

# AHPND Challenge Tests on *L. vannamei* in Biofloc System

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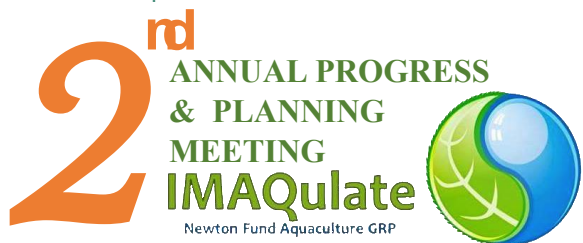
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UNIVERSITY OF  
**STIRLING**



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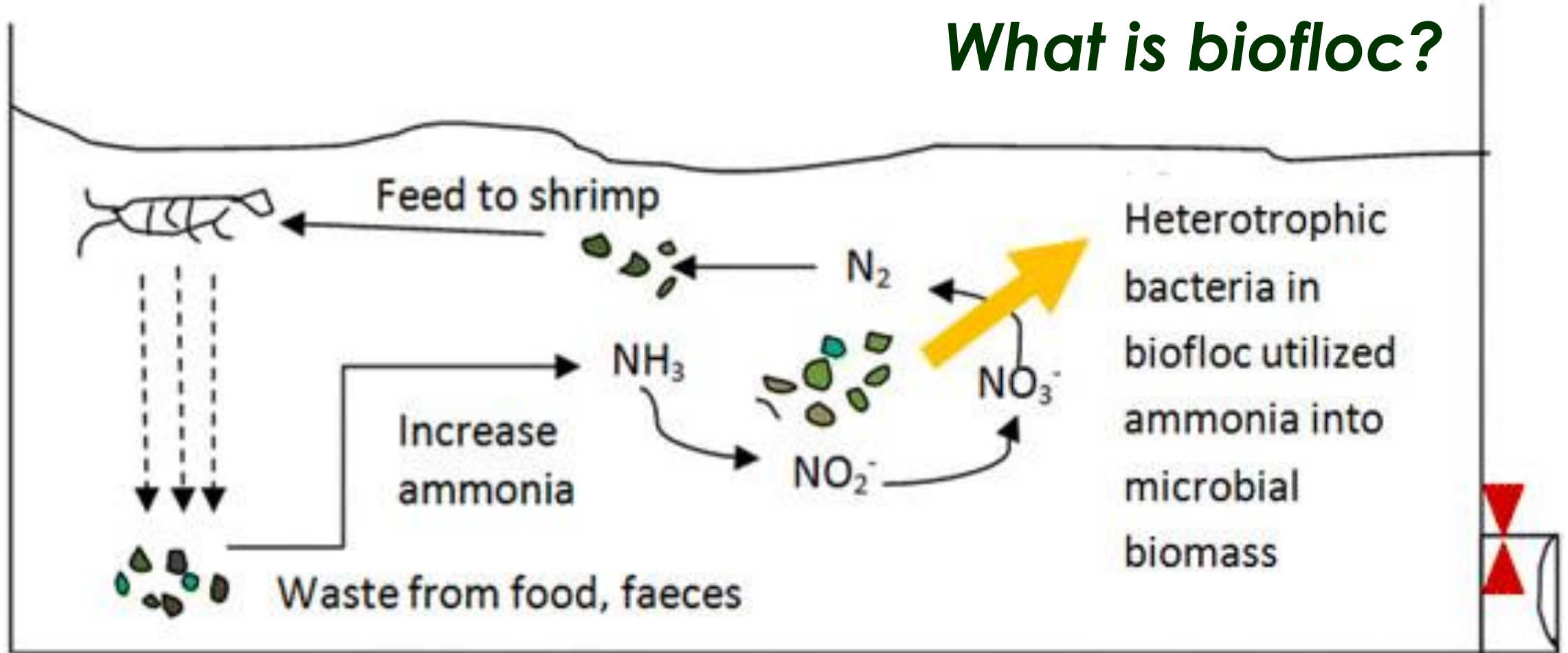
Aquatic disease – major impediment to industry growth.



Disease management – understanding the impacts of the host, the passage of infection within population, its epidemiology widely and the factors associated with infections.

In past 5 months, a studies investigated; **the use of biofloc, including tilapia conditioned water, in reducing magnitude of AHPND losses under controlled laboratory trials.**

# What is biofloc?



Bioremediation process - carried out by microorganisms in the biofloc (bacteria, algae, plankton) to breakdown the hazardous nutrients into the non-toxic substances and can be consumed back as additional protein feed diet for shrimp consumption (Manan, 2016)

# What is AHPND?

**AHPND - acute hepatopancreatic necrosis disease**

Causal agent - ***Vibrio parahaemolyticus*** ( $Vp_{\text{AHPND}}$ ) that carry a plasmid that encodes two Pir-like toxins

OIE listed disease

Early signs - slow growth, a shrunken, pale hepatopancreas, and dead shrimp around the periphery of the pond and result in the entire loss within 30 days (NACA, 2012; Lightner et al., 2012; Zorriehzahra & Banaederakhshan, 2015).



**Gross sign of AHPND, pale hepatopancreas, an empty stomach and guts**

(Lightner, UAZ)



# PROPHYLACTIC HEALTH PROPERTIES OF BIOFLOC AND TILAPIA CONDITIONED WATER IN SHRIMP AQUACULTURE

## Biofloc

Different  
concentrations

## Conditioned greenwater

Different  
salinities

## Biofloc

Different  
stocking density

To investigate the  
potential protective  
effects against  $Vp_{AHPND}$



### Test animal

Healthy *Penaeus vannamei*, ~ 0.4 g



FishVet  
Group

This study was conducted at **Fish Vet Group Asia's Limited (FVGAL)** research aquarium and diagnostic laboratory in Chonburi, **Thailand**.

