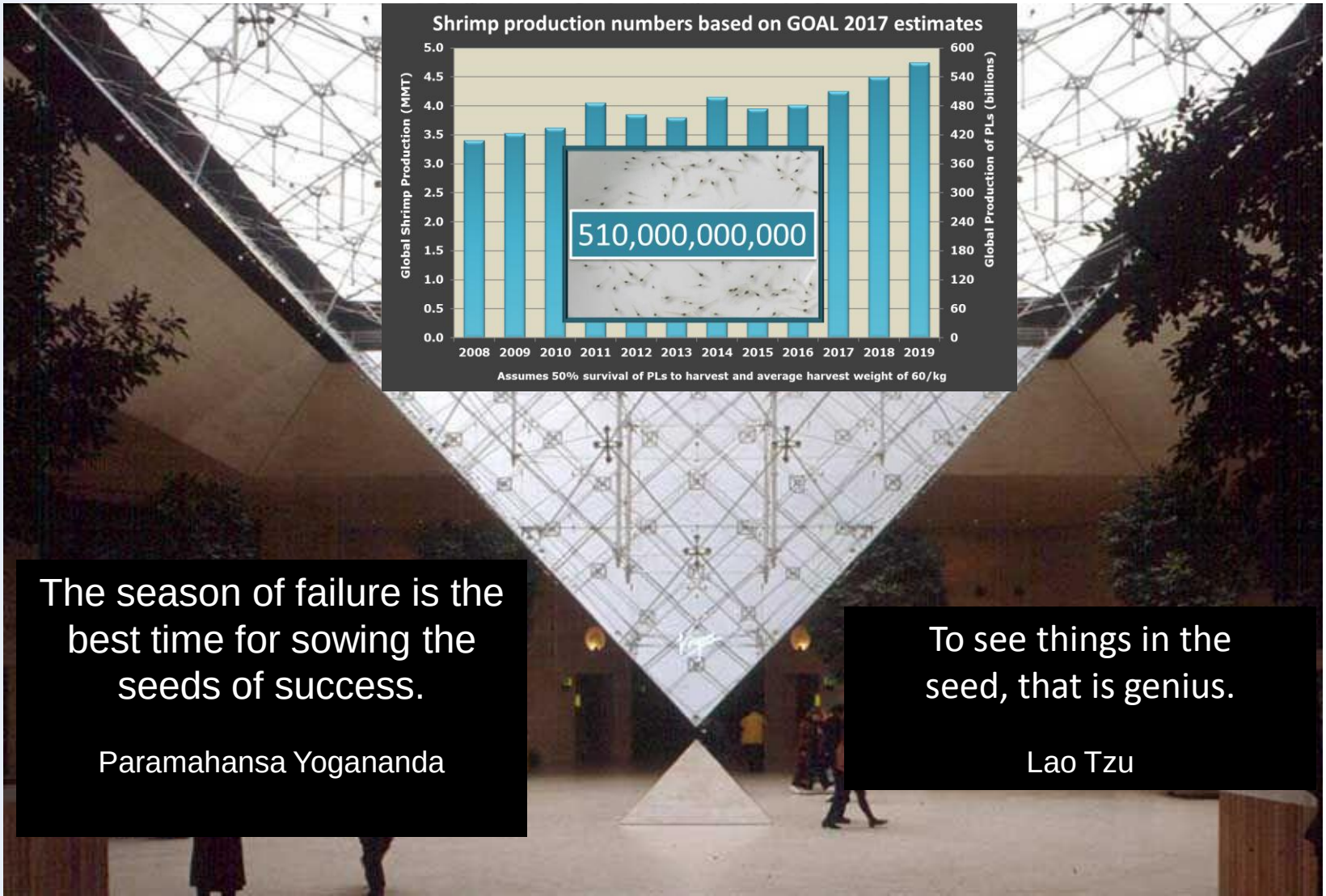
Maximizing production potential through application of biosecure hatchery and nursery technologies

Craig L. Browdy, Diego Flores, Leandro Castro and Peter M. Van Wyk

Look before you leap for as you sow, ye are like to reap.

Samuel Butler



The season of failure is the best time for sowing the seeds of success.

Paramahansa Yogananda

To see things in the seed, that is genius.

Lao Tzu

Genetics

Health

Nutrition

Larval Quality

Hatchery Profits

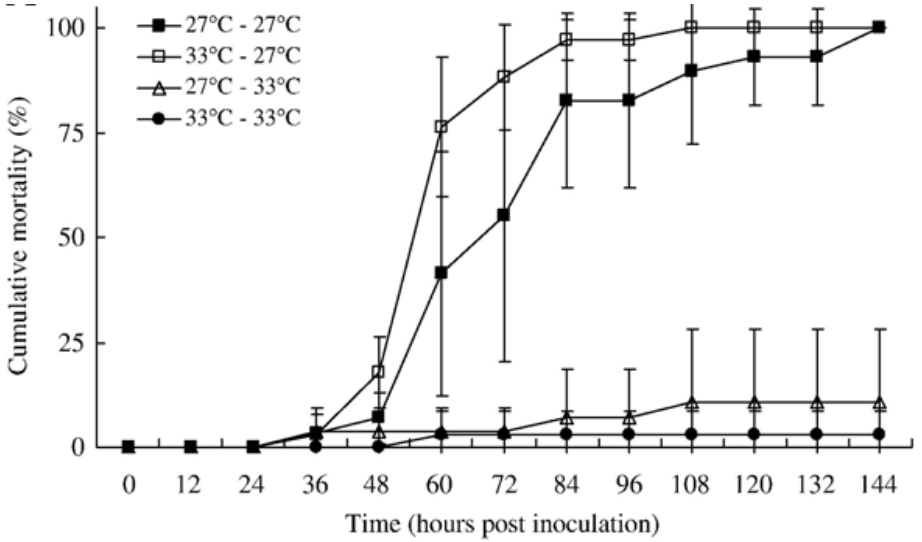
Production Results

Farm Profits

Win / Win

Excludable Pathogens

- Control vertical transmission screening broodstock and washing eggs
- With no host, WSSV is viable in pond water for up to 3–4 days
- Can not detect in PL
- Control vectors
- Pond preparation
- Tolerant stocks

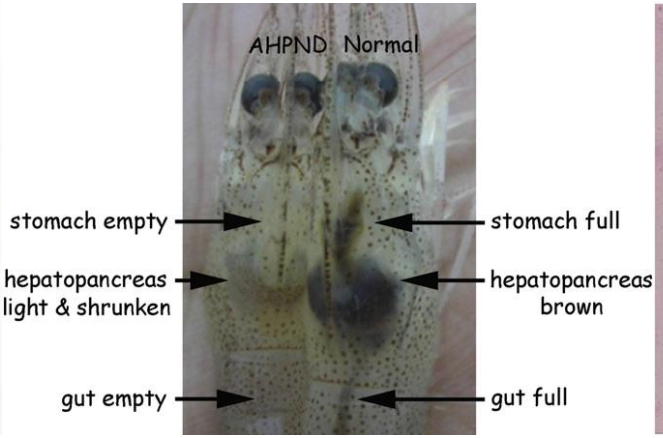


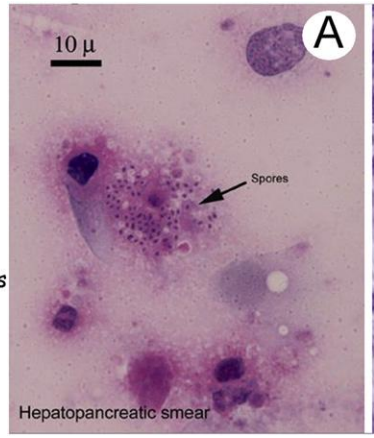
Rahman et al. 2006

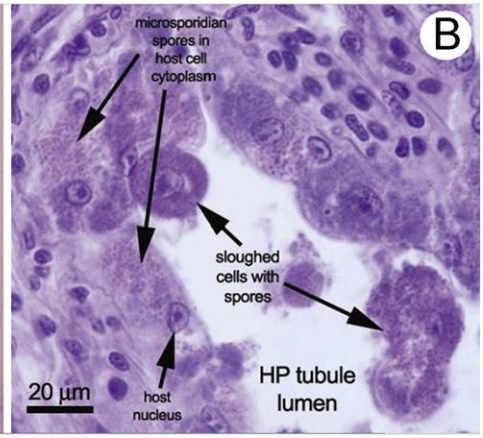
Non Viral Pathogens

More difficult to exclude

- *Vibrio parahemolyticus* - AHPND
  - Bacteria with plasmids coding for virulent toxins
  - Nonobligate, survives and spreads in environment
- *Enterocytozoon hepatopenaei* – EHP
  - Transmitted by cannibalism and cohabitation
  - Spores persist in environment





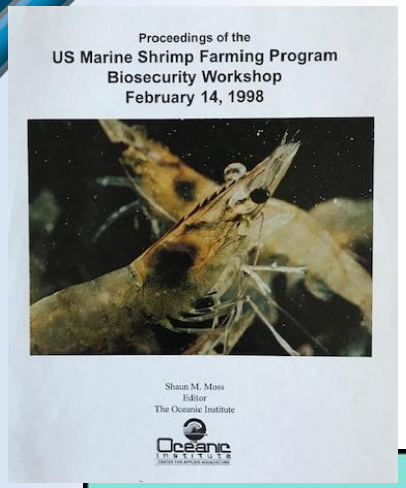


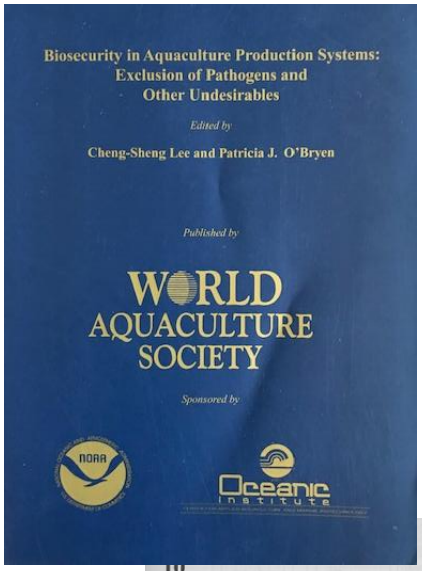
Thitamadee et al. 2016

Rethinking Hygiene and Biosecurity

- More important than ever to control design, practices and standards to maintain cleanliness and reduce opportunities for bacteria and spores to build up
- More important than ever to revisit biosecurity protocols and adapt them to diverse hatchery operational philosophies
- More important than ever to focus on sources of contamination and conditions which favor pathogen numbers and virulence

Hatchery Biosecurity Literature





Application of
Hazard Analysis Critical Control Point (HACCP) Principles
as a Risk Management Tool
to Control Viral Pathogens at Shrimp Production Facilities

Michael L. Jahncke[1], Craig L. Browdy[2], Michael H. Schwarz[1], Al Segars[3], Juan L. Silva[4], David C. Smith[5], and Alvin D. Stokes[3]

[1]**Virginia Seafood Agricultural Research and Extension Center (VSAREC)**
Virginia Tech / Virginia Sea Grant
102 South King Street
Hampton, Virginia 23669 USA

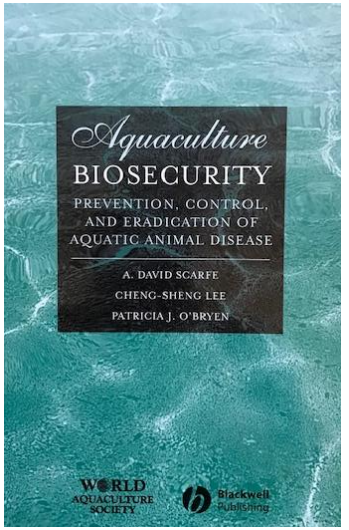
[2]**Marine Resources Resarch Institute**
South Carolina Department of Natural Resources
217 Ft. Johnson Road (P.O. Box 12559)
Charleston, SC 29422 USA

[3]**South Carolina Department of Natural Resources**
Waddell Mariculture Center
P. O. Box 809
Bluffton, SC 29928 USA

[4]**Mississippi State University**
Department of Food Science & Technology, Box 9805
Mississippi State, MS 29762-5953 USA

[5]**Clemson University**
Department of Food Science and Human Nutrition
P. O. Box 189
Beaufort, SC 29901 USA

HACCP



10

Biosecurity measures in specific pathogen free (SPF) shrimp hatcheries

J. Wyban, High Health Aquaculture Inc., USA

DOI: 10.1533/9780857097460.2.32a

Abstract: Global shrimp farming more than tripled production over the last decade. The major driver of that expansion was the introduction, adoption and expansion of farming *P. vannamei* as the shrimp species of choice. A key element determining farmers' preference for *P. vannamei* was widespread availability of High Health post-larvae (PL) produced from specific pathogen free (SPF) broodstock that outperformed other shrimp species in reliability and profitability. Production of High Health PL requires use of SPF broodstock in hatcheries using biosecurity. This chapter reviews key management practices of securing SPF broodstock and biosecurity in *P. vannamei* shrimp hatchery systems.

Key words: shrimp, hatchery, biosecurity, SPF.

10.1 Introduction

A consistent supply of quality seed is essential to all agriculture systems. In shrimp farming, seed are called post-larvae or PL. Production technology for marine shrimp PL is referred to as hatchery technology. Shrimp hatchery technology was first developed in Japan in the 1940s and was subsequently transferred to and refined by the shrimp farming industries in the eastern and western hemispheres during the 1970s/1980s.

