INTEGRATED BIOFLOC SYSTEM WITH SHRIMP (*Litopenaeus vannamei*) AND SEAWEED (*Gracilaria*)

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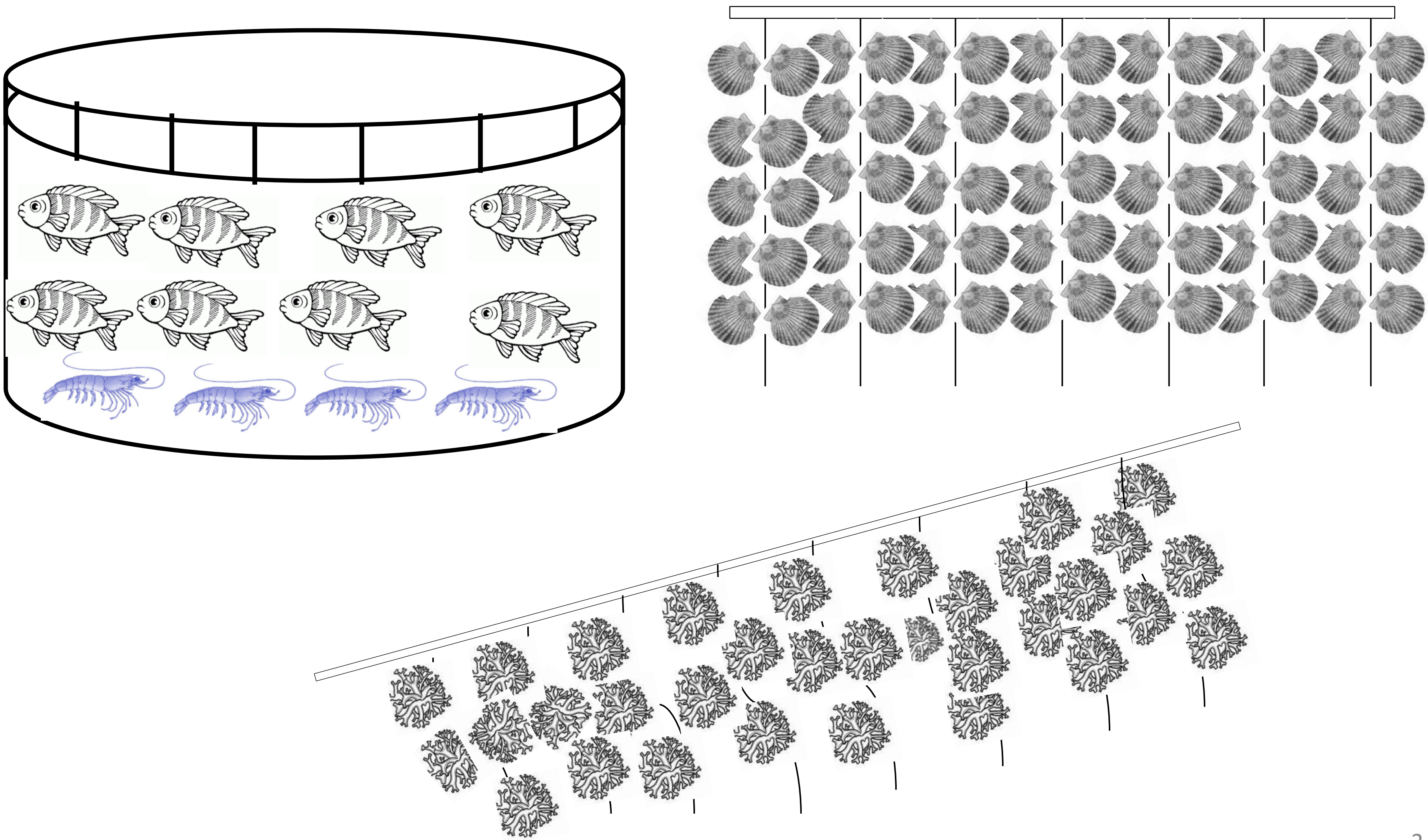
Integrated Multi-Trophic Aquaculture (IMTA)



