



Green water technology for the production of Pacific white shrimp *Penaeus vannamei* (Boone, 1931)

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ABSTRACT

A study was conducted to assess the efficiency of green water culture strategy for production of Pacific white shrimp *Penaeus vannamei* (Boone, 1931) in high density polyethylene (HDPE) lined ponds (300 m²) for a period of 96 days. Green water from a broodstock fish pond was used for rearing shrimp in treatment ponds; whereas, filtered seawater was used for growing shrimp in control ponds, with both treatment and control in triplicates. Shrimp juveniles (specific pathogen free, SPF) of uniform size (1.74±0.46 g) were stocked at the rate of 100 nos. m⁻² per pond. Feeding, water exchange, sampling and water quality analysis were done as per standard shrimp growout procedure. Shrimp health status and microbial load in culture ponds were recorded periodically. Water quality parameters did not show significant variations between control and treatment ponds. Enhanced growth, survival (19.7%) and biomass (23.3%) were observed in treatment ponds when compared to control. Significant reduction in bacterial population and improved shrimp health status were also noticed in treatment ponds. Green water culture technique reduced harmful bacterial population in culture ponds and enhanced shrimp growth, survival, health and production.

Keywords: Green water, Microbial population, *Penaeus vannamei*, Shrimp health

Introduction

Use of green water technology is found to be effective in controlling diseases like early mortality syndrome (EMS) and acute hepatopancreatic necrosis disease (AHPND) in shrimp culture ponds (De Schryver *et al.*, 2014; Zorriezhahra and Banaederakhshan, 2015; Jimenez *et al.*, 2016; Ananda Raja *et al.*, 2017). Green water in tilapia growing pond was found to have high phytoplankton community and was also found effective in controlling luminescent bacteria in culture ponds (De Schryver *et al.*, 2014; Velusamy and Krishnani, 2014). Tilapia produces mucus which contains substances that are inhibitory to a variety of bacteria including vibrios and this is one of the attractive features of green water culture (Zorriezhahra and Banaederakhshan, 2015). Bacteria isolated from green water systems have the ability to nitrify ammonia and nitrate aerobically. Sulphur oxidising bacteria in green water maintain sulphide concentration in a safer level in culture ponds (Velusamy and Krishnani, 2014). Fish metabolic wastes provide nutrients to stimulate phytoplankton (*Nannochloropsis* sp. and *Chlorella* sp.) production (Kokou *et al.*, 2012).

Studies have shown that green water technology helped to decrease white spot syndrome virus (WSSV) load in *Penaeus monodon* culture ponds (Tendencia *et al.*, 2012). Several mechanisms have been linked to the beneficial effect of green water, including the production of antibacterial substances by algae (Kokou *et al.*, 2012) and compounds that inhibit virulence gene (Natrah *et al.*, 2011; 2014). Considering the significance of green water, a study was conducted to test the efficacy of green water from a fish culture pond on the growth, health status and production of Pacific white shrimp, *Penaeus vannamei* (Boone, 1931) an ideal candidate species for coastal aquaculture.

Materials and methods

Design of experiment

The experiment was conducted in 6 high density polyethylene (HDPE) lined ponds (300 m²) at King Abdulaziz University (KAU) Fish Farm, Faculty of Marine Sciences, Obhur, Jeddah for a period of 96 days. Semi-intensive type of culture method was followed for rearing shrimp. Both control and treatment experimental ponds were triplicated. All ponds were sundried and limed

two weeks prior to start of experiment. In control ponds, water preparation was done as per the following procedure. On day 1, culture ponds were filled with filtered seawater (30%) and manured by applying urea (400 g), molasses (1.5 l) and di-ammonium phosphate (200 g) and the dose was repeated on 4th and 8th day of culture. Pond water level was increased to 60% before applying second dose and raised to 90% (*i.e.*, 2 m water column of the pond) before the third dose. Whereas, treatment ponds were filled with green water, *i.e.*, algae rich water from a fish broodstock pond; where tilapia and seabass were grown. For water exchange (10-20%), green water was used for treatment ponds; while seawater from bore well was used in control ponds. After preparation of water, specific pathogen free (SPF) shrimp juveniles of uniform size (1.74 ± 0.46 g) were stocked at the rate of 100 nos. m⁻² in each pond. For pond aeration, two units of aspirator aerator (1 hp) (Force-7, Acquaeo, Italy) were installed at 40 cm below water level with 35 cm angle downwards in each pond.