## Biofloc preparation

400 L tank with 15ppt pre-treated brackish water

Day	1 & 2	3	4 and onwards *
Rice bran (g)	5	-	-
Shrimp feed (g)	1.5	1	1
Sugar (g)	3	3	2.3

\*stocking shrimp once water quality parameters have stabilised

## Greenwater preparation

200 L tank stocked with mixed sex *Oreochromis niloticus* (biomass 960 g tank<sup>-1</sup>; 4.8 kg m<sup>3</sup>) under direct sunlight

Tank	1 (5ppt)	2 ( 10ppt)	3 (15ppt)
Chlorophyll a (mg m <sup>-3</sup> )	1,150	1,917	1,292

Feeding rates: 2% bdy wt d<sup>-1</sup>



**Imhoff cone reading after  
30 mins, > 10 m L<sup>-1</sup>**  
(Hargreaves, 2013)

# CHALLENGE DAY

1 L glass vessels in a temperature controlled room set at 28°C; data loggers set to record every 15 mins

400 mL of test water

Semi-randomised block design

Water samples taken from a random selection of jars in each test group, 10 min after the addition of bacterial for dose confirmations

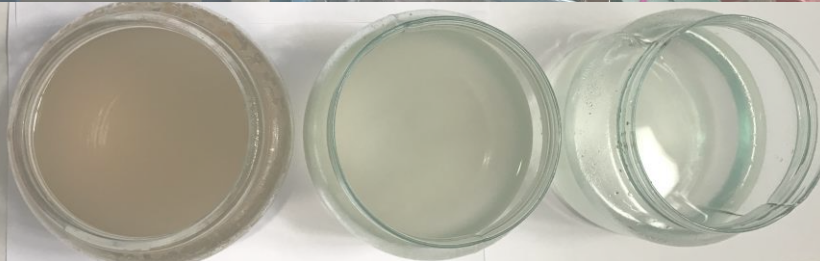
**Morbidity and mortality monitored in each jar every 3 h for 96 h** (until mortality stabilized)



