

# Evaluation of Biofloc Technology Application on Water Quality and Production Performance of Red Tilapia *Oreochromis* sp. Cultured at Different Stocking Densities

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This study evaluated the effect of biofloc technology (BFT) application on water quality and production performance of red tilapia *Oreochromis* sp. at different stocking densities. Three different fish densities were applied, i.e. 25, 50, and 100 fish/m<sup>3</sup>, and for each density there were Control (without external carbon input) and BFT treatments. Mixed sex red tilapia with an initial average body weight  $77.89 \pm 3.71$  g was cultured in 3 m<sup>3</sup> concrete tanks for 14 weeks. Molasses was added on BFT treatments as the organic carbon source at a C/N ratio of 15. Control treatments of each density tested showed more fluctuated water quality parameters throughout the experimental period. The highest TAN and nitrite-nitrogen were observed in control treatment at a stocking density of 100 fish/m<sup>3</sup> (3.97 mg TAN/L and 9.29 mg NO<sub>2</sub>-N/L, respectively). The highest total yield was observed in control treatment at the highest density treatment (43.50 kg), whereas the highest survival was obtained by BFT treatment at a density of 25 fish/m<sup>3</sup> ( $97.78 \pm 0.77\%$ ). Total feed used in BFT was lower than that of control treatments in particular at 50 fish/m<sup>3</sup> density ( $P < 0.05$ ) suggesting that biofloc could be continuously harvested by the fish as other source of food.

Key words: biofloc technology (BFT), biofloc, red tilapia, water quality, growth

## INTRODUCTION

The world demand of tilapia has been steadily increasing, especially in the United States and European countries. This is followed by the progressively growth of world tilapia and other cichlids production from only 107,459 MT in the early eighties to more than 2.5 million MT in 2008 with an average annual growth rate of 11.2% (Food and Agriculture Organization Fisheries and Aquaculture Statistics, 2010). The increasing global population and the limiting global capture fisheries undeniably increase the demand of aquaculture product including tilapia. On the other hand, those will also bring about limitation to aquaculture expansion in particular of land and water utilization. Therefore, productivity enhancement in term of total production per input used becomes one of the major priority in the development of tilapia culture particularly and aquaculture in general (Brune *et al.* 2003; Delgado *et al.* 2003; Piedrahita 2003; Avnimelech *et al.* 2008), and aquaculture intensification is therefore becomes one of the most reasonable way to complete this objective.

An intensive aquaculture system is characterized by the high stocking density which is followed by the needs of high quality and quantity of artificial feed (Piedrahita 2003; Avnimelech *et al.* 2008). As application of high fish biomass and feed input brings about water quality

deterioration, an active water quality management should therefore be regularly performed in an intensive aquaculture system. Avnimelech and Ritvo (2003) noted that fish assimilate only 20-25% of protein in feed, and the remaining is excreted as ammonia and organic nitrogen in faeces and unconsumed feed. At the same time organic nitrogen in faecal matter and unconsumed feed is further mineralized by the decomposing bacteria resulting inorganic nitrogen in the form of ammonia. As fish pellet usually contain protein no less than 25%, the consequence of high feed input in intensive aquaculture system is a high accumulation of ammonia (Brune *et al.* 2003), which is highly toxic for aquatic organism (Stickney 2005). Moreover, if the discharged water of an aquaculture unit is released without any further treatment, it may not only harm aquatic wildlife but also contribute to the eutrophication of surrounding water.

Biofloc technology (BFT) is an aquaculture system which focused on a more efficient use of nutrient input with limited or zero water exchange. The main principle of BFT is to recycle nutrient by maintaining a high carbon/nitrogen (C/N) ratio in the water in order to stimulate heterotrophic bacterial growth that converts ammonia into microbial biomass (Avnimelech 1999). The microbial biomass will further aggregate with other microorganisms and particles suspended in the water forming what has been called "biofloc", which eventually can be consumed in situ by the cultured animals or harvested and processed as a feed ingredient (Avnimelech 1999; Avnimelech 2007;

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Crab *et al.* 2007; De Schryver *et al.* 2008; Kuhn *et al.* 2008; Kuhn *et al.* 2009; Kuhn *et al.* 2010). With this principle, BFT is therefore considered as a promising system for a sustainable and environmentally friendly aquaculture system, and has been applied both at laboratory and commercial scale for various aquaculture species such as tilapia (Avnimelech 2007; Azim & Little 2008; Crab *et al.* 2009), shrimp (Burford *et al.* 2004; Hari *et al.* 2004; Taw 2010), sturgeon and snook (Serfling 2006).

