

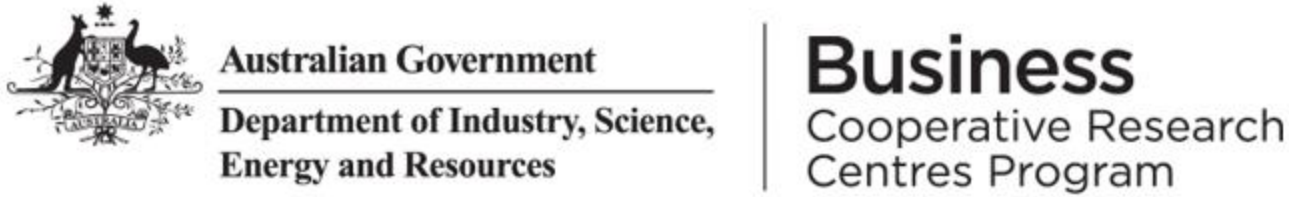
Project : A.3.1718113: **Improving Biosecurity in Australian Prawn Farming**

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Industry Project Committee: Tony Charles, Alistair Dick and Wayne Dibartolo

The CRCNA acknowledges the support of its government partners.







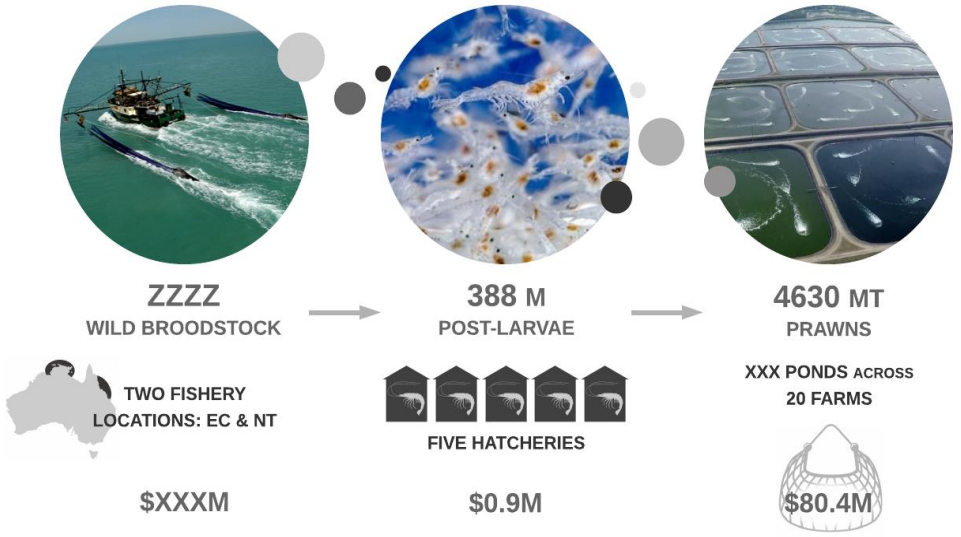






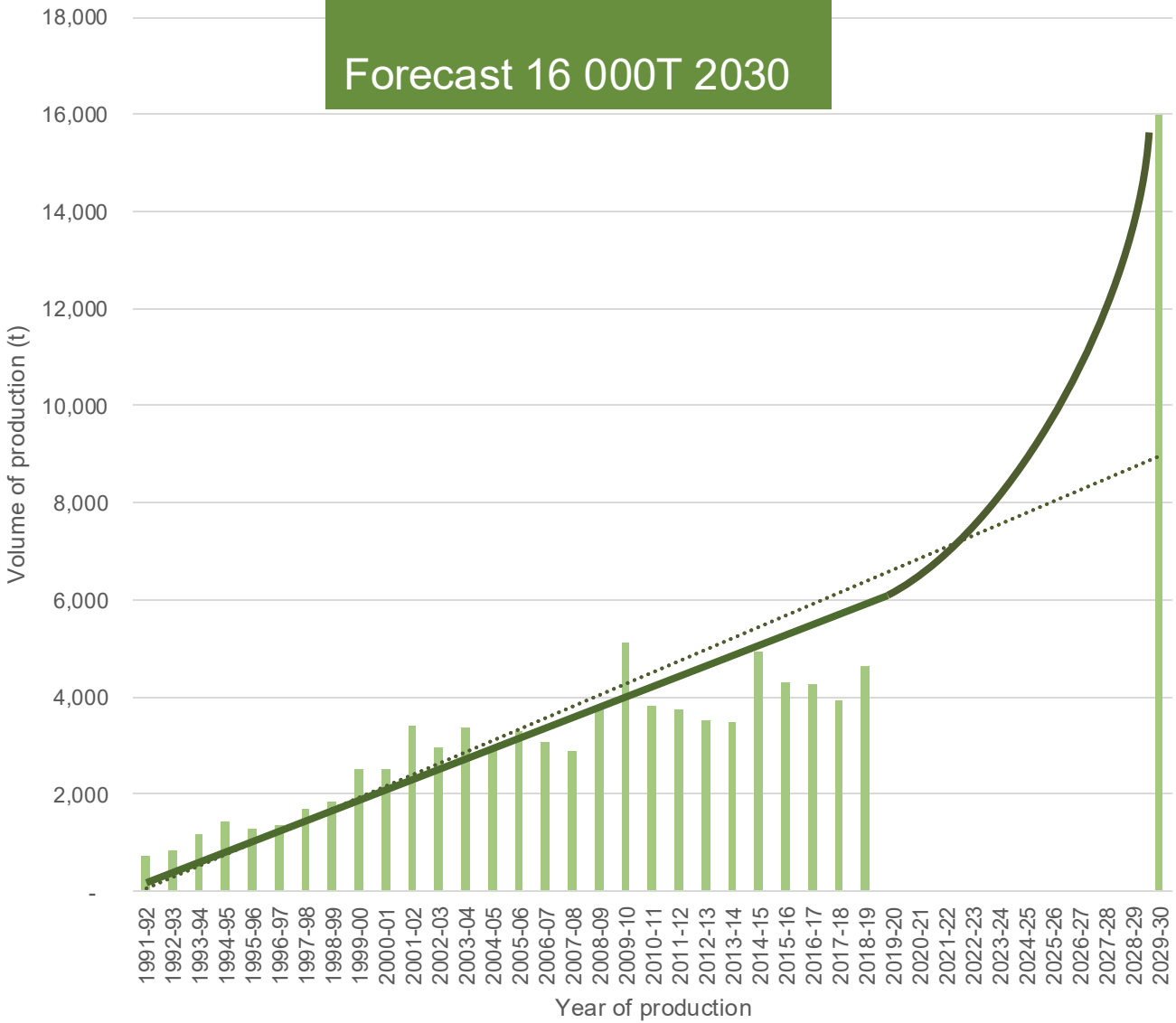
Industry outlook

2018-2019 Summary



9% Annual average increase since 1991-92.

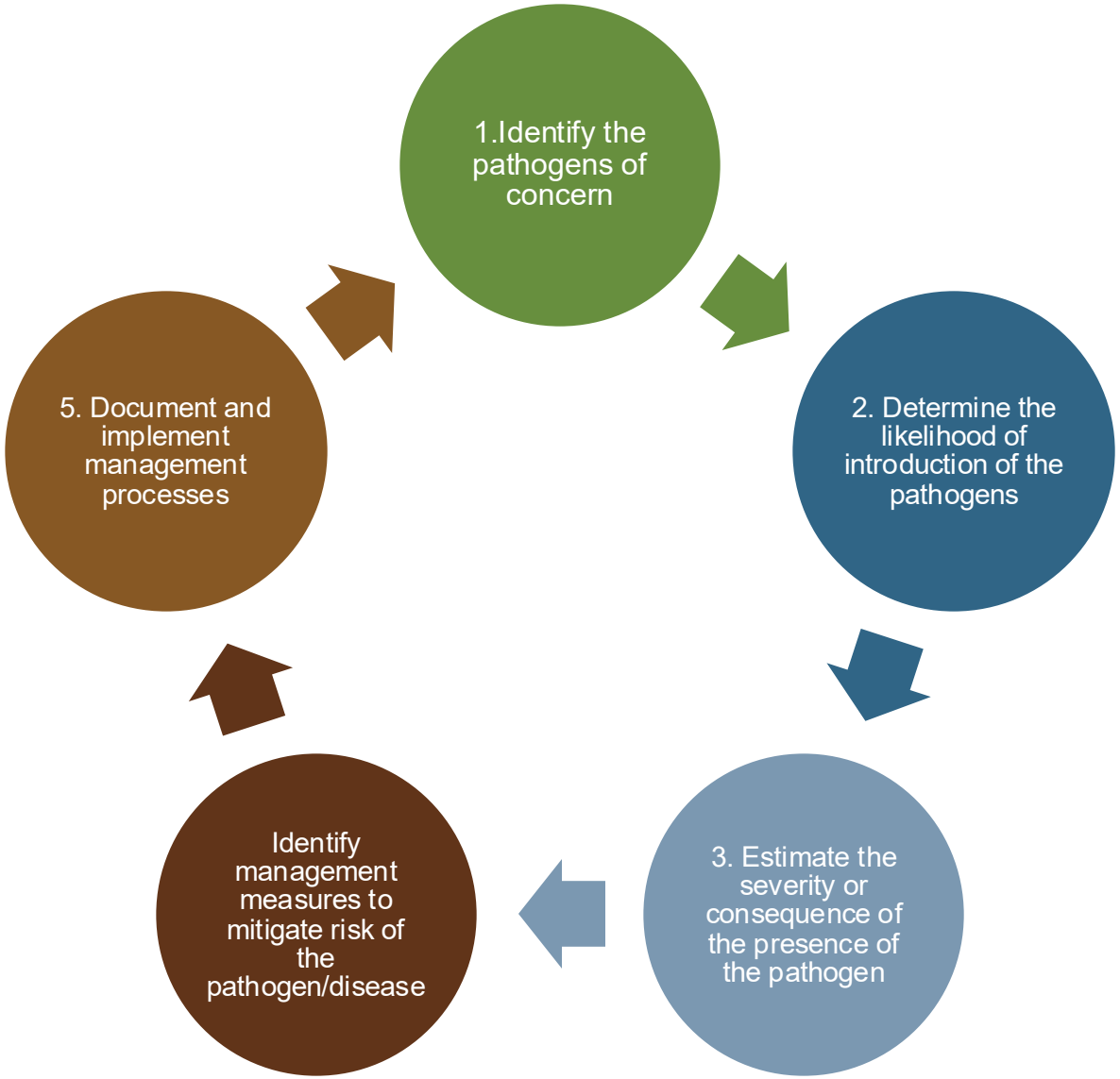
Forecast 16 000T 2030



Data sourced from ABARES, QDAFF and the Lobegeiger Report. 2030 Forecast Cobcroft et al. 2020.

Data to support evidence based decisions to improve biosecurity in Australian prawn farming.

Your General Biosecurity Obligations: The Inputs to a Biosecurity Management Plan



1.Identify the pathogens of concern

Which are the Pathogens of Concern?

OIE Notifiable Diseases: WSSV, YHV-1, TSV, IMNV, EHP, AHPND, Necrotizing hepatopancreatitis, IHHNV, Monodon-baculovirus, Baculovirus penaei.

Australian Notifiable Diseases: WSSV, YHV-1, TSV, IMNV, EHP, AHPND, Necrotizing hepatopancreatitis, IHHNV, Monodon slow growth syndrome.

Disease from Research Literature (in addition to the above): Vibriosis; Decapod Iridescent Virus (DIV-1/SHIV-1/CQIV-1), Hepatopancreas and Digestive tract necrosis virus (HINV; uncharacterized RNA Virus associated with "Glass post-larvae)

Disease from Australian specific cases

Year	Pathogen/Disease	Reference
1987	MBV	Dubrovsky et al., 1988
1991	IHHNV	Owens et al., 1992
1991	Vibriosis	Muir, 1991
1994	Midcrop mortality syndrome (MCMS) GAV and SMV	Owens et al., 1992; Spann et al., 1992
1997	HPV	Spann et al., 1997
2008	IHHNV	OIE, 2008
2011	IHHNV	Moody et al., 2011
2012	Yellowhead Virus-7 (YHV-7)	Cowley et al., 2019
2015	Hepatopancreatitis (Pir-AB like "AHPND")	OIE, 2016; Moody., 2018
2016	WSSV	Glanville et al., 2017
2017	Yellowhead Virus-7 (YHV-7)	Cowley et al., 2019; Moody et al., 2019
2020	WSSV	

2. Determine the likelihood of introduction of the pathogens

Prevalence of detection of prawn pathogens from Australian specific cases (Farm/Hatchery)

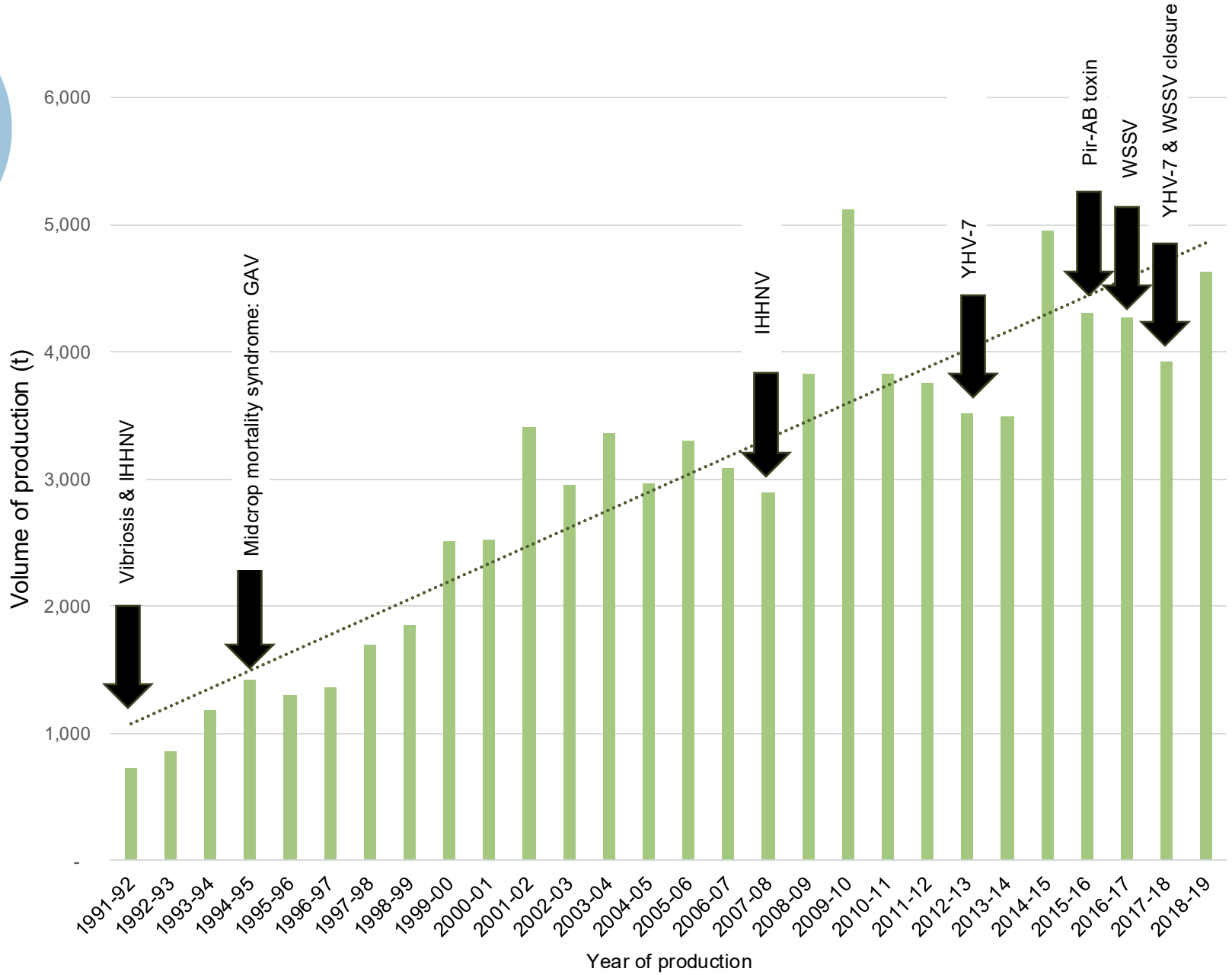
Pathogen/Disease	Summary of detection	Reference
MBV	1 % Broodstock: (n=337) noting pleopod samples are not target tissue for MBV	Cowley et al., 2015
IHHNV	1.39% to 5.3% in ponds (n=1298 samples) no disease 6% in broodstock (n=337)	Moody et al. 2008 Cowley et al., 2015
Vibriosis	Mass Mortality in hatcheries. No prevalence data.	Muir, 1991
SMV	1% Broodstock. SMV genome sequence has 99% homology to Carnobacterium (bacteria)	
HPV	Broodstock: 11 % (n=337) noting pleopod samples are not target tissue for HPV/HDV	Cowley et al., 2015
GAV	Broodstock 43% (n= 493)	Cowley et al., 2015
Yellowhead Virus-7 (YHV-7)	Broodstock 6.5% (n=493)	Cowley et al., 2015
Hepatopancreatitis (Pir-AB)	No published prevalence in detection. Surveillance in 2016 0% detection from 980 samples from 13 farms.	Condon and Bochow 2016
WSSV	No prevalence data. Reported mass mortality	Glanville et al., 2017

3. Estimate the severity or consequence of the presence of the pathogen

- **Unless a pathogen is a notifiable agent there is no database or public record of the impacts of pathogens on production.**

- MCMS mass mortalities up to 80%. GAV and SMV associated
- YHV-7 mass mortalities in 2013: First incidents of YHV-7 associated mortalities.
- Pir-AB mass mortalities in 2015 Cardwell and Bundaberg. First detection of Pir-AB in Australia
- YHV-7 mass mortalities in 2017: “Similar to MCMS”
- WSSV estimated stock losses of $AUD 23 Million but many were destroyed on detection rather than on disease signs.