SPF (specific pathogen free) shrimp are shrimp that have passed through a rigorous pathogen screening and multi-generational quarantine process. They must be certified SPF or free of known disease-causing pathogens by a qualified third party. This technology was developed in the USA in the early 1990s (Wyban *et al.*, 1992). Subsequent commercial production of SPF white shrimp broodstock in Hawaii led to worldwide availability of SPF broodstock which led to the introduction, successful production and widespread adoption of SPF *P. vannamei* in Asia from 1999. Widespread use of

The Israeli Journal of Aquaculture - Bamidgeh, IJA_65.2013.881, 6 pages

Implementation of Biosecurity Measures in Commercial Shrimp Hatcheries in India

S. Raja[1,2]* M. Kumaran[1], P. Ravichandran[1]

[1] *Central Institute of Brackishwater Aquaculture (CIBA), 75 Santhome High Road, Chennai 600028, India*

[2] *Madras Research Centre, Central Marine Fisheries Research Institute (CMFRI), Chennai 600028, India*

(Received 13.7.12, Accepted 2.9.12)

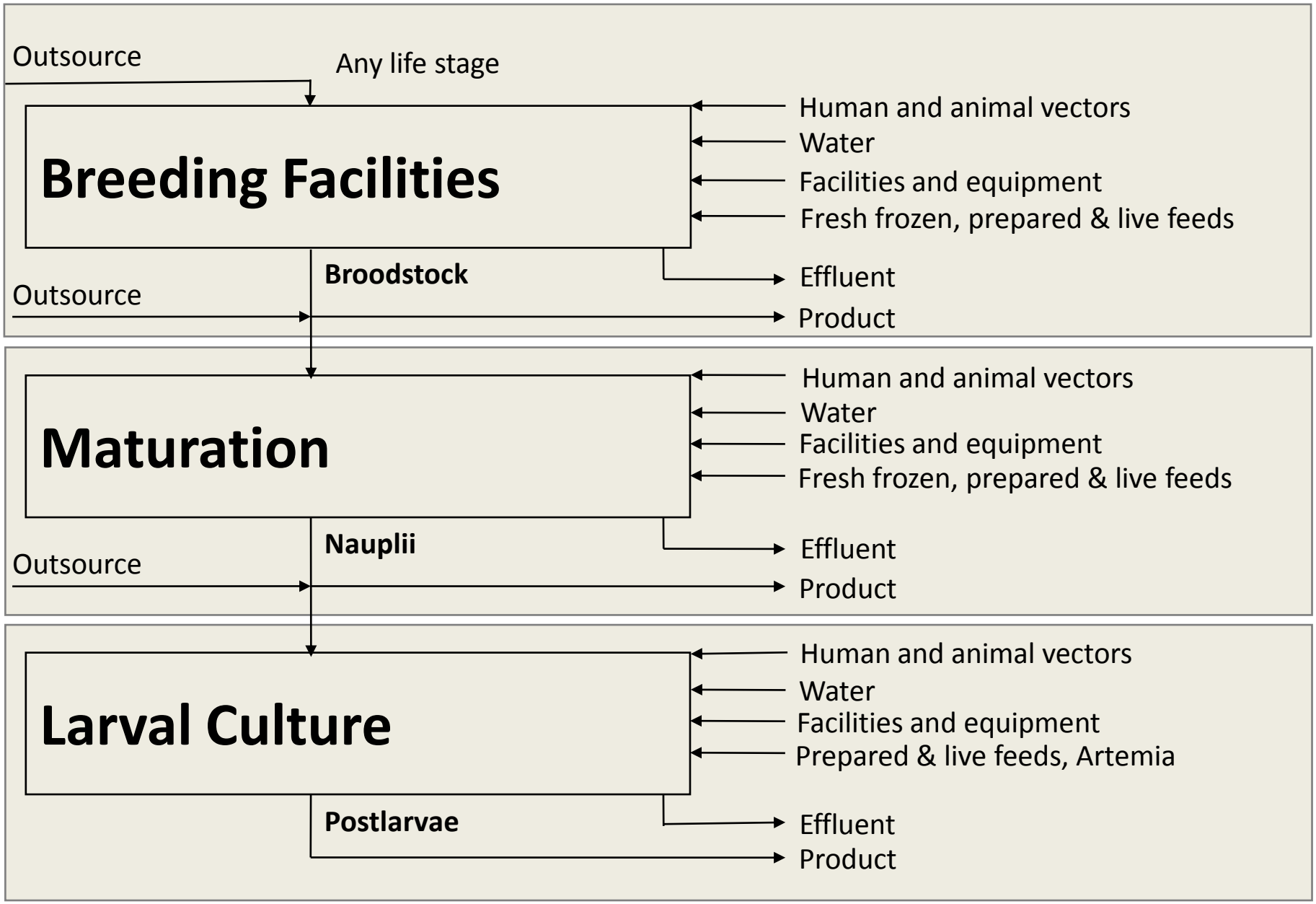
Key words: shrimp hatchery, biosecurity, disease

Abstract

Infectious diseases are a major problem in shrimp aquaculture. Strict biosecurity measures should be implemented to control horizontal and vertical transmission of pathogens. However, implementation of biosecurity measures is neither consistent nor uniform. In this study, we generate baseline information on the variety and degree of adoption of biosecurity measures in shrimp hatcheries in India. Data were collected from 96 hatcheries using a structured questionnaire. Hatcheries were classified as small (<50.6 million seed/annum), medium (50.6-102 million seed/annum), or large (>102 million seed/annum), according to seed production capacity. Biosecurity measures were categorized as personnel, operational, or screening for pathogens in broodfish and live feeds. The highest biosecurity implementation rate of personnel procedures was 50% in small and medium hatcheries and 40% in large hatcheries. The highest implementation rate of operational measures was 63% in small hatcheries, 84% in medium, and 47% in large. The highest rates for screening of pathogens was 50% in small and large hatcheries and 25% in medium. The only measures implemented in all 96 hatcheries were use of foot baths, disinfecting hands after handling brooders/larvae/live feed, and virus screening of broodfish, indicating consistent practice in all hatcheries and an implementation gap of 0%. A lack of understanding, reluctance to implement, and the need for large financial inputs are responsible for the poor implementation of biosecurity measures. The baseline information generated in this study exposes the challenges in implementation of biosecurity in shrimp hatcheries. The collected data can be used to further refine and implement biosecurity practices.

HACCP Principles

- Perform systematic hazards analysis
- Determine critical control points
- Establish critical limits
- Establish monitoring procedures
- Establish record keeping systems
- Establish verification procedures
- Determine appropriate corrective actions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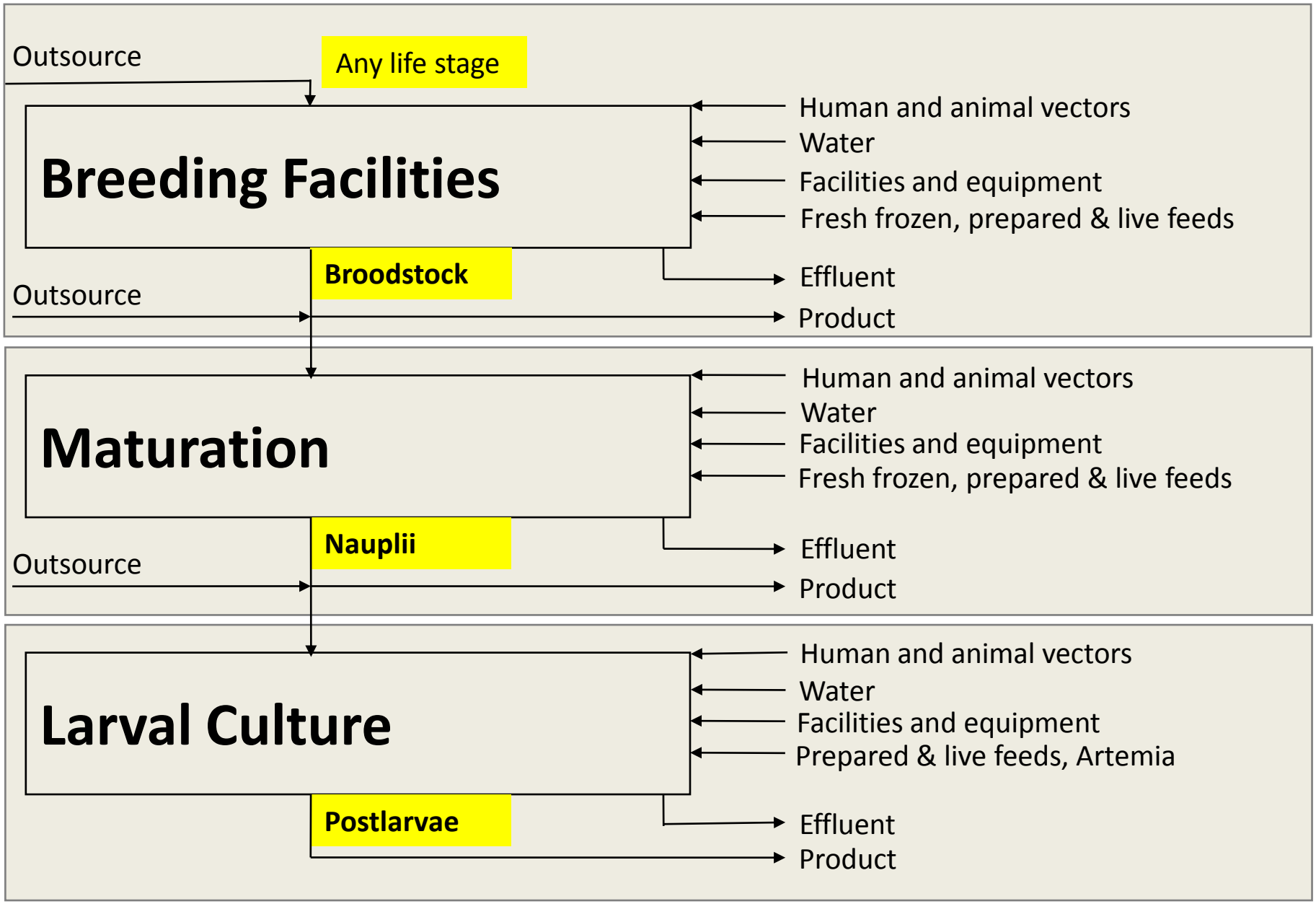
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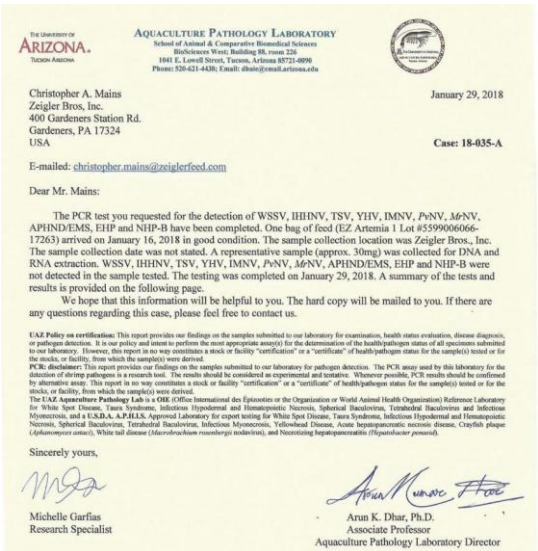
Hazard Analysis Incoming Stocks

ID Potential Hazard	Signif-icant	Justify	Preventive Measures	CCP
Shrimp receipt	Yes	Shrimp may be infective	SPF certification with every shipment. Quarantine procedures and periodic testing for LSP	Yes



AQUACULTURE PATHOLOGY LABORATORY
School of Animal & Comparative Biomedical Sciences
BioSciences West; Building 88, room 226
1041 E. Lowell Street, Tucson, Arizona 85721-0090
Phone: 520-621-4438; Email: dhale@email.arizona.edu

Christopher A. Mains
Zeigler Bros, Inc.
400 Gardeners Station Rd.
Gardeners, PA 17324
USA

January 29, 2018

Case: 18-035-A

E-mailed: christopher.mains@zeiglerfeed.com

Dear Mr. Mains:

The PCR test you requested for the detection of WSSV, IHHNV, TSV, YHV, IMNV, *Pv*NV, *Mr*NV, APHND/EMS, EHP and NHP-B have been completed. One bag of feed (EZ Artemia 1 Lot #5599006066-17263) arrived on January 16, 2018 in good condition. The sample collection location was Zeigler Bros., Inc. The sample collection date was not stated. A representative sample (approx. 30mg) was collected for DNA and RNA extraction. WSSV, IHHNV, TSV, YHV, IMNV, *Pv*NV, *Mr*NV, APHND/EMS, EHP and NHP-B were not detected in the sample tested. The testing was completed on January 29, 2018. A summary of the tests and results is provided on the following page.

We hope that this information will be helpful to you. The hard copy will be mailed to you. If there are any questions regarding this case, please feel free to contact us.

UAZ Policy on certification: This report provides our findings on the samples submitted to our laboratory for examination, health status evaluation, disease diagnosis, or pathogen detection. It is our policy and intent to perform the most appropriate assay(s) for the determination of the health/pathogen status of all specimens submitted to our laboratory. However, this report in no way constitutes a stock or facility "certification" or a "certificate" of health/pathogen status for the sample(s) tested or for the stocks, or facility, from which the sample(s) were derived.

