# Water quality and production performance of catfishprawn co-culture with organic carbon source addition

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Abstract The objective of this study was to determine the effects of prawn juveniles and organic carbon source on the water quality and production performance of catfish. The experiment consisted of three treatments in triplicates, i.e. catfish monoculture as the control (treatment A); catfish-prawn co-culture at a prawn density of 20 prawn/m<sup>2</sup> (treatment B) and catfish-prawn co-culture at a prawn density of 40 prawn/m<sup>2</sup> (treatment C). Catfish was reared in a bamboo cage inside experimental concrete ponds at an initial density of 100/m<sup>2</sup>. Commercial feed pellets were only offered to the fish. In treatments B and C, organic carbon was added at an estimated C/N ratio of 10 to stimulate biofloc growth as the food source for the prawn juveniles. There was no significant difference in catfish growth among treatments. Dissolved inorganic nitrogen concentrations in B and C were generally lower than in the control. Although significantly affected by the initial stocking density, prawn growth was comparable to that fed with formulated feed. Catfish co-culture with prawn with C organic addition resulted in significantly higher feed nitrogen utilization. Overall results suggest that catfish-prawn co-culture with C organic addition may improve total food conversion ratio, reduce nitrogen waste and generate additional income for catfish farmers.

Keywords Catfish · Biofloc · Water quality · Freshwater prawn · Production

## Introduction

In an intensive aquaculture system, fish stocking density is high and consequently, high levels of external feed are added to the system. As fish or shrimp only retain 16–41 % of

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feed nitrogen (Avmimelech 1999; Yi et al. 2003; Hari et al. 2004), and most of aquaculture feed contains more than 25 % protein, high fish stocking density and corresponding high levels of added feed result in high N loading into the water. Nitrogenous waste in an aquaculture system is mostly in the form of ammonia, which is the final result of protein metabolism and decomposition products of faecal matter and unconsumed feed (Crab et al. 2007). Because ammonia is toxic for most aquatic animals, the level of this compound in an aquaculture medium should be maintained at <0.01 mg/L (Wedemeyer 1996) by regular water exchange or by applying water treatment system.

Bioflocs technology is an aquaculture practice based on natural processes occurring in an aquatic ecosystem. In this system, the growth of heterotrophic microbial biomass that assimilates ammonia–nitrogen as their primary nutrient is facilitated by the addition of external organic carbon (Avnimelech 1999; De Schryver and Verstraete 2009; Azim and Little 2008). This microbial biomass may further aggregate with other microorganisms including phytoplankton and zooplankton forming what has been called bioflocs. Bioflocs can be consumed by the cultured organisms themselves or other detritus feeder aquatic organisms (Hari et al. 2004; Azim and Little 2008), or be harvested and processed as a feed ingredient (Kuhn et al. 2010; Bauer et al. 2012). The assimilation of ammonia by the bacteria and the consumption of this microbial biomass by the cultured organisms in this biofloc system may therefore result in higher nitrogen utilization efficiency and at the same time may also maintain the concentration of toxic ammonia at low level.

As most of aquaculture inputs become limited, aquaculture development has been directed towards a culture system that is efficient in nutrient use with less impact on environment (Diana et al. 2013). This includes developing aquaculture feeds with higher digestibility and nutrient-uptake efficiency, an integrated multitrophic aquaculture, as well as better water and effluent treatment technologies (Diana et al. 2013; Verdegem 2013). The application of various aquaculture species in an integrated multitrophic aquaculture and polyculture may improve the nutrient utilization as each species may play different roles in the food chain (Diana et al. 2013). Effluent water treatment technology has been developed not only to maintain good water quality to support the welfare of the cultured species but also to make efficient use of water, to reduce water pollution and thereby the environmental impact of aquaculture, as well as to recycle the wasted nutrient to improve overall feed nutrient utilization (Verdegem 2013).