3. Estimate the severity or consequence of the presence of the pathogen



Alignment of pathogen reports with production data.

Not necessarily a causative relationship but rather indicating time-line alignment.

Data sourced from ABARES, QDAFF and the Lobegeiger Report.

Prioritising Biosecurity Management

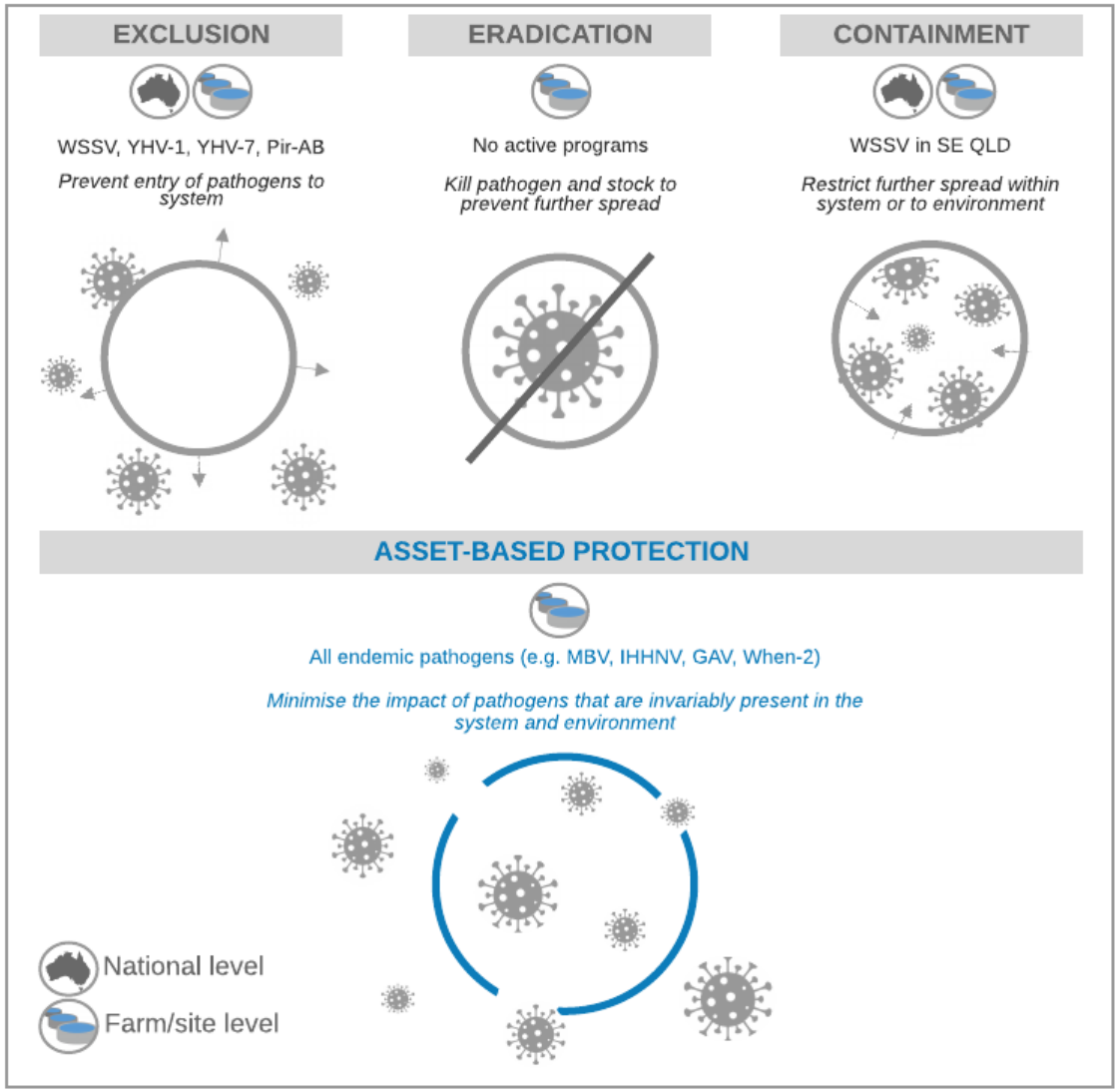


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What is the consequence of Pathogen presence?

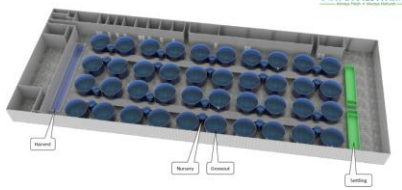
- **Clinical signs**: **“Disease”**
  - Mortality: Low grade 10-20%
  - Mortality: Mass 70-100%
- **Production/Profit reduction:**
  - Loss of crop
  - Reduction in crop
  - + $ Cost to treat
  - + $ Cost to exchange water
  - + $$ Cost of lengthened crop cycle (Wages, Feed, Electricity, certainty to deliver to buyer, certainty to harvest at peak farm gate)
- **Hatchery:**
  - Broodstock direct loss (mortality)
  - Reduced Broodstock performance (#PLs produced)
  - Robustness of PLs
  - Scheduled farm stocking to align with pond filling
  - Scheduled farm stocking to align with seasonal weather
  - Scheduled farm stocking to align with harvest price
  - Scheduled farm stocking to align with market demand

4. Identify management measures to mitigate risk of the pathogen/ disease



Biosecure facilities





High ability to exclude, contain and eradicate pathogens

Non-Biosecure facilities



Lower ability to **exclude,** contain and eradicate pathogens.

YHV-7, Pir-AB containment and eradication have been implemented in farm responses

2. Determine the likelihood of introduction of the pathogens

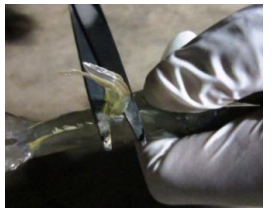
Scheduled

Within aquaculture system			External	
Aquacultured: *P. monodon*			Wild: *Acetes sp. & Palaemon sp.*	
				
Broodstock	Post-larvae	Pond prawns	Within-pond	Farm intake
n = 967	n = 411	n = 666	n = 59	n = 191

Ad-Hoc

Within aquaculture system			External
Aquacultured: *P. monodon*			Imported: *P. vannamei*
			
Broodstock mortalities	Hatchery mortalities	Slow-growth ponds	Imported retail

Detection of putative bacterial toxin genes: Zon occludens, Repeats in Toxin and hemolysin D.



2500+ samples









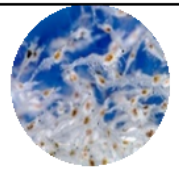
2. Determine the likelihood of introduction of the pathogens

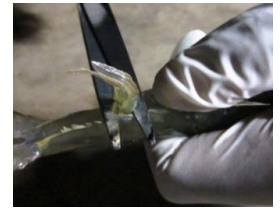








Sample source	Number of Samples analysed	Proportion positive	Most prevalent pathogens	Highest calculated loading (copies per µl of nucleic acid extract)
Hatchery				
Broodstock 	967	93%	GAV (89%) IHHNV (47%) When-2 (32%)	4.2×10^9 1.2×10^8 1.3×10^9
Post larvae 	411	88%	GAV (88%) IHHNV (61%) HDV (19%)	6.4×10^8 1.3×10^8 44
Grow-out Ponds				
P.monodon 	666	99%	GAV (98%) IHHNV (62%) When-2 (22%)	6.5×10^6 1.5×10^7 1.8×10^8
Acetes sp. (glass shrimp) 	59	73%	When-2 (49%) GAV (24%) IHHNV (20%) HDV (20%)	5.3×10^5 3.3×10^4 1.9×10^2 66
Farm intake (prior to prawn ponds)				
Acetes sp. & *Palaemon* sp. (jellyshrimp) 	191	39%	IHHNV (23%) HDV (20%) WSSV (9%) When-2 (1%) Pir-A (1%)	1.8×10^3 6.8×10^3 8.2×10^3 4.2×10^3 18



There was no detection of WSSV or YHV-1 from an aquaculture sample

There is a high likelihood of introduction of pathogens into the production line under current operations

In the current WSSV containment zone.

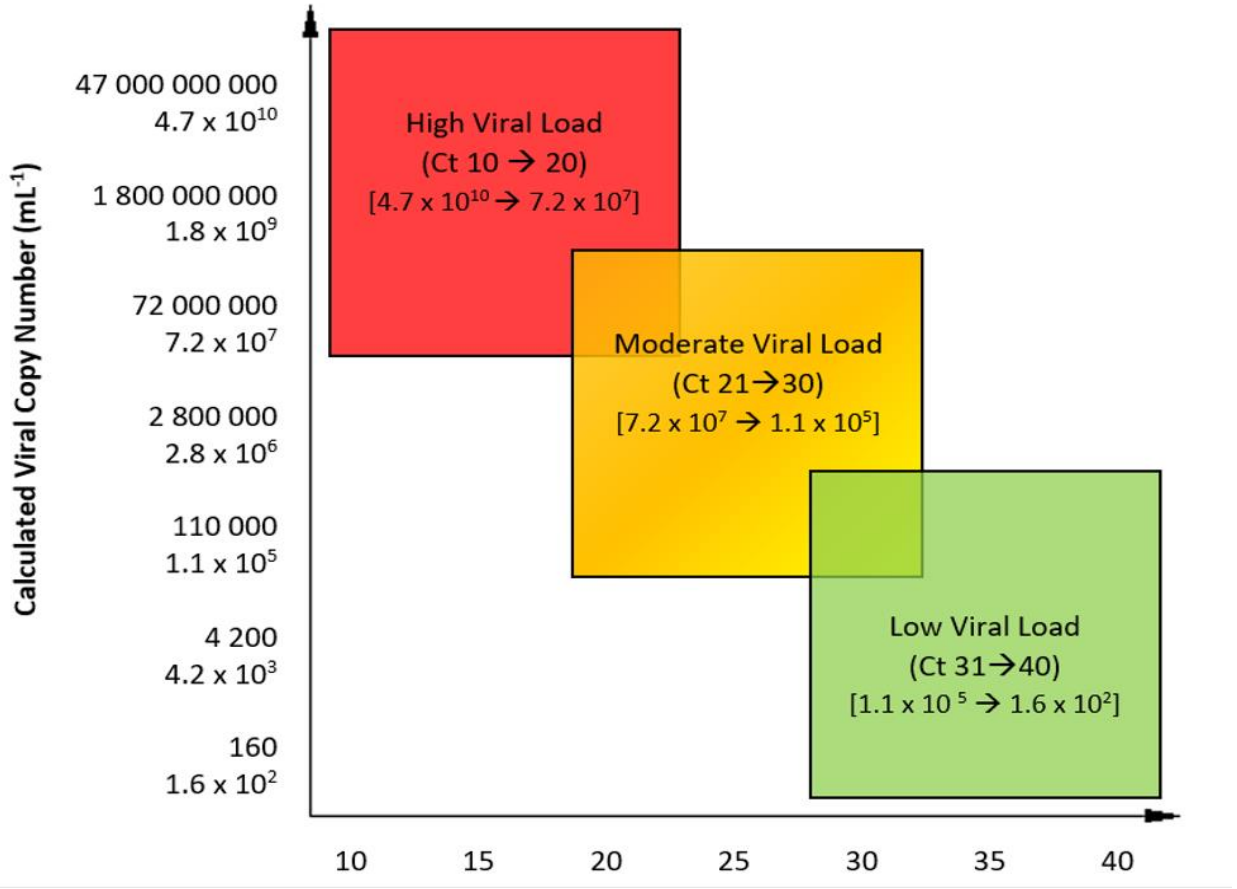
2. Determine the likelihood of introduction of the pathogens

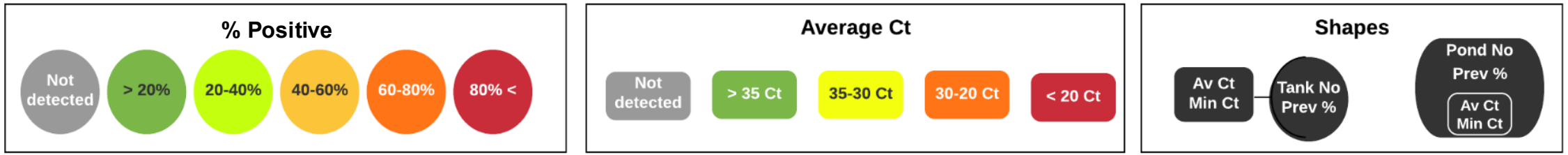
Prevalence of detection of prawn pathogens from imported prawns (this study)

- 42 Pools of 5 animals (tail cuticle and muscle/hind gut section)

Pathogen Target	Summary of detection
Whenzhou Shrimp Virus-2	100% Positive
IHHNV	57% Positive
Zon toxin	2% Positive
Rtx toxin	2% Positive
Not detected	WSSV, YHV-1, YHV-7, GAV, HDV, MBV, Iridescent Virus, Enterocytozoan, Pir-A, hemolysin D.

2. Determine the likelihood of introduction of the pathogens

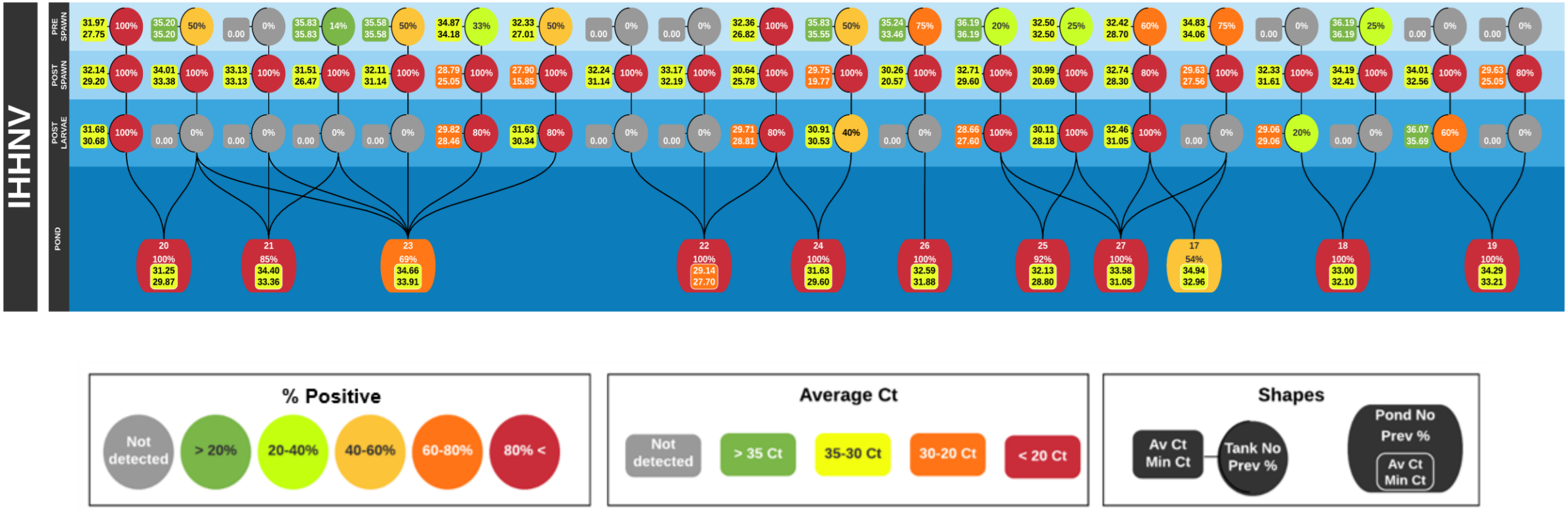




2. Determine the likelihood of introduction of the pathogens

Scheduled sampling:

Tested for : IHHNV, GAV, YHV-7, Pir-A, HDV, When-2, WSSV, YHV-1.