The objective of this experiment was to study the effect of BFT application on water quality and production performance of red tilapia *Oreochromis* sp. cultured at different densities. Additionally, bioflocs primary nutritional parameters as well as plankton identification and abundance measurement were also performed in this study.

## MATERIALS AND METHODS

**Experimental Design.** Twelve units of outdoor rectangular concrete tanks (6 m<sup>2</sup>) at the Department of Aquaculture Research Station, Bogor Agricultural University, Indonesia, were assigned for this experiment. Prior to experiment, tanks were cleaned, dried and filled with freshwater at a volume of 3 m<sup>3</sup> (0.5 m water depth). Aeration was provided by an air blower and installed at 16 lines (5 l/min per line) per tank for the first 7 weeks of culture and 24 lines per tank later on. In order to stimulate biofloc growth in biofloc treatments, two tanks were prepared one week prior to the experiment as the biofloc source of inoculants, and 25 mg/l of N, 3.6 mg/l of (PO<sub>4</sub>)<sup>3-</sup> and 1 mg/l of NaSiO<sub>3</sub>, molasses (53% of C) as the organic carbon source at a C/N ratio of 15 were added.

Mixed sex red tilapia with an average body weight 77.89 ± 3.71 g was used as the experimental animal and cultured for 14 weeks. There were three fish densities applied in this experiment, 25, 50, and 100 fish/m<sup>3</sup>, and for each density there were control (without external C input) and BFT (with external C input) treatments. Thus resulted in 6 different treatments, i.e. BFT 25 (25 fish/m<sup>3</sup> with external C addition), Control 25 (25 fish/m<sup>3</sup>, control without external C addition), BFT 50, Control 50, BFT 100, and Control 100. For each BFT and control treatment, three and two replicates were applied, respectively. Due to the limited availability of tank, there was no replicate applied for the 100 fish/m<sup>3</sup> density treatments. Fish were fed three times a day at satiation with a commercial floating pellet (32% crude protein content). The amount of feed per feeding time was determined based on fish feeding response, i.e. feeding was stopped whenever the fish showed no response to feed. Unconsumed feed was removed and collected using a net, dried and weighed, and not be

included in the daily feed amount that was determined after the last feeding time. As an external organic C source, molasses was added daily to the BFT treatments with a C/N ratio of 15. The amount of molasses addition per day was determined based on the calculation described in Avnimelech (1999). No water replacement was carried out; water addition however was performed to replace water loss due to evaporation.

Sampling for fish growth and biomass monitoring was performed once a week. By the end of the experimental period, total fish number and biomass were counted and calculated to determine survival, growth, total yield, and feed efficiency. With the exception of treatment BFT 100 and Control 100, all data were further statistically analyzed using S.Plus version 8.0.

**Water Analyses.** Some water quality parameters such as temperature, dissolved oxygen (DO) and pH were measured in situ each morning before feeding. Total ammonia nitrogen (TAN), nitrite-nitrogen (NO<sub>2</sub>-N), nitrate-nitrogen (NO<sub>3</sub>-N), total suspended solids (TSS), volatile suspended solids (VSS) and floc volume (FV) were measured biweekly, whereas chlorophyll a (chl-a) concentration was measured on the initial week, week 7 and 14. Temperature and DO were measured using DO meter (HANNA Instrument), whereas other parameters were determined following "Standard Methods for examination of water and wastewater" (APHA 2005).

The density of phytoplankton and zooplankton was observed on the first, seventh, and last week of culture period under a light microscope using a Sedgewick Rafter subsequent to fixation with 1% formaldehyde. Identification of the plankton was also performed and categorized under several major classes based on Prescott (1978).

Proximate analyses of biofloc samples were conducted on the initial and the last culture period following procedures as described in Olvera-Novoa *et al.* (1994) except for total lipid which was determined according to Folch *et al.* (1957).

## RESULTS

**Water Quality.** Temperature and DO in water of all treatments were in optimal condition for fish culture which were ranged from 26.0-29.3 C and 3.26-6.89 mg/l, respectively (Table 1). The range of pH in control treatments at each level of density tested throughout the experimental period seems to be lower than BFT treatment. The tendency of pH drop was markedly observed in Control treatments starting from week 7 to week 12 (Figure 1). In contrast, BFT treatments showed a relatively stable pH level at a range of 6.3-7.5.