The present study investigated the effect of catfish and prawn co-culture with the addition of an organic C source that stimulates the growth of microbial biomass as the food source for the prawn. Water quality, production performance of the system and feed nitrogen utilization efficiency were observed. Additionally, economic comparison between treatments was also performed to evaluate the economical viability of the system.

### Materials and methods

#### Experimental setup

The experiment was performed in the Freshwater Aquaculture Research Station, Sukabumi, and in Bogor Agricultural University, West Java, Indonesia. Catfish *Clarias batracus* (75 days post-hatching) and prawn *Macrobrachium rosenbergii* (105 dph) at average initial body weight of  $10.29 \pm 0.29$  and  $1.07 \pm 0.13$  g, respectively, were used as the experimental animals. The study was conducted with three treatments in triplicates, i.e. catfish monoculture as the control (treatment A); catfish–prawn co-culture at a prawn density of 20 prawn/m<sup>2</sup> (treatment B) and catfish-prawn co-culture at a prawn density of 40 prawn/m<sup>2</sup> (treatment C), and lasted 49 days.

Nine units of concrete tanks with a dimension of  $5 \times 3 \times 1.5 \text{ m}^3$ , each with a bamboo cage ( $1.5 \times 1 \times 1 \text{ m}^3$ ), were used for the experiment (Fig. 1). Catfish juveniles were placed in the bamboo cages, whereas the prawns were distributed in the water outside the cages. The cages were placed 20 cm above the tank bottom and were supported by bamboo at each of their four sides. Prior to experimentation, the ponds were cleaned and filled with 12 m<sup>3</sup> of water. The water was subsequently disinfected using calcium hypochlorite at a concentration of 30 mg/L followed by strong aeration for neutralization. Water addition was applied during the culture period only to replace losses due to evaporation.

Feed containing 32 % of crude protein was offered only to the catfish, twice a day, at a level range of 5-3 % of the fish biomass. The feeding level was regularly adjusted according to fish biomass estimations following weekly sampling. Molasses was used as the organic carbon source in the co-culture treatments (B and C) at a dose of 72.5 % of the total feed given to the catfish (De Schryver et al. 2008). To promote floc formation, 1 mg/L of sodium silicate was daily added to the tanks with biofloc during the first week of culture period.

Sampling was performed weekly to determine fish feeding level and growth, whereas survival was calculated based on daily monitoring of fish mortality. Total bacterial count was weekly measured in pond water samples using tryptic soy agar (TSA) medium with 24-h incubation at 37 °C. The water quality parameters, such as dissolved oxygen (DO), pH and temperature, were measured in situ using hand-held pH meter, and DO meter. Total ammonia nitrogen, nitrite-N, nitrate-N, alkalinity, carbon dioxide, biological oxygen demand (BOD), volatile suspended solids (VSS) and total suspended solids (TSS) concentrations were measured using procedures as described in APHA (2005). Protein content of fish and shrimp was measured according to Kjeldahl method (Takeuchi 1988).

Specific growth rate, survival and feed efficiency were calculated according to Huisman (1987). Food conversion ratio (FCR) was calculated according to Eq. (1):

$$FCR = Total feed(g)/Total biomass gain(g)$$
 (1)

Nitrogen retention was calculated according to Eq. (2):



Fig. 1 Experimental pond unit with a bamboo cage

N Recovery(%) = 
$$(\Delta N_{\text{Fish}} / N_{\text{feed}}) \times 100$$
 (2)

where both N in fish and in feed are in grams. Gross and net productivity were calculated according to Huisman (1987).

Statistical analyses

The data were statistically analysed using one-way analyses of variance using SPSS statistics software version 13 (SPSS Inc., Chicago, USA) at 95 % confidence level. Post hoc analyses using Fisher's least significant difference test were performed for any significant difference.

## Results

Water quality

The water quality parameters observed in this experiment are presented in Table 1. There was no significant difference observed in most water quality parameters except in  $CO_2$ , TSS and VSS concentrations. Carbon dioxide concentrations were significantly higher in co-culture treatments than those in the control. Similarly, total and volatile suspended solids levels in co-culture treatments were also higher than in the control; however, the differences were only significant in co-culture treatment with high prawn density.