PCR: disclaimer: This report provides our findings on the samples submitted to our laboratory for pathogen detection. The PCR assay used by this laboratory for the detection of shrimp pathogens is a research tool. The results should be considered as experimental and tentative. Whenever possible, PCR results should be confirmed by alternative assay. This report in no way constitutes a stock or facility "certification" or a "certificate" of health/pathogen status for the sample(s) tested or for the stocks, or facility, from which the sample(s) were derived.

The **UAZ Aquaculture Pathology Lab** is a **OIE** (Office International des Epizooties or the Organization or World Animal Health Organization) Reference Laboratory for White Spot Disease, Taura Syndrome, Infectious Hypodermal and Hematopoietic Necrosis, Spherical Baculovirus, Tetrahedral Baculovirus and Infectious Myonecrosis, and a **U.S.D.A. A.P.H.I.S.** Approved Laboratory for export testing for White Spot Disease, Taura Syndrome, Infectious Hypodermal and Hematopoietic Necrosis, Spherical Baculovirus, Tetrahedral Baculovirus, Infectious Myonecrosis, Yellowhead Disease, Acute hepatopancreatic necrosis disease, Crayfish plague (*Aphanomyces astaci*), White tail disease (*Macrobrachium rosenbergii* nodavirus), and Necrotizing hepatopancreatitis (*Hepatobacter penaei*).

Sincerely yours,

Michelle Garfias
Research Specialist

Arun K. Dhar, Ph.D.
Associate Professor
Aquaculture Pathology Laboratory Director

New Diagnostic Options

Sample <5 mm^2 tissue

70% Lab Grade Ethanol

info@genics.com

3 days

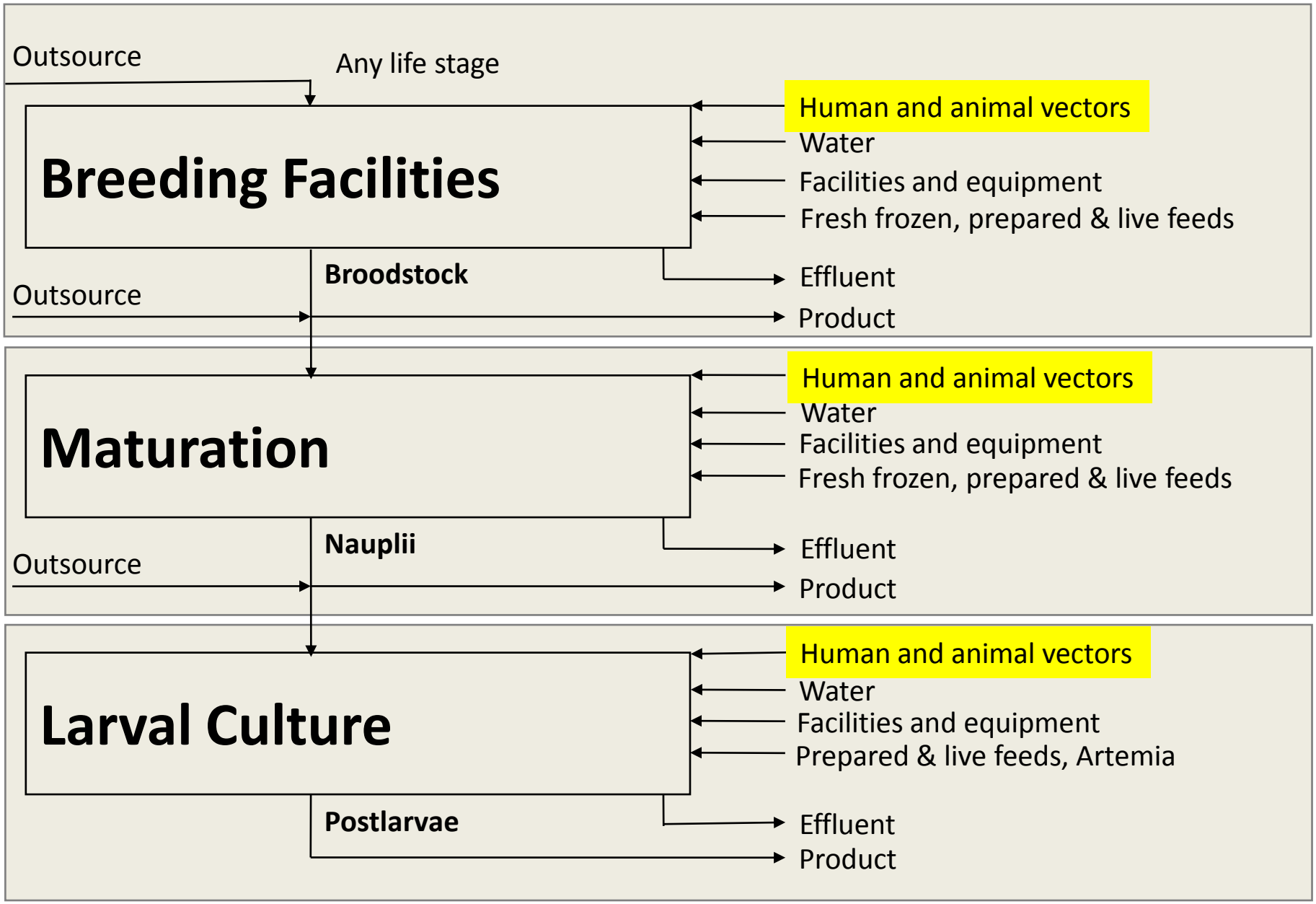
Shrimp **Multi**Path

13 Quality Controlled Pathogen Assays

WSSV	LSNV
IMNV	YHV
IHHNV	GAV
MBV	EHP
TSV	NHP
AHPND	MoV
	HPV

48 hours

Shrimp MultiPath Report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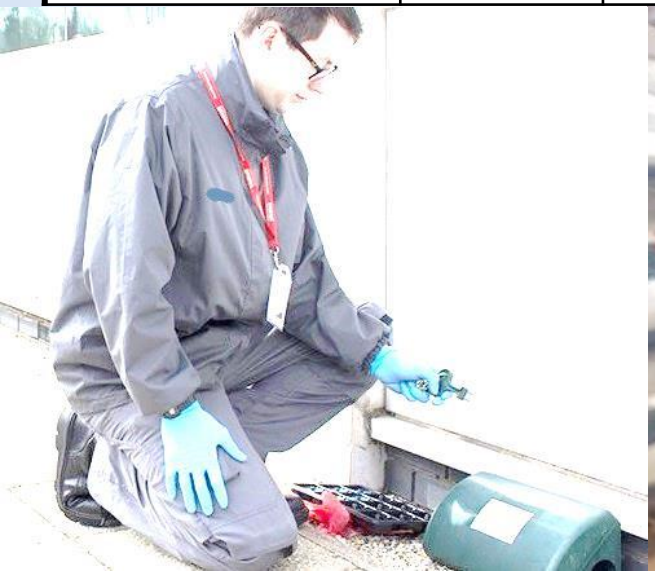
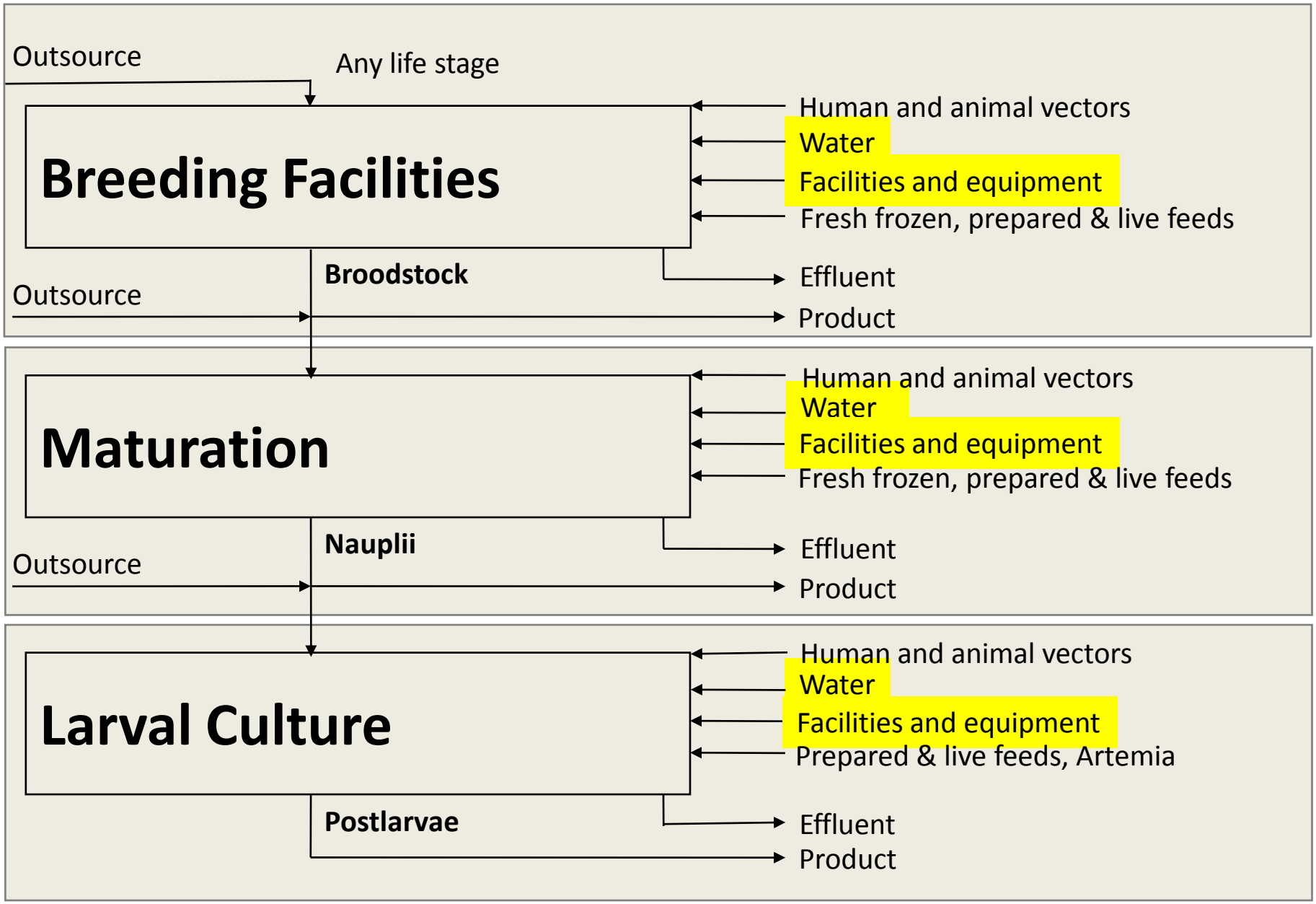
Hazard Analysis
Human and Animal vectors

ID Potential Hazard	Significant	Justify	Preventive Measures	CCP
Humans	Yes	Human vectors may transfer pathogens	Controlled by SOPs	No
Pests	Yes	Pests may transfer pathogens	Controlled by SOPs	No

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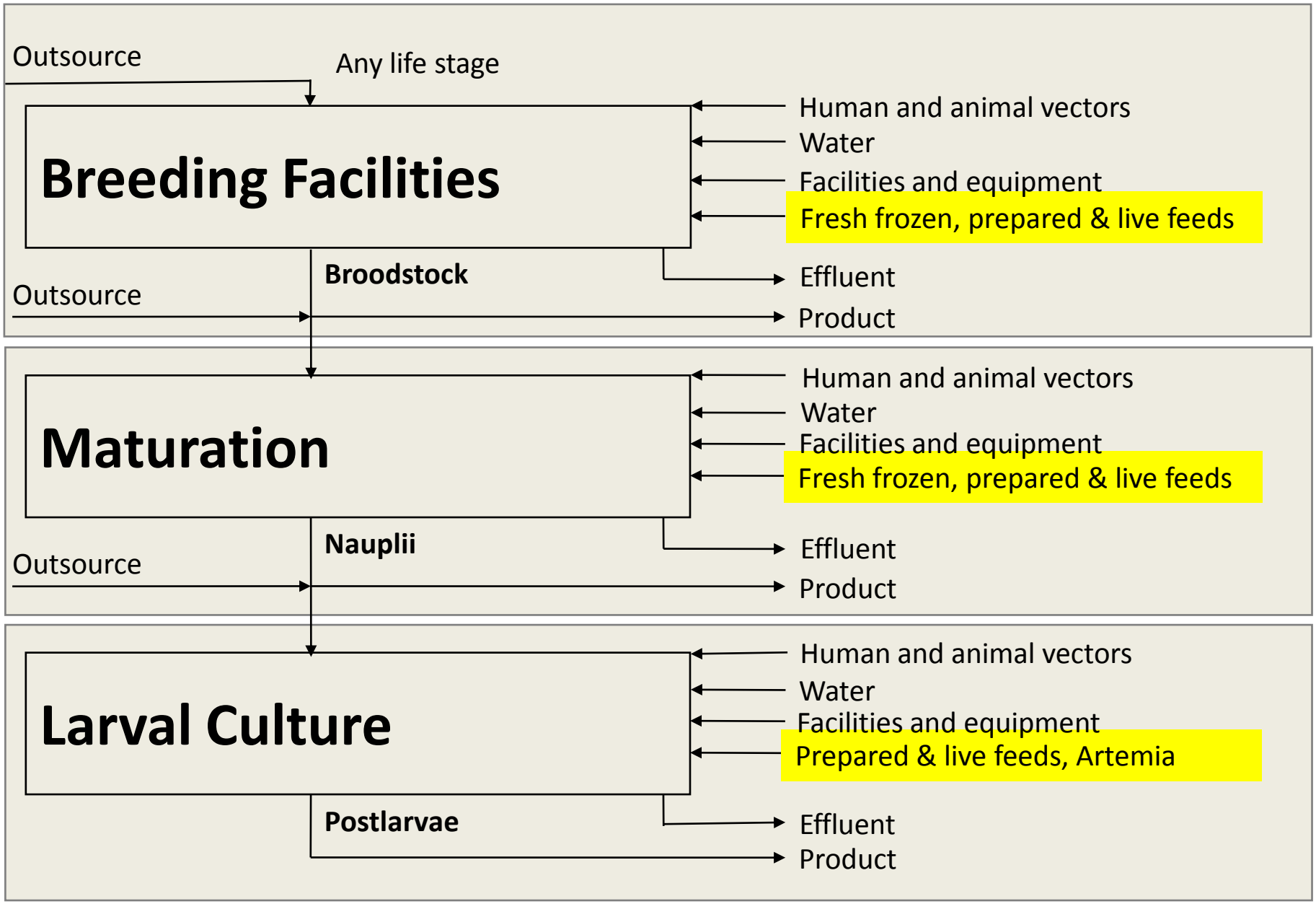
Effluent
Product