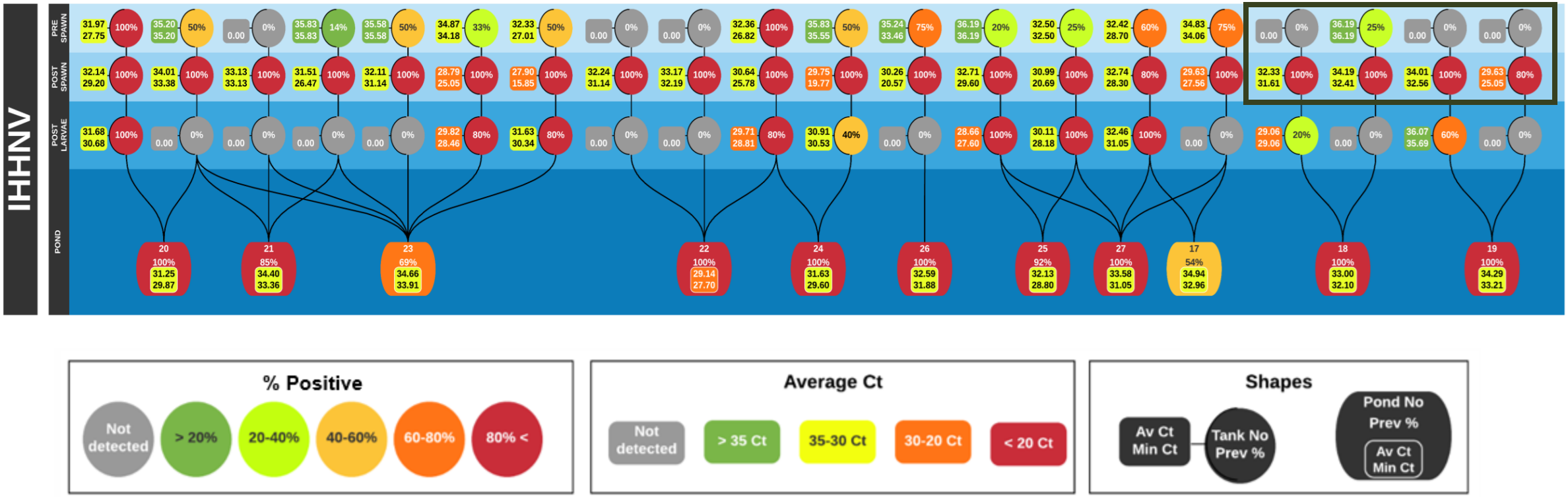
2. Determine the likelihood of introduction of the pathogens

Sources:

Broodstock to Broodstock (in maturation tanks)

Increase in prevalence and copy number from Pre-spawn to Post-spawn

- Stress leading to increase in load from not-detectable to detectable load
- Transfer from other positive broodstock in the maturation tank
- Fresh feeds

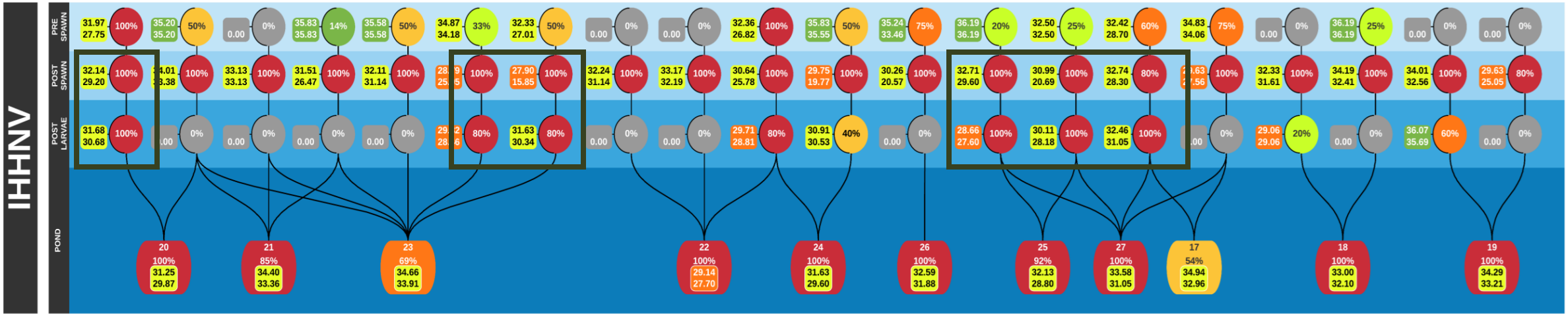


2. Determine the likelihood of introduction of the pathogens

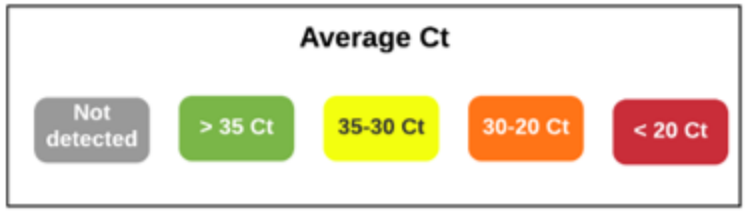
Sources of introduction:
Broodstock to PLs

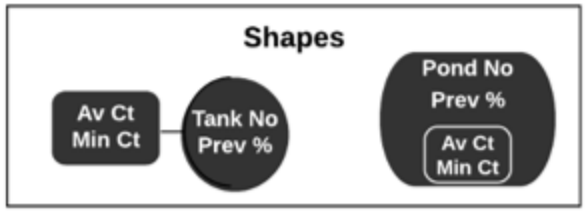
- Egg surface contamination
- Trans-ovarian to egg. (variable with copy number of the adult).
- Low copy number less transfer

Live feeds: Artemia, insects, algae, probiotics.
PL tanks are high density, high nutrient load, high microbial ecosystem.





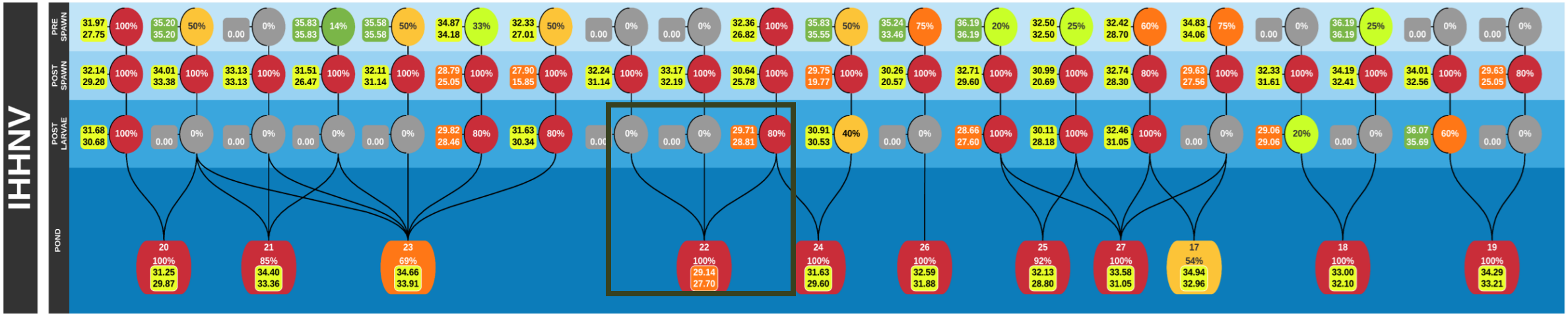




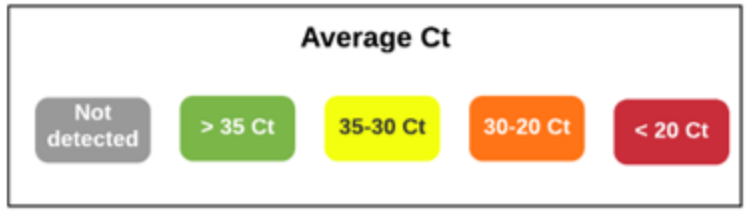
2. Determine the likelihood of introduction of the pathogens

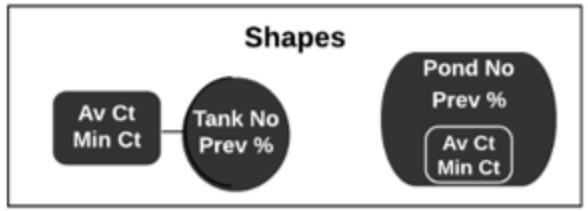
Sources of introduction:

- PLs to PLs ? Transfer from one tank of 80% positive to 100% positive in the pond.
- Contamination from the environment



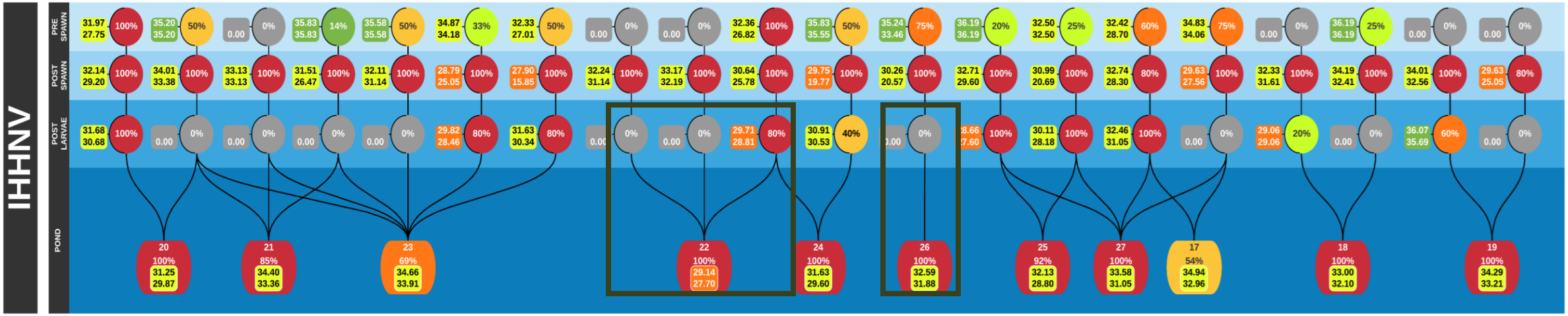


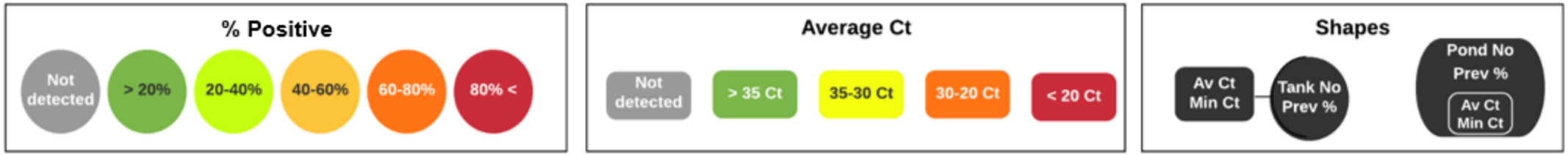




2. Determine the likelihood of introduction of the pathogens

Sources of introduction:
Contamination from the environment





Identify management measures to mitigate risk of the pathogen/ disease

Recommendation: Industry to actively pursue domestication of *P.monodon* to remove the annual intake of wild broodstock with unknown pathogen potential.

- ***SPF: Specific Pathogen Free****: wild-sourced Founders (high health HH):* Currently free of many of the pathogens on the SPF list. However, high prevalence of IHHNV and GAV in broodstock.
- Higher percentage of females positive v males
- Data set is time restricted September 2018-February 2019.

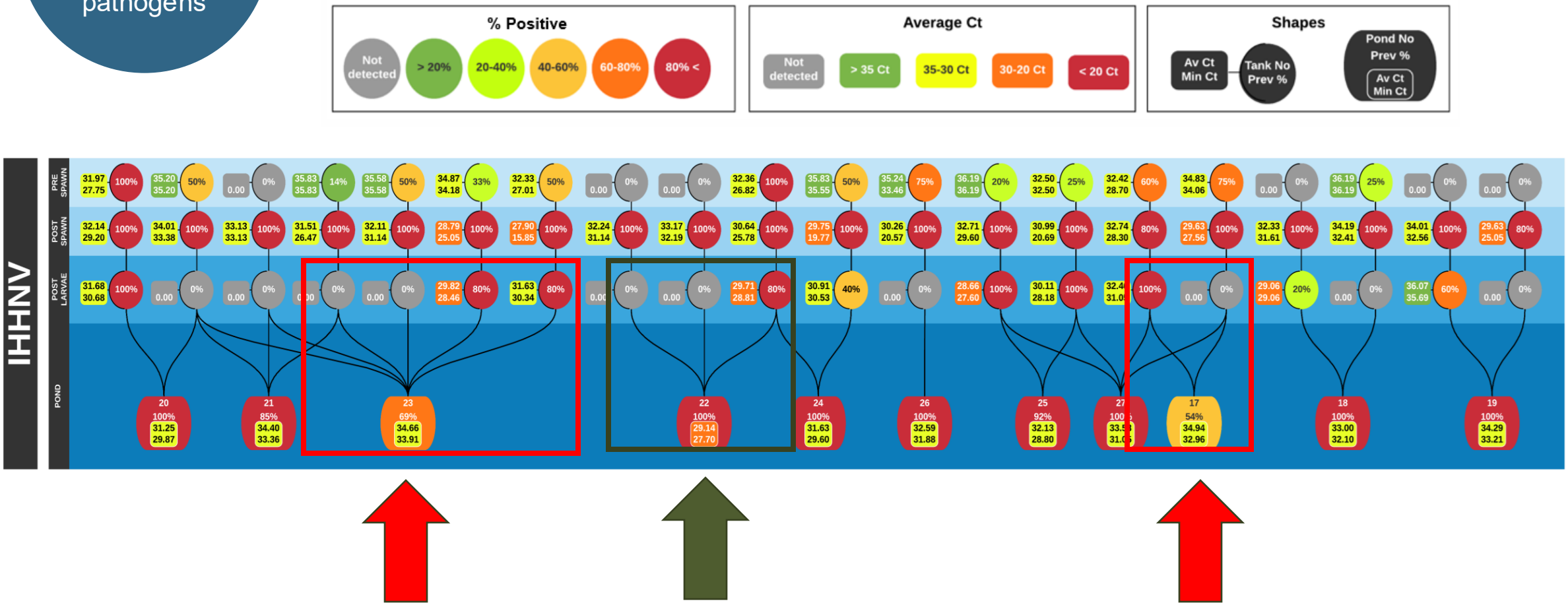


- Pond data indicates SPF will be exposed to pathogens.
- ***Specific Pathogen Resistant***: higher survival when exposed to a pathogen. Some remain refractory to infection. (0% infected after exposure)
- ***Specific Pathogen Tolerant***: carry a high load without growth affected.

SPF, SPT, SPR are all specific to particular pathogens: Which ones to target?

2. Determine the likelihood of introduction of the pathogens

What does Resistance look like?