Figure 2 presents diurnal dissolved oxygen concentrations in the culture water on week 5. It can be seen that diurnal DO concentration and fluctuation in the control tend to be higher than those in the co-culture treatments. Diurnal oxygen concentration on the fifth week of culture showed strong reductions of dissolved oxygen in the treatments with organic C addition.

While there were no significant differences among treatments in TAN and  $NO_2$ -N concentrations when considering the whole experimental period, higher values in the control were recorded by the end of the culture period (Fig. 3). A higher concentration of  $NO_3$ -N was also noticed in the control on day 21.

Total heterotrophic bacteria in the water of treatments B and C were two log units higher (P < 0.05) than the treatment without any addition, with a tendency to increase during the culture period (Fig. 4).

#### Production and economic performance

No significant differences among treatments were observed in growth, survival and FCR of catfish (Table 2). Prawn harvesting average body weight, growth and survival were significantly higher in the treatment with lower prawn density, respectively 43, 49 and 14 % higher. In terms of prawn biomass, no significant differences were observed in production and productivity. However, when calculated as the number of juveniles produced, the productivity of treatment C, 1.5 million juveniles/ha/year, was significantly higher than that of treatment B (900,000 juveniles/ha/year). Significantly lower FCR was observed in the co-culture treatments than in the control (Table 2).

In comparison with catfish monoculture, catfish co-culture with prawn juvenile resulted in higher profit (Table 3). Furthermore, when the prawn density increased from 20 to

**Table 1** The range of water quality parameters in pond water of catfish monoculture (treatment A) and catfish–prawn co-culture at prawn densities of  $20/m^2$  (treatment B) and  $40/m^2$  (treatment C) throughout the culture period

Parameter	Average (range)			
	A	В	С	
Temperature (°C)	28 (27–30) <sup>a</sup>	28 (27–30) <sup>a</sup>	28 (27–30) <sup>a</sup>	
pH	6.8 (6.1–7.7) <sup>a</sup>	6.9 (6.3–7.5) <sup>a</sup>	7.0 (6.4–7.6) <sup>a</sup>	
Alkalinity (mg/L CaCO <sub>3</sub> eq)	116 (101–140) <sup>a</sup>	155 (122–196) <sup>a</sup>	154 (132–173) <sup>a</sup>	
DO (mg/L)	4.21 (2.24-8.14) <sup>a</sup>	2.65 (1.48–4.79) <sup>a</sup>	2.89 (1.16-5.59) <sup>a</sup>	
CO <sub>2</sub> (mg/L)	12.92 (11.88–15.94) <sup>a</sup>	20.12 (16.83-23.92) <sup>b</sup>	23.84 (19.80-29.89) <sup>b</sup>	
TAN (mg/L)	0.31 (0-0.81) <sup>a</sup>	0.26 (0–0.44) <sup>a</sup>	0.26 (0-0.50) <sup>a</sup>	
NH <sub>3</sub> (mg/L)	0.005 (0-0.023) <sup>a</sup>	0.003 (0-0.009) <sup>a</sup>	0.003 (0-0.011) <sup>a</sup>	
NO <sub>2</sub> (mg/L)	0.240 (0.003–0.726) <sup>a</sup>	0.075 (0.025–0.223) <sup>a</sup>	0.040 (0.016–0.066) <sup>a</sup>	
NO <sub>3</sub> (mg/L)	0.404 (0.128–0.860) <sup>a</sup>	0.214 (0.109-0.338) <sup>a</sup>	0.282 (0.150-0.368) <sup>a</sup>	
BOD <sub>5</sub> (mg/L)	4.73 (3.64–7.30) <sup>a</sup>	5.33 (3.87–7.60) <sup>a</sup>	5.40 (4.03-7.50) <sup>a</sup>	
TSS (mg/L)	35.68 (20.80-46.00) <sup>a</sup>	48.70 (43.47–53.40) <sup>ab</sup>	85.10 (52.40–161.40) <sup>b</sup>	
VSS (mg/L)	3.34 (0.80-6.32) <sup>a</sup>	7.74 (5.76–9.52) <sup>ab</sup>	17.22 (9.55–11.63) <sup>b</sup>	