Some reasons for using Integrated Multi-Trophic Aquaculture - IMTA

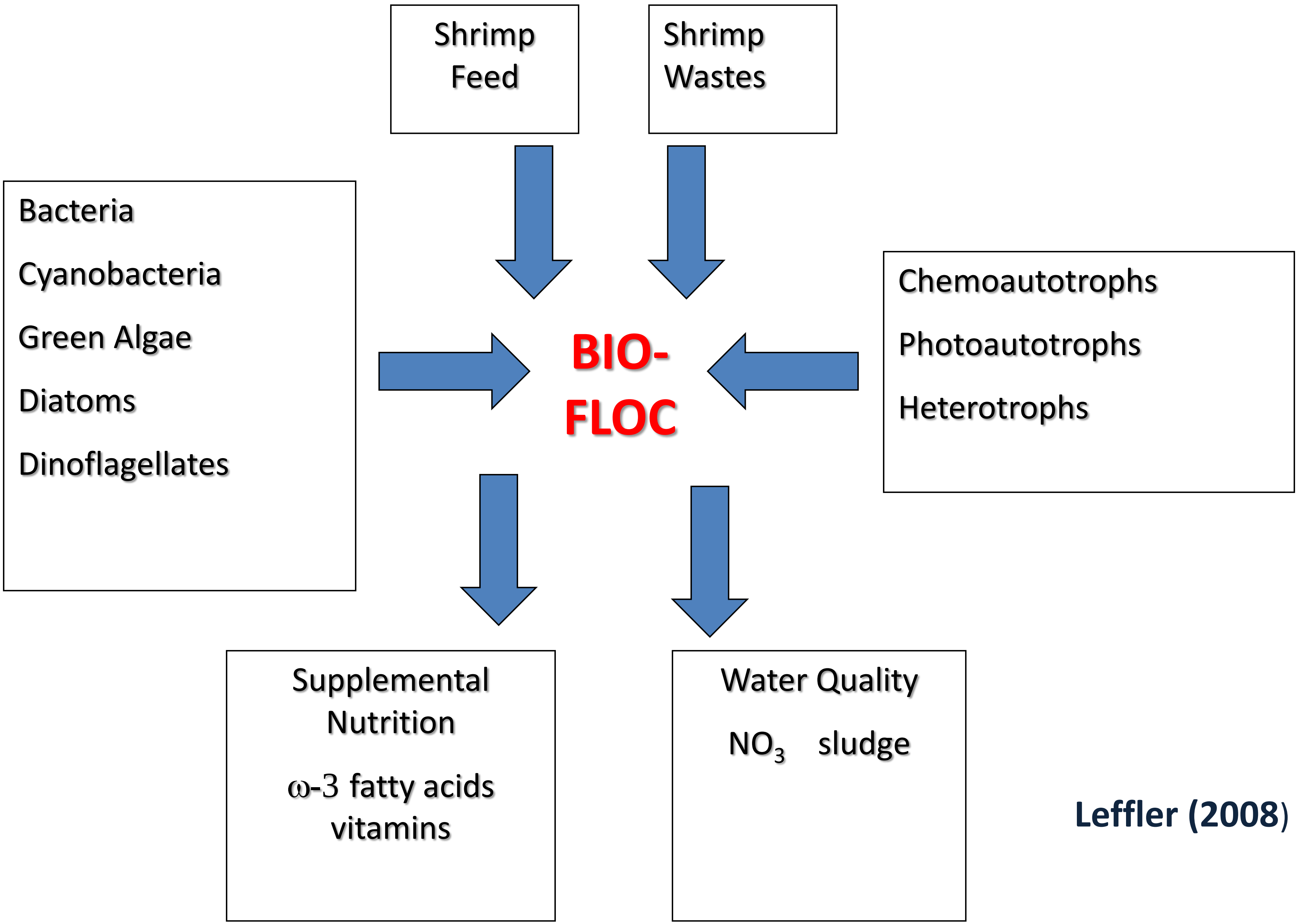
- Disease prevention in marine shrimps.