Table 1. Water quality parameters

Treatment	pH	Dissolved oxygen (mg/l)	TAN (mg/l)	NO <sub>2</sub> -N (mg/l)	NO <sub>3</sub> -N (mg/l)
BFT 25	6.8-7.5	4.19-6.89	0.01-1.13	0.00-2.09	0.00-2.92
Control 25	5.0-6.3	4.37-6.23	0.23-3.78	0.00-6.07	0.00-2.87
BFT 50	6.3-7.3	3.60-5.96	0.14-0.75	0.00-3.32	0.00-2.93
Control 50	5.5-6.0	3.96-6.53	0.21-1.80	0.00-4.96	0.00-2.57
BFT 100	6.3-7.5	3.26-5.54	0.11-1.04	0.00-5.85	0.00-2.57
Control 100	5.3-5.8	2.43-5.75	0.33-3.97	0.00-9.29	0.00-3.04

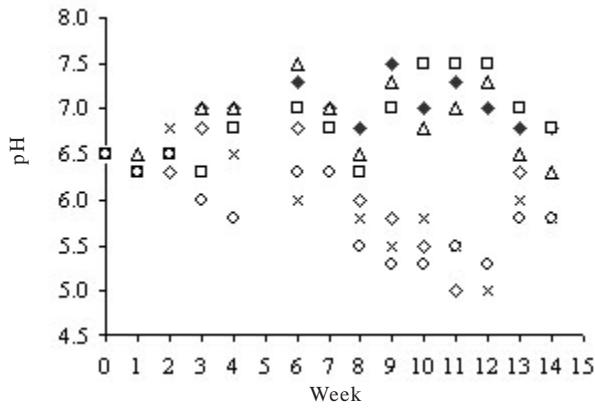


Figure 1. pH observed in different treatments throughout the experimental period. ◆: BFT 25, ◇: Control 25, △: BFT 50, ×: Control 50, □: BFT 100, ○: Control 100.

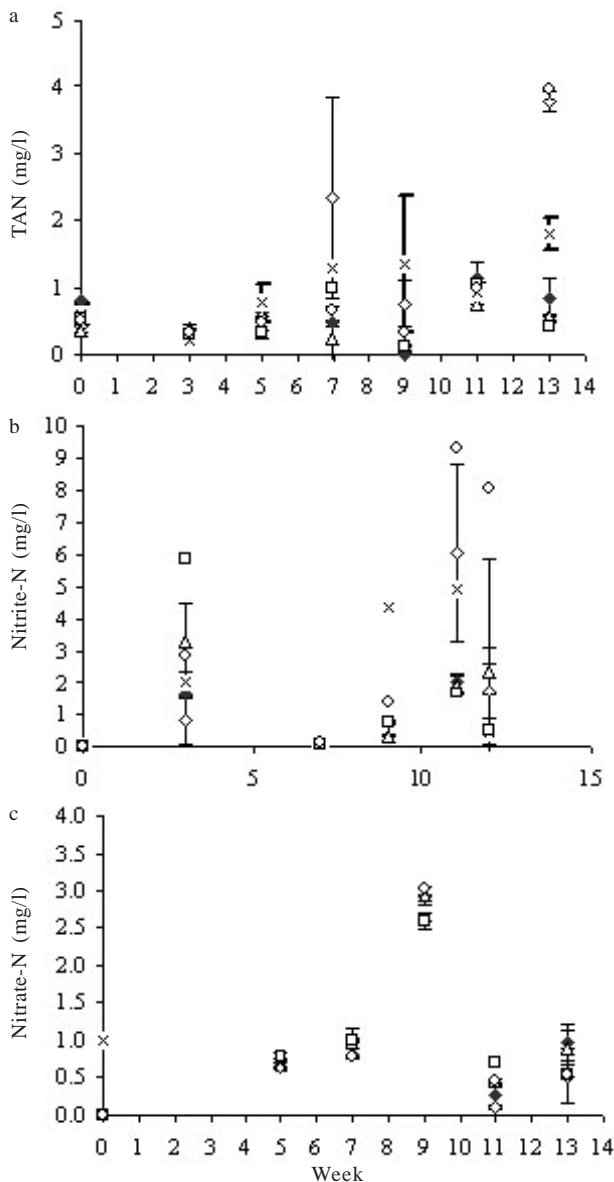


Figure 2. (a) TAN, (b) NO<sub>2</sub>-N, and (c) NO<sub>3</sub>-N concentrations in different treatments throughout of the experimental period. ◆: BFT 25, ◇: Control 25, △: BFT 50, ×: Control 50, □: BFT 100, ○: Control 100.