Mean values (n = 3) within the same row marked with a different superscript are significantly different (P < 0.05)

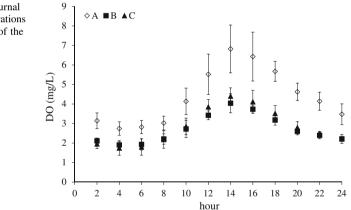


Fig. 2 Mean values of diurnal dissolved oxygen concentrations in pond water on week 5 of the culture period

 $40/m^2$ , the total net return increased 37 % and 2.3 times higher than that of catfish monoculture.

Feed nitrogen utilization

There was no significant difference in feed N recovery by catfish among treatments (Table 4). However, as prawn recovered 3.25-3.81 % of the total feed N, co-culture of catfish with prawn resulted in significantly higher total feed N recovery (P < 0.05).

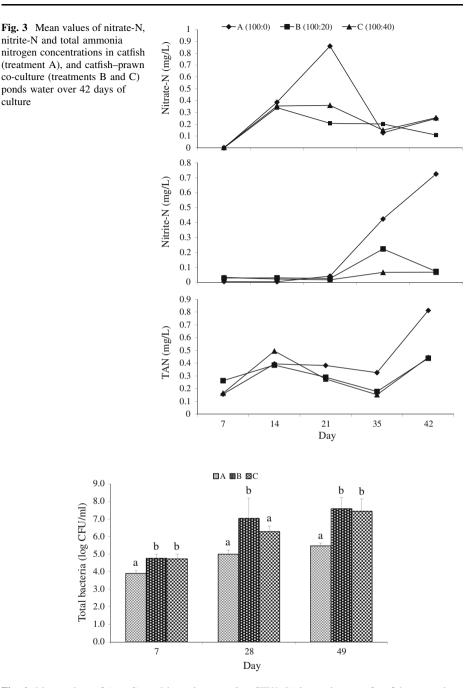


Fig. 4 Mean values of (n = 3) total bacteria count (log CFU/mL) in pond water of catfish monoculture (treatment A) and catfish-prawn co-culture at prawn densities of 20/m<sup>2</sup> (treatment B) and 40/m<sup>2</sup> (treatment C). Bars marked with a different superscript in each sampling point are significantly different (P < 0.05)

culture

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	А	В	С	Significance
Catfish grow out				
Stocking				
Density per cage (fish/m <sup>3</sup> )	100	100	100	
Average fish weight (g/fish)	10.0	10.2	10.6	
Total biomass (kg)	1.51	1.53	1.59	
Harvest				
Survival (%)	98.7 <sup>a</sup>	98.7 <sup>a</sup>	98.7 <sup>a</sup>	NS*
Average fish weight (g/fish)	88.2 <sup>a</sup>	90.1 <sup>a</sup>	92.5 <sup>a</sup>	NS
SGR (%/day)**	4.54 <sup>a</sup>	4.55 <sup>a</sup>	4.53 <sup>a</sup>	NS
Total biomass (kg)	13.1 <sup>a</sup>	13.3 <sup>a</sup>	13.7 <sup>a</sup>	NS
Total feed (kg)	9.74 <sup>a</sup>	9.76 <sup>a</sup>	9.78 <sup>a</sup>	NS
FCR (%)***	$0.85^{a}$	0.83 <sup>a</sup>	0.81 <sup>a</sup>	NS
Net productivity (ton/ha/year)	38.5 <sup>a</sup>	39.5 <sup>a</sup>	$40.5^{a}$	NS
Gross productivity (ton/ha/year)	43.5 <sup>a</sup>	44.5 <sup>a</sup>	45.5 <sup>a</sup>	NS
Prawn nursery				
Stocking				
Density/pond (prawn/m <sup>2</sup> )		20	40	
Average body weight (g/prawn)		1.07	1.07	
Total biomass (kg)		0.32	0.64	
Harvest				
Survival (%)		88.3 <sup>a</sup>	77.2 <sup>b</sup>	P < 0.05
Average body weight (g/prawn)		3.68 <sup>a</sup>	2.57 <sup>b</sup>	P < 0.01
SGR (%/day)		2.98 <sup>a</sup>	2.10 <sup>b</sup>	P < 0.01
Total biomass (kg)		$0.98^{\rm a}$	1.19 <sup>a</sup>	NS
Juvenile productivity (prawn/ha/year)		900,000 <sup>a</sup>	1,550,000 <sup>b</sup>	P < 0.01
Net productivity (ton/ha/year)		$2.2^{\mathrm{a}}$	1.85 <sup>a</sup>	NS
Gross productivity (ton/ha/year)		3.25 <sup>a</sup>	3.95 <sup>a</sup>	NS
FCR catfish and prawn	0.85 <sup>a</sup>	0.78 <sup>b</sup>	0.77 <sup>b</sup>	P < 0.05