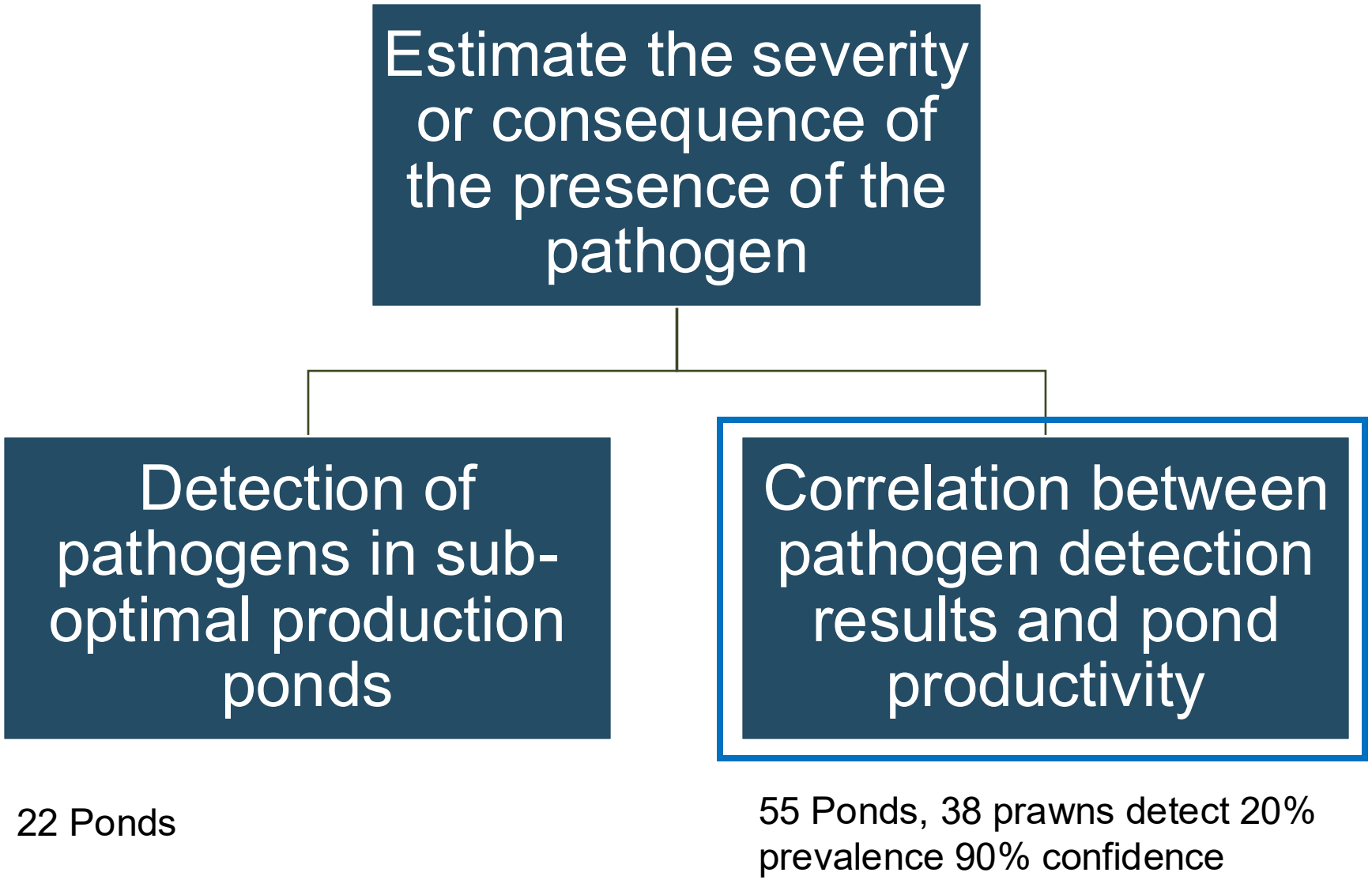


6 PL tanks
69% positive.

3 PL tanks
100% positive.

2 PL tanks
54% positive.

3. Estimate the severity or consequence of the presence of the pathogen




IHHNV
FARM A
1 92% 32.58 30.70
2 42% 32.72 31.50
3 58% 33.40 31.19
4 42% 33.85 33.06
5 15% 32.77 32.41
6 0% nd 0.00
7 0% nd 0.00
8 0% nd 0.00
9 67% 33.16 31.82
10 92% 33.79 32.64
11 83% 33.61 31.41
12 42% 33.55 32.42
13 70% 32.23 30.70
14 8% 34.50 34.50
15 0% nd 0.00
16 0% nd 0.00
FARM B
17 54% 34.94 32.96
18 100% 33.00 32.10
19 100% 34.29 33.21
20 100% 31.25 29.87
21 85% 34.40 33.36
22 100% 29.14 27.70
23 69% 34.66 33.91
24 100% 31.63 29.60
25 92% 32.13 28.80
26 100% 32.59 31.88
27 100% 33.58 31.05
FARM C
28 77% 31.68 26.87
29 8% 35.33 35.33
30 15% 32.30 29.84
31 0% nd 0.00
32 0% nd 0.00
33 0% nd 0.00
34 85% 33.80 32.61
35 0% nd 0.00
36 92% 30.01 25.78
37 100% 31.50 26.03
38 100% 32.17 27.15
39 100% 31.85 28.60
FARM D
40 55% 33.98 31.88
41 82% 33.29 30.60
42 100% 34.92 33.83
43 27% 36.15 35.95
44 27% 35.82 35.41
45 100% 27.60 18.55
46 100% 22.32 18.82
47 100% 29.80 28.71
48 45% 33.40 30.55
49 73% 34.01 30.75
50 73% 32.74 29.57
51 100% 32.79 31.83
52 100% 30.87 28.82
53 100% 34.64 33.85
54 91% 32.91 30.13
55 73% 34.39 33.42
% Positive
Not detected
> 20%
20-40%
40-60%
60-80%
80% <
Average Ct
Not detected
> 35 Ct
35-30 Ct
30-20 Ct
< 20 Ct
Shapes
Av Ct Min Ct
Tank No Prev %
Pond No Prev %
Av Ct Min Ct

GAV

FARM A

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
50%	67%	100%	100%	100%	100%	100%	92%	100%	100%	100%	100%	100%	100%	100%	83%
35.53	35.95	35.11	35.35	33.96	34.92	34.84	36.00	35.27	35.06	34.24	35.27	35.01	34.62	34.31	34.56
31.05	32.35	28.69	34.58	27.91	33.23	28.53	33.55	34.13	31.51	31.81	32.50	29.89	32.73	31.85	30.92

FARM B

17	18	19	20	21	22	23	24	25	26	27
100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%
32.51	33.33	32.08	31.69	30.41	29.98	32.55	33.27	31.83	30.01	31.99
30.92	28.69	26.37	25.79	25.38	26.02	30.53	31.90	28.38	25.46	26.66

FARM C

28	29	30	31	32	33	34	35	36	37	38	39
100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%
31.82	29.28	30.08	29.46	29.88	28.65	30.05	28.19	29.98	32.03	31.37	32.16
23.83	25.14	28.01	24.65	25.27	24.74	27.53	24.67	25.71	28.24	27.50	27.24

FARM D

40	41	42	43	44	45	46	47	48	49	50	51	52	53	54	55
100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	91%	100%	100%	100%	100%	100%
33.83	32.87	33.74	32.07	33.14	32.69	32.30	34.93	31.62	31.35	34.12	31.16	32.65	33.49	32.52	34.75
29.84	30.09	30.53	27.75	28.50	28.59	28.62	31.34	26.20	26.95	30.25	28.05	27.98	29.07	29.84	33.00

% Positive

Not detected | > 20% | 20-40% | 40-60% | 60-80% | 80% <

Average Ct

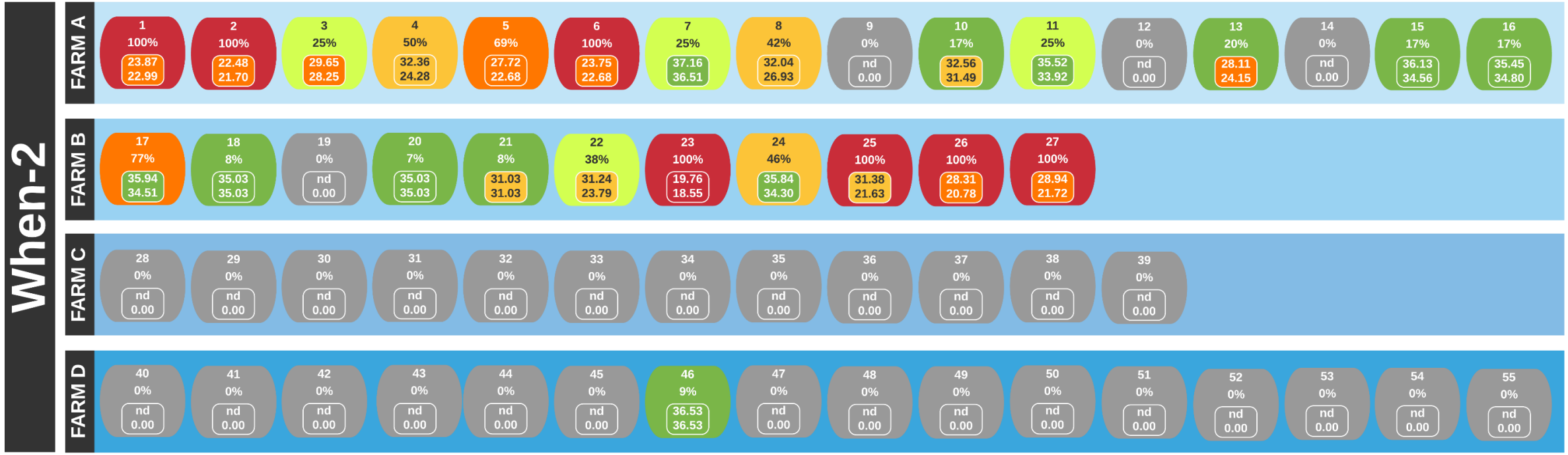
Not detected | > 35 Ct | 35-30 Ct | 30-20 Ct | < 20 Ct

Shapes

Av Ct / Min Ct — Tank No / Prev %

Pond No / Prev %

Av Ct / Min Ct


When-2
FARM A
1 100% 23.87 22.99
2 100% 22.48 21.70
3 25% 29.65 28.25
4 50% 32.36 24.28
5 69% 27.72 22.68
6 100% 23.75 22.68
7 25% 37.16 36.51
8 42% 32.04 26.93
9 0% nd 0.00
10 17% 32.56 31.49
11 25% 35.52 33.92
12 0% nd 0.00
13 20% 28.11 24.15
14 0% nd 0.00
15 17% 36.13 34.56
16 17% 35.45 34.80
FARM B
17 77% 35.94 34.51
18 8% 35.03 35.03
19 0% nd 0.00
20 7% 35.03 35.03
21 8% 31.03 31.03
22 38% 31.24 23.79
23 100% 19.76 18.55
24 46% 35.84 34.30
25 100% 31.38 21.63
26 100% 28.31 20.78
27 100% 28.94 21.72
FARM C
28 0% nd 0.00
29 0% nd 0.00
30 0% nd 0.00
31 0% nd 0.00
32 0% nd 0.00
33 0% nd 0.00
34 0% nd 0.00
35 0% nd 0.00
36 0% nd 0.00
37 0% nd 0.00
38 0% nd 0.00
39 0% nd 0.00
FARM D
40 0% nd 0.00
41 0% nd 0.00
42 0% nd 0.00
43 0% nd 0.00
44 0% nd 0.00
45 0% nd 0.00
46 9% 36.53 36.53
47 0% nd 0.00
48 0% nd 0.00
49 0% nd 0.00
50 0% nd 0.00
51 0% nd 0.00
52 0% nd 0.00
53 0% nd 0.00
54 0% nd 0.00
55 0% nd 0.00
% Positive
Not detected
> 20%
20-40%
40-60%
60-80%
80% <
Average Ct
Not detected
> 35 Ct
35-30 Ct
30-20 Ct
< 20 Ct
Shapes
Av Ct
Min Ct
Tank No
Prev %
Pond No
Prev %
Av Ct
Min Ct



RtX Toxin

FARM A	1 100% 33.83 32.72	2 100% 34.71 33.98	3 25% 38.62 37.92	4 33% 34.63 24.80	5 62% 37.35 35.92	6 64% 37.91 36.13	7 58% 36.76 34.99	8 58% 35.92 29.04	9 83% 35.61 32.72	10 42% 36.86 35.61	11 83% 37.15 35.10	12 25% 37.15 35.59	13 20% 37.05 35.15	14 83% 35.62 33.26	15 75% 36.80 35.30	16 75% 36.39 35.09
FARM B	17 46% 37.89 34.84	18 15% 37.84 37.17	19 15% 37.72 36.85	20 79% 36.86 35.41	21 92% 36.18 35.09	22 100% 34.77 33.20	23 23% 38.09 36.23	24 31% 37.41 35.80	25 92% 35.69 33.99	26 100% 34.24 33.15	27 100% 35.21 32.22					
FARM C	28 100% 34.31 33.05	29 100% 33.63 31.70	30 100% 33.31 28.85	31 100% 33.22 32.22	32 100% 34.08 33.05	33 100% 32.68 30.89	34 100% 32.96 25.73	35 100% 28.57 17.28	36 100% 33.82 32.65	37 100% 33.82 32.65	38 100% 34.53 33.46	39 85% 33.16 32.40				
FARM D	40 0% nd 0.00	41 0% nd 0.00	42 18% 36.87 34.51	43 0% nd 0.00	44 0% nd 0.00	45 18% 36.00 34.97	46 9% 36.44 36.44	47 18% 36.49 35.70	48 0% nd 0.00	49 0% nd 0.00	50 0% nd 0.00	51 82% 36.20 34.89	52 0% nd 0.00	53 0% nd 0.00	54 0% nd 0.00	55 9% 37.33 37.33

Hemo Toxin

FARM A	1 58% 38.09 35.99	2 42% 38.21 37.46	3 0% nd 0.00	4 8% 24.92 24.92	5 15% 37.11 35.33	6 27% 38.25 37.94	7 8% 37.68 37.68	8 8% 32.32 32.32	9 25% 37.47 36.01	10 8% 37.68 37.68	11 17% 37.60 37.20	12 0% nd 0.00	13 0% nd 0.00	14 50% 37.05 35.89	15 8% 39.57 39.57	16 0% nd 0.00
FARM B	17 15% 37.22 36.92	18 0% nd 0.00	19 0% nd 0.00	20 0% nd 0.00	21 0% nd 0.00	22 0% nd 0.00	23 0% nd 0.00	24 0% nd 0.00	25 0% nd 0.00	26 8% 38.09 38.09	27 8% 37.72 37.72					
FARM C	28 62% 37.00 35.69	29 46% 36.36 34.47	30 92% 35.89 31.30	31 85% 35.59 34.70	32 46% 36.77 35.62	33 100% 35.50 33.63	34 92% 35.17 28.33	35 92% 32.07 20.77	36 92% 35.54 34.22	37 77% 36.12 35.16	38 54% 36.66 35.31	39 77% 35.20 34.62				
FARM D	40 0% nd 0.00	41 0% nd 0.00	42 0% nd 0.00	43 0% nd 0.00	44 0% nd 0.00	45 0% nd 0.00	46 0% nd 0.00	47 0% nd 0.00	48 0% nd 0.00	49 0% nd 0.00	50 0% nd 0.00	51 0% nd 0.00	52 0% nd 0.00	53 0% nd 0.00	54 0% nd 0.00	55 0% nd 0.00



HDV

FARM A

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
8%	25%	8%	17%	8%	0%	0%	17%	8%	0%	0%	17%	10%	17%	17%	17%
37.81 / 37.81	37.40 / 37.14	38.85 / 38.85	38.23 / 37.66	37.29 / 37.29	nd / 0.00	nd / 0.00	37.45 / 37.38	36.18 / 36.18	nd / 0.00	nd / 0.00	38.21 / 37.57	37.67 / 37.67	37.30 / 36.66	36.67 / 35.74	36.70 / 36.08

FARM B

17	18	19	20	21	22	23	24	25	26	27
0%	8%	15%	14%	8%	23%	15%	8%	23%	15%	8%
nd / 0.00	39.41 / 39.41	37.01 / 36.58	36.70 / 35.70	37.79 / 37.79	38.75 / 38.16	38.28 / 37.77	37.85 / 37.85	38.46 / 37.41	38.91 / 38.89	37.42 / 37.42

FARM C

28	29	30	31	32	33	34	35	36	37	38	39
15%	23%	62%	62%	38%	23%	46%	15%	46%	77%	38%	31%
37.41 / 37.40	37.10 / 34.80	37.43 / 36.03	38.01 / 37.20	37.75 / 36.13	38.21 / 37.45	37.01 / 36.01	38.68 / 38.41	37.54 / 36.85	38.17 / 37.04	38.06 / 35.79	38.09 / 36.42

FARM D

40	41	42	43	44	45	46	47	48	49	50	51	52	53	54	55
0%	9%	18%	9%	0%	27%	0%	27%	45%	18%	36%	9%	18%	9%	36%	0%
nd / 0.00	38.46 / 38.46	38.72 / 38.39	39.10 / 39.10	nd / 0.00	39.50 / 38.39	nd / 0.00	39.96 / 39.26	40.63 / 39.29	39.17 / 39.04	41.32 / 39.44	39.05 / 39.05	40.45 / 39.40	39.37 / 39.37	39.33 / 38.34	nd / 0.00