Total ammonia nitrogen concentrations in BFT treatments, regardless the density, remained stable at below 1.1 mg/l throughout the culture period (Figure 2). Control treatments on the other hand showed peak relatively fluctuated TAN concentrations. The highest peak of TAN concentrations of all treatments was observed in control treatments on week 13 of culture period (3.97 mg/l). Nitrite nitrogen concentrations in all treatments throughout the culture period appear to be fluctuated. Nevertheless, more prominent fluctuations of NO<sub>2</sub>-N concentrations were observed in control treatments (Figure 2b). The highest level of NO<sub>2</sub>-N concentration was observed in Control 100 treatment on week 11 which was 9.29 mg/l. All treatments showed a similar trend of NO<sub>3</sub>-N concentration throughout the experimental period. On the first 9 weeks of culture period all treatments showed a tendency of NO<sub>3</sub>-N build up with the highest levels observed on week 9. On week 11 however nitrate-nitrogen concentrations of all treatments abruptly decreased before raised again on week 13.

The averages TSS of BFT treatments at 25, 50, and 100 fish/m<sup>3</sup> were 418, 586, and 726 mg/l, respectively, which were constantly higher than their corresponding control treatments which were 253, 366, and 399 mg/l. There was no significant difference observed in FV in between treatments ( $P > 0.05$ ) for the first 4 weeks of culture. Significant variation on the other hand was observed starting from week 7 onward, that FV in BFT treatments were significantly higher than Control ( $P < 0.05$ ). Floc volume of BFT 25, 50, and 100 on week 14 were respectively  $138 \pm 14$ ,  $113 \pm 0$ ,  $147 \text{ ml/l}$ , which were higher than their corresponding control treatments, i.e.  $90 \pm 5$ ,  $83 \pm 5$ , and  $93 \text{ ml/l}$ .

Chlorophyll-a concentrations observed in all treatments was at a range of 389-1,718 mg/m<sup>3</sup> (Figure 3). With the exception of Control 100, this parameter appears to be relatively stable at a level less than 1,000 mg/m<sup>3</sup> throughout the culture period. The highest chl-a concentration was observed in Control 100 on week 12 which was 1,718 mg/m<sup>3</sup>. Phytoplankton abundance observed in BFT treatments ( $1.7\text{-}9.7 \times 10^7 \text{ ind/l}$ ) was almost one log unit lower those of control treatments ( $2.1\text{-}5.8 \times 10^8 \text{ ind/l}$ ) (Figure 4). Bacillariophyceae mostly dominated

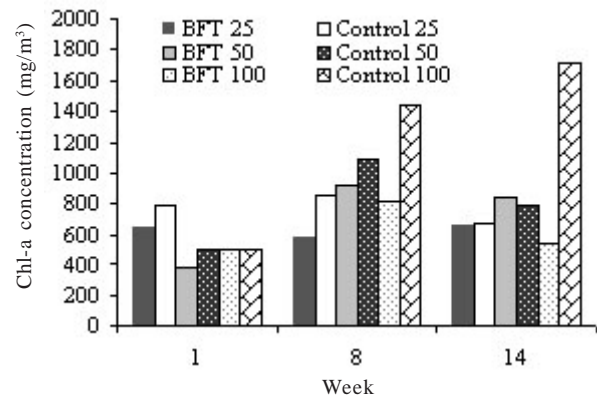


Figure 3. Chlorophyll-a concentrations in different treatments measured on week 2, 8, and 12 of experimental period.

phytoplankton community in BFT treatments (34-39% of total abundance), whereas Chlorophyceae was found to be the most abundant (16-27%) phytoplankton class in control treatments. Moreover, *Fragilaria* sp. ( $2.4-15.2 \times 10^7/\text{ml}$ ) and *Scenedesmus* sp. ( $4.8-15 \times 10^7/\text{ml}$ ), respectively, appear to be the most dominant genus in BFT and control systems.