**Table 2** Production performance of catfish (*Clarias* sp.) and prawn (*Macrobrachium rosenbergii*) in catfish monoculture (treatment A) and catfish–prawn co-culture at prawn densities of  $20/m^2$  (treatment B) and  $40/m^2$  (treatment C)

Mean values (n = 3) within the same row marked with a different superscript are significantly different (P < 0.05)

\* NS not significant

\*\* SGR specific growth rate

\*\*\* FCR food conversion ratio

## Discussion

The ranges of water temperature and pH were similar between treatments and within acceptable ranges. Diurnal oxygen concentration on the fifth week of culture showed that there were significant reductions of dissolved oxygen observed in the treatments with organic C addition. The reduction of DO towards the end of the culture period and the increase of  $CO_2$  concentrations in treatments B and C indicate that the addition of prawn

Table 3 Economic comparison   of catfish monoculture and cat-		А	В	С
fish–prawn co-culture with organic C addition $(1,000 \times IDR)$ per ha production unit	Operational cost			
	Catfish juvenile	15,000	15,000	15,000
	Prawn post-larvae	_	8,000	16,000
	Feed	258,825	258,545	257,985
	Molasses	_	39,890	39,803
	Electricity	6,000	6,000	6,000
	Labour	27,000	27,000	27,000
	Total operational cost	306,825	354,435	361,788
	Harvest			
	Catfish	522,000	534,000	546,000
	Prawn	_	180,000	310,000
	Total revenue	522,000	714,000	856,000
	Net return	215,175	359,565	494,212

biomass and the stimulation of heterotrophic bacteria growth by C organic addition resulted in higher respiration activities in the prawn–catfish culture system, mainly under higher prawn density. The high nitrogenous waste loaded into the water and the addition of organic C stimulated heterotrophic microbial growth in treatment B and especially so in treatment C, as shown by the levels of total heterotrophic bacteria, TSS and VSS in the water of the respective treatments. The high total bacterial count in treatment C confirms previous studies in the application of biofloc technology that showed that the addition of an external organic carbon source resulted in an increase in total heterotrophic bacteria (Hari et al. 2004; Azim and Little 2008). Total and volatile suspended solids are two physical characteristics that are frequently used as the main parameters for the characterization of floc formation in biofloc-based aquaculture systems (De Schryver et al. 2008). Highly suspended solids were particularly noticed in treatment C that was typical for a biofloc system where suspended solids may reach a value of more than 200 mg/L (Azim and Little 2008).

