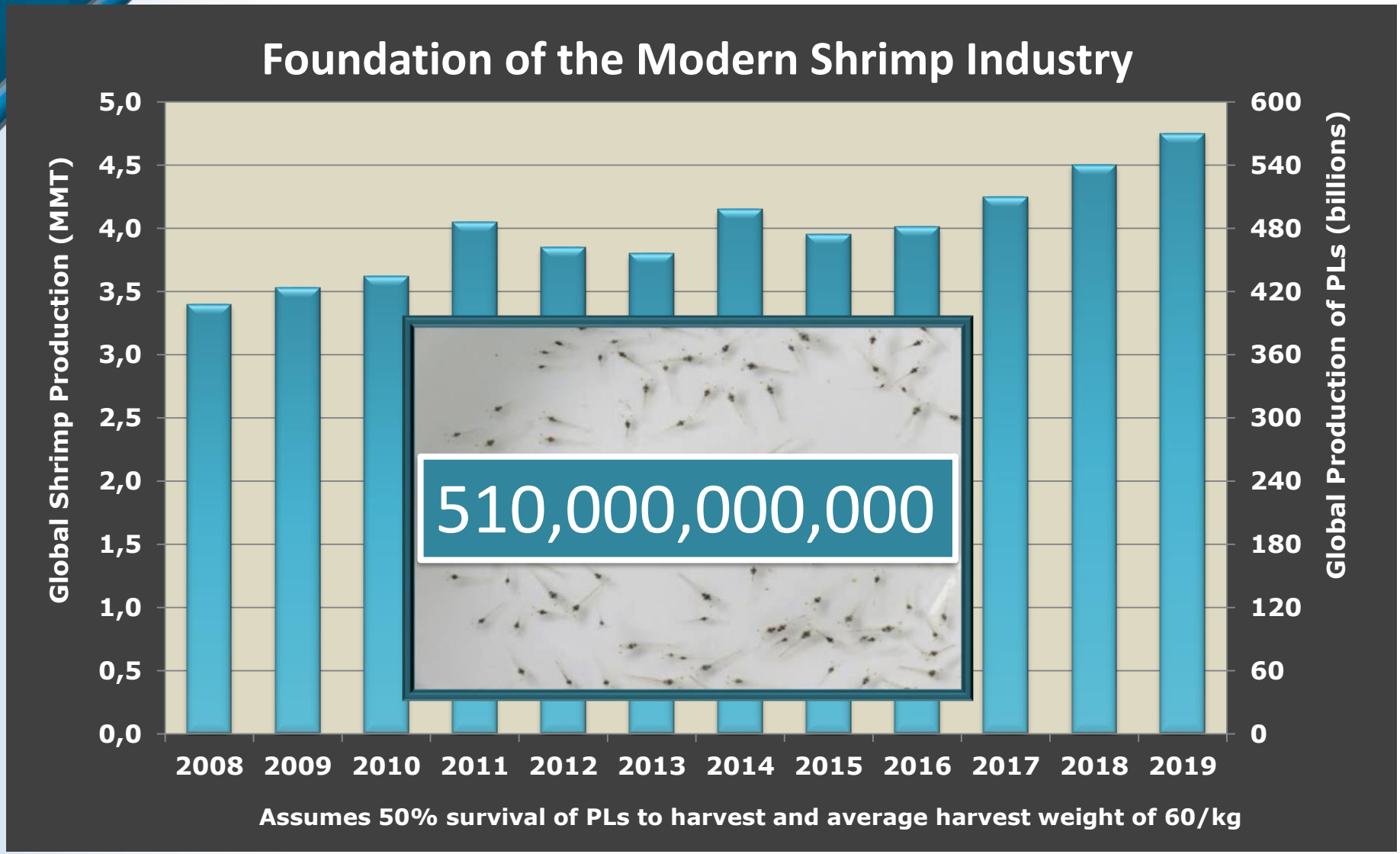
Shrimp Farm Risk Management and Biosecurity Starts with the Hatchery

Craig L. Browdy, Diego Flores,
Peter M. Van Wyk

Global PL Production



Production numbers based on GOAL 2017 estimates

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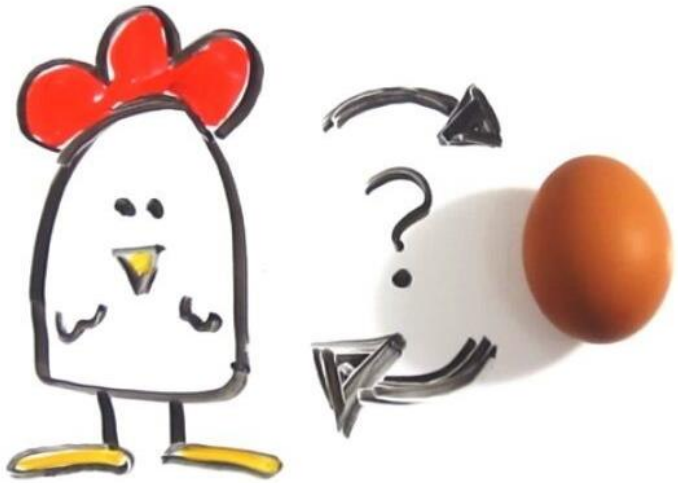
Hatchery