For both 25 and 50 fish/m<sup>3</sup> density treatments, the abundance of zooplankton in BFT treatments ( $4.6 \times 10^6$  ind/l and  $9.0 \times 10^5$  ind/l) were higher than the corresponding Control treatments ( $0.5 \times 10^6$  ind/l and  $3.4 \times 10^5$  ind/l), whereas at fish density of 100 fish/m<sup>3</sup>, Control treatment ( $1.6 \times 10^6$  ind/l) showed a higher zooplankton abundance than BFT treatment ( $5.1 \times 10^5$  ind/l) (Figure 5). Protozoan seems to be the most dominant zooplankton types in all treatments (30-33% of total zooplankton abundance) with testate amoeboid genera, *Arcella*, *Centrophyxis*, *Difflugia*, and *Euglypha* sp. as the dominant genera.

Proximate composition of biofloc collected on harvest day was not significantly different (Table 2). The range of crude protein content of biofloc was 39-48%, whereas biofloc crude lipid and ash contents were considerably high with ranges of 12-24% and 25-28%, respectively.

**Fish Production.** Fish survival appears to be affected by fish density, i.e. lower density showed a higher survival

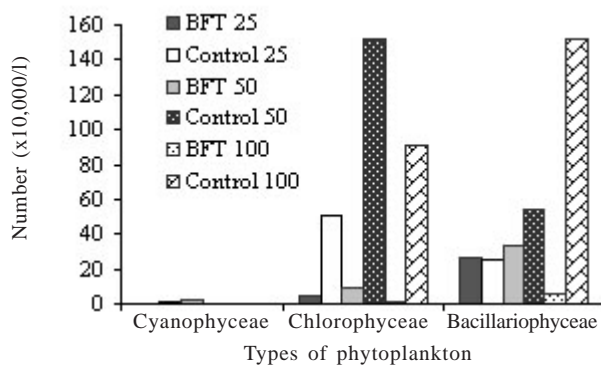


Figure 4. Phytoplankton abundance observed at the closing day of experimental period.

(Table 3). BFT treatments showed slightly higher survival, nevertheless the difference was insignificant. The average individual fish weight at harvest of all treatments was not significantly different with a range of 129-216 g/fish. The total harvested biomass seems to be influenced by fish density being treatments with higher stocking density showed a higher total harvested biomass. Nonetheless, the total harvested biomass of BFT 50 ( $22.60 \pm 1.93$  kg) treatment was not significantly different from the lower density treatments, BFT 25 ( $14.00 \pm 1.00$  kg) and Control 25 ( $15.75 \pm 1.06$  kg), as well as to its counterpart Control 50 ( $28.75 \pm 1.93$  kg). Fish density and BFT treatments apparently influenced the total feed given in each treatment. Higher fish density resulted in higher amount of feed input regardless BFT or control treatment. At the same time, BFT treatments seem to utilize lower amount of

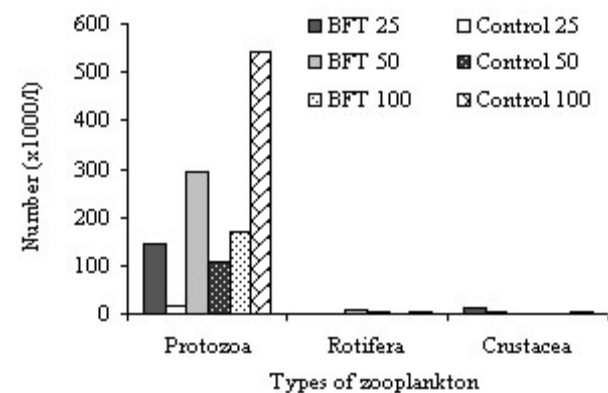


Figure 5. Zooplankton abundance observed at the closing day of experimental period.