  Vibrio spp (Fu et al. 2007; Huynh et al. 2011; Kanjana et al. 2011; Selvin et al. 2011)

  WSSV (Lin et al. 2011; Immanuel et al. 2012).

- Reduction of nitrogenated and fosfated compounds

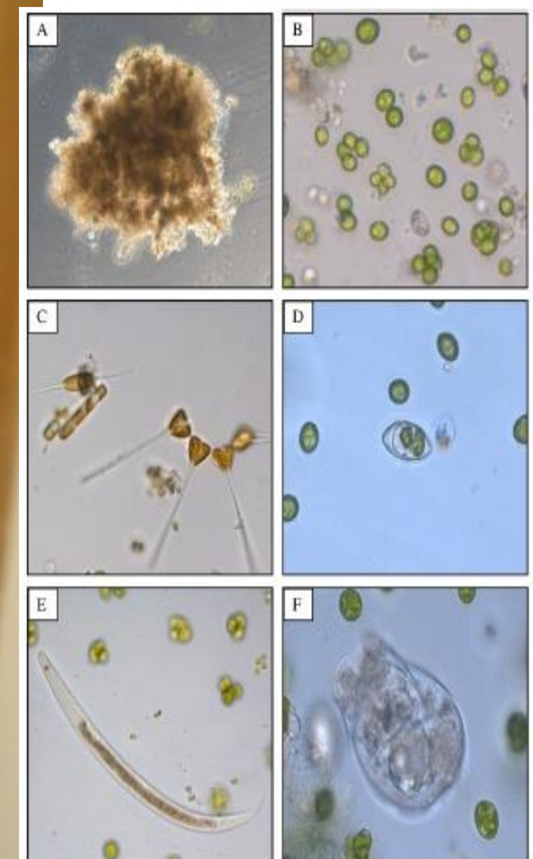
- Enviromental sustainability.


Shrimp Feed
Shrimp Wastes
Bacteria
Cyanobacteria
Green Algae
Diatoms
Dinoflagellates
BIO-FLOC
Chemoautotrophs
Photoautotrophs
Heterotrophs
Supplemental Nutrition
ω-3 fatty acids
vitamins
Water Quality
NO_3 sludge
Leffler (2008)

Reduction of nitrogenated compounds and assimilation of microbial protein by shrimp or fish cultured (Avnimelech, 2009; Crab et al., 2012)

Balance C:N

Microbial community



Intensive system with zero or minimal water exchange presents:

- Large amounts of nitrogen (dissolved organic) and phosphorus (dissolved inorganic and organic phosphate) (Silva *et al*. 2013a).

- Large amounts of total suspended solids, volatile suspended solids (Vinatea et al. 2010)

- Biochemical and chemical oxygen demand (Samocha et al., 2007)

- Large amounts of sludge (Coyle et al. 2011),

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Gracilaria sp

- Tropical weather Rodophyta

- Agar agar
- Carragenan
- β-glicans
- Sulphated Polysaccharides

Objective

Evaluate the integrated biofloc system with *Litopenaeus vannamei* and *Gracilaria*, in relation to water quality and shrimp growth.

FIRST EXPERIMENT

Use the two *Gracilaria* species in integrated biofloc system with *Litopenaeus vannamei*