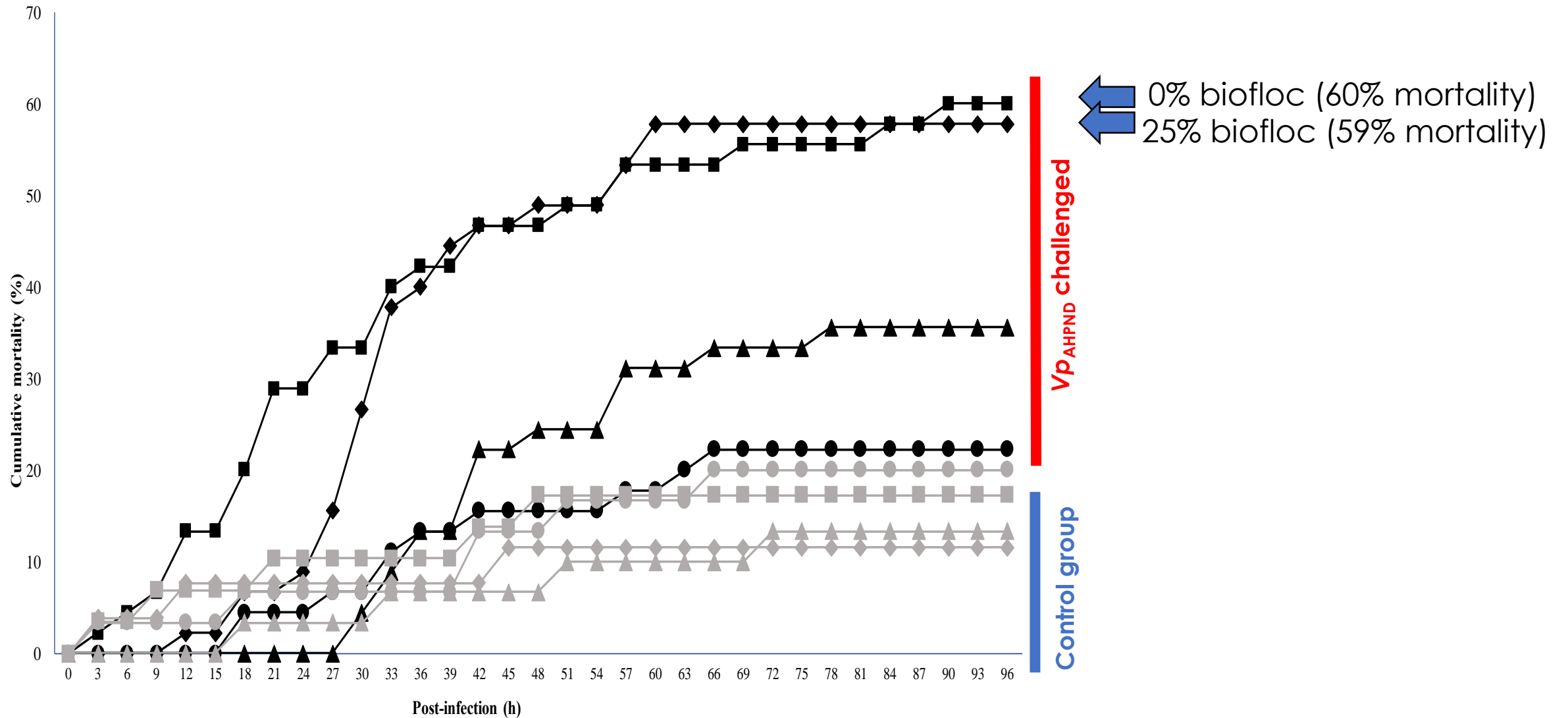




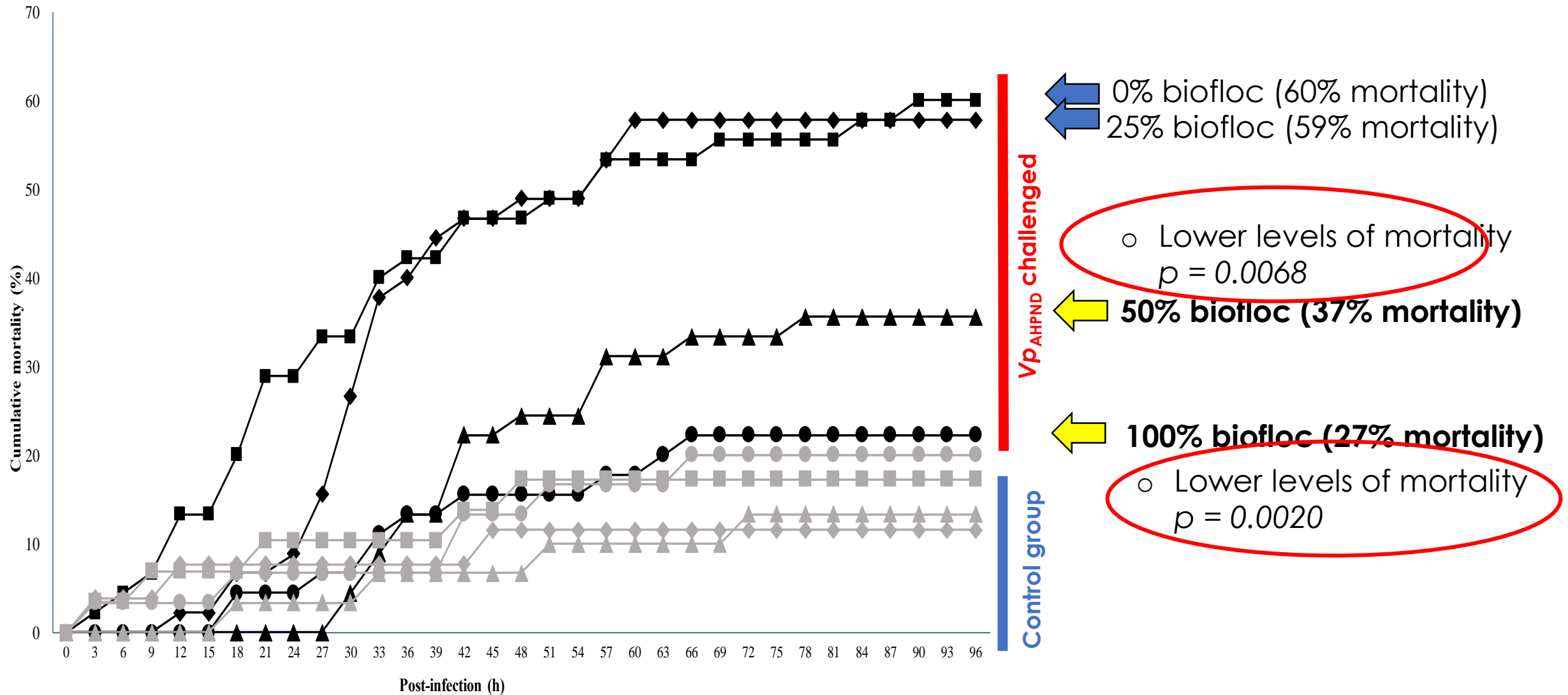
# RESULTS