Table 2. Proximate parameters (mean  $\pm$  SD) of bioflocs collected from BFT treatments at different fish density

Composition (% DM)	BFT 25	BFT 50	BFT 100
Crude protein	39.71 $\pm$ 1.89	44.12 $\pm$ 0.38	48.13
Crude lipid	24.33 $\pm$ 1.69	21.27 $\pm$ 0.31	12.56
Crude fiber	3.07 $\pm$ 0.41	3.32 $\pm$ 0.22	4.48
Ash	26.92 $\pm$ 2.45	25.18 $\pm$ 3.61	28.72

Table 3. Production performance of red tilapia *Oreochromis* sp. with BFTI at different density

	Treatment					
	25 fish/m <sup>3</sup>		50 fish/m <sup>3</sup>		100 fish/m <sup>3</sup>	
	BFT	Control	BFT	Control	BFT	Control
<b>Stocking</b>						
Total no. of fish	75	75	150	150	00	300
Mean weight (g/fish)	75.98 $\pm$ 3.02	78.20 $\pm$ 7.64	78.56 $\pm$ 3.10	78.20 $\pm$ 1.79	77.80	74.03
Total weight (kg)	5.70 $\pm$ 0.23	5.87 $\pm$ 0.23	11.78 $\pm$ 0.47	12.22 $\pm$ 0.27	23.21	22.21
<b>Harvest</b>						
Mean weight (g/fish)	190.86 $\pm$ 12.34	215.63 $\pm$ 6.17	161.04 $\pm$ 13.05	216.46 $\pm$ 50.01	129.03	165.40
Total weight (kg)	14.00 $\pm$ 1.00a	15.75 $\pm$ 1.06a	22.60 $\pm$ 1.93ab	28.75 $\pm$ 1.93b	36.00	43.50
Total feed (kg)	11.96 $\pm$ 1.39a	16.92 $\pm$ 0.56ab	19.05 $\pm$ 1.87b	23.21 $\pm$ 5.05c	26.64	46.22
Feed efficiency (%)	68.99 $\pm$ 1.49	58.12 $\pm$ 7.48	55.49 $\pm$ 7.13	47.83 $\pm$ 26.45	44.80	44.15
Survival (%)	97.78 $\pm$ 0.77a	97.33 $\pm$ 3.77ab	93.56 $\pm$ 2.69ab	88.00 $\pm$ 4.7b	93.00	87.67
<b>Gain</b>						
Mean weight gain (g/fish)	114.88 $\pm$ 9.94	137.43 $\pm$ 13.81	82.48 $\pm$ 11.76	135.00 $\pm$ 51.80	51.35	91.37
Mean daily gain (g/day)	1.16 $\pm$ 0.10	1.39 $\pm$ 0.14	0.83 $\pm$ 0.12	1.36 $\pm$ 0.52	0.52	0.92
Total weight gain (kg)	8.30 $\pm$ 0.81	9.89 $\pm$ 1.63	10.82 $\pm$ 1.60	16.53 $\pm$ 8.40	12.70	21.29
Net Yield (kg/m <sup>3</sup> )	2.77 $\pm$ 0.27	3.3 $\pm$ 0.54	3.61 $\pm$ 0.53	5.51 $\pm$ 2.80	4.23	7.10

mean value in the same row with different superscript differ significantly (P < 0.05).



feed than their control treatments. This tendency was clearly shown at a fish density of 50 fish/m<sup>3</sup>, where BFT50 treatment used significantly lower amount of feed (19.05 ± 1.87 kg) than Control 50 (23.21 ± 5.05 kg). Although not statistically confirmed, the difference was also noticed at the highest density tested (100 fish/m<sup>3</sup>), being BFT100 required 73% less feed than Control 100. The difference in total feed input however was not reflected in feed efficiency, as there was no significant difference shown by all treatments in this particular parameter.

There was no significant difference observed in growth as well as production parameters. The average individual and daily gain were ranged from 51 to 137 g/fish and 0.52 to 1.39 g/day, respectively whereas the range of total weight gain and net yield were correspondingly 8.30-21.29 kg and 2.77-7.10 kg/m<sup>3</sup>.

## DISCUSSION

Temperature range (26.0-29.3 °C) observed throughout this experimental period was in an optimal range. Dissolved oxygen depletion along with the increasing density was noticed in particular during the second half experimental period. The situation however had been anticipated by the addition of aeration lines from 16 to 24 lines.

Photosynthesis and nitrification processes that likely to occur in control system possibly resulted in pH fluctuation, as these processes are likely to alter CO<sub>2</sub> concentration and buffering capacity in water (Ebeling *et al.* 2006). Ebeling *et al.* (2006) also suggested that nitrogen uptake by heterotrophic process that likely to dominate BFT system consumes alkalinity half than nitrification (3.57 g alkalinity/g NH<sub>4</sub><sup>+</sup>-N). As alkalinity concentration relates to the buffering capacity of water, thus it could be suggested that in BFT system, the effect of the high concentration of CO<sub>2</sub> resulted from fish and microbial respiration on water pH could sufficiently buffered.