Water quality analysis

Physical parameters of water such as temperature, dissolved oxygen, salinity and pH were recorded at 06 00 and 15 00 hrs daily; whereas, transparency, ammonia and alkalinity were recorded daily at 10 00 hrs (APHA, 1995). Chemical parameters of water such as nitrite, nitrate, phosphate and calcium were tested fortnightly (Ananda Raja *et al.*, 2014).

Feeding and water exchange

Standard shrimp feed (NAQUA) having 35% protein was supplemented to shrimp based on a feeding schedule in the farm. Shrimp sampling was done every week to assess growth such as average body weight (ABW), average weekly growth (AWG) and survival. Water exchange was done daily at the rate of 10-20% based on algal transparency in the ponds.

Sampling

For growth assessment, shrimp sampling was done fortnightly with a nylon cast net (6.5 m²) at four sites of the pond and individual weight of 100 shrimps sampled randomly from each pond was recorded to estimate ABW and survival. Quantity of feed was re-adjusted according to the weight increment of shrimp.

Growth performance of shrimp

Survival, biomass and shrimp quality after 96 days of culture were compared between control and treatment. Specific growth rate (SGR) was calculated as:

$$\text{SGR (\%)} = \frac{\log_8 W_2 - \log_8 W_1}{T_2 - T_1} \times 100$$

where W1 = Weight of fish (g) at time T1 (Day 1) and W2 = Weight of shrimp at time T2 (Day 96). Feed conversion ratio (FCR) was calculated as:

$$\text{FCR (\%)} = \frac{\text{Feed consumption (kg)}}{\text{Weight gain (kg)}} \times 100$$

Shrimp health analysis

Bacterial load as colony forming unit per ml (cfu ml⁻¹) in terms of total bacteria count (TBC), yellow *Vibrio* count (YVC) that forms yellow colonies on thiosulphate citrate bile salts sucrose (TCBS) agar, green *Vibrio* count (GVC) of vibrios that form green colonies on TCBS agar and luminescent bacteria count (LBC) of luminescent bacterial colonies, in the treatment and control ponds were assessed biweekly (Biswas *et al.*, 2012). Shrimp health was assessed every month. For physical evaluation, 50 shrimps were collected randomly from each control and treatment ponds and brought to the laboratory (Ananda Raja *et al.*, 2012). Shrimps were subjected to check abnormalities on body colour, shell necrosis, gut, behaviour, hepatopancreas and body deformities. Wet mount test for gills and hepatopancreas was done to identify gill melanisation/fouling and lipid vacuolisation in hepatopancreas (HP) and gregarine infestation. Health status was graded as normal (G0), low (G1), moderate (G2), high (G3) and severe (G4) and the values were expressed in per cent (Naqua, 2010).

Statistical analysis

t-test was employed to find out the statistical difference of water quality parameters and final mean weight of shrimp between control and treatment (Snedecor and Cochran, 1989).

Results

Water quality parameters

Overall data on physical and chemical water quality parameters recorded during the culture period is depicted in Table 1. The parameters were found to be within the range suitable for shrimp growth and there was no significant difference ($p > 0.05$) in mean values between control and treatment ponds.

Shrimp health analysis

Details on physical quality and wet mount test done are presented in Table 2. Shrimp behaviour, body, tail, gut and gill colour, hepatopancreas size and melanisation were found to be normal during the period of culture in both control and treatment. Hard shell percentage and gut fullness were high in treatment ponds when compared to control. Gill melanisation, fouling and hepatopancreas melanisation were found to be normal in both control and