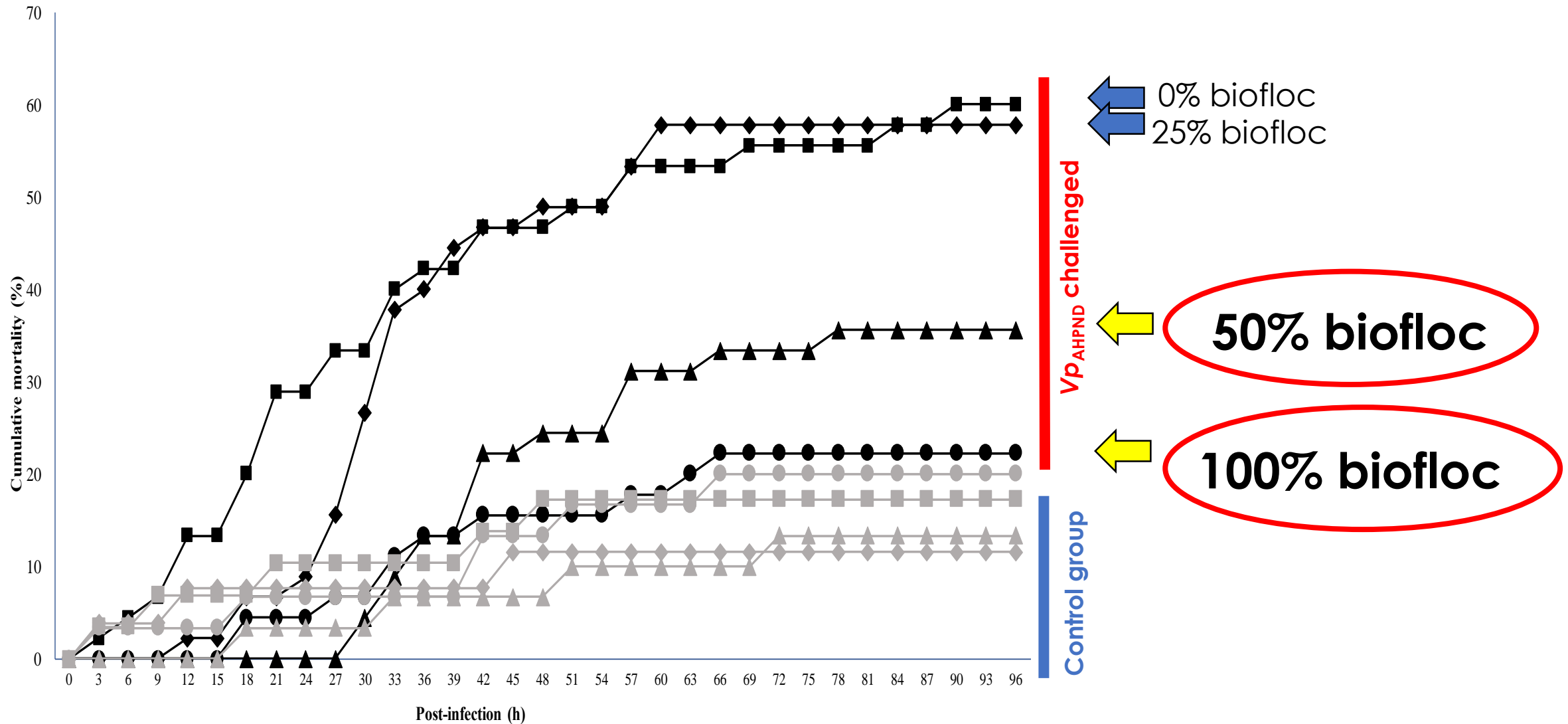
# TRIAL 1 The impact of three different levels of biofloc on challenges with $Vp_{AHPND}$