Hazard Analysis
Water Facilities Equipment

ID Potential Hazard	Significant	Justify	Preventive Measures	CCP
Incoming water	Yes	Water or waterborne particles may be infective	Periodic testing for pathogens, sentinel system, disinfection	Yes
Facilities and equipment	Yes	Facilities and equipment may become contaminated	Controlled by SOPs	No

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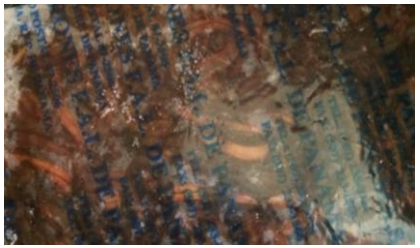
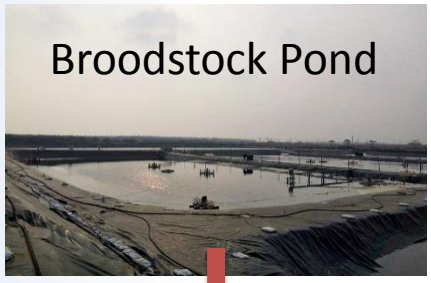
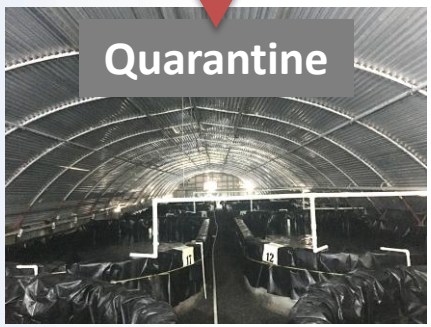


ID Potential Hazard	Significant	Justify	Preventive Measures	CCP
Live feeds	Yes	Water or waterborne particles with live feeds may be infective	Certificate of compliance (COC) ensuring virus free feed with every shipment. Periodic testing of feeds for LSP	Yes
Fresh frozen feeds, prepared feeds, and Artemia cysts	Yes	Feeds, Artemia, and fertilizers may be infective	Certificate of compliance (COC) ensuring virus free feed with every shipment. Periodic testing of feeds for LSP	Yes

Broodstock Contamination





GLOBAL GEN

Primo Broodstock
Genetically Superior

Molokai Broodstock Company

SHRIMP IMPROVEMENT SYSTEMS

AMERICAN PENAEID

SEA PRODUCTS DEVELOPMENT

KONA BAY

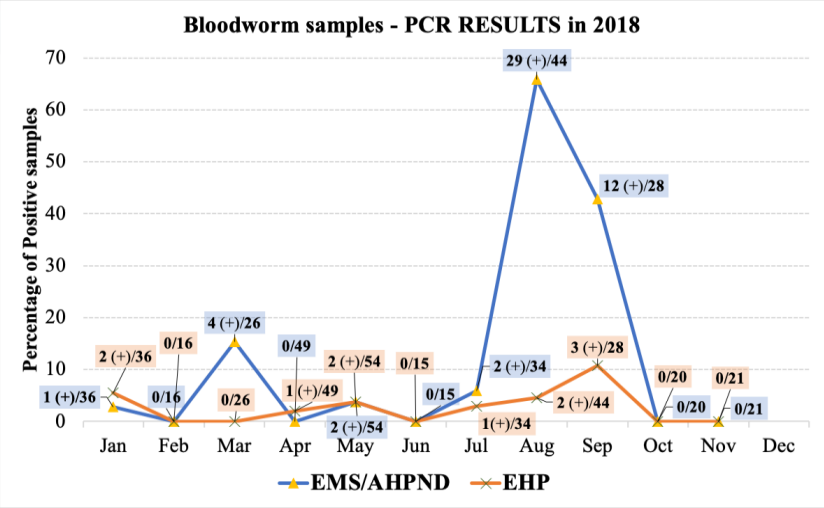
EMS testing

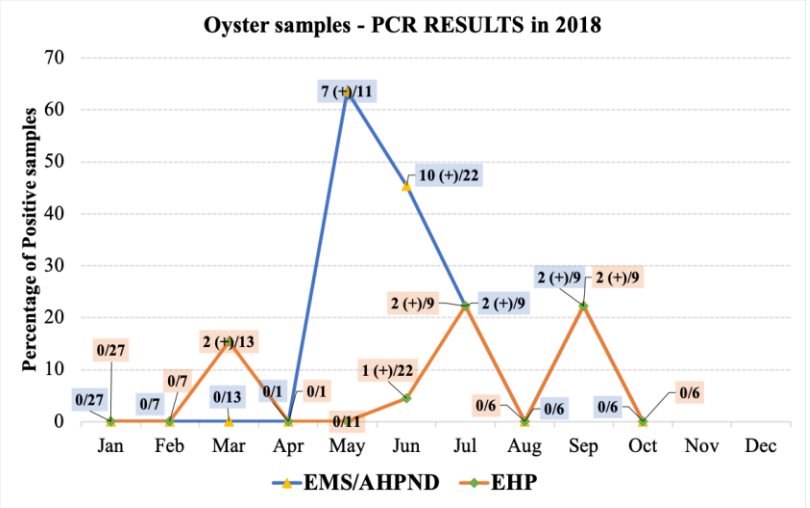
PCR results from AP2 PCR detection with enrichment specimens

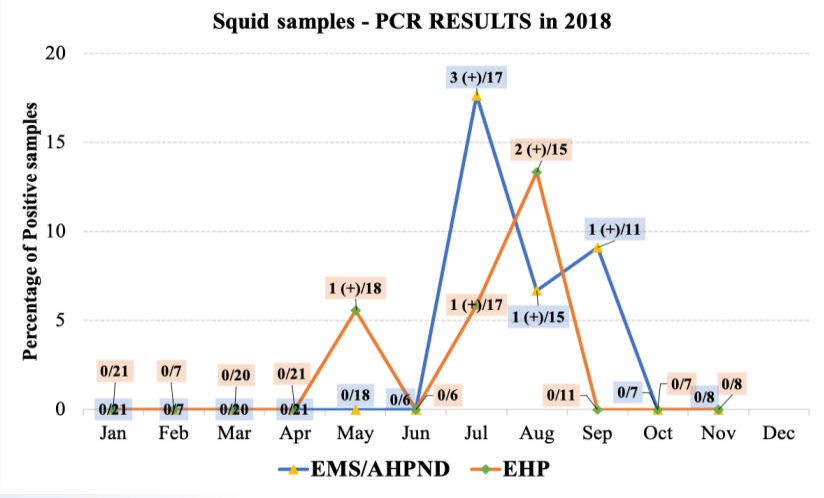
Sources	Province (Positive/Total tested)			Totals %
	Songkhla	Trad	Rayong	
Broodstock feces	2 / 5	8 / 15	14 / 24	24 / 44 (55%)
Nauplii	1 / 1	0 / 5	3 / 8	4 / 14 (29%)
Polychaetes	1 / 2	2 / 3	2 / 3	5 / 7 (71%)
Squid	1 / 1	0 / 3	3 / 5	4 / 9 (44%)
Artemia	1 / 1	-	0 / 1	1 / 2 (50%)
Oysters	0 / 1	-	0 / 2	0 / 3 (0%)
Clams	1 / 1	1 / 1	-	2 / 2 (100%)

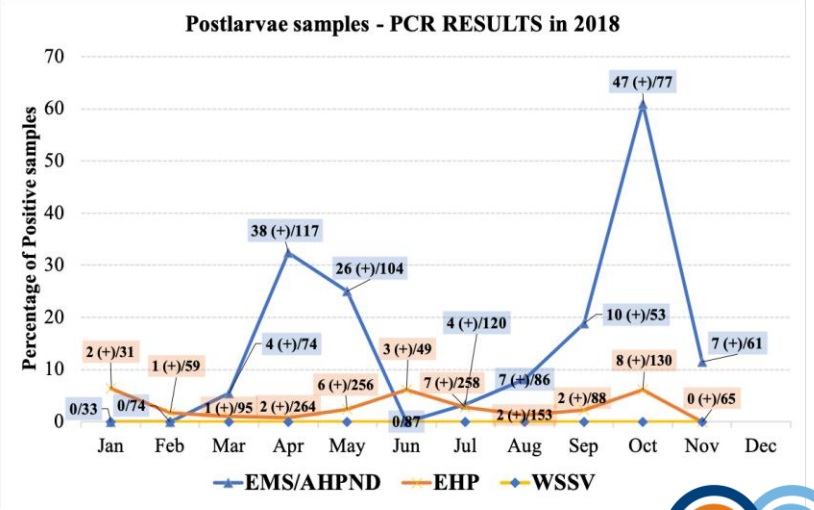
Flegel et al. TARS 2014

Fresh Feed and PL Diagnostics









EMS/AHPND: Early Mortality Syndrome/Acute Hepatopancreatic Necrosis Disease
EHP: *Enterocytozoon hepatopenaei* **WSSV:** *White Spot Syndrome Virus*

SHRIMPVET

Loc Tran - ShrimpVet (2018)

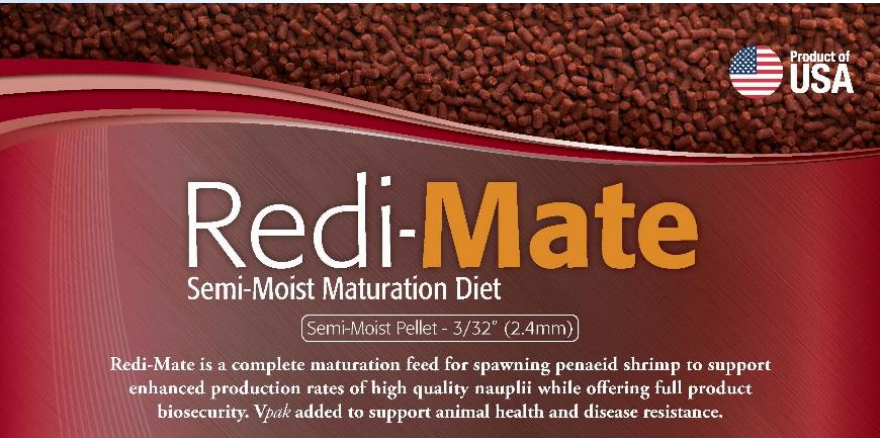
Feed

- Critical limits - No detectable LSP
- Monitoring

What	How	Frequency	Who
No LSPs in feed	Batch testing	Continuous	Third Party Lab
Supplier acquisition and handling protocols	Periodic site visits and or review of protocols	Yearly	Facility manager
Certificate of compliance LSP free	Documentation	Every shipment	Facility manager

Replacement of Live and Fresh Frozen Maturation Feeds

- Improving technologies
- 100% pathogen free
- Nutritionally complete, optimal HUFA levels, pigments, antioxidants, attract ants, health supplements
- Easier storage
- No need to freeze
- Long shelf-life



Aquatic Animal Health Laboratory
Harbor Branch Oceanographic Institute at FAU
Ft. Pierce FL 34946