Zon Toxin

FARM A

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
83%	75%	0%	0%	0%	0%	0%	8%	0%	8%	17%	0%	0%	8%	25%	8%
37.98 / 36.33	38.46 / 36.67	nd / 0.00	nd / 0.00	nd / 0.00	nd / 0.00	nd / 0.00	33.52 / 33.52	nd / 0.00	39.75 / 39.75	39.62 / 39.49	nd / 0.00	nd / 0.00	39.13 / 39.13	39.06 / 38.25	39.71 / 39.71

FARM B

17	18	19	20	21	22	23	24	25	26	27
92%	54%	38%	0%	62%	100%	23%	0%	0%	100%	92%
37.80 / 35.98	37.88 / 36.19	38.15 / 37.69	nd / 0.00	38.95 / 37.77	35.92 / 35.03	39.02 / 38.55	nd / 0.00	nd / 0.00	34.09 / 32.95	35.45 / 34.51

FARM C

28	29	30	31	32	33	34	35	36	37	38	39
8%	8%	8%	38%	23%	100%	31%	23%	0%	15%	0%	0%
37.71 / 37.71	29.24 / 29.24	35.69 / 35.69	38.65 / 36.86	38.64 / 37.56	38.10 / 37.37	38.49 / 36.56	35.65 / 30.04	nd / 0.00	39.25 / 38.76	nd / 0.00	nd / 0.00

FARM D

40	41	42	43	44	45	46	47	48	49	50	51	52	53	54	55
9%	9%	18%	0%	0%	0%	0%	0%	18%	0%	0%	0%	0%	9%	9%	0%
39.07 / 39.07	39.07 / 39.07	38.58 / 37.83	nd / 0.00	nd / 0.00	nd / 0.00	nd / 0.00	nd / 0.00	37.38 / 35.74	nd / 0.00	nd / 0.00	nd / 0.00	nd / 0.00	38.77 / 38.77	39.86 / 39.86	nd / 0.00

3. Estimate the severity or consequence of the presence of the pathogen



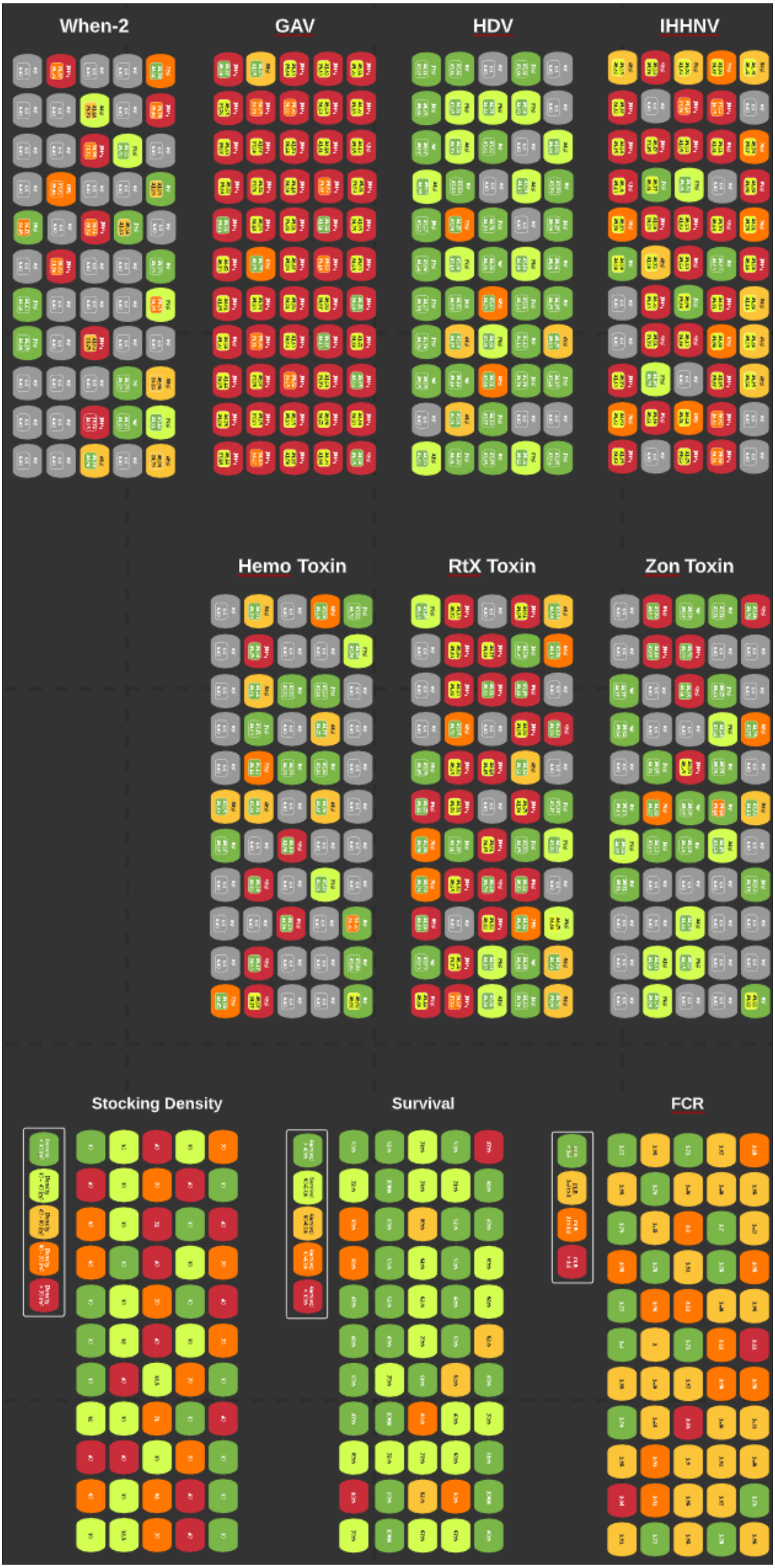
Stocking density range: 30-80/m^2



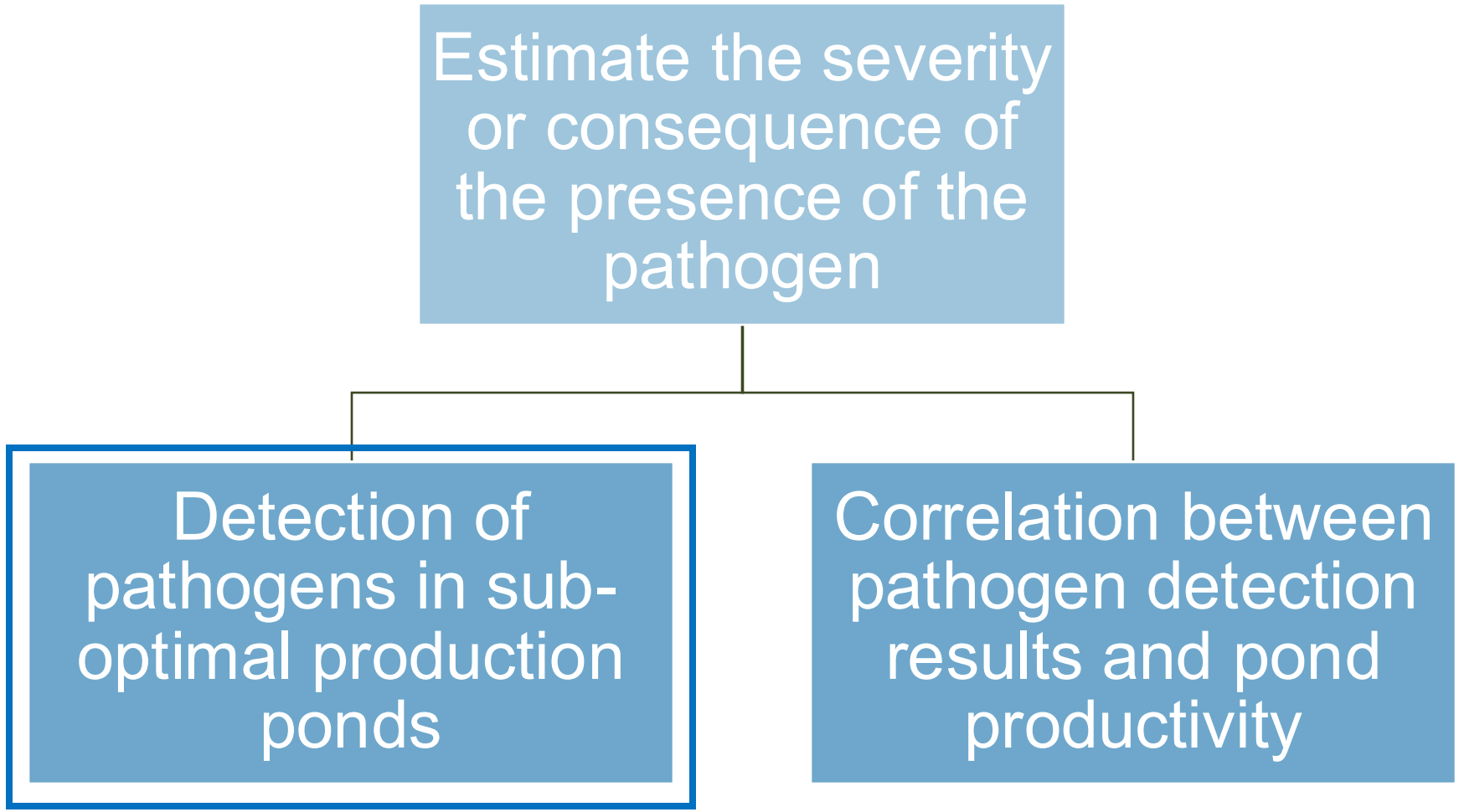
Survival range: 27-100%



FCR range: 1.72 - 2.34


When-2
GAV
HDV
IHHNV
Hemo Toxin
RtX Toxin
Zon Toxin
Stocking Density
Survival
FCR

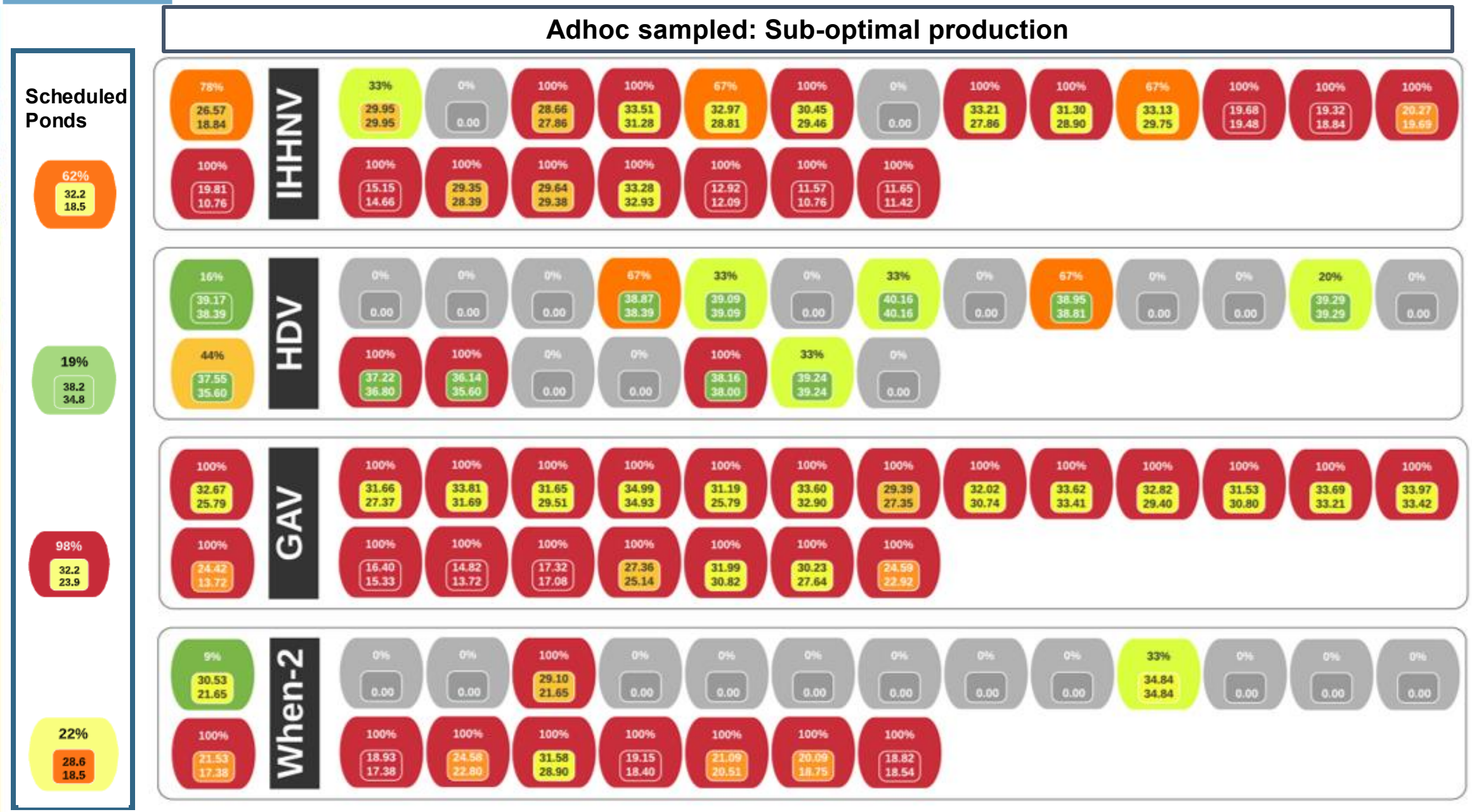
3. Estimate the severity or consequence of the presence of the pathogen



22 Ponds. 10-38 samples pools 3.

Correlation between pathogen detection results and pond productivity

Pathogen detection associated with ponds with sub-optimal production



3. Estimate the severity or consequence of the presence of the pathogen

Correlation between pathogen detection results and pond productivity

Adhoc sampled: Sub-optimal production

Scheduled Ponds

21%
37.3
29.3

57%
34.7
17.3

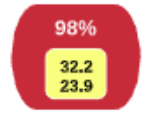
23%
35.9
20.7



Correlation between pathogen detection results and pond productivity

Pathogen detection in ponds with sub-optimal production: adhoc samples not standardised

Pathogen Target	Percentage of Ponds with 100% positive detection (%)	
	Scheduled sampling	Sub-optimal production
GAV	100	100
IHHNV	30.9	75
When-2	12.7	40
HDV	0	15
Rtx toxin gene	29	50
Zon toxin gene	5.4	40
Hemo toxin gene	1.8	10
*No detection of WSSV, YHV-1, YHV-7 or Pir-A from any of the pond collected samples		



An influence of multi-pathogen presence on health and production.

- Association studies NOT causative conclusions.

Correlation between pathogen detection results and pond productivity

Pathogen detection in ponds with sub-optimal production: adhoc samples not standardised



Some ponds not particularly high prevalence and copy number

High throughput sequencing

Homology to	Type of viral genome	Project sample ref	Number of blast matches with viral genome records	Range of Length of blast alignments (amino acid)	Average length of alignment (amino acids)	% identity (by amino acid)
Endogenous nimavirus	DNA	#8 DNA	100	12 to 6118	123	26-100%
		#9 DNA	488	19 to 6114	266	24-100%
		#8 RNA	832	16-3032	365	22-100%
		#2 RNA	1205	24-5995	355	19-100%
Densovirus	DNA	#9 DNA	6	25-143	63	30-62%
		#8 RNA	10	46-96	70	27-96%
		#2 RNA	19	31-111	66	23-45%
		#8 DNA	3	53-78	62	28-24%
Nuclopolyhedrovirus	DNA	#9 DNA	44	17-593	76	23-59%
		#8 RNA	134	27-122	58	21-55%
		#2 RNA	361	18-209	63	21-66%
		#8 DNA	87	25-153	65	22-50%
Iridescent virus	DNA	#9 DNA	465	17-209	58	14-62%
		#8 RNA	215	25-142	63	20-64%
		#2 RNA	383	24-459	71	19-63%
		#8 DNA	1096	20-177	78	21-70%
Iflavivirus	RNA	#9 DNA	24	26-124	63	25-42%
		#8 RNA	21	50-89	55	38-43%
		#8 DNA	10	37-162	64	22-58%
		#2 RNA	76	26-139	65	22-56%
Picornavirus	RNA	#9 DNA	256	21-168	64	20-61%
		#8 RNA	243	13-144	60	21-69%
		#2 RNA	858	17-177	67	20-65%
Vibrio phages	Both	#9 DNA	61	28-694	210	23-100%
		#8 DNA	13	44-104	70	28-45%
		#8 RNA	13	38-80	54	29-42%
		#2 RNA	90	27-203	77	25-64%
Phage	Both	#9 DNA	428	21-776	100	22-75%
		#2 RNA	530	22-326	88	20-63%
		#8 DNA	65	27-141	62	22-55%
Granulovirus	DNA	#2 RNA	168	13-262	66	20-100%
		#8 DNA	29	12-360	68	18-100%

Investigation on these sequences continues beyond this project.