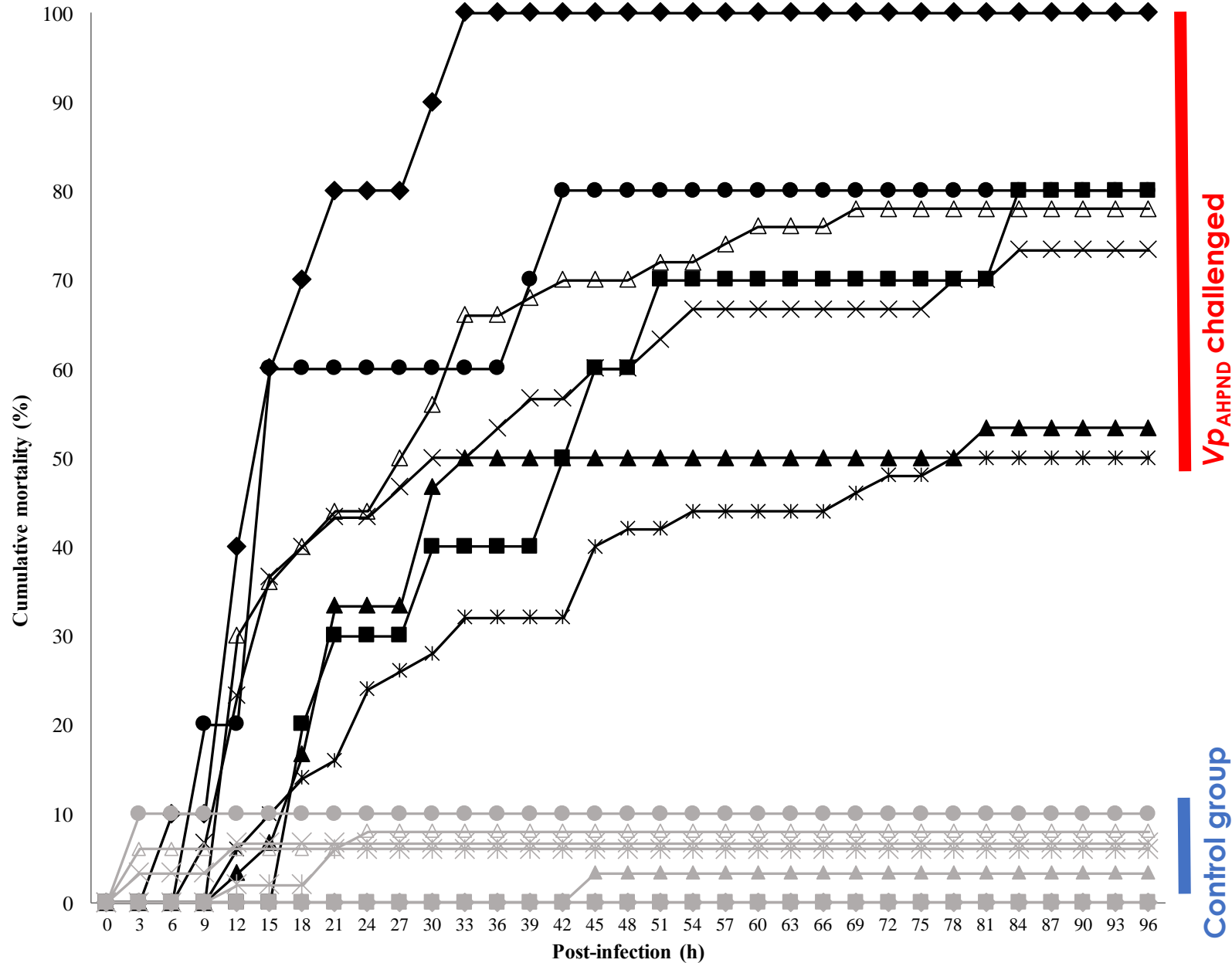
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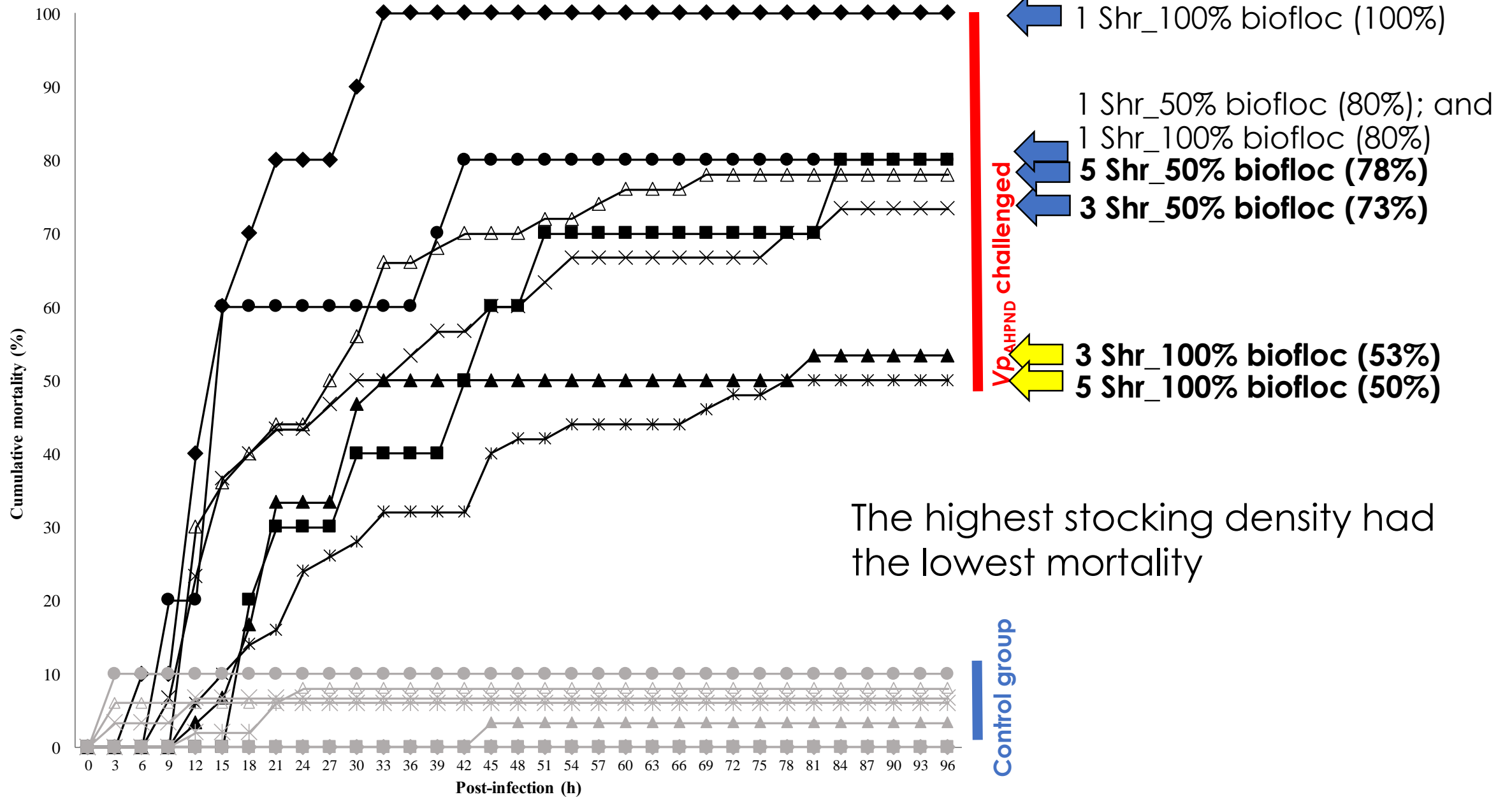