CERTIFICATE OF ANALYSIS

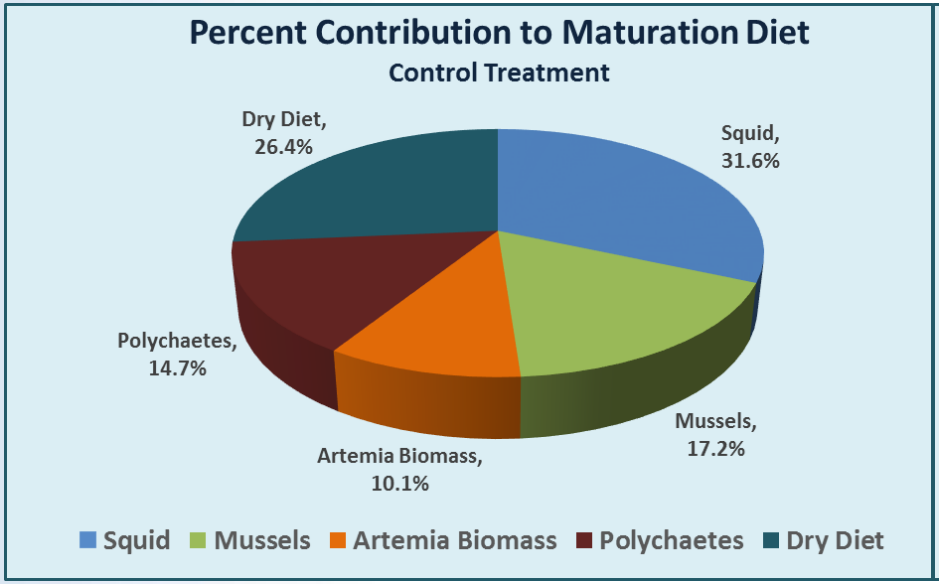
To Whom It May Concern:

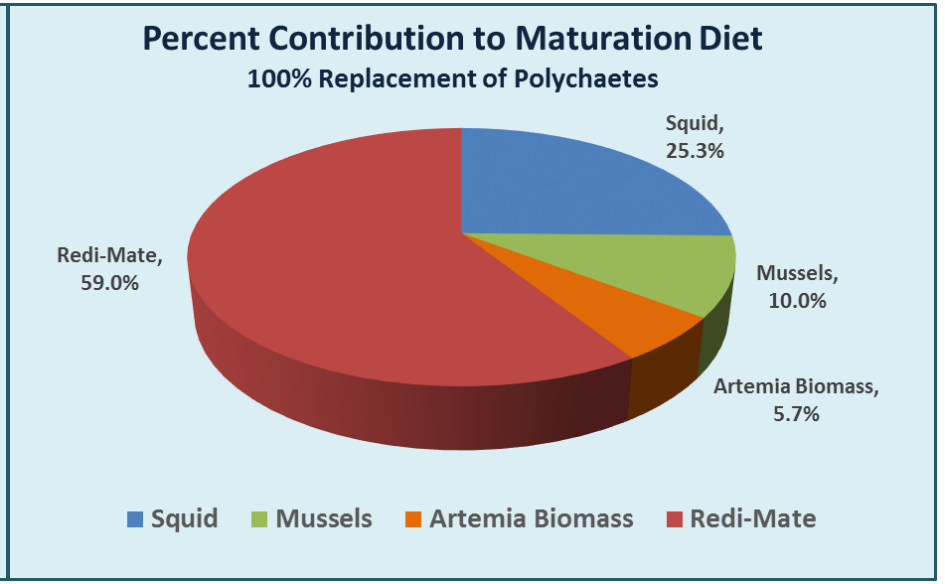
This is to certify that fourteen (14) samples of Shrimp Diet were received from *Zeigler Bros., Inc.* on *11/17/2017* to be tested for the presence of the following OIE invertebrate pathogens of concern: EHP, IHHNV, WSSV, NHP, EMS, TSV, YHV, IMNV, WTD and crayfish plague.

Let it be known that the samples tested and identified below was negative for the aforementioned pathogens by the Polymerase Chain Reaction (PCR) test (viruses) and by isolation on mycological media (crayfish plague).

Photographs of the gel electrophoresis patterns made from the PCR tests may be obtained from the Aquatic Animal Health Lab, upon written request.

Frozen Polychaete Replacement

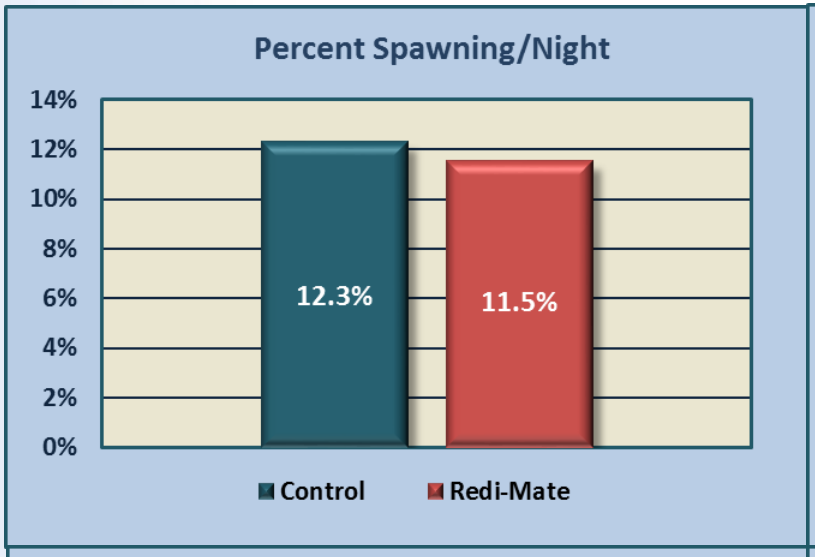


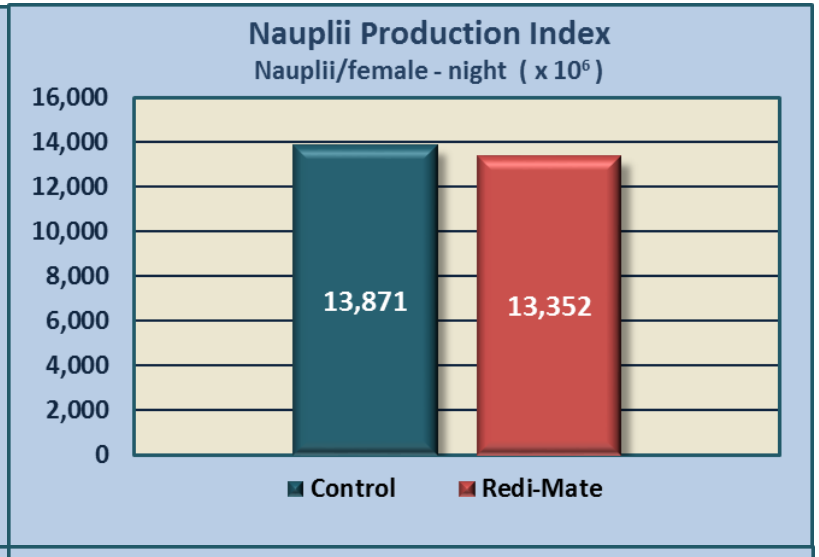


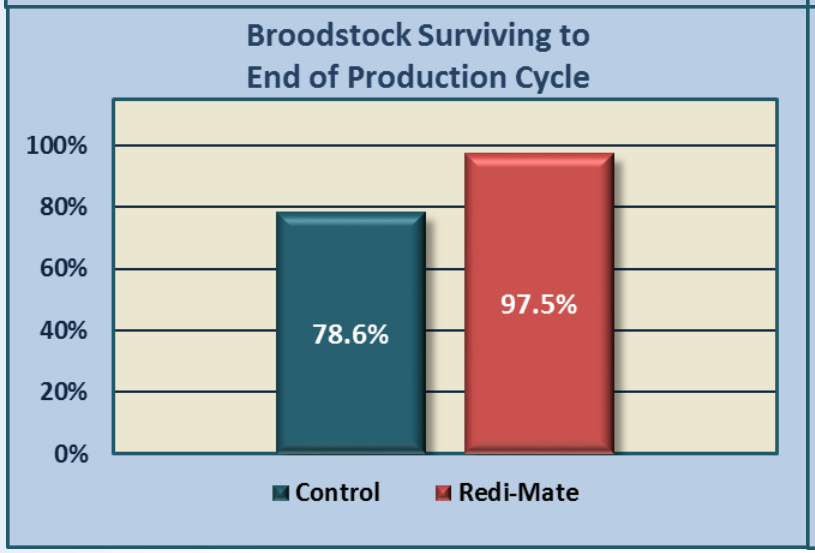
Feed	% BW/day (wet weight)	g dry wt per g wet wt	% BW /day (dry weight)	Percentage of Dry Diet
Squid	11.0%	20%	2.20%	31.6%
Mussels	6.0%	20%	1.20%	17.2%
Artemia Biomass	7.0%	10%	0.70%	10.1%
Polychaetes	6.0%	17%	1.02%	14.7%
Dry Diet	2.0%	92%	1.84%	26.4%
Total per day	**32.0%**		**6.96%**	**100.0%**

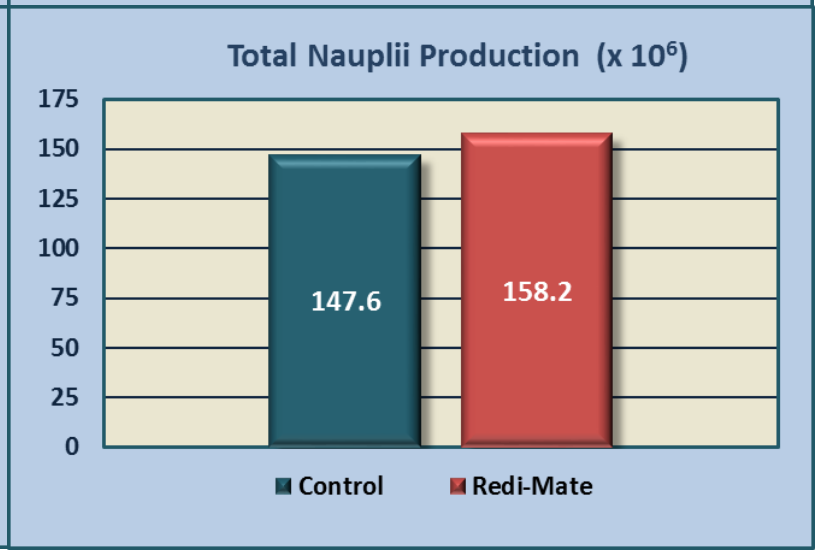
Feed	% BW/day (wet weight)	g dry wt per g wet wt	% BW /day (dry weight)	Percentage of Dry Diet
Squid	8.8%	20%	1.76%	25.3%
Mussels	3.5%	20%	0.70%	10.0%
Artemia Biomass	4.0%	10%	0.40%	5.7%
Polychaetes	0.0%	17%	0.00%	0.0%
Redi-Mate	5.6%	73%	4.10%	59.0%
Total per day	**21.9%**		**6.96%**	**100.0%**

Maturation Trial 1 Results









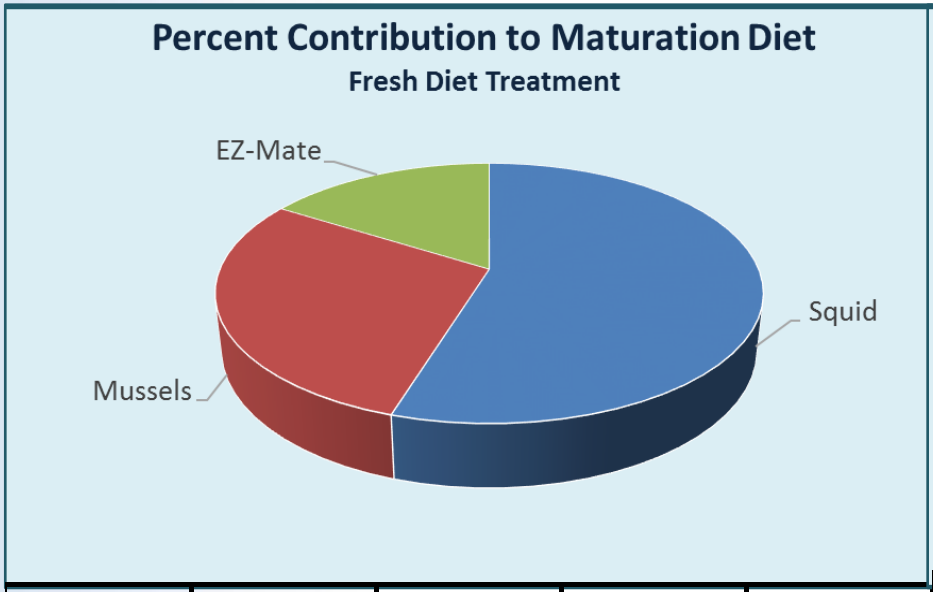
Replacement of Polychaetes

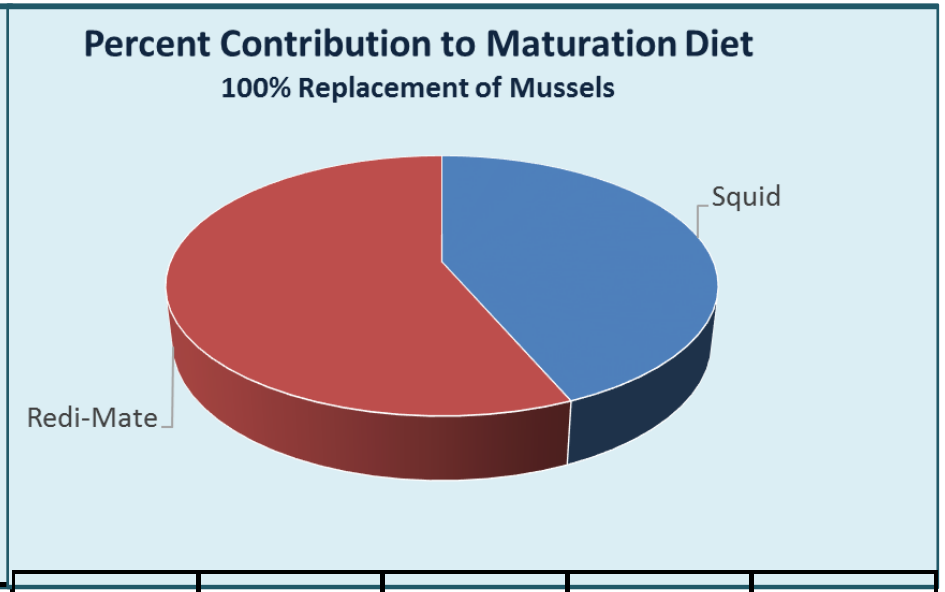
- More nauplii produced
- Improved nauplii quality
  - More yolk
  - More active phototactic
  - Better larval survival