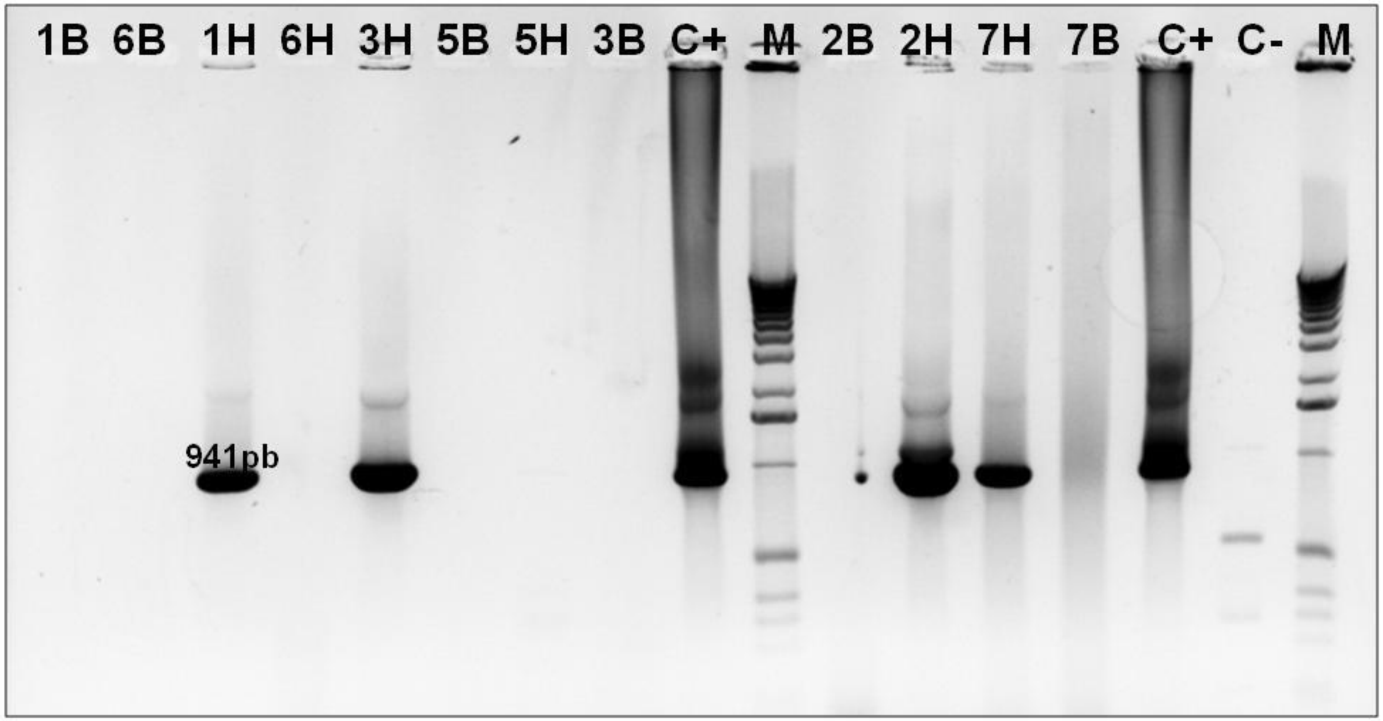
SECOND EXPERIMENT

Use the different *Gracilaria* biomass in integrated biofloc system with *Litopenaeus vannamei*

Methods

Sustainable Mariculture Laboratory (LAMARSU) , Pernambuco Federal Rural University (UFRPE), Recife, Brazil.

- Phytoplankton and Cyanobactéria (Pereira-Neto et al. 2008)
- WSSV – Shrimp (hemolinfa e brânquias) (Lo et al., 1996).



Gel de agarose a 0,9% corado com brometo de etídio. Amostras de camarões 1H, 3H, 2H e 7H são positivas para WSSV; C+, controle positivo; C-, controle negativo (água ultra-pura) e M, marcador de peso molecular de 1kb (Invitrogen,USA).

First experiment 28 days and second experiment 42 days

Preparação do biofloco: 30 dias antes

Fontes de Carbono (melaço);

Nitrogênio (ração 35% P.B);

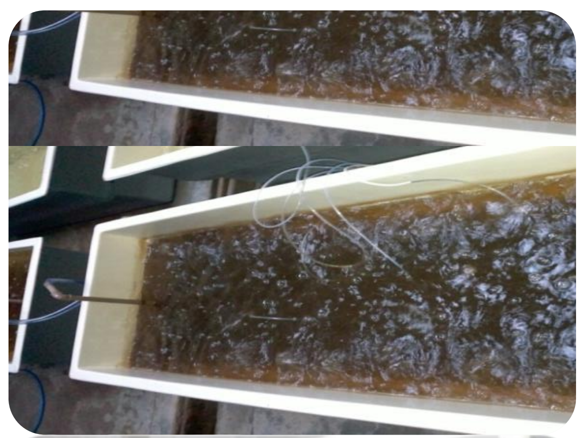
Relação 12:1

(Avnimelech, 2009)