The difference in TAN concentrations between control and BFT treatments was expected as ammonia conversion rate in control treatments were slower than by heterotrophic bacteria in BFT treatments (Hargreaves 1998). The presence of NO<sub>2</sub>-N and NO<sub>3</sub>-N in both control and BFT treatments indicates the occurrence of nitrification processes in both culture systems. While NO<sub>2</sub>-N concentration in BFT treatments seems to be relatively stable, the opposite was observed in control treatments which might be explained by the higher rate of nitrification processes in these treatments. For the first 9 weeks of experimental period, NO<sub>3</sub>-N accumulation was observed in all treatments which were followed by a sharp decline on week 13. This decrease probably relates to NO<sub>3</sub>-N uptake by phytoplankton in both treatments in particular when there is limited ammonia-nitrogen available in the water (Hargreaves 1998). As most of ammonia in the culture system is up taken by heterotrophic bacteria, the availability of NO<sub>3</sub>-N in BFT system thus allows the phytoplankton to grow (Kirchman 1994; Middelburg & Nieuwenhuize 2000).

It should also be noted that the highest TAN concentrations observed in BFT treatments at 50 fish/m<sup>3</sup> (0.75 mg/l) and 100 fish/m<sup>3</sup> (1.04 mg/l) in this experiment were comparable to that reported from red tilapia culture in RAS with similar stocking densities and culture period (1.41 and 1.13 mg/l, respectively) (Suresh & Lin 1992). A different result however noticed with tilapia culture with BFT application in indoor tanks (Azim & Little 2008) where the inorganic nitrogen concentrations in RAS system was lower and more stable than that of BFT treatments.

Chl-a concentrations and phytoplankton abundance suggested that the rate of photoautotrophic nitrogen conversion in the control systems was higher than in BFT systems. There was a trend that chl-a concentrations increased at higher stocking densities which reflected the increasing level of nutrient waste as the culture became more intensified. The different class of phytoplankton that dominated control (Chlorophyceae) and BFT treatments (Bacillariophyceae) was possibly be caused of the regular addition of sodium silicate (1 mg/l) in BFT treatments that was aimed to stimulate biofloc formation (Zita & Hermansson 1994), which apparently also stimulated diatom growth in the system.

The high density of food (phytoplankton and bacteria) in both control and BFT treatments stimulate the growth of zooplankton which was dominated by non-specific feeder testate amoebas (Finlay & Esteban 1998). Madoni *et al.* (1993) reported that there was a correlation between the occurrence and abundance of protozoa species with the activated sludge operational performance, and *Arcella* and *Euglypha* were found to be directly associated with nitrifying condition in an activated sludge system.

The crude protein content of biofloc collected from BFT treatments was within the range of what have been previously studied (Azim & Little 2008; De Schryver & Verstraete 2009; Crab *et al.* 2010; Ekasari *et al.* 2010). Protein requirement for grow out culture of red tilapia seems to be varied from 20 to 42% (Hepher *et al.* 1983; Clark *et al.* 1990; Watanabe *et al.* 1990), indicating that protein level of biofloc in this study had met protein requirement of red tilapia. Crude lipid content with a range of 25-28% was by far higher than what has been measured in other studies that ranged from 2 to 5% (Azim & Little 2008; Azim *et al.* 2008; Crab *et al.* 2010). The reason for the high content of lipid could not be clearly explained, but it may relate to the biofloc biological composition. Ju *et al.* (2008) suggested that biofloc biological composition might influence its biochemical composition, whereas (Shifrin & Chisholm 1981) reported that diatom could contain lipid up to 25%. For that reason, it may be suggested that the high diatom density associated in bioflocs contributed to the high lipid content of biofloc. With regard to tilapia lipid requirement, Lim *et al.* (2009) noted that optimum dietary lipid requirement of tilapia is in a range of 5-12% suggesting that the lipid content of biofloc in this study was more than sufficient. High level of ash in biofloc (40%) was also reported in De Schryver and Verstraete (2009) when sodium acetate was used as

the carbon source and appeared to be affected by the source of organic carbon. The maximum level of ash content in fish feed seems to depend on the target fish species (Shearer *et al.* 1992; Gomes *et al.* 1995; Millamena 2002), several authors however generally suggested that the ash content of fish feed should be less than 13% (Tacon 1988; Craig & Helfrich 2009).