Similarly a large number of tiger prawn gene sequences will be investigated further.



Identify management measures to mitigate risk of the pathogen/disease

Strategic Recommendations & Key Outcomes

Identify management measures to mitigate risk of the pathogen/disease

1. Strengthen National Border control: Keep Exotic Pathogens Exotic

2. Strengthen Regional capacity and capability in Biosecurity response

3. Domesticated Black tiger prawn, *P.monodon*

4. Increase focus on prawn health: Consider the influences of host-pathogen-environment

5. Increase education in prawn health and biosecurity.

Identify management measures to mitigate risk of the pathogen/disease

1. Strengthen National Border control: Keep Exotic Pathogens Exotic

1. Strengthen conditions on imported commodity prawns

Exclusion presents a high cost benefit ratio. (DAFF $100 return for every $1 invested.)

Consequence:
The import of frozen commodity prawns presents a clear path allowing introduction and then uncontrolled release of material potentially containing exotic pathogens. Potential release into the environment is uncontrolled and not containable. Once established in the environment, biosecurity measures are a permanent cost to production.

Review underway:
Prawn IRA is under review and operating an heightened testing: 100% of batches for WSSV and YHV. Prawn IRA has also included need for deveined tails due to heightened risk of EHP

Current Appropriate Level of Protection (ALOP) is “very low but not zero”. Previous testing regime was designed to detect a 5% prevalence of non-compliant product.

Identify management measures to mitigate risk of the pathogen/disease

1. Strengthen National Border control: Keep Exotic Pathogens Exotic

Stentiford et al., (2020) “Close attention to national and transboundary spread of hazards-particularly via trade-must extend beyond live animals and include the risk of distributing pathogens via end-products , even those destined directly for human consumptions that would not normally interact further with the environment”

Previous reports of Nunan *et al*., 1998, Durand *et al*., 2000; McColl *et al*., 2004 and Scott-Orr et al., 2017

1a. Allowing cooked product would mitigate the uncooked pathway.

Identify management measures to mitigate risk of the pathogen/disease

Current Appropriate Level of Protection (ALOP) is "**very low but not zero**". Previous testing regime was designed to detect a 5% prevalence of non-compliant product.

Since July 2018:

145 consignments / 10770 (1.3%) have failed import testing > Positive WSSV

Estimate a consignment (one pond) = 1 x 10T container = speculatively 1450T Positive WSSV

~ 30% of 2018-2019 Total Aquaculture Production:

"A very low but not zero % but is this a very low but not zero volume ?"

https://www.agriculture.gov.au/import/goods/uncooked-prawns/uncooked-prawn-consignments

1b. Recognise the volume of material to define ALOP and testing to detect non-compliant product.

Identify management measures to mitigate risk of the pathogen/disease

- WSSV and YHV-1 are not the only pathogens that can cause severe impacts.
- IMNV, TSV, Covert Mortality Nodavirus are all listed in Australia's nationally reportable diseases of crustaceans.
- *Enterocytozoan hepatopenaei* (EHP)
- Decapod Iridescent Virus -1 (DIV-1 *syn.* SHIV-1, CqIV-1) currently causing 80% mortalities. Estimated to have caused a decline of 0.3 Million Tonne decline in China (Huifeng, 2020) is not listed for exclusion. and no requirement to test in current protocols. **64 X Australian total aquaculture production**
- Recognise different strains. Recognised in the terrestrial animal health space. Dhar (2020)

"A significant variation in the genome sequence of IHHNV in prawn populations in the Gulf of California has been detected and the monitoring of genetic variance in IHHNV was recommended because slight changes in genome sequence can lead to increased virulence of viral pathogens".

1c. The target pathogen list: Needs to be extended and more regularly updated to include pathogens quickly after they are identified.

Identify management measures to mitigate risk of the pathogen/disease

1. Strengthen National Border control: Keep Exotic Pathogens Exotic









Data to Support this Recommendation:
Tested 42 pools of 5 tail imported uncooked product (cuticle and muscle/gut wedge).

Detected:

- **IHHNV 54%** of samples
- **Whenzhou Shrimp Virus-2 (When-2): 100%** of samples.
- **Not detected:** YHV-1, YHV-7, GAV, WSSV, MBV, Pir-A, HPV, Enterocytozoan, Iridescent virus.

When-2: detected in
- China (2015). No prevalence data.
- HTS in Australian *P. monodon* (Huerlimann et al., 2018).
- Some prevalence data from this project.
- Only detected in EC QLD sourced *P. monodon*.
- Detected in glass shrimp (pre-pond and pond)

Was When-2 an incidental introduction of uncharacterized unlisted virus with imported prawns or has it been here all the time?

Identify management measures to mitigate risk of the pathogen/disease

A potential difficulty to adding additional pathogens is the lack of data to support claims of freedom of the added pathogens.

1d. Leverage current broodstock translocation testing to demonstrate proof of freedom.

- The National survey to demonstrate proof of freedom to WSSV & YHV-1 (East et al. 2015) tested 3,081 samples.
- Industry testing of broodstock for WSSV within translocation testing estimate ~8000+. Sample collection and testing is currently industry funded.
- Can this process be leveraged with a Government-Test provider collaboration to extend to rapidly emerging pathogens? Co-contribution from Government-test providers to cover the cost of running an additional analysis. No impact on industry costs and efficient use of government funds (doing things more efficiently $$).

Identify management measures to mitigate risk of the pathogen/disease

1. Strengthen National Border control: Keep Exotic Pathogens Exotic

Benefits:

- Avoid repeated losses from an exotic pathogen (cooked)

- Provides strong data to support claims of Freedom (nationally and internationally)
- Cost effective: removes duplication of costs of travel, capture, sampling.
- Targets surveillance to the primary input to the production line
- Provides an early indication of endemic variants of the pathogens (early warning)
- Puts industry at the forefront of biosecurity surveillance

With policy change to extend testing to current providers:

- Increases aquatic emergency disease preparedness (prawns).
- Increases national capacity for detection of exotic prawn pathogens.
- Enables continuity of service to non-affected areas if an emergency occurs
- Partial validation of assay for negative detection, identifies any assay issues prior to an outbreak if a published assay is not suitable for Australian application e.g IHHNV

Risk:

- Unintended consequence of detection? **PCR alone is not a diagnosis.**

Requires presence of disease, confirmation by sequencing, other diagnostic assay.

Identify management measures to mitigate risk of the pathogen/disease

2. Strengthen Regional capability and capacity in prawn biosecurity response.

Successful Eradication and Containment are also more cost effective than asset based protection. Estimates $56 to $15 saving for every $1.

Successful Containment and Eradication relies on:

- **Rapid detection of** "abnormal"
- **Rapid pathogen detection** & diagnosis
- **Rapid deployment** of appropriate knowledge/ workforce and resource to contain and eradicate.

"Abnormal":

- Some level of mortality is expected in hatchery and farm. The delay in differentiating "unusual" = 1-2 days.

Non-state government laboratories have permission to test for **exotics for translocation NOT in response to disease.**

- Diagnosis requires samples to BQ and confirmation at AAHL.
- High prevalence of IHHNV/GAV may lead to wrong interpretation of qPCR results.

Two thirds of industry in Northern Australia is located regionally. Logistical impediment to rapid deployment. The lack of rapid access to everything is exacerbated under COVID.

Identify management measures to mitigate risk of the pathogen/disease

2. Strengthen Regional capability and capacity in prawn health and biosecurity response.

2a. Establish regional industry-embedded training and capability in emergency disease response

- Scenario training for the most immediate emergency responses by industry.
- Extend permission to test for exotics to suspect disease incidents. **In addition to, not in place of**, sending samples to government laboratories.
- Enable industry providers of testing to send duplicate samples to BQ and AAHL at the same time rather than sequential.

Identify management measures to mitigate risk of the pathogen/disease

2. Strengthen Regional capability and capacity in prawn health and biosecurity response.

Data to Support this Recommendation:

WSSV 2016: Farm in SE QLD (~50km from BSL)
During the WSSV incidents in SE QLD, samples for genetic testing were collected on Infected property 1 (IP1) on Thursday 22nd and 25th November 2016 and disease testing occurred on the Wednesday 30th November (OIE, 2016 Ref 21737).

This project: (From Northern Queensland: Townsville)
WSSV positive glass shrimp were detected in this project.
Detected JCU: **Friday 15th May** 2020.
Sent to BSL: **Mon 18th May** 2020 (O/N priority by 12)
Received BSL: **Wed 20th May** 2020 (am)
Received at AAHL: **Thursday 21st May** 2020
Confirmed AAHL: **Friday 22nd May** 2020

One week from detection to confirmation:
Not rapid Freight delivery exacerbated if Thursday/Friday detection

Identify management measures to mitigate risk of the pathogen/disease

2. Strengthen Regional capability and capacity in prawn health and biosecurity response.

Establish regional industry-embedded emergency disease response and training.

Benefits:

Training policy:

- Provides farmers with direct training to “responsibly manage”. GBO under Biosecurity Act.
- Empowers those most impacted by the disease to respond to the disease.
- Removes mistakes or delays in response due to responders not having local knowledge of networks/geography.

Testing policy:

- Reduces a risk of delay due to government response agents being in high COVID risk zones
- Reduces a risk of delay in diagnosis and response due to isolation and regionalization.
- Reduces time to alert if current industry providers deliver an early positive result.
- Provides some insulation against future government staff cuts. (COVID impact on budgets)

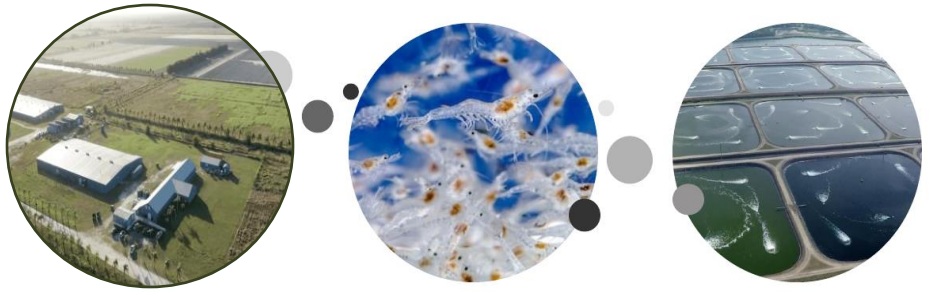
Risks:

- Potentially still insufficient workforce for a response.
- Staff turnover leads to knowledge not being retained by industry.
- Potential “False positives” escalated to AAHL. Samples cancelled or tested anyway as enables open comparison of quality of service between industry providers and State and National laboratories.

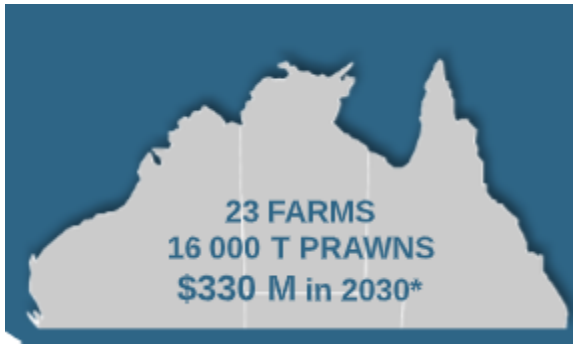
Identify management measures to mitigate risk of the pathogen/disease

3. Domestication of Black tiger prawn, *P.monodon*





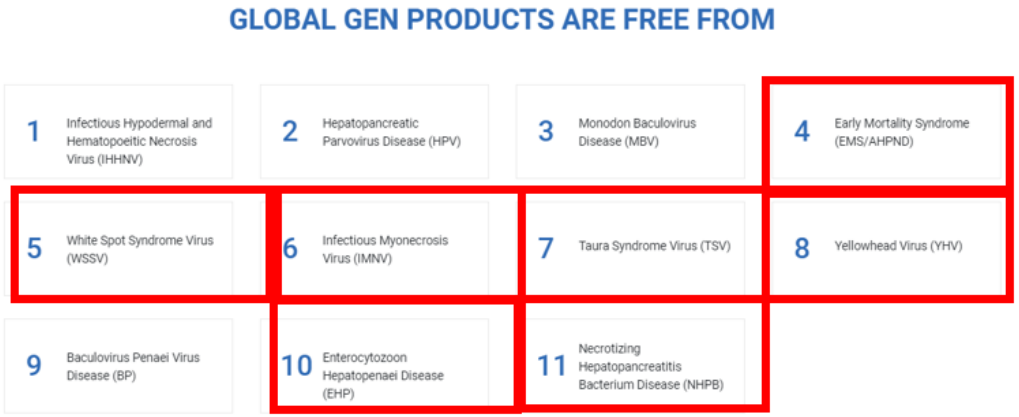
Closed life-cycle to remove uncertainty created by wild collection of broodstock → Consistent supply of high health post-larvae to support industry → Resilient, Consistent Sustainable, Profitable Australian Farmed Prawn Production



Identify management measures to mitigate risk of the pathogen/ disease

3. Industry to actively pursue domestication of *P.monodon* to remove the annual intake of wild broodstock with unknown pathogen potential.

- ***SPF: Specific Pathogen Free****: wild-sourced Founders (high health HH):* Currently free of many of the pathogens on the SPF list.
- However, high prevalence of IHHNV and GAV in broodstock.
- Higher percentage of females positive v males
- Data set is time restricted September 2018-February 2019.



- Pond data indicates SPF will be exposed to pathogens.
- ***Specific Pathogen Resistant***: higher survival when exposed to a pathogen. Some remain refractory to infection. (0% infected after exposure)
- ***Specific Pathogen Tolerant***: carry a high load without growth affected.

SPF, SPT, SPR are all specific to particular pathogens: Which ones to target?

Identify management measures to mitigate risk of the pathogen/disease

3. Domestication of *P.monodon:*

Removal and annual intake of pathogens via wild broodstock*:*

- *SPF*
- *SPT*
- *SPR*

Which pathogens to target?

- IHHNV and GAV are the most prevalent
- YHV-7 and Pir-A have been historically associated with high mortality & experimentally reproduced.
- IHHNV has been associated with slow growth & deformity
- GAV has been associated with mortality
- When-2 is unknown
- WSSV is highly pathogenic and present in wild (Logan River Area)
- Other emergent pathogens? EHP, DIV-1, HINV. YHV-7/IHHNV variant, Iflavirus?

Identify management measures to mitigate risk of the pathogen/disease

3. Domestication of *P.monodon:*

Removal and annual intake of pathogens via wild broodstock*:*

SPF

SPT

SPR

Which pathogens to target?