The Missing Link

Genetics

Biosecurity

Nutrition



Larval Quality

Production Results

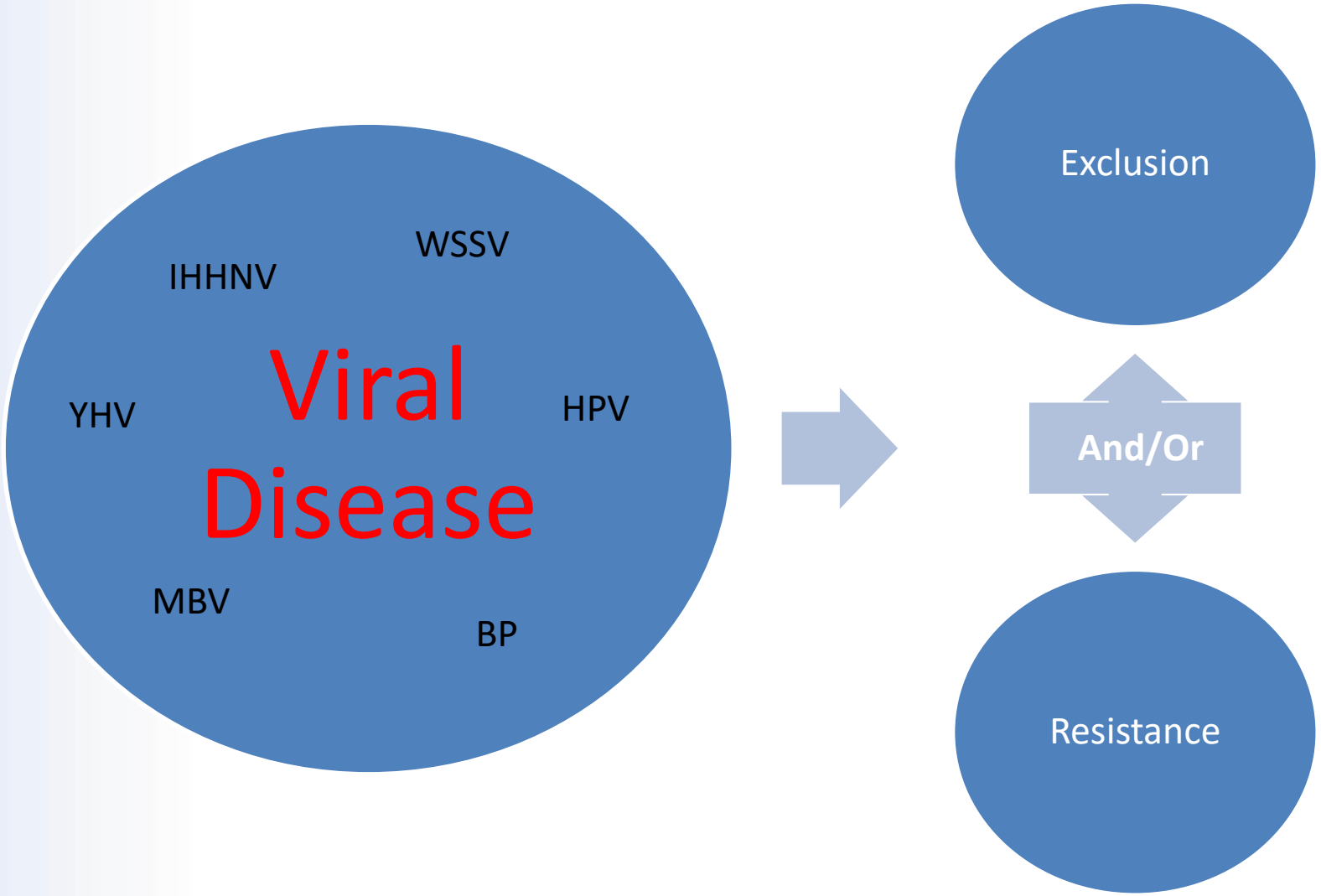
Farm Profits

Biosecurity

"Biosecurity is defined as the implementation of measures that reduce the risk of disease agents being introduced and spread. It requires that people adopt a set of attitudes and behaviors to reduce risk in all activities involving domestic, captive/exotic, and wild animals and their products"

(FAO/OIE/World Bank 2008).

Shrimp Disease Challenges



Breeding Programs SPF

- SPF – Specific Pathogen Free
  - Began in early 1990s in response to IHHNV
  - Very effective in excluding known viruses
  - Diverse founding gene pool, avoid inbreeding
  - Individual selection for growth
- SPR – Specific Pathogen Resistant
  - Based on family selection
  - Very effective for TSV, less for other viruses
- Breeding centers, biosecurity

Ecuadorian Breeding Model

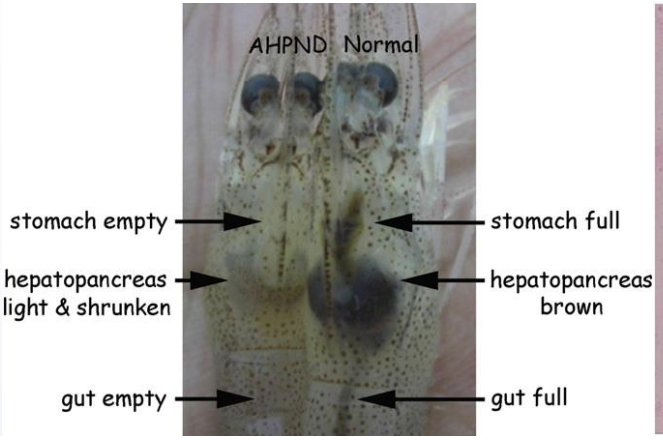
- APE – All pathogen exposed
  - Microsatellite based genetic selection schemes
  - Avoid inbreeding typical of mass selection
  - Family and interfamily selection growth, survival, disease resistance, commercial traits
  - Controlled spawning, commercial larval and pond culture, sampling top 5%, genetic analysis, selection multiplication elite families