Table 1. Water quality parameters recorded during culture period

Parameters	Control (Mean)	Treatment (Mean)	p(T<=t) two-tail @ alpha 0.05
Temperature (°C)	23.231	23.274	0.743
Dissolved oxygen (mg l ⁻¹)	4.177	4.498	0.001
pH	7.762	7.805	0.274
Salinity (g l ⁻¹)	42.650	42.250	0.214
Alkalinity ((mg l ⁻¹)	135.573	125.832	0.006
Nitrate (mg l ⁻¹)	5.091	4.818	0.082
Nitrite (mg l ⁻¹)	0.482	0.261	0.008
Orthophosphate (mg l ⁻¹)	0.126	0.025	0.117
Ammonia (mg l ⁻¹)	0.011	0.006	0.016
Calcium (mg l ⁻¹)	385.182	414.000	0.421

Table 2. Details on shrimp health (physical and wet mount test) status

Parameters	Status	Control (Mean ±SD)	Treatment (Mean ±SD)
<i>Physical test</i>			
Behaviour	Normal	100±0.0	100±0.0
Body colour	Normal	98±4.5	100±0.0
	Reddish	0.0	0.0
Melanisation	Normal	100±0.0	100±0.0
Shell	Hard	78.8±11.5	81.8±17.3
	Soft	14.2±2.5	7.4±3.2
	Loose	7.2±4.4	9±4.2
Gut fullness	Full	66±9.0	77.2±14.1
	Medium	33.4±10.0	22.2±11.2
	Empty	0.6±0.1	0.6±0.1
Gut colour	Brown	86.8±10.8	94±8.9
	Black	13.2±7.8	6±2.0
Gill colour	Normal	100±0.0	100±0.0
Tail colour	Normal	93.4±6.5	94±8.9
	White	0.6±0.3	0.6±0.1
	Red	6.0±1.0	5.4±1.3
Hepatopancreas size	Normal	100±0.0	100±0.0
Deformities	None	99.4±1.3	100±0.0
	Bent rostrum	0.6±0.3	0.0
<i>Wet mount test</i>			
Gill melanisation	G0	100±0.0	100.0±0.0
Gill fouling	G0	100±0.0	100.0±0.0
Hepatopancreas tubular constriction	G0	76.0±13.6	83.3±10.0
	G1	18.0±9.6	10.7±4.3
	G2	6.0±1.5	6.0±1.9
Hepatopancreas melanisation	G0	100.0±0.0	100.0±0.0
Hepatopancreas lipid vacuolisation	High	58.0±15.0	70.0±11.1
Gregarine infestation	Medium	41.3±16.4	29.3±5.2
	Low	0.7±0.5	0.7±0.7
	G0	100.0±0.0	100.0±0.0

G0: Normal, G1: Low, G2: moderate, G3: high, G4: Severe

treatment shrimps. However, tubular constriction and lipid vacuolisation in hepatopancreas were found to be high in treatment shrimps; which indicated that treatment shrimps were healthier than control.

Bacterial load

Data on bacteriology of water sampled from fish broodstock pond, control and treatment ponds during the culture period is presented in Table 3. Microbial load was found to be low in the green water (fish pond) throughout the culture period. In shrimp culture ponds, total bacteria count (TBC), yellow *Vibrio* count (YVC), green *Vibrio* count (GVC) and luminescent bacteria count (LBC) were high in control when compared to fish pond and treatment pond water showed the lowest bacterial load.

Table 3. Microbial load of pond water

Days	Ponds	TBC (cfu ml ⁻¹) (Mean±SD)	YVC (cfu ml ⁻¹) (Mean±SD)	GVC (cfu ml ⁻¹) (Mean±SD)	LBC (cfu ml ⁻¹) (Mean±SD)
18	Green water	130,120±6,000	700±100	10±0	100±10
33	(Fish pond)	79,543±9,100	960±120	10±0	10±0
47		133,553±2,000	600±100	50±10	100±10
62		110,000±8,000	330±150	77±20	10±0
82		100,000±2,000	467±120	10±0	10±0
Culture ponds					
18	Control	730,000±35,000	7,367±5,08	100±25	1,000±100
	Treatment	513,333±32,000	1,200±700	10±0	200±10
33	Control	159,000±10,000	3,467±218	320 ±40	100±10
	Treatment	129,333±20,500	1,767±420	100±10	10±0
47	Control	630,000±20,000	12,000±900	833±90	3,000±200
	Treatment	233,333±22,000	1,700±200	250±20	2,000±400
62	Control	720,000±40,000	5,967±890	763±20	100±10
	Treatment	510,000±28,000	3,833±450	367±20	10±0
82	Control	283,333±12,300	1,567±200	100±10	100±10
	Treatment	105,000±5,000	1,467±190	10±0	10±0