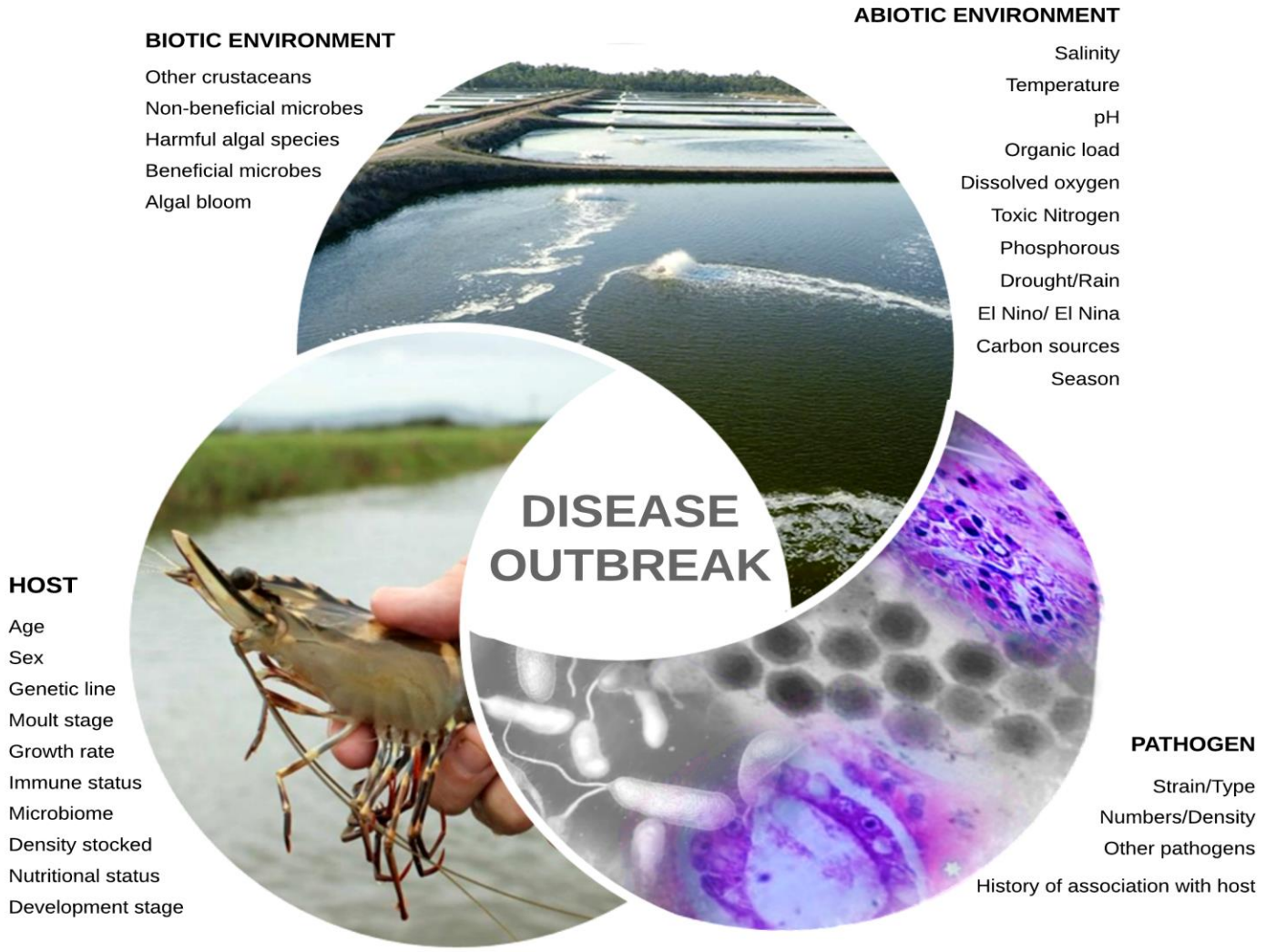
- IHHNV and GAV are the most prevalent
- YHV-7 and Pir-A have been historically associated with high mortality
- IHHNV has been associated with slow growth & deformity
- GAV has been associated with mortality
- When-2 is unknown
- WSSV is highly pathogenic and present in wild (Logan River Area)
- Other emergent or exotics in future?

4. Increase focus on prawn health: Consider the influences of host-pathogen-environment

Effect of global and human activity on the biotic environment:
- Pesticides
- Antibiotic resistance
- Impact of microbes

Increasing demand for wild caught broodstock & PLs:
- impediment to stocking
- Increased cost
- Sourced from less ideal season/location

Hope for the best, plan for the worst.



Global climate change: more frequent and more extreme weather.

Recognise industry has Tropical & Temperate located systems

Past 5 years:
YHV-7, Pir-AB, WSSV.

Prevalence in this project is higher than previous reports.

Viral purification and more robust science on experimental challenges:
- Purified
- Sequenced
- HTS
- Immune
- Water temp & salinity
- Different life stages

5. Increase education in prawn health and biosecurity.

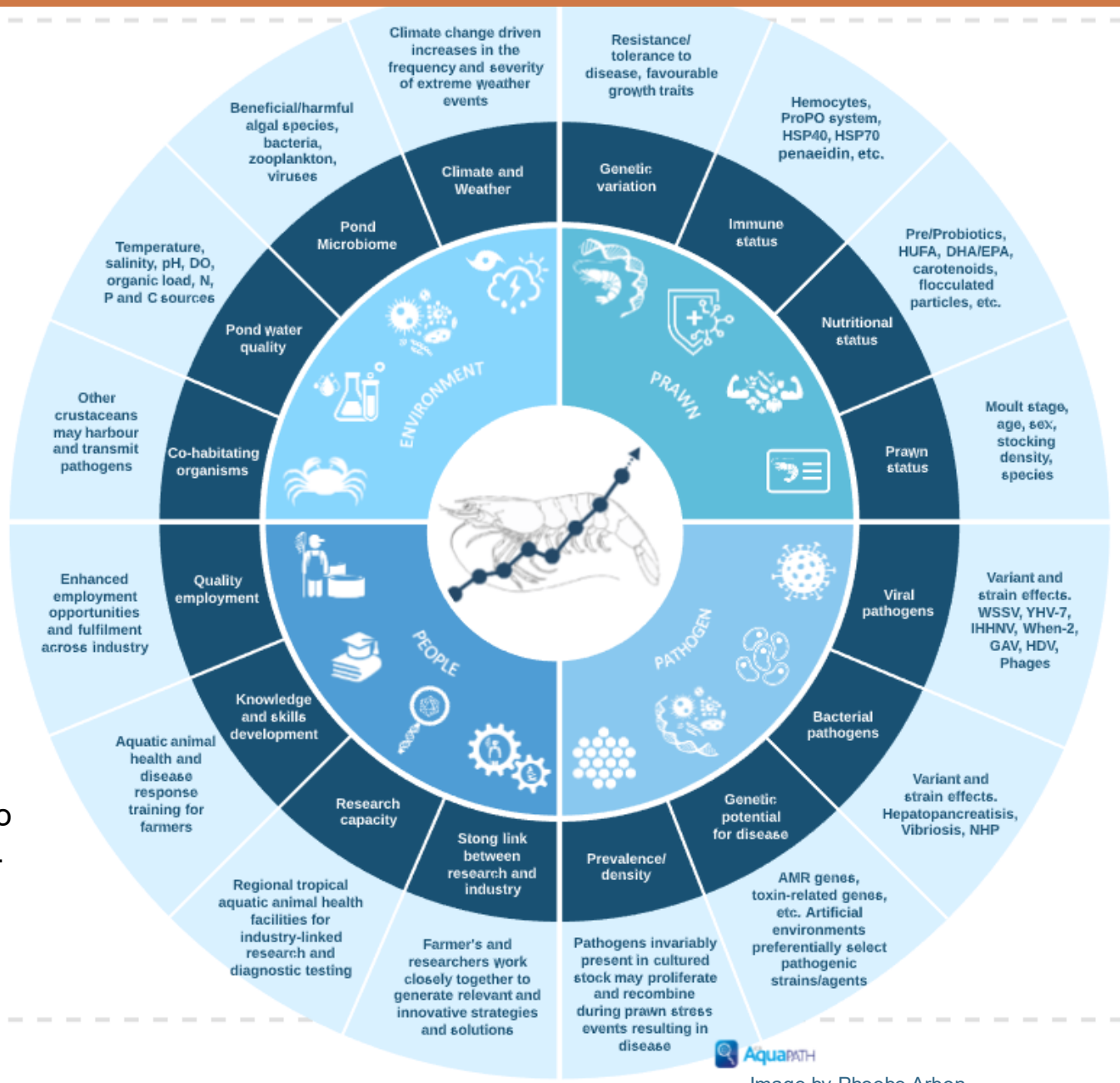


Image by Phoebe Arbon

Increase collaborative research with proximity providers & implement prawn specific training and education.

Create some resilience to regionalization & COVID. Reduce reliance on metropolitan centres and reduce reliance on government funded departments.

Aim for outcome of

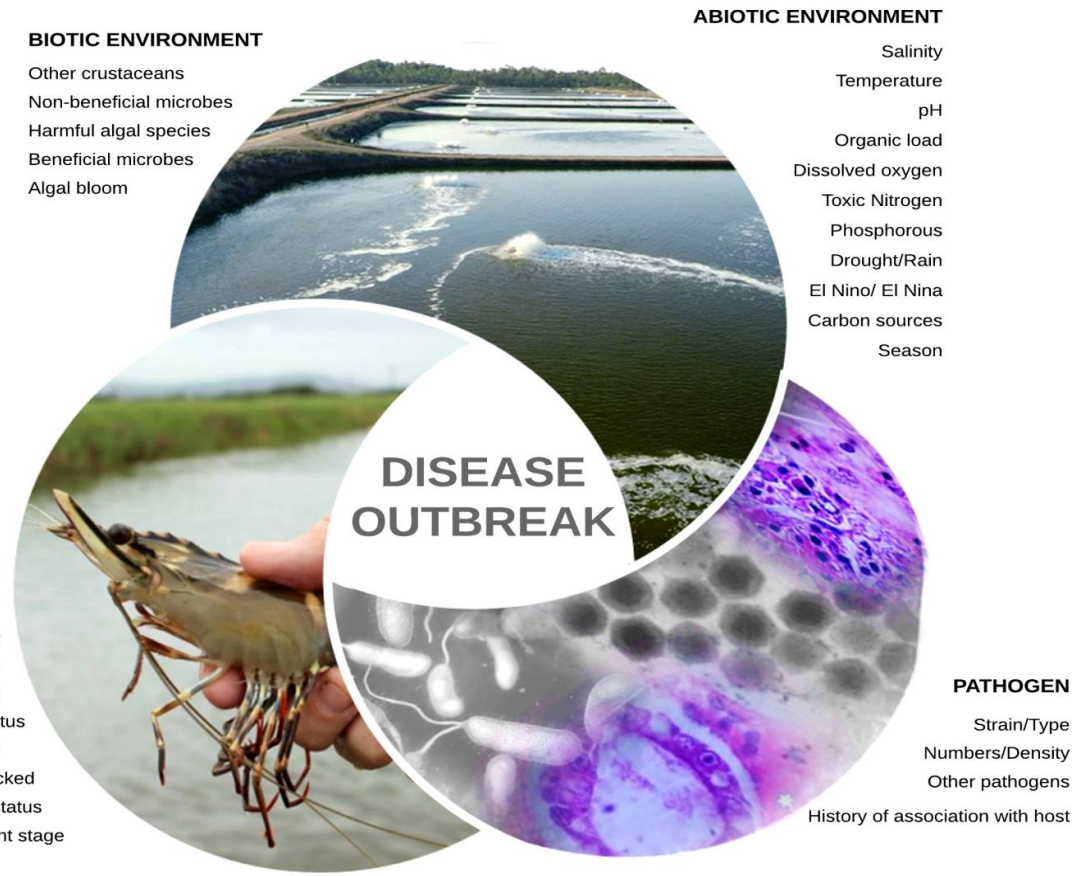
High Health Prawns

Adopt a multidiscipline approach to health research

Multi-factorial approach:

Access to technology:

- High throughput sequencing (HTS)
- Internet of Things (IOT)
- Machine Learning (ML)



Article

The use of machine learning to predict acute hepatopancreatic necrosis disease (AHPND) in shrimp farmed on the east coast of the Mekong Delta of Vietnam

May 2020 · Fisheries Science
DOI: 10.1007/s12562-020-01427-z

Nguyen Minh Khiem · Yuki Takahashi · Dang Thi Hoang Oanh · Show all 6 authors · Nobuo Kimura

Abstract

Predicting the outbreak of disease is essential when managing shrimp farms. Acute hepatopancreatic necrosis disease (AHPND) caused by Vibrio parahaemolyticus is a serious disease in shrimp. It is essential that shrimp farmers on the east coast of the Mekong Delta detect the disease as early as possible, because the mortality rate can reach 100%. Here, we used machine learning to predict AHPND development based on data collected since 2010 from shrimp farms in Tra Vinh, Ben Tre, Bac Lieu, and Ca Mau provinces. We initially hypothesized that the dependent variable, AHPND, was affected by 31 independent variables, but ultimately used 15 key variables to train the models. Logistic regression, artificial neural network, decision tree, and K-nearest neighbor analyses were performed, and the accuracy of the predictions was evaluated using hold-out and cross-validation tests. Logistic regression, as the most stable algorithm, was thus used to predict AHPND outbreaks in shrimp farms.

viruses

MDPI

Article

Iflavirus Covert Infection Increases Susceptibility to Nucleopolyhedrovirus Disease in *Spodoptera exigua*

Arkaitz Carballo [1,2], Trevor Williams [3], Rosa Murillo [1,2,*] and Primitivo Caballero [1,2]

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Received: 15 April 2020; Accepted: 1 May 2020; Published: 5 May 2020

check for updates

Abstract: Naturally occurring covert infections in lepidopteran populations can involve multiple viruses with potentially different transmission strategies. In this study, we characterized covert infection by two RNA viruses, Spodoptera exigua iflavirus 1 (SeIV-1) and Spodoptera exigua iflavirus 2 (SeIV-2) (family Iflaviridae) that naturally infect populations of *Spodoptera exigua*, and examined their influence on susceptibility to patent disease by the nucleopolyhedrovirus Spodoptera exigua multiple nucleopolyhedrovirus (SeMNPV) (family Baculoviridae). The abundance of SeIV-1 genomes increased up to ten-thousand-fold across insect developmental stages after surface contamination of host eggs with a mixture of SeIV-1 and SeIV-2 particles, whereas the abundance of SeIV-2 remained constant across all developmental stages. Low levels of SeIV-2 infection were detected in all groups of insects, including those that hatched from surface-decontaminated egg masses. SeIV-1 infection resulted in reduced larval weight gain, and an unbalanced sex ratio, whereas larval developmental time, pupal weight, and adult emergence and fecundity were not significantly affected in infected adults. The inoculation of *S. exigua* egg masses with iflavirus, followed by a subsequent infection with SeMNPV, resulted in an additive effect on larval mortality. The 50% lethal concentration (LC_{50}) of SeMNPV was reduced nearly 4-fold and the mean time to death was faster by 12 h in iflavirus-treated insects. These results suggest that inapparent iflavirus infections may be able to modulate the host

viruses

MDPI

Article

Effects of a Covert Infection with Phthorimaea operculella granulovirus in Insect Populations of *Phthorimaea operculella*

Andreas Larem, Saoussen Ben Tiba, Eva Fritsch, Karin Undorf-Spahn, Jörg T. Wennmann and Johannes A. Jehle *

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* Correspondence: Johannes.Jehle@julius-kuehn.de; Tel.: +49-6151-407-220

Received: 11 December 2018; Accepted: 4 April 2019; Published: 9 April 2019

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Abstract: Virus infections of insects can easily stay undetected, neither showing typical signs of a disease, nor being lethal. Such a stable and most of the time covert infection with Phthorimaea operculella granulovirus (PhopGV) was detected in a *Phthorimaea operculella* laboratory colony, which originated from Italy (Phop-IT). This covert virus (named PhopGV-R) was isolated, purified and characterized at the genetic level by full genome sequencing. Furthermore, the insect colony Phop-IT was used to study the crowding effect, double infection with other PhopGV isolates (CR3 and GR1), and co-infection exclusion. An infection with a second homologous virus (PhopGV-CR3) activated the covert virus, while a co-infection with another virus isolate (PhopGV-GR1) led to its suppression. This study shows that stable virus infections can be common for insect populations and have an impact on population dynamics because they can suppress or enable co-infection with another virus isolate of the same species.

Keywords: PhopGV; *Baculoviridae*; *Betabaculovirus*; potato tuber moth; genome sequencing; sublethal infection; co-infection

Genes (Basel). 2020 Jan; 11(1): 94.
Published online 2020 Jan 14. doi: 10.3390/genes11010094
PMCID: PMC7016691
PMID: 31947590

The Complete Genome of an Endogenous Nimavirus (*Nimav-1_LVa*) From the Pacific Whiteleg Shrimp *Penaeus* (*Litopenaeus*) *Vannamei*

Weidong Bao,[1,*] Kathy F. J. Tang,[2] and Acacia Alcivar-Warren[3,4,*]

▸ Author information ▸ Article notes ▸ Copyright and License information Disclaimer

Associated Data

▸ Supplementary Materials

Abstract

Go to:

White spot syndrome virus (WSSV), the lone virus of the genus *Whispovirus* under the family *Nimaviridae*, is one of the most devastating viruses affecting the shrimp farming industry. Knowledge about this virus, in particular, its evolution history, has been limited, partly due to its large genome and the lack of other closely related free-living viruses for comparative studies. In this study, we reconstructed a full-length endogenous nimavirus consensus genome, *Nimav-1_LVa* (279,905 bp), in the genome sequence of *Penaeus* (*Litopenaeus*) *vannamei* breed Kehai No. 1 (ASM378908v1). This endogenous virus seemed to insert exclusively into the telomeric pentanucleotide microsatellite $(TAACC/GGTTA)_n$. It encoded 117 putative genes, with some containing introns, such as *g012* (inhibitor of apoptosis, IAP), *g046* (crustacean hyperglycemic hormone, CHH), *g155* (innexin), *g158* (Bax inhibitor 1 like). More than a dozen *Nimav-1_LVa* genes are involved in the pathogen-host interactions. We hypothesized that *g046*, *g155*, *g158,* and *g227* (semaphorin 1A like) were recruited host genes for their roles in immune regulation. Sequence analysis indicated that a total of 43 WSSV genes belonged to the ancestral/core nimavirus gene set, including four genes reported in this study: wsv112 (dUTPase), wsv206, wsv226, and wsv308 (nucleocapsid protein). The availability of the *Nimav-1_LVa* sequence would help understand the genetic diversity, epidemiology, evolution, and virulence of WSSV.