Initial 35 Days

Diet Treatment	Total Spawns	Nauplii/ Spawn	Total Nauplii
Replacement	1,389	116,882	158.2 M
Control	1,327	114,271	147.6 M

Frozen Mussel Replacement

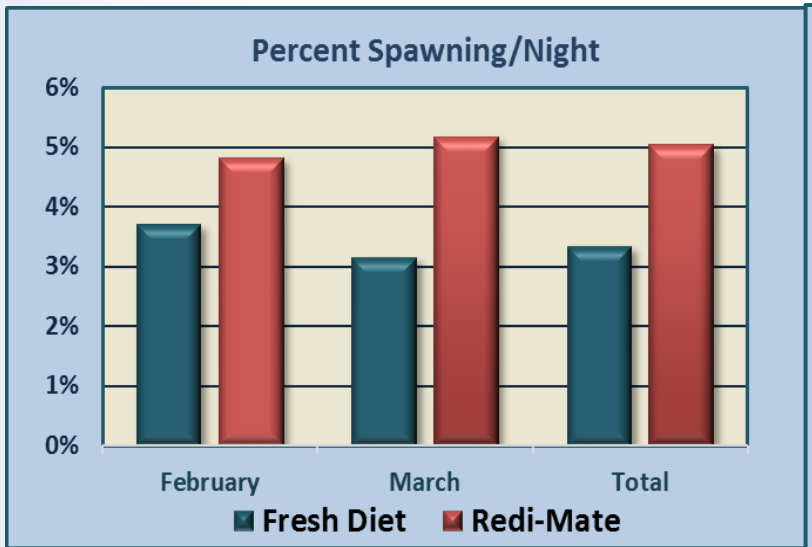


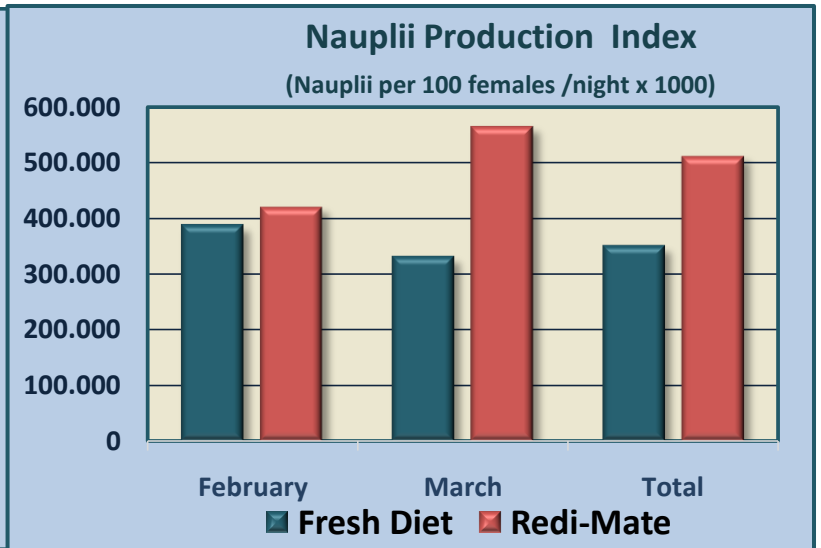


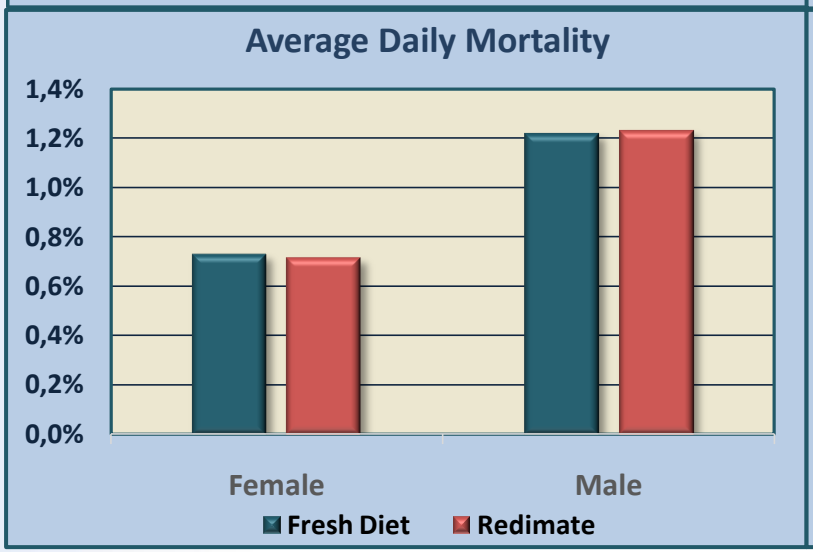
Feed	% BW / day (Wet weight)	g dry wt per g wet wt	% BW / day (Dry weight)	Percentage of Dry Diet
Squid	17%	20%	3.4%	54.8%
Mussels	9%	20%	1.8%	29.0%
EZ-Mate	2%	50%	1.0%	16.1%
Total per day	**28%**		**6.2%**	**100.0%**

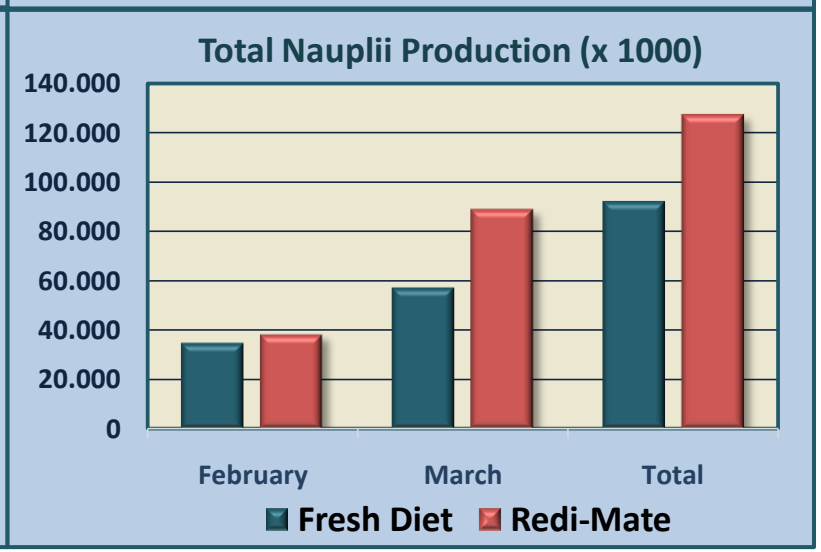
Feed	% BW / day (Wet weight)	g dry wt per g wet wt	% BW / day (Dry weight)	Percentage of Dry Diet
Squid	13.50%	20%	2.7%	43.5%
Mussels	0%			
Redi-Mate	5.00%	70%	3.5%	56.5%
Total per day	**18.50%**		**6.2%**	**100.0%**

Maturation Trial 2 Results









From Hatchery to Farm

PL Quality Assurance

The farmer should be responsible for carrying out:

- Verification of PL counts
- Determination of the average weight and coefficient of variation of the PLs stocked
- PCR testing for excludable pathogens
- Hatchery source evaluation
- Microscopic observations
- Stress tests

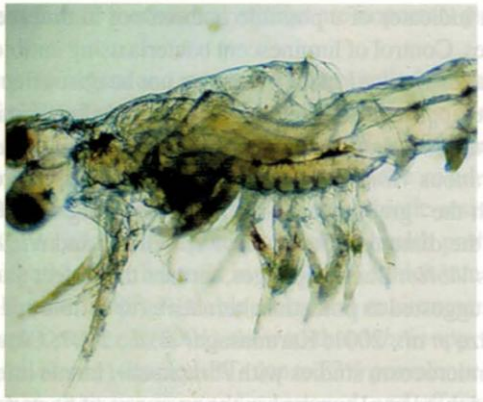


Figure 8. Postlarval blue shrimp (*Penaeus stylirostris*) with vibriosis. Necrosis of several appendages (pleopods and pereiopods) is indicated by melanized foci or tips. A dark oral region is indicative of bacterial colonization of the cuticle of the oesophagus and mouth appendages. Wet-mount; no stain. Magnification 50X. Photo courtesy of DV Lightner, University of Arizona, Aquaculture Pathology Laboratory.

Selecting PL – Evaluating quality

- Level 1
  - Behavioral - activity
  - Culture performance, yields, size/age
- Level 2
  - Physical observations – size, gills, fouling, muscle/gut ratio, uniformity
  - Stress test survivability
- Level 3
  - Disease diagnostics

Juarez and Fegan in GAA global shrimp ops 2002

Intensive Nursery Systems

- High biosecurity systems for post-larvae grown at high densities from 2 mg to sizes as large as 3 g, resulting in healthy, strong and uniform juveniles with significant potential for compensatory growth after transfer to the growout system.

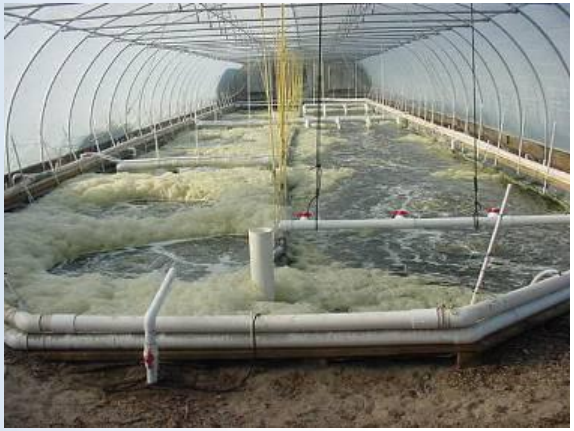
Quarantine and Stocking

- Water should be held for at least 3 days and free of carriers to assure no WSSV
- Water prepared with a stable bloom before stocking
- Acclimation for temperature and salinity
- Feed should be in the system as the animals are added
- Overfeed 5x the first two days from proven feeding tables
- Feeds used in hatchery should make up at least half of the daily feed requirements of transferred animals for at least the first three days.
- Quantify transport and acclimation mortality
    - Stock animals on a tray or in a small net cage in tank
    - Eliminate batches of PL with poor survivability

Biofloc Technology

Biofloc technology is an intensive approach to aquaculture production that relies on elevated suspended solids concentration to provide water treatment and supplementary nutrition for cultured animals.