TBC: Total bacteria count, YVC: Yellow *Vibrio* count, GVC: Green *Vibrio* count. LBC: Luminescent bacteria count

Table 4. Growth and production of shrimp

Parameters	Control (Mean ±SD)	Treatment (Mean ±SD)
Initial weight (g)	1.01±0.01	1.01±0.01
Final weight (g)*	14.1±1.5	14.52±0.9
Weight gain (g)	13.0±0.63	13.5±0.22
AWG (g)	0.95±0.05	0.99±0.02
SGR (%)	1.14±0.01	1.15±0.04
Survival (%)	56.2±5.60	67.3±4.03
Biomass (kg)	237.7±9.5	293.3±23.2
TFC (kg)	402±16.5	446±19.5
FCR	1.69±0.09	1.52±0.12

*p<0.05

AWG: Average weekly growth, SGR: Specific growth rate, TFC: Total feed consumption, FCR: Feed conversion ratio

Shrimp growth performance and production

Growth performance and production of shrimp during the culture period is presented in Table 4 and Fig.1. Shrimp growth (ABW) was found to be improved in treatment ponds and differed significantly (p<0.05) from control (Table 5). Considerable difference in average weekly growth (AWG) rate and SGR (%) were not observed between control and treatment. Enhanced survival (19.7%) and biomass (23.3%) were observed in treatment ponds when compared to control; which might be due to influence of green water used for culture. High biomass recorded in treatment ponds were due to the enhanced survival of shrimp. FCR was found to be lowered in treatment ponds from that of the control.

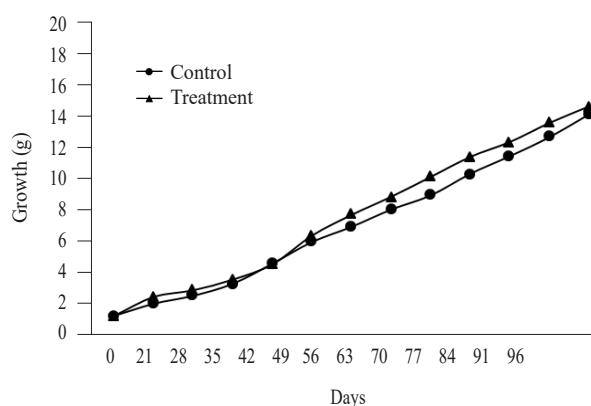


Fig. 1. Growth performance of shrimp during culture period

Table 5. t-Test analysis for shrimp growth comparison between control and treatment

Parameters	Control	Treatment
Mean	14.1	14.52
Variance	2.297627	0.947988
Observations	100	100
Pearson Correlation	0.086986	
Hypothesised mean difference	0	
df	99	
t Stat	-2.18413	
p(T<=t) one-tail	0.015657	
t Critical one-tail	1.660391	
p(T<=t) two-tail	0.031314	
t Critical two-tail	1.984217	

Discussion

Results of the experiment show that use of green water (fish pond water) helps to improve growth, survival and health of Pacific white shrimp *P. vannamei*. Studies have revealed that green water technology has lowered the incidence of EMS/AHPND in aquaculture (De Schryver *et al.*, 2014). According to Tendencia *et al.* (2015) green water systems are characterised by a mature microalgal community and found to decrease *Vibrio* levels in *Penaeus monodon* culture ponds. A study reports that when green water-shrimp growout method was applied, outbreak of luminous vibriosis could be prevented in tiger shrimp culture ponds (Rosa, 2004; Corre *et al.*, 2005; Jimenez *et al.*, 2016). The mechanism behind the beneficial effect of green water includes the algal production of antibacterial substances as well as production of compounds that inhibit virulence gene regulation for e.g., quorum sensing inhibitors (Natrah *et al.*, 2011; Kokou *et al.*, 2012). Therefore, it is evident that the green water had the ability to control harmful *Vibrio* population in the pond and this can be attributed as a reason for the enhanced survival rate recorded in treatment ponds.