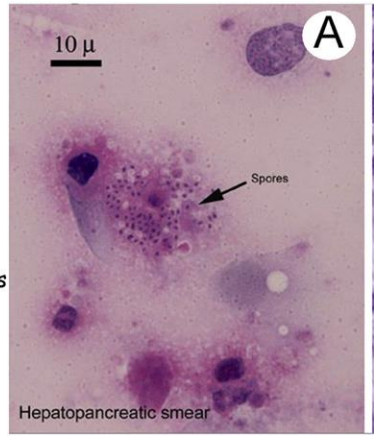
Perez and Harris 2014

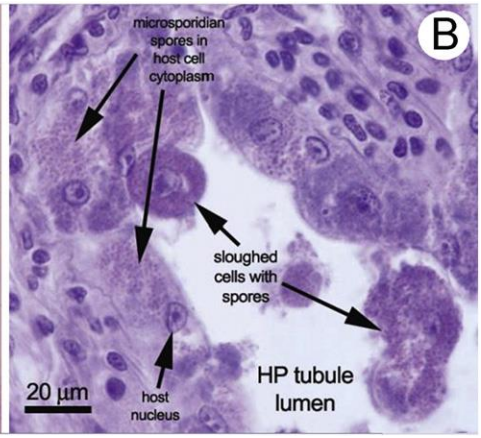
Non Viral Pathogens

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

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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]

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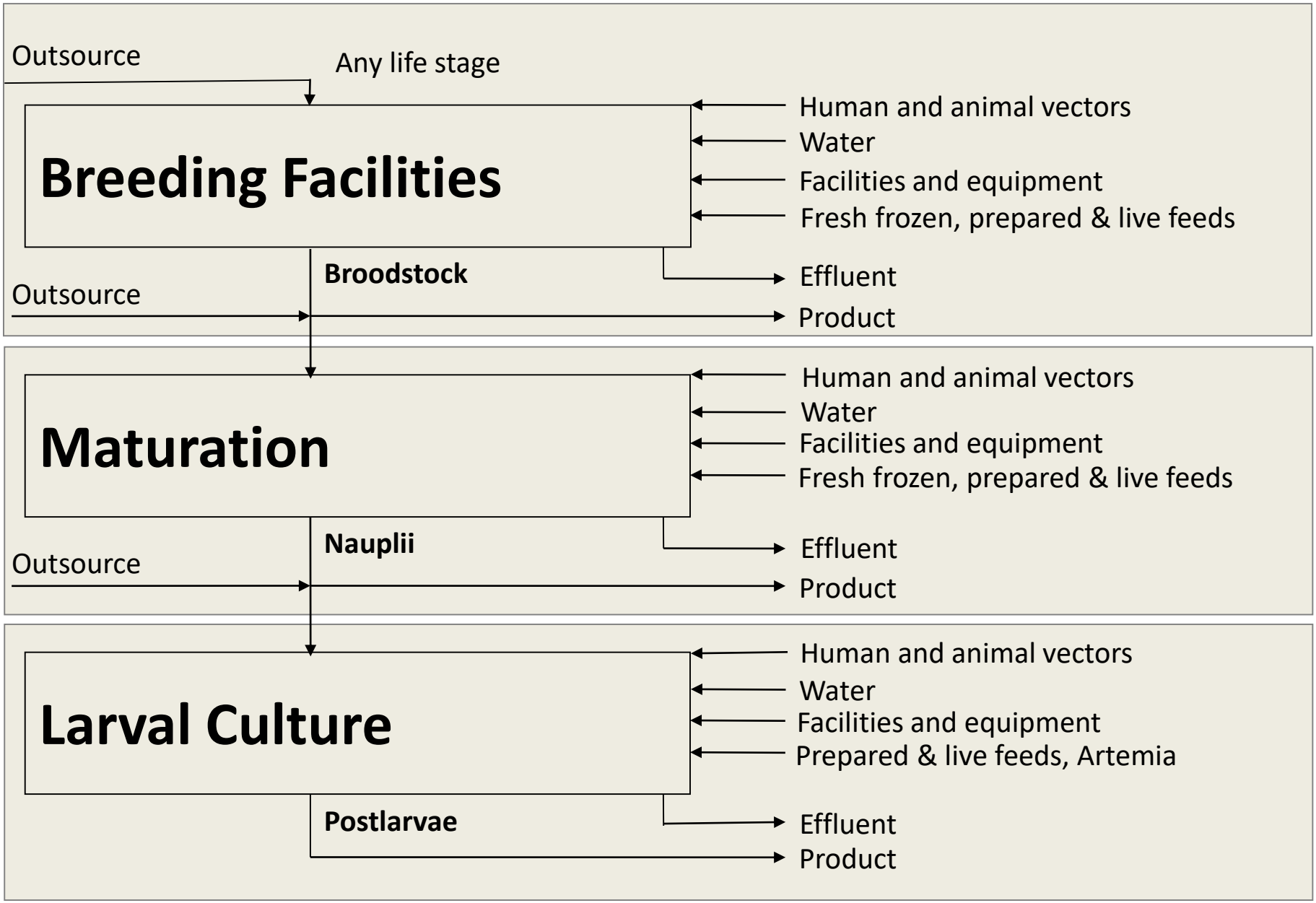
P. O. Box 189
Beaufort, SC 29901 USA

Jahncke, M.L., C.L. Browdy, M.H. Schwarz, A. Segars, J.L. Silva, D.C. Smith, and A.D. Stokes. 2002. Application of Hazard Analysis Critical Control Point (HACCP) Principles as a Risk Management Tool to Control Viral Pathogens at Shrimp Production Facilities. Publication Number VSG-02-10. Virginia Sea Grant. Charlottesville, VA, USA. 33pp.