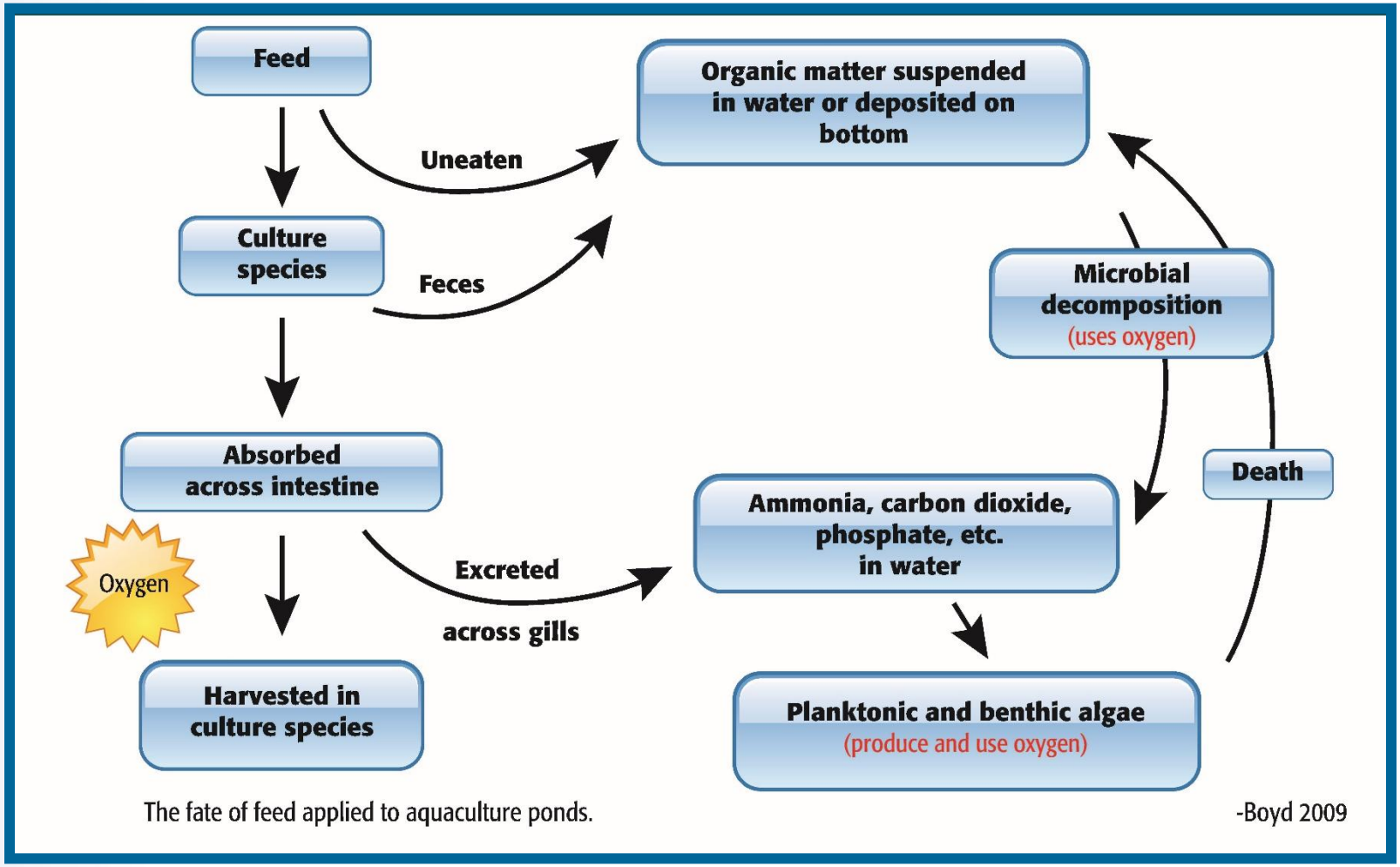
Super-intensive Systems



Nursery Systems

Intensive ponds

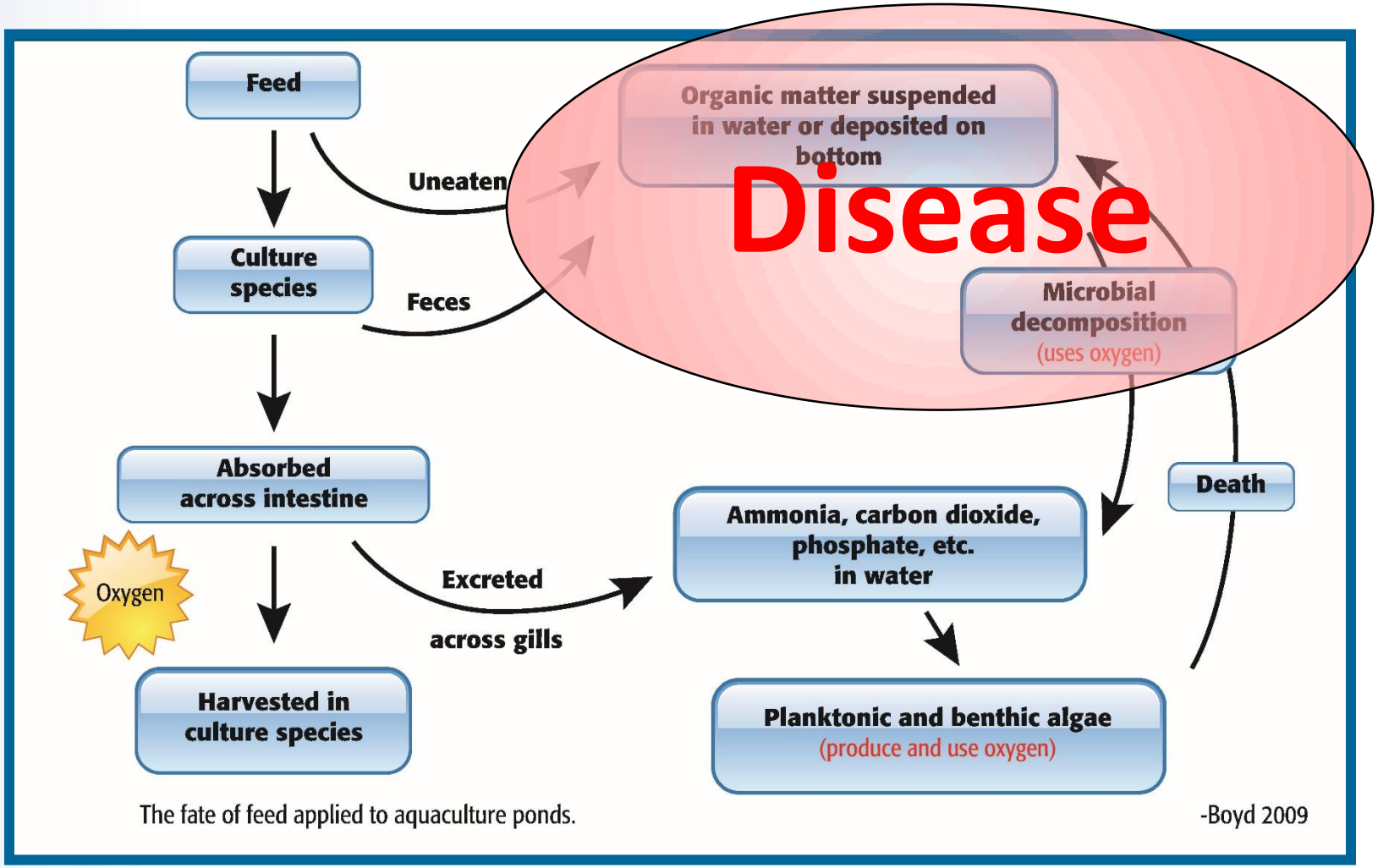
Feed Drives the System



The fate of feed applied to aquaculture ponds.

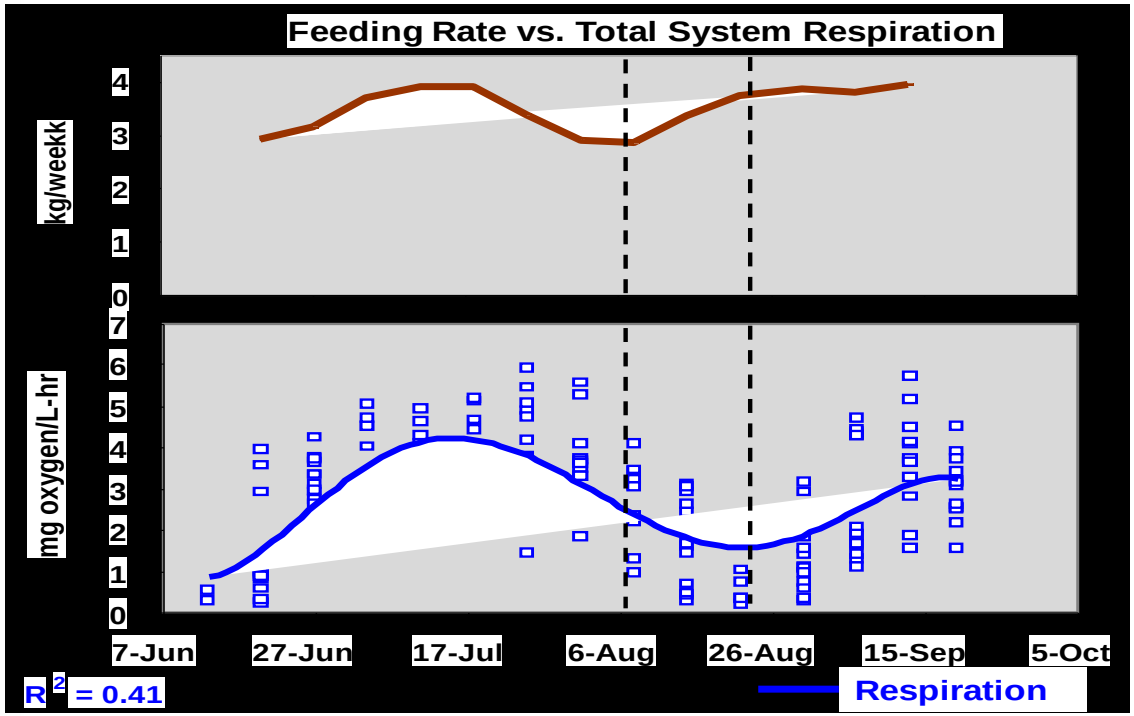
-Boyd 2009

Feed Drives the System



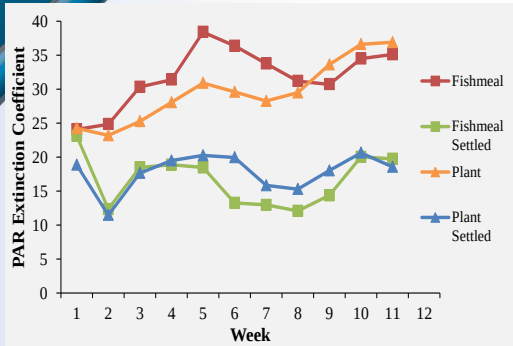
Feed Drives the System

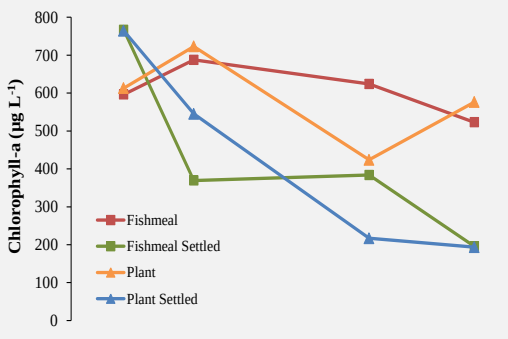
Microbial Community and Shrimp Metabolism

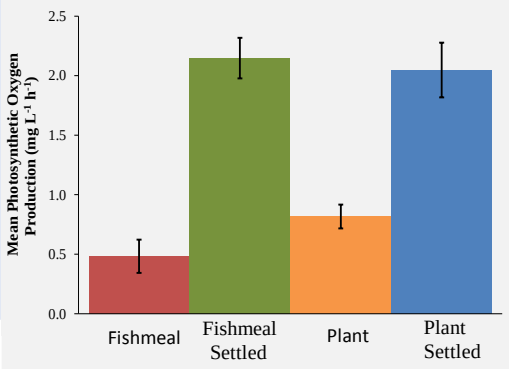


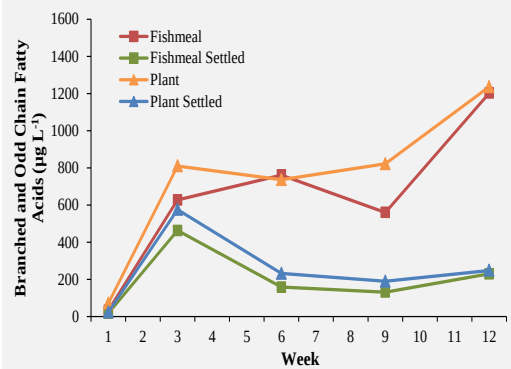
Feed drives the microbial community metabolism as well as the shrimp

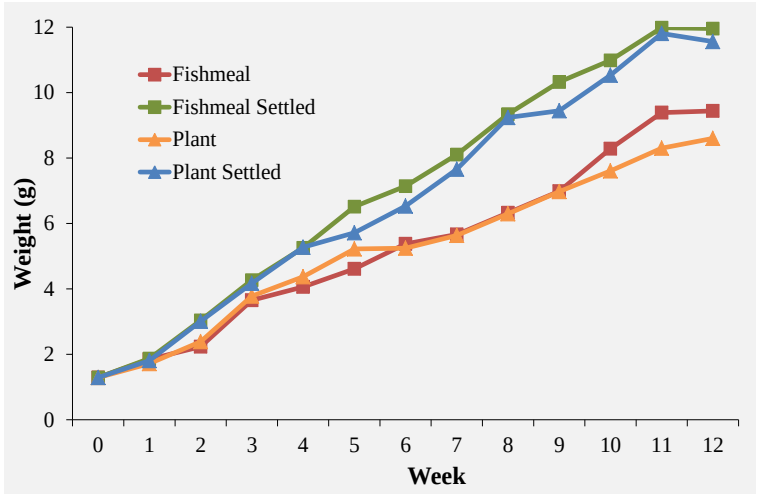
Solids Management and Microbial Communities











- Effects of settling chambers
  - 47% ↓photosynthetically active radiation extinction coefficient
  - 200% ↑ photosynthetic oxygen production
  - 65% ↓ final chlorophyll-a
  - 80% ↓ fatty acid bacterial indicators

- **Shrimp Production**
  - **No difference in survival**
  - **28% ↑ growth rate**
  - **41% ↑ biomass**
  - **26% ↓ FCR**

Ray et al 2012 - Aquaculture

Precision Feeding

Precision Feeding Concept:

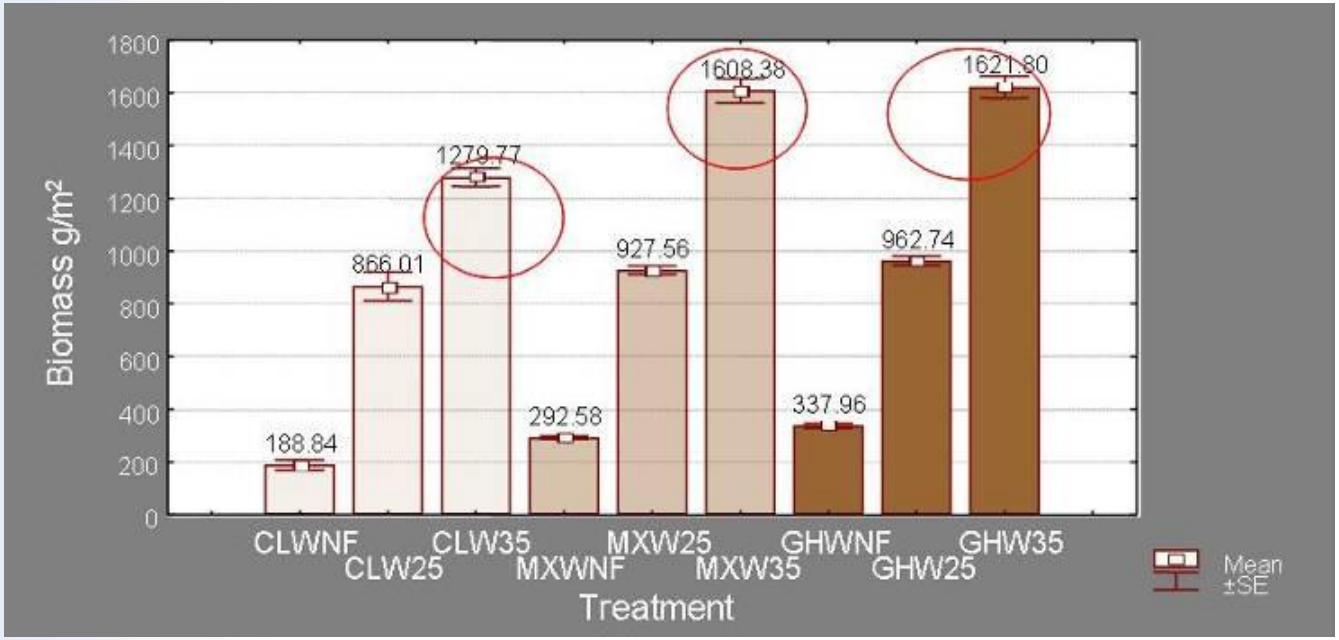
Provide each Animal with:

- the exact quantity of feed that it can consume,
- when the animal is ready to consume it,
- the exact nutrition that the animal requires,
- the correct feed particle sizes and optimum texture,
- In the location where the animal is located

“With the objective to optimize the desired results”

Biofloc Influence on Shrimp Growth

- No food, 25% protein diet, 35% protein diet
- UV filtered water, heterotrophic biofloc water, 50:50 mix
- 300 shrimp/m^2; stocked at 1.82±0.71 g



Significantly higher weight gain in biofloc water than clear water with good diet.

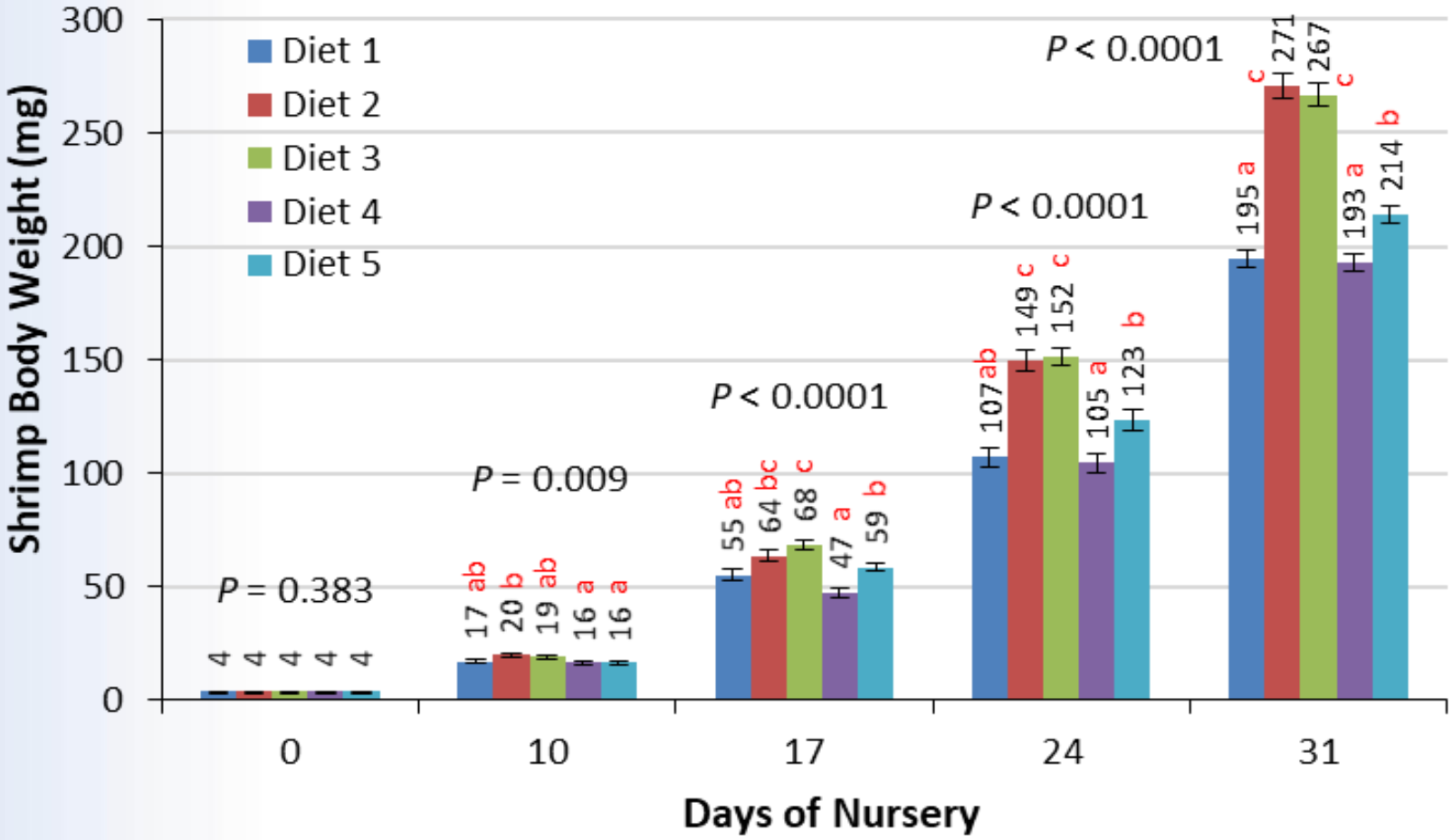
Wasielesky, W., et al., 2006. Aquaculture 258:396-403.

Feed Programs

- Feeds should be designed for shrimp not for Biofloc.
- Feeds are the driver of nutrient inputs into the system
  - Physical characteristics, leaching
  - Nutrient quality, digestibility
  - Nutrient density, formulations
  - Ingredient costs
  - Feeding timing, frequency, amounts
- Design feeds to efficiently meet shrimp requirements
  - High protein nutrient dense formulations
  - Tight control of feeding rates
  - Supplemental carbon addition as necessary
  - Avoid waste nutrient buildup, Phosphorus, Minerals etc.

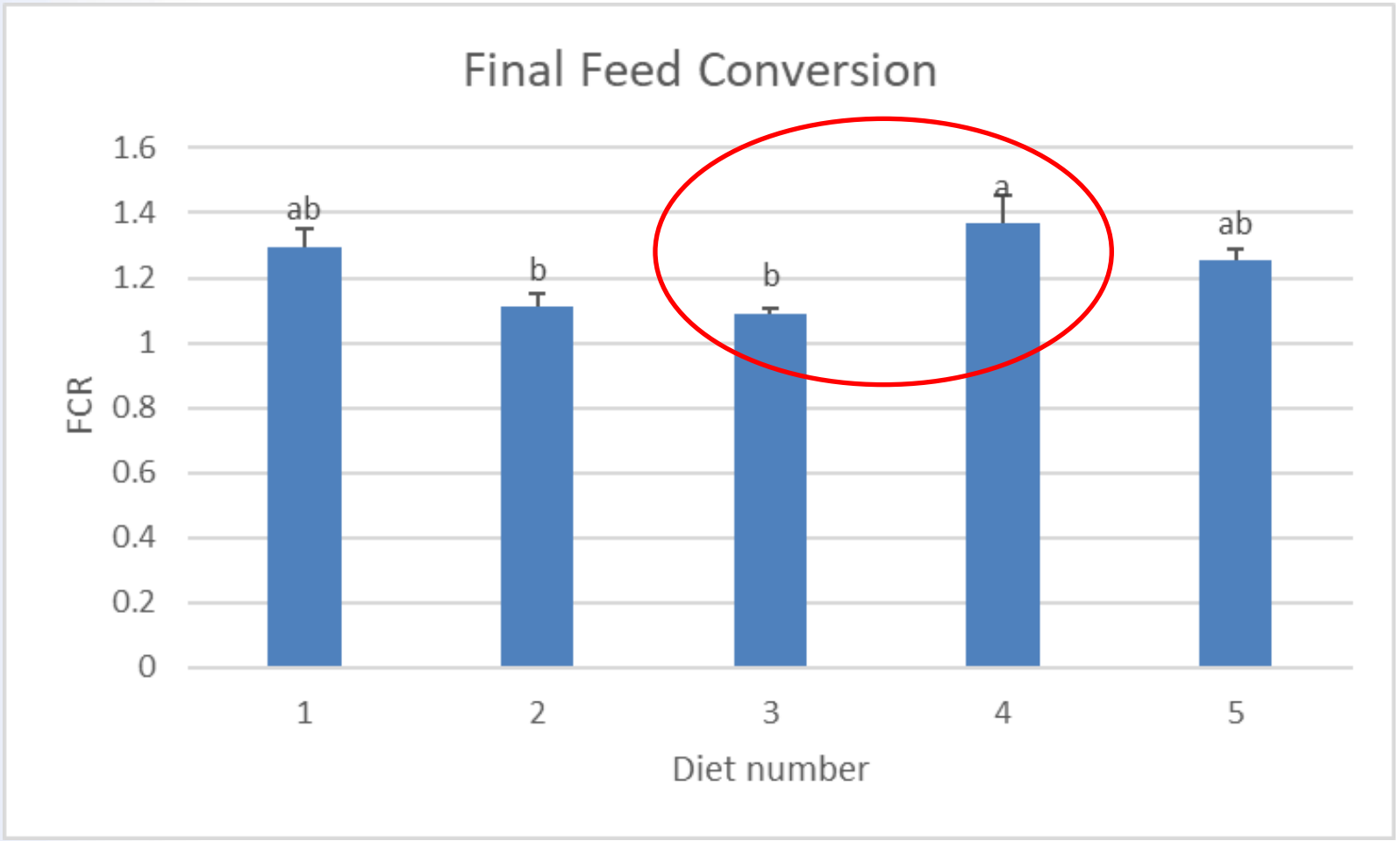
Feed Performance

Comparison of 5 nursery feeds available in Brazil from well respected internationally recognized suppliers



Feed Drives the System

Up to 18.7% higher feed conversion efficiency



A More Biosecure Future

The Past

- PL price and hatchery costs prioritized over biosecurity and sustainability
- Attachment to traditional approach to feeding with live and natural feeds
- Billions of dollars lost due to diseases resulting from these practices

The Future

- A mature industry that invests in hatcheries and nurseries, prioritizing biosecurity
- Adoption of new approaches to hatchery nutrition based on biosecure prepared feeds
- A new era of sustainability and profitability for the industry

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XVI FENACAM'19
12 a 15 de novembro de 2019

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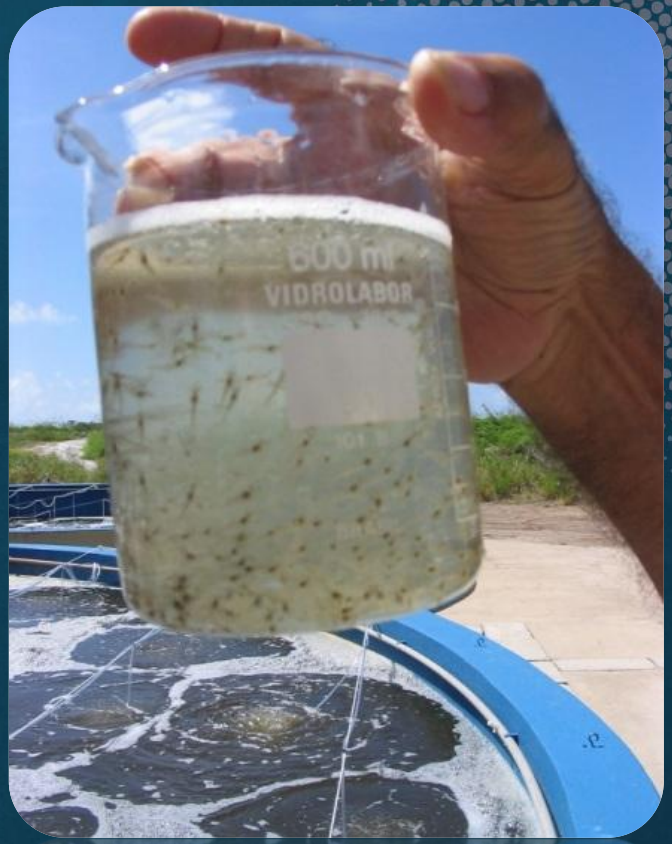
To Success!

Thank You



The law of harvest is to reap more than you sow.
Sow an act, and you reap a habit.
Sow a habit and you reap a character.
Sow a character and you reap a destiny.
James Allen