All of the Methods, Results, Key Findings and Recommendations of this project are in the NA CRC reports:

- Final Report is under review from: APFA project team & NA CRC Board.
- Will be released on-line (CRC NA website) and to APFA.

- Individual component reports have been included in the final report and are also currently available (already approved)
    - Hatchery: Broodstock specific data
    - Pond: Pond specific data

- The pathogen tracking linked maps for each pathogen are at the end of this presentation.

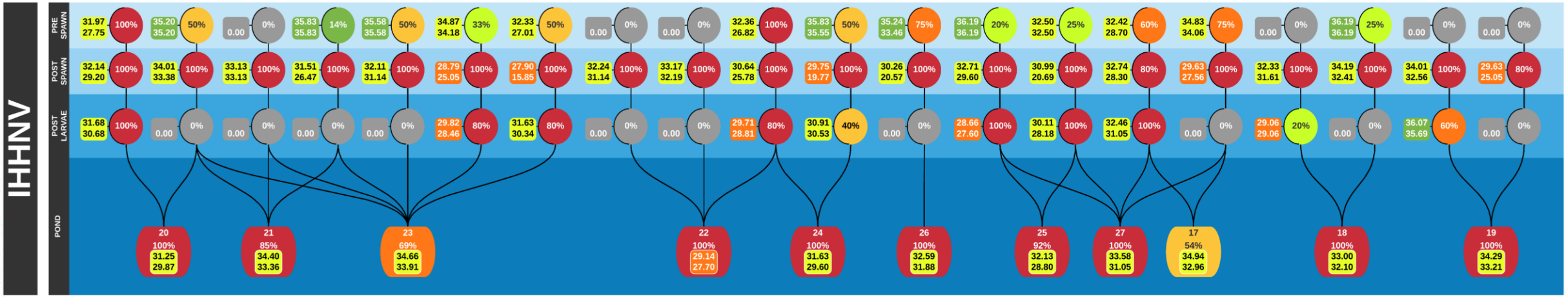
- Email: Kelly.condon@jcu.edu.au or Dean.jerry@jcu.edu.au

- FRDC APF Training workshop in Prawn disease. Postponed to be completed by June 30 2021.

Sincere thank you to the APFA and members for the cash contribution and in-kind contribution of staff time and stock to this project:

Akimasa Omori, Alistair Dick, Andrew Smith, Bambang Julianto, Bastien Finet, Brad Calcott, Courtney Remilton, Cristina Gordon, Dallas Donovan, Danielle Harman, Dave Owen, Erika Giardina, Jairo Llanos, Jordan Hack, Kathiga Kumanan, Kristian Mulholland, Matt West, Patrick Seincho, Ryan Lowrey, Skye Tara Lewis, Tony Charles and Wayne Dibartolo.

2500+ samples ~25 000 tests, Most number of samples analysed for multi-pathogen assays ever conducted, prevalence data on When-2, detection of Pir-A using pleopods, productivity data with pathogen detection, novel bacterial toxin detection and qPCR assays, detection of prawn pathogens in glass shrimp, pathogen presence does not inevitably involve disease outbreaks, some pathogens don't transfer to efficiently to PL, most detections involve multiple pathogens, NO Detection of WSSV or YHV-1 from Broodstock, Information from HTS.

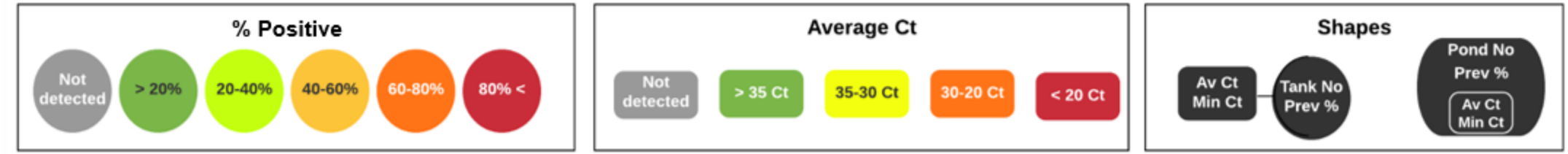


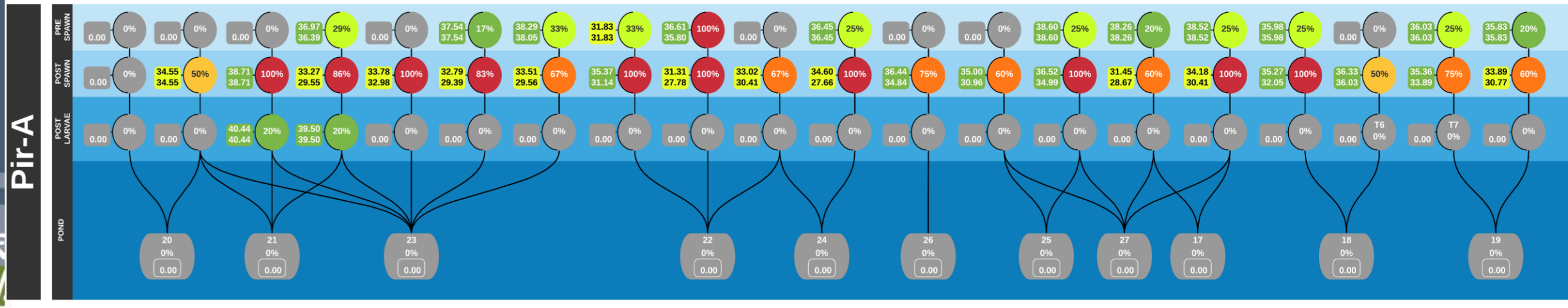
Prevalence and loading increases with pre v post spawn. (Days 1-45 spawns 1-4)

Larval Ct mostly similar to Pre-Spawn. Neg Larval from Neg Broodstock or Ct greater than 34. (Strain specific this pattern doesn't apply to a more recent detection of IHHNV: Type 2)

Ponds typically reflective of the PL combination except 17 and 23 (17 had poor survival)

IHHNV potentially more manageable at hatchery level.





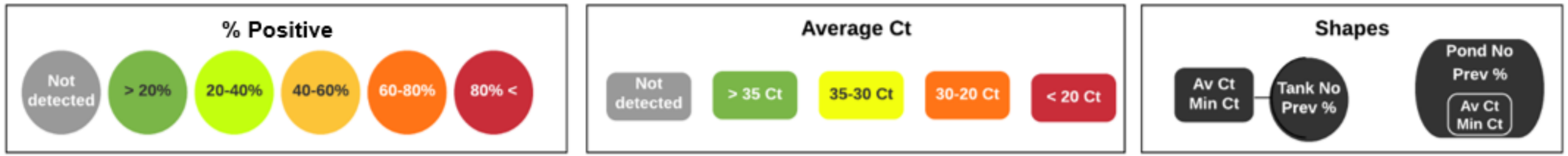
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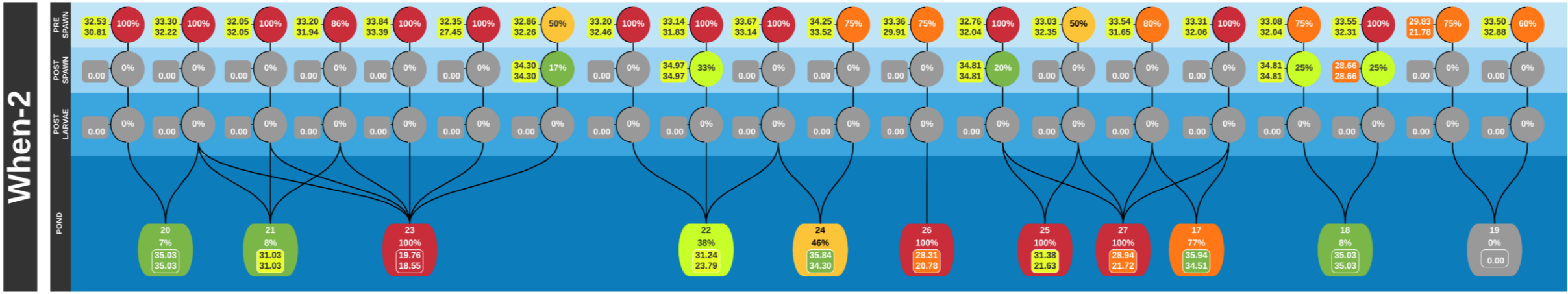
Low/no detected in PLs. (low prevalence and load or not present?)

Variable amplification in ponds. Influenced by Pond management.
Detected in glass shrimp. (1% outside, seasonally variable (outside was April collection)

Excludable and treatable in hatchery, poor transfer to PLs.

Can be in the environment with high risk conditions



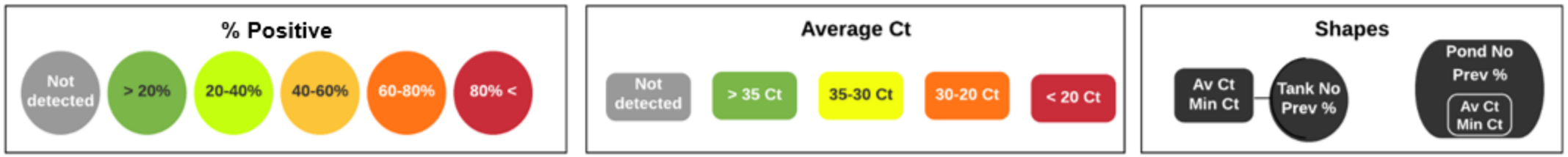


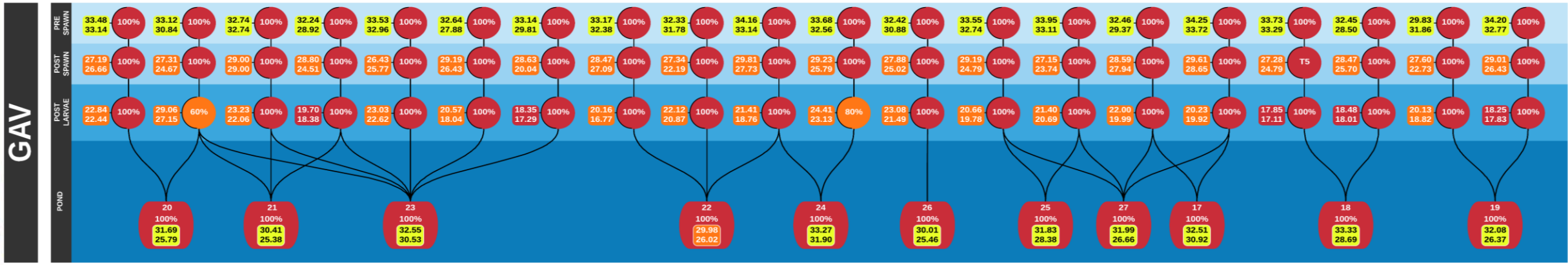
Prevalence and loading decreases with pre v post spawn. (Days 1-45 spawns 1-4).

Not detected in PLs. (low prevalence and load or not present?)

Variable distribution across farms.
High prevalence in glass shrimp. (49% inside 1% outside, seasonally variable (outside was April collection)

32% Potentially excludable, need to determine if transmitted in hatchery (enveloped virus). Management: Resistant strains as likely will be exposed in the farm ponds if can't exclude glass shrimp. 100% detection in imported commodity prawns





Prevalence and loading increases with pre v post spawn. (Days 1-45 spawns 1-4)

Larval Ct decreases = GAV loading increases ~10-100x sometimes 1000x

Ponds reduce loading of GAV.

GAV ubiquitous, highest prevalence in broodstock. Potentially more manageable to select for tolerance. (test adults at harvest or select strong growers that you know were high load GAV as PLs.

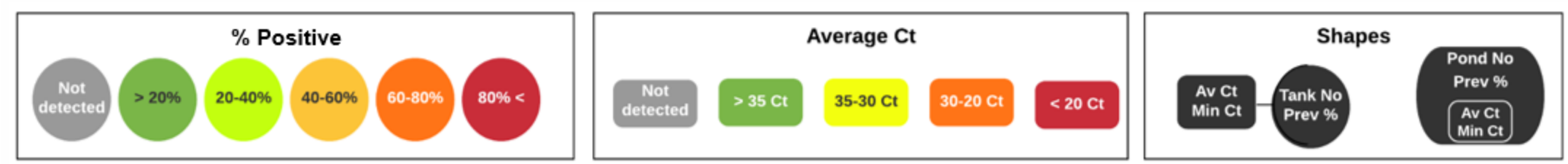


Table 8 List of Vibrio strains in the NCBI database collected from shrimp samples that contain the Zon occludens toxin gene. Table includes bacterial species, host species, country of origin, bacterial strain if provided, NCBI database number and any disease that was associated with the accession.

ND= not defined, BRS=Bright Red syndrome

Species	Host species	Country of origin	Strain	NCBI ref	Disease
V.campbelli	shrimp-undefined	Thailand	VIB391	MNLF01000022.1	ND
V.campbelli	shrimp-undefined	Thailand	VIB392	MNLF01000043	ND
V.campbelli	*P.monodon*	Thailand	1114GL	CP019634.1	ND
V.cholerae	shrimp pond sediment	Bangladesh	CRA_S9	QJSG01000030	ND
*V.harveyi**	L.vannamei	Mexico	CAIM 1792	CP033144.1	BRS
V.owensii	L.vannamei	China	V180403	CP033144.1	AHPND
V.parahaemolyticus	*P.monodon*	Malaysia	MSR17	Ahmed et al., 2019	ND
V.parahaemolyticus	shrimp-undefined			LFYM01000076.1	ND
V.parahaemolyticus	shrimp-undefined	Vietnam	13-028/A2	NZ_JOKT01000001.1	AHPND
V.parahaemolyticus	*L.vannamei*	South Korea	SM4	NZ_QPQE01000002.1	AHPND
V.parahaemolyticus	shrimp pond water	Malaysia	NA1	POBW01000024.1	AHPND
V.parahaemolyticus	*L.vannamei*	Malaysia	ND13	POBH01000024.1	AHPND
V.parahaemolyticus	*L.vannamei*	Malaysia	ND12	POBI01000025.1	AHPND
V.parahaemolyticus	*L.vannamei*	Malaysia	ND11	POBJ01000025.1	AHPND
V.parahaemolyticus	*L.vannamei*	Malaysia	ND16	POBE01000022.1	AHPND
V.parahaemolyticus	*L.vannamei*	Malaysia	ND15	POBB01000005.1	AHPND
V.parahaemolyticus	*L.vannamei*	Malaysia	ND22	POAYO01000025.1	AHPND
V.parahaemolyticus	*L.vannamei*	Malaysia	ND24	POAWO1000025.1	AHPND
V.parahaemolyticus	shrimp-undefined	Vietnam	M1-1	PDDQ01000038.1	AHPND
V.parahaemolyticus	*L.vannamei*	Thailand	NCKU_TV_3HP	JPKS00000000	AHPND
V.parahaemolyticus	*L.vannamei*	Thailand	NCKU_TV_5HP	JPKT00000000.1	AHPND
V.parahaemolyticus	*L.vannamei*	China	NCKU_CV_CHN	JPKU00000000	AHPND
V.parahaemolyticus	*P.japonicus*	China	20140722001-1	CP034292.1	AHPND
V.parahaemolyticus	shrimp-undefined	China	CHN25	CP010884.1	ND
V.parahaemolyticus	shrimp-undefined	China	PB1937	CPO22243	AHPND
V.vulnificus	shrimp-undefined	China	Vv004	QCYB01000004.1	ND
V.xuii	shrimp culture water	China	DSM 17185	LHPK01000016	ND