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


Outsource
Any life stage
Breeding Facilities
Human and animal vectors
Water
Facilities and equipment
Fresh frozen, prepared & live feeds
Broodstock
Effluent
Outsource
Product
Maturation
Human and animal vectors
Water
Facilities and equipment
Fresh frozen, prepared & live feeds
Nauplii
Effluent
Outsource
Product
Larval Culture
Human and animal vectors
Water
Facilities and equipment
Prepared & live feeds, Artemia
Postlarvae
Effluent
Product

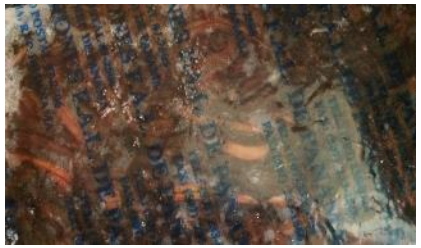
Hazard Analysis

ID Potential Hazard	Significant	Justify	Preventive Measures	CCP
Humans	Yes	Human vectors may transfer LSP	Controlled by SOPs	No
Facilities and equipment	Yes	Facilities and equipment may become contaminated w/LSP	Controlled by SOPs	No

Hazard Analysis

ID Potential Hazard	Signif-icant	Justify	Preventive Measures	CCP
Incoming water	Yes	Water or waterborne particles may be infective	Periodic testing for pathogens, sentinel system, disinfection of water	Yes
Shrimp - receipt	Yes	Shrimp may be infective	SPF certification with every shipment. Quarantine procedures and periodic testing for LSP	Yes

Hazard Analysis



ID Potential Hazard	Significant	Justify	Preventive Measures	CCP
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
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

Hatchery Feeds
Live and Fresh

Maturation Feeds:

- Polychaetes
- Squid
- Marine bivalves
  - Mussels
  - Oysters
  - Clams
- Artemia biomass
- Krill

Larval Feeds:

- Live marine algae
  - *Chaetoceros*
  - *Thalassiosira*
  - *Tetraselmis*
- Artemia nauplii

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%)
Acetes	-	-	0 / 2	0 / 2 (0%)
Blood worms	-	-	0 / 2	0 / 2 (0%)

- : no specimens

Flegel et al. TARS 2014

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

Feed

- Verification
  - LSP free feed history documented by feed receiving form records
  - Periodic review of shrimp shipment receipt records
  - Periodic site visits with records of visits
  - Periodic testing of feed, diagnostic records
- Corrective actions
  - If certificate of compliance does not accompany incoming feed shipment, reject shipment

Replacement of Live and Fresh Frozen Maturation Feeds

- Improved formulated maturation feeds
- Progress towards 100% replacement but..
  - smaller spawn size,
  - lower nauplii productivity
  - nauplii quality issues
- Up to 80% dry weight replacement

1. Weigh

2. Add Equal Parts Water

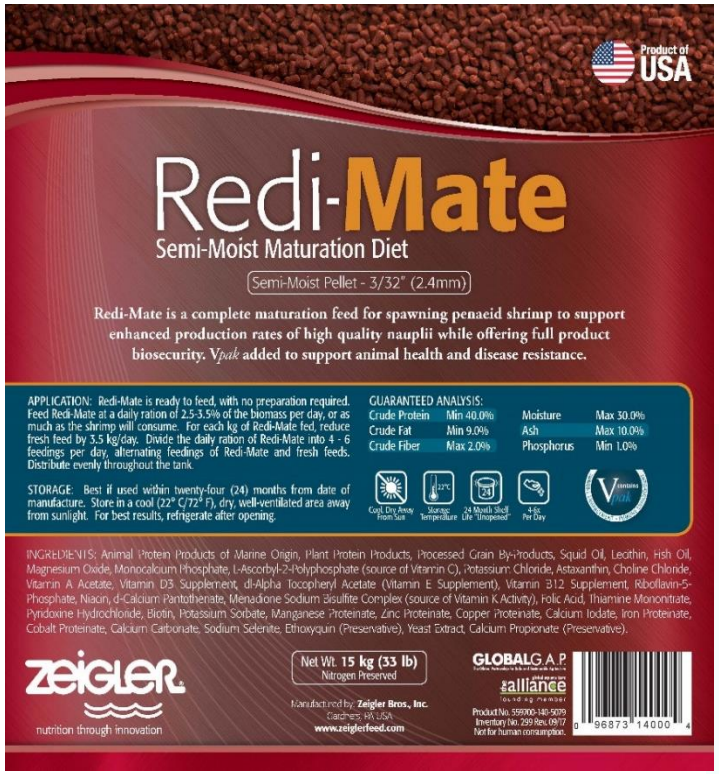
3. Mix

4. Shape and Dry

Prepared Feeds

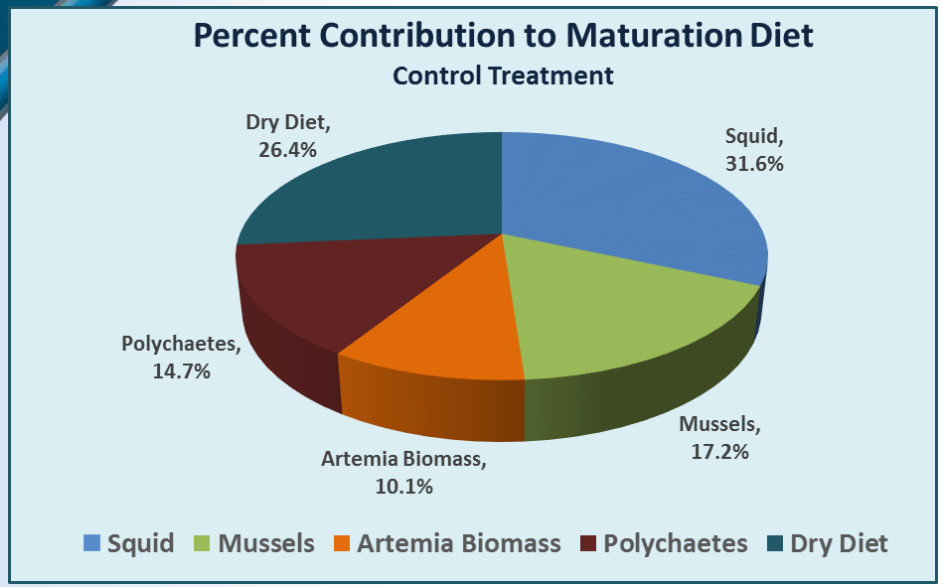
Advantages:

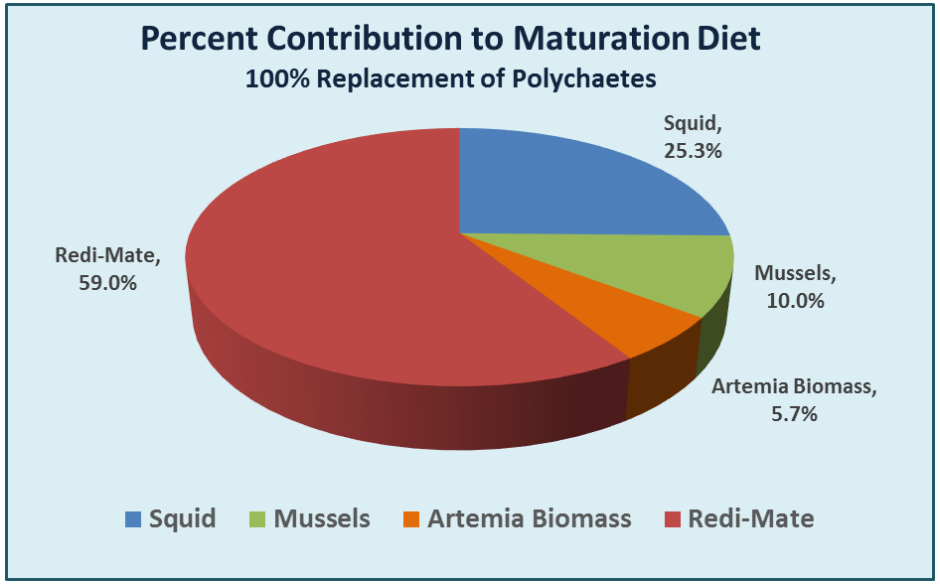
- 100% pathogen free -
- Formulated as nutritionally complete diets containing optimal levels of HUFAS, pigments, and nutrients essential for supporting the metabolic demands of maturation and spawning
- Can be supplemented with immune system stimulants, probiotics
- Easier storage
  - No need to freeze
  - Long shelf-life



V pak™

Fresh Maturation Feed Replacement

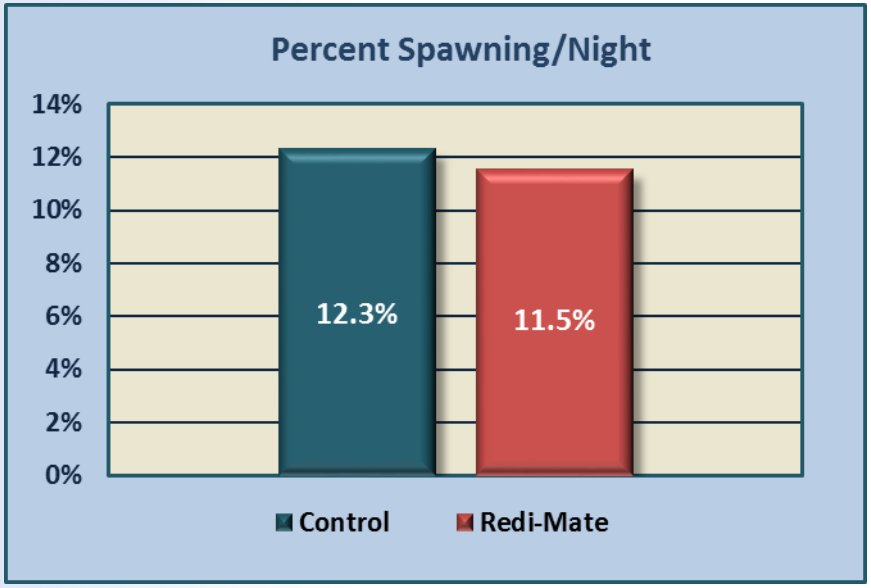


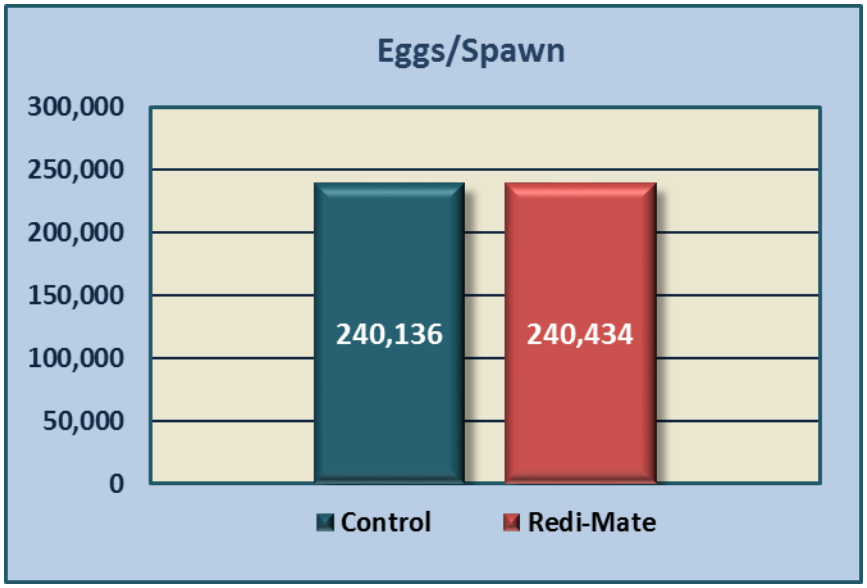


Feed	% BW/day (wet weight)	g dry wt per g wet wt	% BW /day (dry weight)	Percentage of Dry Diet
Squid	11.0%	20%	2.20%	31.6%
Mussels	6.0%	20%	1.20%	17.2%
Artemia Biomass	7.0%	10%	0.70%	10.1%
Polychaetes	6.0%	17%	1.02%	14.7%
Dry Diet	2.0%	92%	1.84%	26.4%
Total per day	**32.0%**		**6.96%**	**100.0%**