Juvenis *L. vannamei*:

(2.63 g)

425 /m3



Trat.		**Seaweed biomass ($Kg\ m^{-3}$)**
Control	Shrimp monoculture	-
SB	Integrated biofloc system (Shrimp and *G. birdiae*	2
SD	Integrated biofloc system (Shrimp and *G. Domingensis*	2

Trat.		**Seaweed biomass ($Kg\ m^{-3}$)**
Control	Shrimp monoculture	-
IBS 2.5	Integrated biofloc system (Shrimp and *G. birdiae*	2.5
IBS 5.0	Integrated biofloc system (Shrimp and *G. birdiae*	5.0
IBS 7.5	Integrated biofloc system (Shrimp and *G. birdiae*	7.5

Water quality parameters

- Temperature
- Salinity
- pH
- Dissolved oxygen
- Total ammonia
- Nitrite – nitrogen
- Nitrate - nitrogen
- Ortophosphate
- **Settleable solids**
- Alkalinity
- **Total suspended solids**

Zootechnical parameters

- **Final weigth** .
- SGR
- Survival
- **FCR**

Statistic

- Cochran & Shapiro-Wilk Test.
- ANOVA bi factorial.
- **Kruskal-Wallis (alkalinity)**
- Student Test ($p<0,05$)
- Tukey Test ($p<0,05$)

Results

First experiment

Control

SB

SD

Table 1 Water quality parameters of Pacific white shrimp *Litopenaeus vannamei* in an integrated biofloc system with red seaweed *Gracilaria* with the presence of WSSV during the 28-days experiment period

Parameters	Treatments[1]		
	Control	SB	SD
Dissolved oxygen (mg L^{-1})	6.37 ± 0.26^a	6.15 ± 0.30^a	6.20 ± 0.05^a
Temperature (°C)	25.04 ± 0.46^a	25.05 ± 0.47^a	24.78 ± 0.86^a
Salinity (ppt)	36.17 ± 2.47^a	35.61 ± 1.50^a	36.22 ± 0.84^a
Alkalinity (mg $CaCO_3$ L^{-1})	88.26 ± 12.06^a	91.30 ± 3.92^a	82.26 ± 12.60^a
pH	7.58 ± 0.13^a	7.75 ± 0.22^a	7.56 ± 0.08^a
TSS (mg L^{-1})	283.67 ± 25.91^a	275.21 ± 47.60^a	251.59 ± 41.79^a
SS (mg L^{-1})	9.5 ± 0.5^b	12.0 ± 2.0^{ab}	14.5 ± 0.5^a
PO_4^3-P (mg L^{-1})	2.42 ± 0.08^a	2.55 ± 0.14^a	2.53 ± 0.11^a
TAN (mg L^{-1})	0.10 ± 0.03^a	0.17 ± 0.05^a	0.14 ± 0.09^a
NO_2-N (mg L^{-1})	0.53 ± 0.01^a	0.61 ± 0.04^a	0.50 ± 0.08^a
NO_3-N (mg L^{-1})	2.25 ± 0.22^a	1.64 ± 0.31^a	2.25 ± 0.68^a

Results from repeated ANOVA measures and Tukey's test. Mean values in the same row with different superscripts differ significantly ($P < 0.05$)

Control (shrimp monoculture in biofloc); SB (shrimp and *Gracilaria birdiae* in an integrated biofloc system) and SD (Shrimp and *Gracilaria domingensis* in an integrated biofloc system)

[1] The data correspond to the mean ± standard deviation

TSS total suspended solids, *SS* settleable solids, *PO_4^3-P* orthophosphate, *TAN* total ammonia nitrogen, *NO_2-N* nitrite-nitrogen, *NO_3-N* nitrate-nitrogen

Table 2 Mean phytoplankton density (cells mL^{-1}) of Pacific white shrimp *Litopenaeus vannamei* in an integrated biofloc system with red seaweed *Gracilaria* with the presence of WSSV presence during the 28-day experiment period

