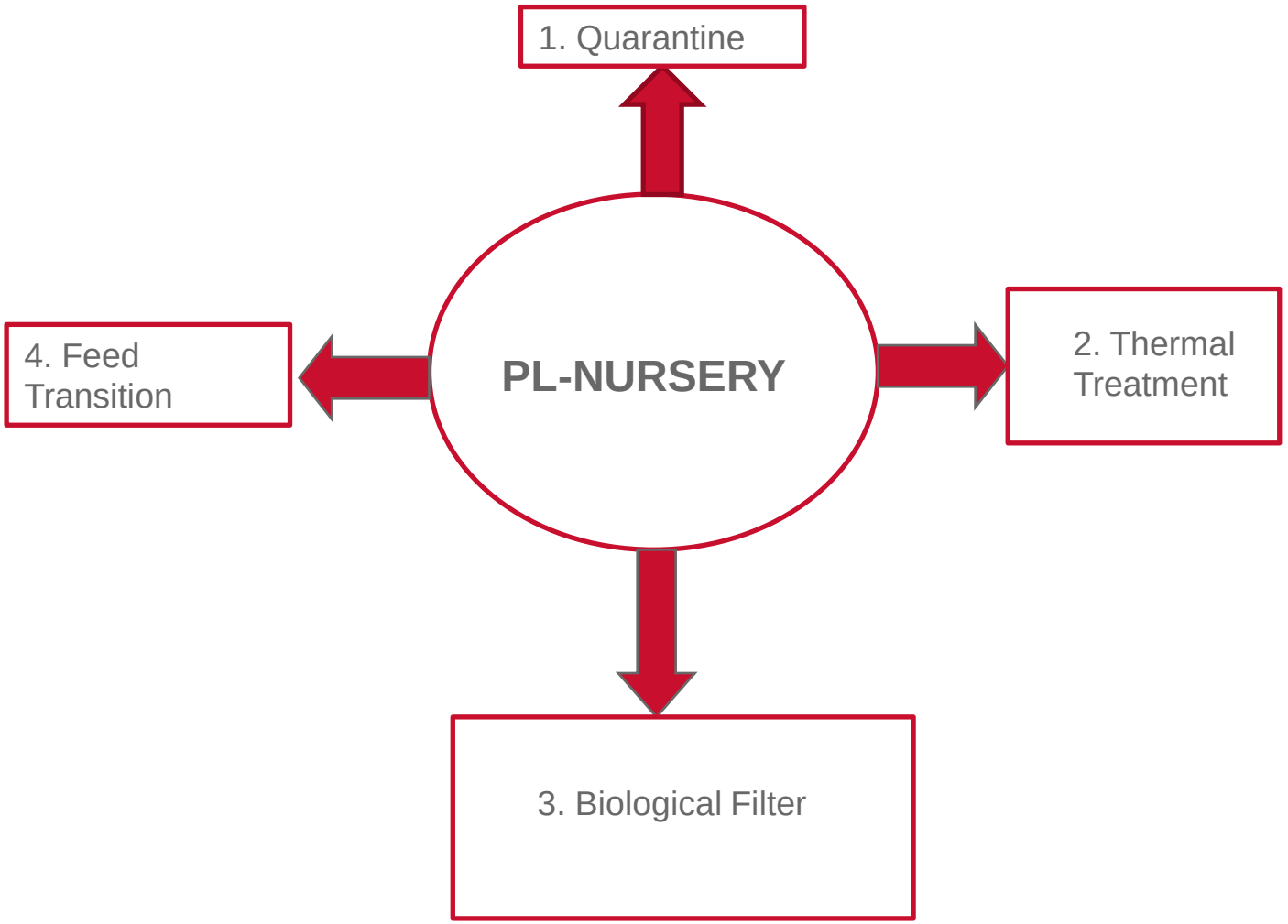
PL-Nursery Functions:



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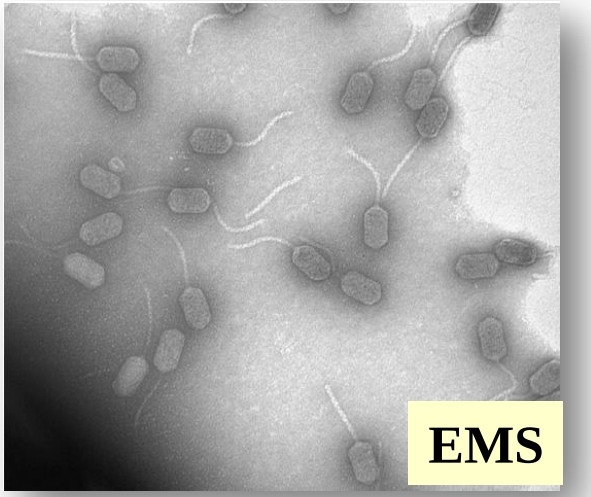
Quarantine

Virus

Bacteria



Quarantine:

Before animals are stocked in the pond animals are screened once more for major disease, as well as overall fitness.

Only after passing the health check they will be stocked in the ponds.

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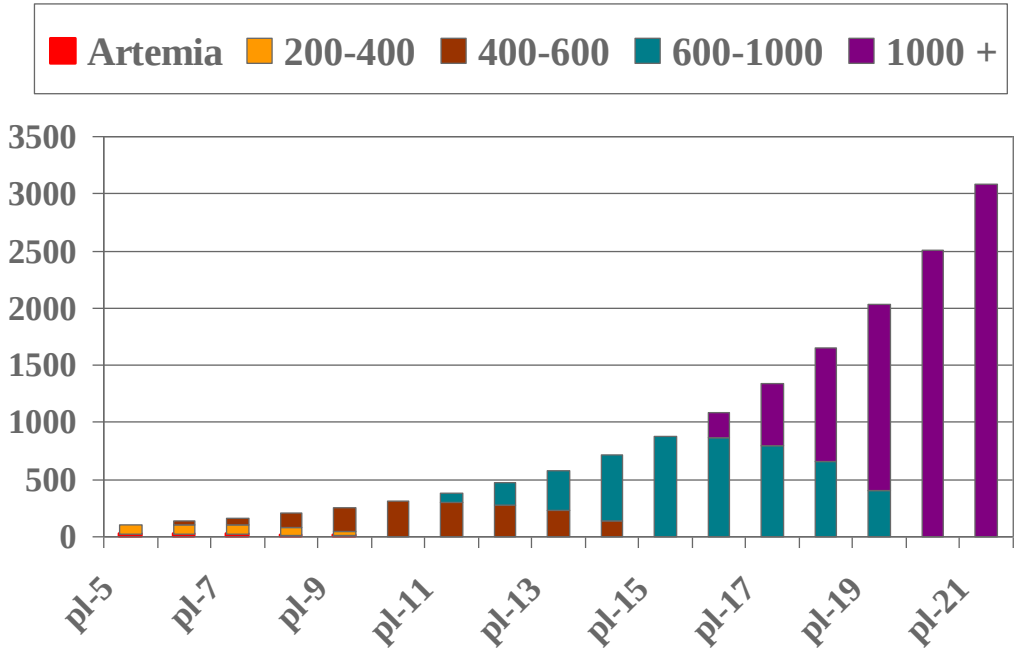
Transition Feeds



Transition: From hatchery feeds to grow out feeds.

Making animals have imediate acceptance of the grow out feed in the ponds.

Feeding ratio STARTS at 25% of ABW and decreases as stages get bigger.

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Conclusions:

1. Make sure your post-larva is not a WSSV vector.
2. Thermal Treatment can eliminate WSSV from our Post-Larva
3. Use of Nursery tanks to quarantine, thermal treatment and check health status of big animals
4. Transition feeds from larval diets to grow out diets for 100% acceptance in pond environment at stocking.
5. Stock bigger and stronger PL's into the grow out ponds. Eliminate any diseased and/or weak animals in nursery tanks. Which will represent a saving in the farm as incidence of flushed ponds should decrease.
6. Their is compensatory growth after harvesting juveniles from the nurseries.
7. Less culture time in the grow out ponds.

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Questions?

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