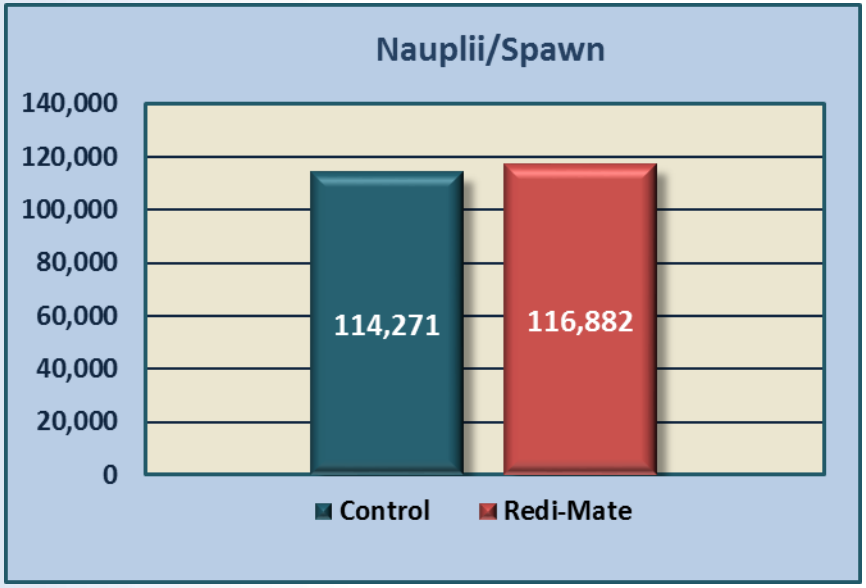
Feed	% BW/day (wet weight)	g dry wt per g wet wt	% BW /day (dry weight)	Percentage of Dry Diet
Squid	8.8%	20%	1.76%	25.3%
Mussels	3.5%	20%	0.70%	10.0%
Artemia Biomass	4.0%	10%	0.40%	5.7%
Polychaetes	0.0%	17%	0.00%	0.0%
Redi-Mate	5.6%	73%	4.10%	59.0%
Total per day	**21.9%**		**6.96%**	**100.0%**

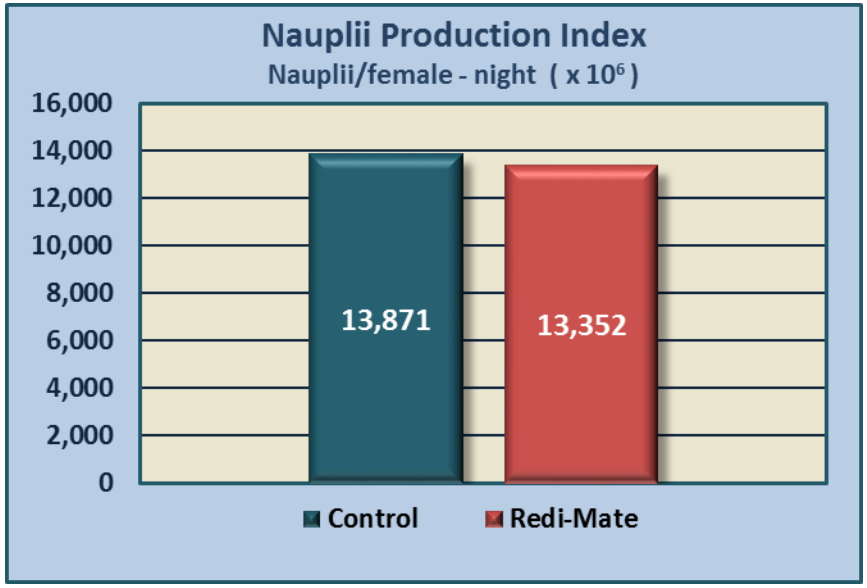
Replacement of Maturation Fresh Feeds





Replacement of Maturation Fresh Feeds





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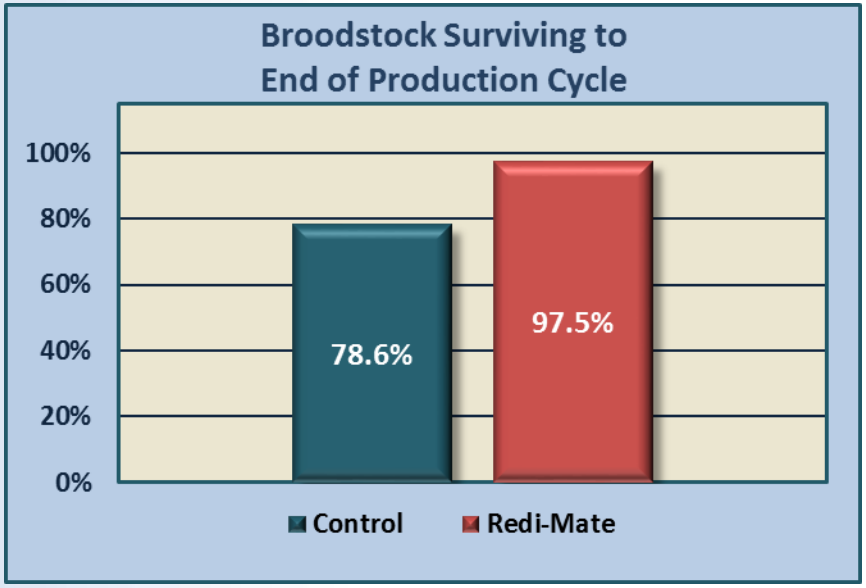