Groups	Genera	Initial	Control	SB	SD
Cyanobacteria	*Anabaena*	339.34	216.74	185.64	245.41
	Anabaenopsis	0	11.54	0.00	0.00
	Aphanizomenon	0	0.00	0.00	18.75
	Aphanocapsa	256.62	12,088.62	3,163.19	569.12
	Dactylococcopsis	46.94	12.60	14.33	463.32
	Merismopedia	0	0.00	79.35	14.43
	Microcystis	1,058.02	834.39	877.67	531.41
	Oscillatoria	12,234.53	18,405.90	17,976.68	10,498.19
	Plectonema	99.26	54.97	85.23	8,139.43
	Pseudanabaena	320.1	220.69	234.83	165.29
	Raphidiopsis	0	4,210.92	2,123.92	1,284.63
	Schizothrix	3,961.22	5,337.60	5,268.93	4,956.72
	Synechocystis	448.21	136.29	123.02	2,841.74
	Synechococcus	0	0.00	0.00	16.45
	Spirulina	191.6	47.90	47.90	47.90
	Cells mL^{-1}	18,955.84	41,578.17	30,180.69	29,792.77
	%	81.36	92.53[a]	76.71[b]	76.96[b]

Table 3 Performance parameters of Pacific white shrimp *Litopenaeus vannamei* in an integrated biofloc system with red seaweed *Gracilaria* with the presence of WSSV during the 28-day experiment period

Parameters	Treatments[1]		
	Control	SB	SD
Final weight (g)	5.42 ± 0.19^{c}	6.57 ± 0.02^{a}	5.75 ± 0.08^{b}
Weight gain (g)	2.74 ± 0.12^{c}	3.92 ± 0.29^{a}	3.22 ± 0.07^{b}
Yield (kg m^{-3})	1.30 ± 0.25^{a}	1.40 ± 0.09^{a}	1.37 ± 0.38^{a}
Survival (%)	56 ± 9^{a}	50 ± 3^{a}	56 ± 15^{a}
FCR	2.89 ± 0.53^{a}	2.06 ± 0.19^{a}	2.26 ± 0.45^{a}
Growth $week^{-1}$	0.69 ± 0.03^{c}	0.98 ± 0.07^{a}	0.81 ± 0.02^{b}
SGR (% day^{-1})	1.56 ± 0.07^{c}	2.12 ± 0.12^{a}	1.81 ± 0.03^{b}

Results from one-way ANOVA and Tukey's test. Mean values in the same row with different superscripts differ significantly ($P < 0.05$)

Control (shrimp monoculture in biofloc); SB (shrimp and *Gracilaria birdiae* in an integrated biofloc system) and SD (Shrimp and *Gracilaria domingensis* in an integrated biofloc system)

SGR (% day^{-1}) = $100 \times$ [ln final weight (g) − ln initial weight (g)]/time

FCR = feed supplied (dry matter)/biomass gain

[1] The data correspond to the mean of three replicates ± standard deviation

Results

Second experiment

Control
IBS 2.5
IBS 5.0
IBS 7.5

Table 1 Water quality parameters in an integrated biofloc system (IBS) with *Litopenaeus vannamei* and *Gracilaria birdiae*, during a 42-day experimental period