Dissolved inorganic nitrogen including TAN, NO<sub>2</sub>-N and NO<sub>3</sub>-N fluctuated over the culture period. There was a tendency towards the end of the culture period that the concentrations of dissolved inorganic nitrogen in the control without external C addition were higher than in the co-culture treatments with C addition. The concentrations of TAN, NO<sub>2</sub>-N and NO<sub>3</sub>-N that represent nitrification processes in the systems showed that during the first 3 weeks of culture, nitrification occurred in all treatments. After day 21, on the other hand, nitrification in the control was not completely working as indicated by the accumulation of TAN and NO<sub>2</sub>-N but not of NO<sub>3</sub>-N. Nevertheless, the range of inorganic N was appropriate for normal growth of both catfish and prawn (Peteri et al. 1992; New 2002).

As it was expected, the growth of prawn juvenile stocked at lower density  $(20/m^2)$  in treatment B was significantly higher (P < 0.05) than that of treatment C  $(40/m^2)$ . The growth performance of prawn juvenile in the present study was comparable to that of a still unpublished stocking density experiment performed in the Freshwater Aquaculture Research Station, Sukabumi. In that experiment, prawn juveniles fed with 28 % crude protein feed at a stocking density of  $20/m^2$  resulted in a specific growth rate of 3 %/day, survival of 91 % and FCR of 1.4. At a stocking density of  $40/m^2$ , on the other hand, slower growth rate (2.58 %/day) and higher FCR (1.9) were observed. In this regard, the growth

Treatment	Feed N recovery by catfish (%)	Feed N recovery by prawn (%)	Total feed N recovery (%)
A (100:0)	$63.6 \pm 2.0$	_	$63.6 \pm 2.0^{a}$
B (100:20)	$64.9 \pm 0.9$	$3.81 \pm 0.49$	$68.7\pm1.1^{\rm b}$
C (100:40)	$66.5 \pm 2.8$	$3.25 \pm 0.64$	$69.7\pm2.4^{\text{b}}$

**Table 4** Feed N utilization by catfish and prawn in catfish monoculture (treatment A) and catfish–prawn co-culture at prawn densities of  $20/m^2$  (treatment B) and  $40/m^2$  (treatment C)

Mean values (n = 3) within the same column marked with a different superscript are significantly different (P < 0.05)

and survival of prawn juveniles in the present experiment, where external feeding for prawn was not provided, was supported by the availability of bioflocs as the food source in the system. This result supports a previous study by Crab et al. (2010), who demonstrated that prawn post-larvae could utilize bioflocs grown with different carbon sources as their food. The similar growth of prawn juveniles in the present experiment and in that of prawn fed with artificial feed also indicates that the nutritional quality of the bioflocs available continuously in the system was comparable to that of artificial feed. Previous studies showed that bioflocs contain considerably high protein and lipid levels as well as other essential nutrients, such as essential amino acids and fatty acids, carotenoids, exogenous digestive enzymes and other bioactive compounds (Ju et al. 2008; Ekasari et al. 2010; Crab et al. 2010; Xu et al. 2012; Xu and Pan 2012).

Although survival was higher in treatment B, prawn juvenile productivity (prawn/ha/ year) was noticeably higher in treatment C. This may indicate that even though higher mortality occurred at higher prawn density, the total surviving prawn juveniles was still higher than that at the lower prawn density. As prawn nursery productivity is determined by the number of juvenile produced, treatment C resulted in higher economical viability than treatment B (Table 3). Overall FCR of catfish–prawn co-culture regardless of the prawn density was significantly lower than that of catfish monoculture, which can be attributed to the utilization of bioflocs as food by the prawn. Furthermore, co-culture and C source addition in treatments B and C also contribute to the increase in total feed N utilization. The present study showed that the addition of prawn and organic C might recover approximately 10 % of the unutilized N from the feed provided to the catfish or 3 % of the total feed N input.

## Conclusion

The water quality of catfish–prawn co-culture with organic C addition was generally better than the catfish monoculture. Co-culture with prawn with C source addition in catfish culture also resulted in significantly lower total FCR and significantly higher feed nitrogen utilization. Overall results suggest that catfish co-culture with prawn nursery with C organic addition would reduce nitrogen waste and also generate additional income for the catfish farmer.

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