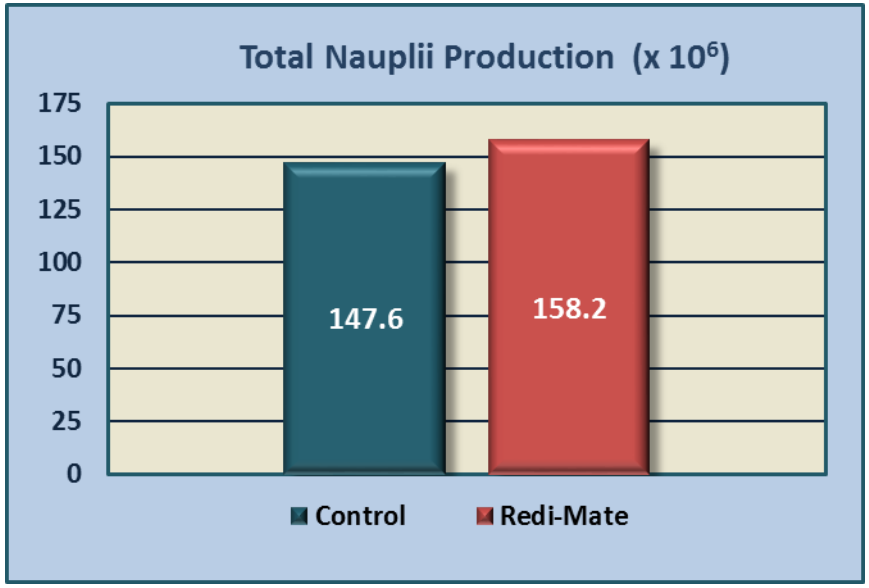

100% replacement of frozen polychaetes

- More nauplii produced
- Improved nauplii quality
  - More yolk
  - More active
  - Better phototaxis

March 1 – April 4, 2018

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

Towards Algae Replacement

- Algae can be a significant source of contamination and cause ZII syndrome
- Liquid feeds with optimal digestibility, cold processed for nutritional integrity, water stability, excellent performance
- Successful use in hatcheries with Algae contamination or production bottlenecks

Artemia nauplii are an important vector for *Vibrio*

- High *Vibrio* loads associated with hatched nauplii are an important pathway for Vibrio introduction into larval tanks
- Glycols released from the cysts during hatching is like a nutrient broth for culturing *Vibrio*
- Disinfection and decapsulation of cysts prior to hatching doesn't solve the problem because *Vibrio* is seeded into hatching tanks from biofilms

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Artemia Replacement

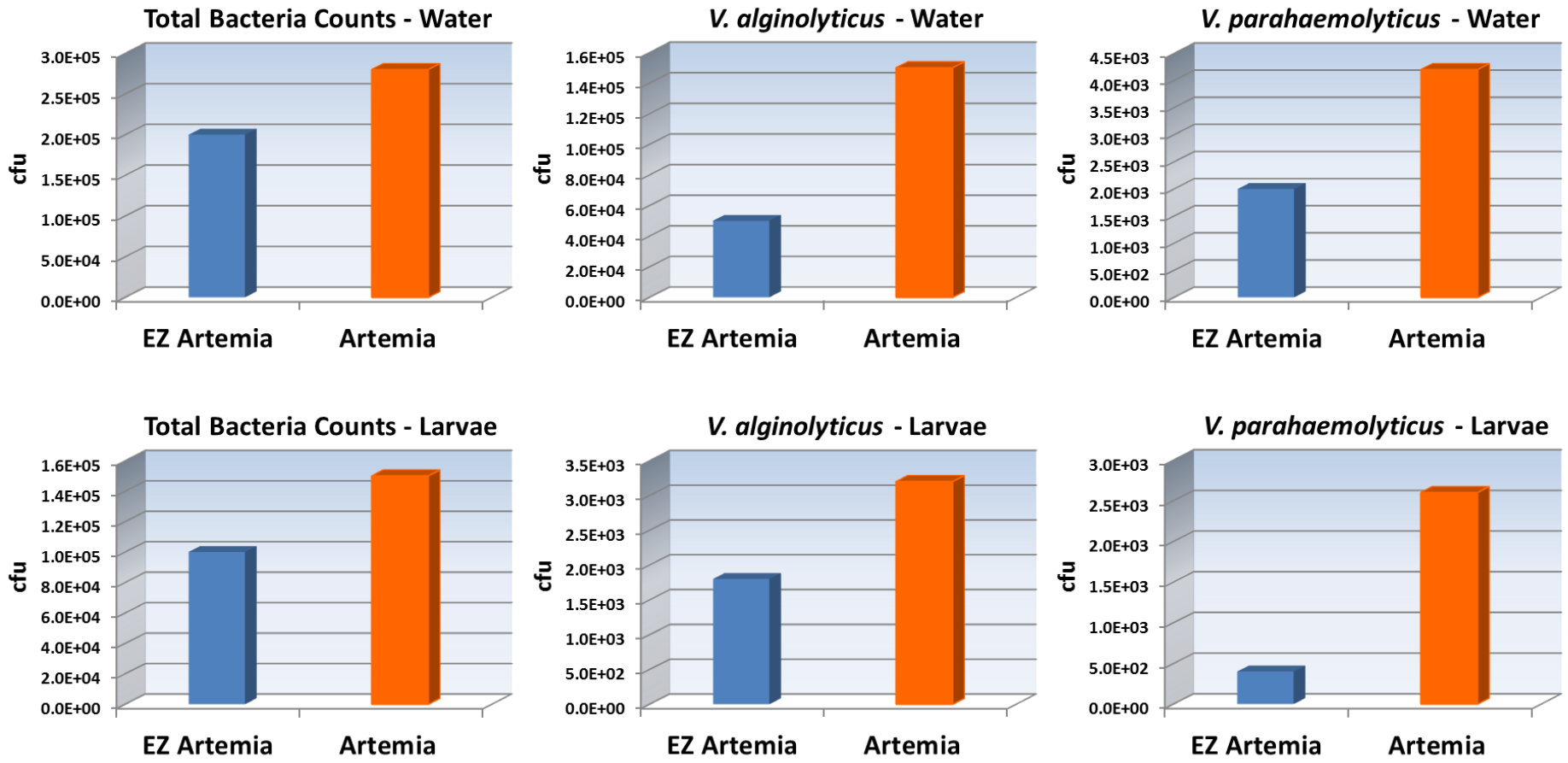
- Liquid diets designed to replace up to 100% of *Artemia* in hatcheries
- Formulated to match or exceed the nutritional profile of enriched *Artemia*
- 2 Sizes (50-200 & 300-500 micron)
- Can be fed from Zoea to PL
- Stable supply and costs significantly less than *Artemia*
- Each lot certified Biosecure