# TRIAL 2 The impact of biofloc and shrimp stocking on challenges with $Vp_{AHPND}$

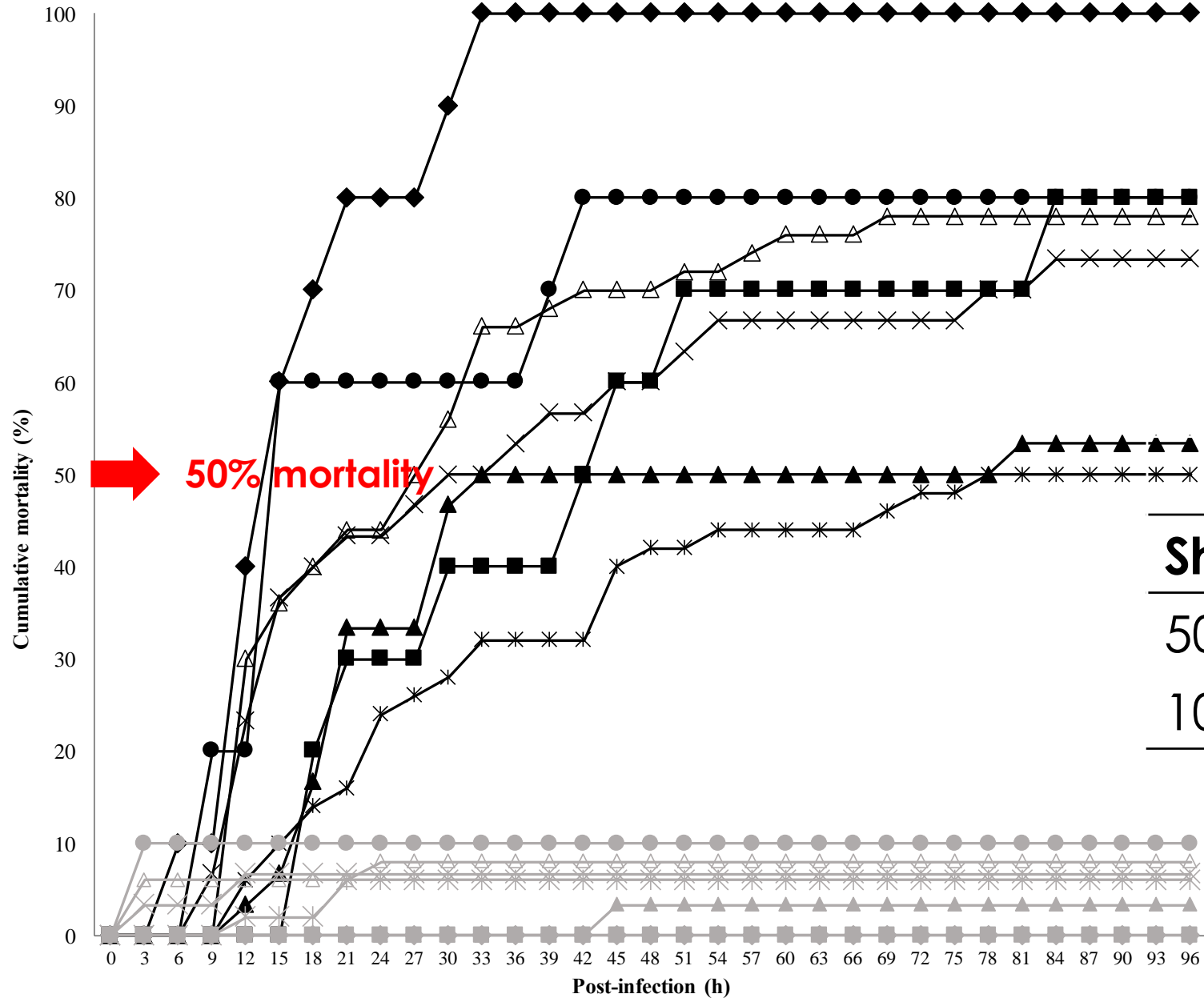


No statistical difference between mortality rates of different stocking densities and biofloc concentrations

# TRIAL 2 The impact of biofloc and shrimp stocking on challenges with $Vp_{AHPND}$



# TRIAL 2 The impact of biofloc and shrimp stocking on challenges with $Vp_{AHPND}$



$Vp_{AHPND}$  challenged

50% population to die

Hours

Shrimp (Shr)