Variables	Treatments			
	Control	IBS 2.5	IBS 5.0	IBS 7.5
Morning temperature (°C)	26.08 ± 0.03[a]	26.04 ± 0.06[a]	26.07 ± 0.16[a]	26.07 ± 0.08[a]
Afternoon temperature (°C)	27.48 ± 0.14[a]	27.43 ± 0.16[a]	27.60 ± 0.46[a]	27.42 ± 0.04[a]
Morning dissolved oxygen (mg L^{-1})	6.38 ± 0.04[a]	6.41 ± 0.08[a]	6.31 ± 0.16[a]	6.32 ± 0.08[a]
Afternoon dissolved oxygen (mg L^{-1})	6.62 ± 0.11[a]	6.65 ± 0.01[a]	6.57 ± 0.09[a]	6.55 ± 0.09[a]
pH	7.82 ± 0.04[a]	7.88 ± 0.09[a]	7.87 ± 0.03[a]	7.89 ± 0.05[a]
Salinity (g L^{-1})	36.01 ± 0.12[a]	35.83 ± 0.22[a]	35.95 ± 0.11[a]	36.26 ± 0.41[a]
Dissolved inorganic nitrogen (mg L^{-1})	4.73 ± 0.61[a]	3.83 ± 0.92[ab]	3.23 ± 0.36[b]	3.12 ± 0.70[b]
Total ammonia nitrogen (mg L^{-1})	0.39 ± 0.14[a]	0.21 ± 0.04[a]	0.28 ± 0.30[a]	0.15 ± 0.14[a]
Nitrite–nitrogen (mg L^{-1})	0.40 ± 0.02[b]	0.45±0.04[ab]	0.54 ± 0.05[a]	0.50 ± 0.03[a]
Nitrate-nitrogen (mg L^{-1})	3.93 ± 0.69[a]	3.16 ± 0.89[ab]	2.40 ± 0.34[b]	2.46 ± 0.84[b]
Orthophosphate (mg L^{-1})	1.97 ± 0.09[a]	1.98 ± 0.37[a]	2.19 ±0.13[a]	2.20 ± 0.15[a]
Alkalinity (mg $CaCO_3$ L^{-1})	126.1 ± 8.9[b]	140.7 ± 17.0[ab]	160.6 ± 13.3[a]	160.5 ± 12.9[a]
Total suspended solids (mg L^{-1})	213.3 ± 22.5[a]	253.8 ± 42.9[a]	223.1 ± 39.0[a]	240.3 ±70.1[a]
Settleable solids (mg L^{-1})	4.59 ± 0.60[a]	5.85 ± 0.76[a]	6.58 ± 0.77[a]	5.11 ± 1.03[a]

The data correspond to the mean ± SD. Results were analysed by performing repeated ANOVA measures and the Tukey's test. Mean values in the same row with different superscripts differ significantly ($P < 0.05$). Control (monoculture *L. vannamei*); IBS 2.5 (*L. vannamei* and 2.5 kg wet weight m^{-3} of *G. birdiae*); IBS 5.0 (*L. vannamei* and 5.0 kg wet weight m^{-3} of *G. birdiae*) and IBS 7.5 (*L. vannamei* and 7.5 kg wet weight m^{-3} of *G. birdiae*).

Table 2 Proximate composition of *Litopenaeus vannamei* (whole body) in an integrated biofloc system (IBS) with *Gracilaria birdiae* during a 42-day experimental period

	Control	IBS 2.5	IBS 5.0	IBS 7.5
Moisture (%)*	81.5 ± 0.4^{a}	81.2 ± 0.3^{a}	82.1 ± 0.1^{a}	81.8 ± 0.7^{a}
Crude protein	12.1 ± 0.1^{b}	13.7 ± 0.1^{a}	13.5 ± 0.1^{a}	13.2 ± 0.4^{a}
Crude lipids	1.6 ± 0.2^{a}	1.5 ± 0.2^{a}	1.4 ± 0.1^{a}	1.6 ± 0.2^{a}
Ash	2.4 ± 0.1^{a}	2.6 ± 0.1^{a}	2.2 ± 0.1^{a}	2.1 ± 0.1^{a}

*Except for moisture (%), the other values are in terms of wet weight (g 100 g^{-1} wet weight).

The data correspond to the mean of three replicates ± SD. Results from one-way ANOVA and Tukey's test. Mean values in the same column with different superscripts differ significantly ($P < 0.05$).