Shrimp health analysis showed major difference in gut fullness as well as lipid vacuolisation in hepatopancreas between control and treatment shrimps. Since high lipid vacuolisation is considered as an index of shrimp health and immunity, it could be assumed that shrimp grown in treatment ponds (green water) acquired better immune power to resist infection of harmful bacteria in the pond (Lio-Po *et al.*, 2005; Tendencia *et al.*, 2015). Microbial load of pond water showed reduction of total bacteria population particularly disease causing yellow, green and luminescent *Vibrio* colonies. Similar reduction in bacterial population was also reported in tiger shrimp culture ponds when green water technology was applied (Tendencia and Verreth, 2011). The reduction of bacteria population noticed in treatment pond water could be attributed to

the effect of green water which contains antibacterial substances from fish mucus as suggested by Zorriehzahra and Banaederakhshan (2015).

Green water from tilapia (*Oreochromis mossambicus*) broodstock ponds inhibited the proliferation of luminous *Vibrio* colonies (Huervana *et al.*, 2006). Lio-Po *et al.* (2005) endorsed the effectiveness of green water system to the presence of anti-luminous *Vibrio* factors in bacterial, fungal and skin mucus of tilapia. The ability of skin mucus and faeces collected from a red hybrid tilapia culture pond and of bacteria isolated from fish mucus and faeces to reduce the growth of luminous *Vibrio harveyi* was also reported by Tendencia and Dela Pena (2010). Results of the present study, showed lower concentration of harmful *Vibrio* population and high amount of green algae such as *Chlorella* and *Nitzschia* in the green water pond when compared to shrimp culture ponds. The high *Vibrio* load in the control ponds may be due to the lack of fish mucus and green algae, *Chlorella*; as it received filtered seawater from a nearby bore well. Therefore it is suggested that the reduction of bacteria (yellow, green and luminous *Vibrio*) population observed in treatment ponds could be due to the effect of antimicrobial compounds present in the fish mucus and green algae in the green water of fish pond.

The higher *Chlorella* levels observed in green water ponds is in accordance with the observation of Cremen *et al.* (2007). *Chlorella* has been reported to improve pond water quality (Hernandez *et al.*, 2009). Apart from its effect on water quality, the abundance of *Chlorella* in green water ponds might have helped to improve the shrimp's immune competence to resist disease thus resulting in higher survival (Ponprateep *et al.*, 2011; Tendencia *et al.*, 2012). It has been reported that hydrolysed *Chlorella vulgaris* promotes the release of interferons (IFN) in mice (Kim *et al.*, 2010). Furthermore, IFNs have protective immuno-modulatory function against pneumonia virus in mice leading to high survival (Rigaux *et al.*, 2012). Higher *Chlorella* observed in the green water ponds suggested that the use of the green water technique to culture *P. vannamei* could improve water quality of culture ponds.

In this study, the general pattern of the ammonia, nitrite and nitrate levels in pond water was similar. However, the ammonia, nitrite and nitrate concentrations in the treatment ponds were lower during most part of the experimental period (Ananda Raja *et al.*, 2014) and this could be correlated to the denitrification of nitrogenous compounds due to the presence of heterotrophic bacteria in the green water. During the nitrification process, hydrogen ions are produced and released into the water column (Henriksen and Kemp, 1988), which is likely to cause the reduction of pH in the rearing environment. In this study also, similar trend was observed. The pH in the treatment

ponds decreased with increase in rearing time. Wasielesky *et al.* (2006) suggested that the increase in turbidity of the water column could be the result of increase in levels of suspended particles and the addition of large amounts of artificial feed in the rearing environment. In this study, the water transparency in the treatment ponds was higher than the control group in the beginning. This is likely to be due to the presence of green algae in the green water used for culture. Physical water quality parameters such as dissolved oxygen, temperature, salinity, alkalinity as well as nutrients such as phosphate and calcium in culture ponds were not influenced by the use of green water.

Results of the present study clearly indicate that use of green water discharged from fish ponds helps improve water quality, shrimp growth, health and survival of *P. vannamei*. The data provides base line information for the use of green water culture strategy for culture of *P. vannamei* in semi arid lands.

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