1

3

5

50% biofloc

15

30

27

100% biofloc

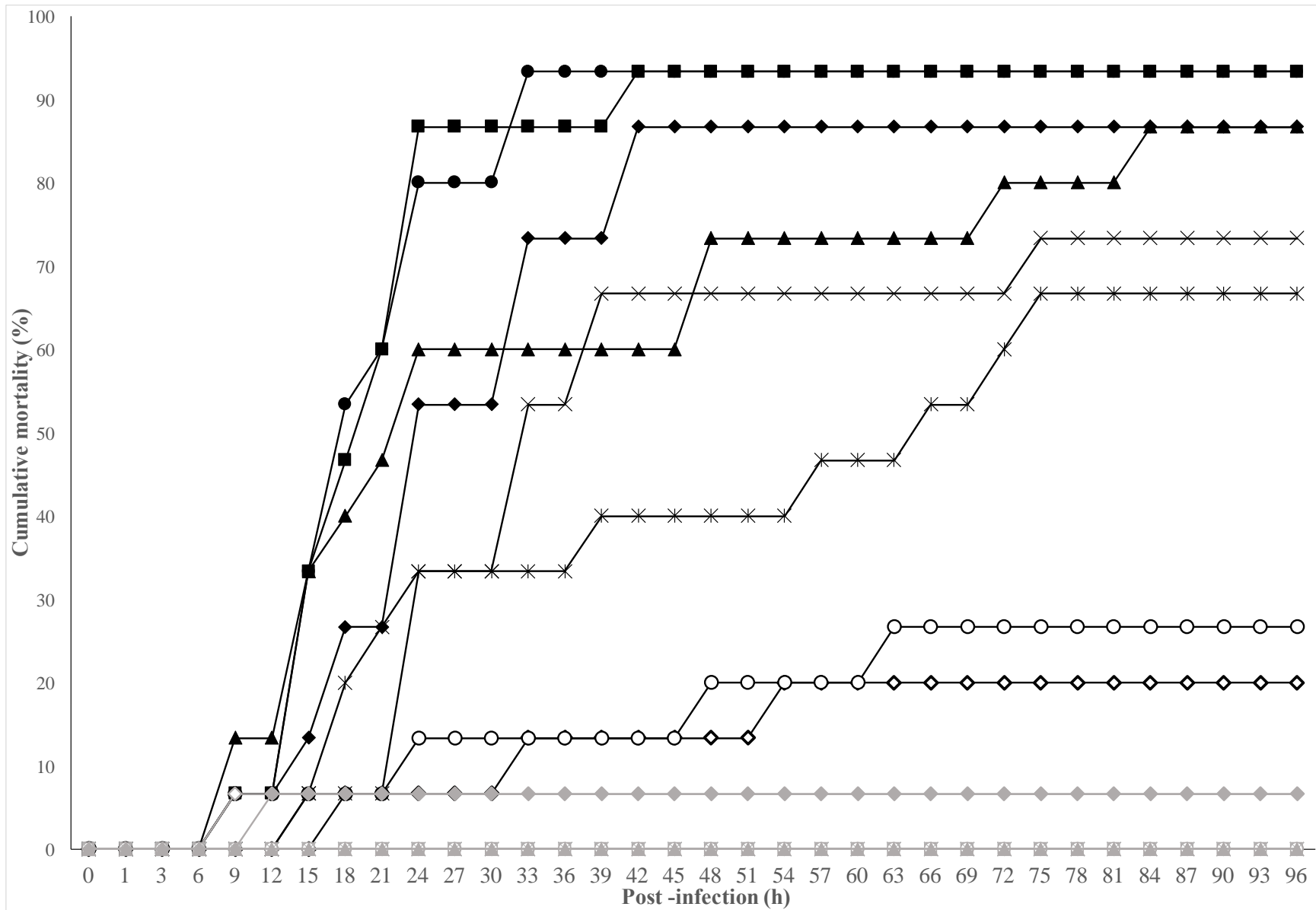
42

33

78

Control group

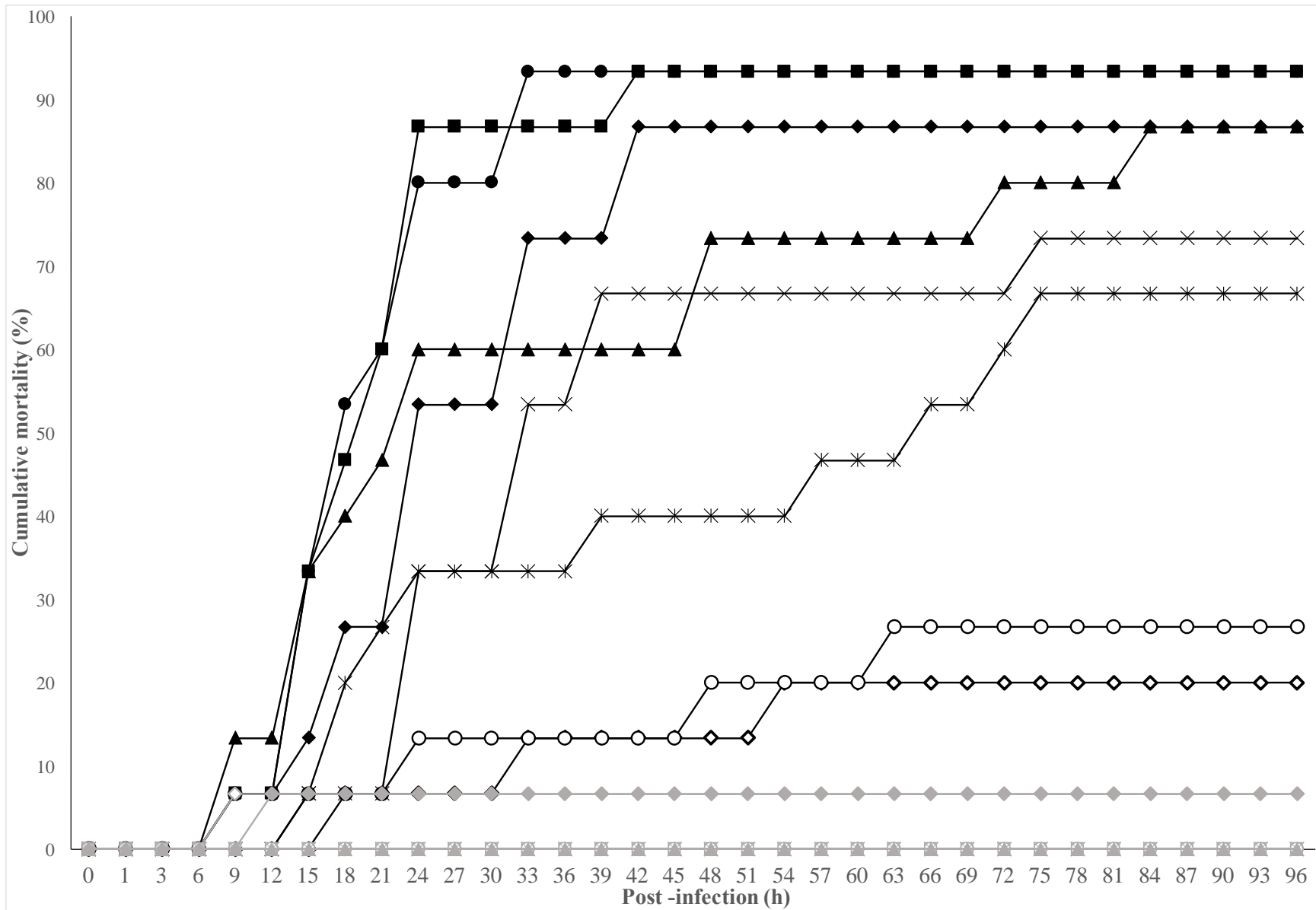
# TRIAL 3 Nile tilapia-conditioned greenwater and *Chaetoceros* on challenges with $Vp_{AHPND}$



- ← 10 ppt & 15ppt clear water (94%)
- ← *Chaetoceros* 1 x 10<sup>5</sup> cell ml<sup>-1</sup> (80%); and 15 ppt greenwater
- ← *Chaetoceros* 1 x 10<sup>5</sup> cell ml<sup>-1</sup> (73%)
- ← 15ppt clear water (67%)
- Vp<sub>AHPND</sub> challenged**
- ← 10ppt greenwater (27%)
- ← 5ppt greenwater (20%)
- Control group**