Table 4 Performance parameters of *Litopenaeus vannamei* reared in an integrated biofloc system (IBS) with *Gracilaria birdiae* during a 42-day experimental period

	Treatment			
Performance parameters	**Control**	**IBS 2.5**	**IBS 5.0**	**IBS 7.5**
Final weight (g)	3.12 ± 0.25^{b}	4.12 ± 0.04^{a}	3.97 ± 0.24^{a}	3.90 ± 0.04^{a}
Survival (%)	90.0 ± 5.0^{a}	95.00 ± 5.00^{a}	91.67 ± 5.77^{a}	89 ± 2.89^{a}
Yield (kg m^{3})	1.41 ± 0.18^{b}	1.96 ± 0.09^{a}	1.82 ± 0.21^{a}	1.72 ± 0.06^{a}
Biomass Gain (g)	56.27 ± 7.35^{b}	78.22 ± 3.73^{a}	72.95 ± 8.49^{a}	68.93 ± 2.34^{a}
Weight Gain (g)	2.80 ± 0.23^{b}	3.77 ± 0.06^{a}	3.64 ± 0.22^{a}	3.56 ± 0.01^{a}
Weekly growth (g $week^{-1}$)	0.47 ± 0.04^{b}	0.63 ± 0.01^{a}	0.61 ± 0.03^{a}	0.59 ± 0.01^{a}
FCR	1.74 ± 0.25^{b}	1.20 ± 0.06^{a}	1.30 ± 0.16^{a}	1.37 ± 0.05^{a}
SGR (% day^{-1})	5.42 ± 0.35^{a}	5.86 ± 0.18^{a}	5.90 ± 0.20^{a}	5.85 ± 0.27^{a}

The data correspond to the mean of three replicates ± SD. Results from one-way ANOVA and Tukey's test. Mean values in the same row with different superscripts differ significantly ($P < 0.05$). Control (monoculture *L. vannamei*); IBS 2.5 (*L. vannamei* and 2.5 kg wet weight m^{-3} of *G. birdiae*); IBS 5.0 (*L. vannamei* and 5.0 kg wet weight m^{-3} of *G. birdiae*) and IBS 7.5 (*L. vannamei* and 7.5 kg wet weight m^{-3} of *G. birdiae*).

Discussion

- The data for FCR (2.06–2.26) and weekly growth (0.81–0.98) in the biofloc system with seaweed were similar to those observed by Ray et al. (2010, 2012) and Schveitzer et al. (2013), with densities near 460 shrimp m-3.

- The increased results of final weight (6–21 %), weight gain (17–43 %), SGR (16–36 %) and decreased FCR (21–28 %) in an integrated treatments (shrimp and seaweed) indicate that the presence of seaweed improves shrimp performance in a biofloc system.

- Cyanobacteria groups can negatively affect water quality by producing compounds that are toxic to some aquatic animals because of their production of toxins (Ju et al. 2008; Yusoff et al. 2010).

- The treatments with *Gracilaria* showed a decrease in Cyanobacteria density of about 16 % compared with the control.

Discussion

With the addition of seaweeds, NO_3-N was the main form of inorganic nitrogen in the tanks, followed by NO_2-N and TAN which facilitates its removal, since NO_3-N and NH_4^+ are the main forms of inorganic nitrogen removed by red seaweed (Abreu, Pereira, Buschmann, Sousa-Pinto & Yarish 2011).

The higher crude protein content of shrimp in the IBS as compared to monoculture was recorded in our study, similar to that previously reported by Cruz-Suarez, Leon, Peña -Rodrıguez, Rodrıguez- Peña, Moll and Ricque-Marie (2010).

A positive effect of seaweed biomass on the increased final weight, decreased FCR, and increased yield was recorded in our study, similarly to Brito, Arantes et al. (2014), using *U. lactuca* and *L. vannamei* in a biofloc system.

Conclusions

The addition of *Gracilaria* to biofloc system can improve suspendes solids (SS) concentration, decrease Cyanobacteria density and FCR, and increase shrimp growth

The presence of *Gracilaria birdiae* (2.5 and 5.0 kg wet weight m^{-3}) in biofloc systems improve water quality by reducing dissolved inorganic nitrogen (DIN), NO_3-N and *Vibrio* density, furthemore it increase shrimp growth and body protein content

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Water quality, phytoplankton composition and growth of *Litopenaeus vannamei* (Boone) in an integrated biofloc system with *Gracilaria birdiae* (Greville) and *Gracilaria domingensis* (Kützing)

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Water quality, *Vibrio* density and growth of Pacific white shrimp *Litopenaeus vannamei* (Boone) in an integrated biofloc system with red seaweed *Gracilaria birdiae* (Greville)

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