EZArtemia
Liquid *Artemia* Replacement Diet

100% Replacement of Artemia nauplii - Results from 5 countries

Parameters	Country 1 (2014)	Country 1 (2015)	Country 2 (2015)	Country 3 (2015)	Country 4 (2016)	Country 5 (2016)
Stocking Density (nauplii/L)	185	85	200	300	190	117
EZ Artemia Feed Rate (kg/million PLs)	2.75	4.5	1.66	4	2.02	1.68
Culture Cycle	10 days Z1 - PL4	12 days Z1 - PL4	11 days Z1 - PL4	15 days Z1 - PL8	20 days Z1 - PL13	12 days Z1 - PL6
Survival (%)	85%	70%	69%	50%	70%	92%
Harvest Weight	0.87 mg	-----	0.72	1.00 mg	5.50 mg	1.1-1.2 mg

Bacterial Counts Ecuadoran Hatchery



Note: All tanks were fed with 100% EZ Artemia up until 2 days before harvest. Several larval tanks were fed Artemia instead of EZ Artemia for the last two days before harvest. These counts compare the bacterial counts just before harvest of the tanks switched to artemia with those fed only with EZ Artemia.

Testing and Certification

THE UNIVERSITY OF ARIZONA. TUCSON ARIZONA

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: dhuie@email.arizona.edu

January 29, 2018

Case: 18-035-A

ection of WSSV, IHHNV, TSV, YHV, IMNV, *Pv*NV, *Mr*NV,
completed. One bag of feed (EZ Artemia 1 Lot #5599006066-
condition. The sample collection location was Zeigler Bros., Inc.
representative sample (approx. 30mg) was collected for DNA and
V, IMNV, *Pv*NV, *Mr*NV, APHND/EMS, EHP and NHP-B were
was completed on January 29, 2018. A summary of the tests and

e helpful to you. The hard copy will be mailed to you. If there are
l free to contact us.

n the samples submitted to our laboratory for examination, health status evaluation, disease diagnosis,
ost appropriate assay(s) for the determination of the health/pathogen status of all specimens submitted
ck or facility "certification" or a "certificate" of health/pathogen status for the sample(s) tested or for

es submitted to our laboratory for pathogen detection. The PCR assay used by this laboratory for the
ld be considered as experimental and tentative. Whenever possible, PCR results should be confirmed
facility "certification" or a "certificate" of health/pathogen status for the sample(s) tested or for the

ional des Épizooties or the Organization or World Animal Health Organization) Reference Laboratory
mal and Hematopoietic Necrosis, Spherical Baculovirus, Tetrahedral Baculovirus and Infectious
r export testing for White Spot Disease, Taura Syndrome, Infectious Hypodermal and Hematopoietic
tious Myonecrosis, Yellowhead Disease, Acute hepatopancreatic necrosis disease, Crayfish plaque
bergii nodavirus), and Necrotizing hepatopancreatitis *(Hepatobacter penaeid)*.

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

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.

PCR and Bioassay Analysis:

Log	Rec.	Product ID	Lot No.	Results
2529	11/17/17	Shrimp Grower 35 2.4mm	3274533381-17307	Negative
2529	11/17/17	Shrimp Grower 35 2.4mm	3274423381-17315	Negative
2529	11/17/17	Shrimp Grower 35 2.4mm	3274423381-17315	Negative
2529	11/17/17	Shrimp Grower 35 2.4mm	3274423381-17315	Negative
2529	11/17/17	Shrimp Grower 35 2.4mm	3274533381-17317	Negative
2529	11/17/17	Shrimp Maturation 2.4mm	3446413397-17318	Negative
2529	11/17/17	PL Raceway 40-9 1.0mm	3847709244-17312	Negative
2529	11/17/17	PL Raceway 40-9 VPak 1.5mm CS	3234016144-17317	Negative
2529	11/17/17	Brine Shrimp Flake Black	7347426589-17313	Negative
2529	11/17/17	Brine Shrimp Flake Black	7347426589-17317	Negative
2529	11/17/17	Larva Diet Z Plus Flake	3847316589-17313	Negative
2529	11/17/17	Larva Z Plus Flake	3847316589-17318	Negative
2529	11/17/17	Z Plus Flake	3847316589-17319	Negative
2529	11/17/17	EZ Larva 250-600	5579003166-17317	Negative

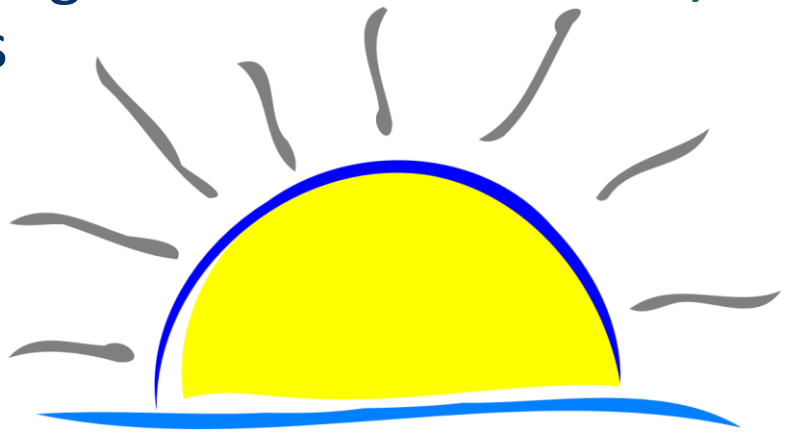
A More Biosecure Future

The Past

- Hatchery productivity 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 prioritizes biosecurity
- Adoption of new approaches to hatchery nutrition based on biosecure prepared feeds
- A new era of sustainability and profitability for the industry



ZARC
zeigler-aquaculture.research.center

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Wishing you all great success!