# TRIAL 3 Nile tilapia-conditioned greenwater and *Chaetoceros* on challenges with $Vp_{AHPND}$



Vp<sub>AHPND</sub> challenged

Control group

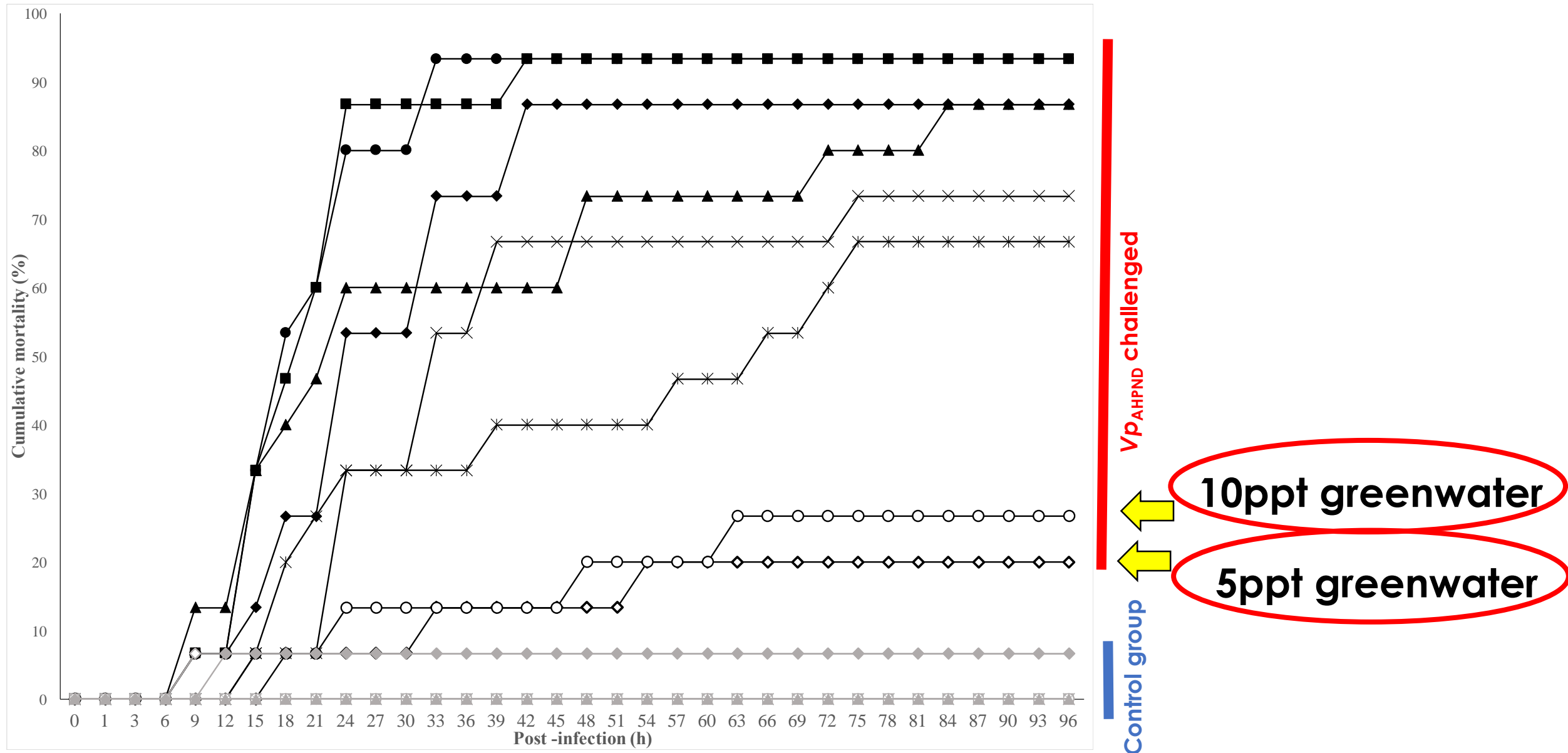
Lower levels of mortality  
 $p = <0.0001$

← 10ppt greenwater

← 5ppt greenwater

Lower levels of mortality  
 $p = <0.0001$

# TRIAL 3 Nile tilapia-conditioned greenwater and *Chaetoceros* on challenges with $Vp_{AHPND}$



# DISCUSSION

- Beneficial effects of biofloc - resistance against pathogens (Crab *et al.*, 2010; Haslun *et al.*, 2012; Moss *et al.*, 2012; Zhao *et al.*, 2012, Dash *et al.*, 2017) **Where is the threshold of protection ?**
- Complexity and the concentration of the bacterial community in the water dictates the magnitude of shrimp mortality in a population.  
**What are the impacts on shrimp health?** *e.g. gill condition, stress*
- Different salinities of tilapia conditioned greenwater and the potential effects it may have on bacterial pathogens such as  $Vp_{APHND}$ . **Anti-Vibrio isolates have been found from tilapia skin mucus and gut production** (Lio-Po *et al.*, 2005; Dash *et al.*, 2017).

# Conclusion

The study concludes that 14-day old **50% biofloc containing  $>0.25 \text{ g L}^{-1}$  dry organic matter** and / or  **$>14 \text{ d Nile tilapia-conditioned greenwater}$**  at **salinities of 5 ppt and 10 ppt** can result in statistically significant lower mortalities of whiteleg shrimp when challenged with a pathogenic isolate of  $Vp_{\text{AHPND}}$ .



Low cost strategies in shrimp industry

# ***Randomised Controlled Trials (RCT)***

Carefull management such as supply of oxygen and water exchange to avoid potentially crashes in biofloc

For greenwater technology - mixing tilapia and shrimp is not a good idea!

A new design for tilapia water to flow into shrimp pond.