Overall water quality parameters suggested that control systems was likely to be dominated by photoautotrophic and to some extent chemoautotrophic microbial nitrogen conversion pathways. This was shown by the high concentration of chl-a ( $> 250 \text{ mg/m}^3$ ) and phytoplankton abundance as well as the presence of  $\text{NO}_2\text{-N}$  and  $\text{NO}_3\text{-N}$  in the water (Hargreaves 1998; Ebeling *et al.* 2006). In BFT systems on the other hand, though organic carbon source seems to stimulate heterotrophic bacterial nitrogen conversion, the presence of photoautotrophic and chemoautotrophic microbial processes were also evidenced by a considerable concentrations of chl-a and nitrification products. Similar findings were also observed in BFT application in commercial shrimp ponds in Belize, Central America, where manipulating C/N ratio did not increase heterotrophy (Burford *et al.* 2003).

The negative correlation between stocking density of tilapia with growth as well as other production parameters has been reported in previous study (Suresh & Lin 1992). Stickney (2005) noted that fish mortality at high stocking density may be caused by the accumulation of waste metabolites and dissolved oxygen limitation which relate to the high feeding input. BFT treatments, in particular at 50 and 100 fish/ $\text{m}^3$ , showed a higher survival than the control. Additionally, the survival differences between densities in BFT treatments was not as many as in Control, suggesting that the water quality in BFT treatments were better than control. Suresh and Lin (1992) also reported that the survival of red tilapia cultured a recirculating aquaculture system (RAS) at stocking densities of 50 and 100/ $\text{m}^3$  were 87.37 and 85.35 %, respectively, which were lower than the BFT treatments (93.56 and 93.00%, respectively) but comparable to the control (88.00 and 87.67%) in the present study.

The mean individual fish weight, total harvested biomass, growth, and production parameters of BFT treatments were relatively lower than control. Nevertheless, the differences were not statistically significant. The use of mixed sex red tilapia as the tested animal apparently resulted in an unexpected and uncontrolled breeding in the culture system which was observed in all treatment after the first month of culture. The larvae and offspring obtained from each treatment was then collected and counted (Figure 6), and revealed to be different between BFT and control treatments. The averages seed number in BFT treatments at all density tested were higher than what have been observed in control. The effect of biofloc on reproduction of aquatic organism has been recorded recently, where blue shrimp broodstock cultured in bioflocs system showed a better spawning performance than that of earthen pond (Emerenciano *et al.* 2011). The high reproductive activity in fish in BFT treatments may

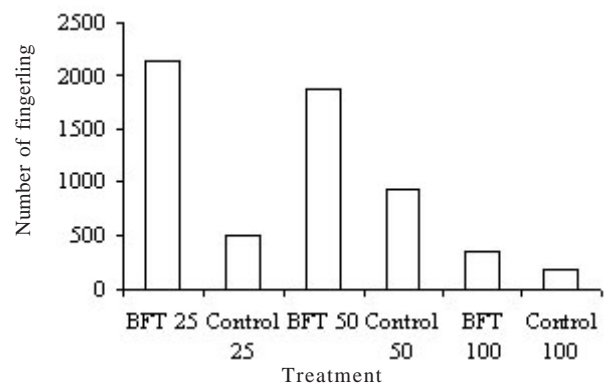


Figure 6. Red tilapia *Oreochromis* sp. offsprings collected from different treatments tanks throughout the experimental period.

explain the relatively lower fish growth in comparison to the control, as breeding process occurred most of the energy obtained from feeding will be allocated for gonad development.

In general, the value of feed efficiency in BFT treatments were better than control. The availability of biofloc in the tank was expected as a food source for the fish so that less commercial feed would be required in the biofloc system (Hari *et al.* 2004; Avnimelech 2007). This was also observed in the present experiment where the total feed input in BFT treatments was significantly lower than the controls. The lesser total feed used in BFT treatments observed in this experiment may be related to two possible reasons. First, the high suspended solids visually prevent the fish to consume their feed as what has been suggested by Azim and Little (2008). Secondly, the fish has been continuously fed on biofloc in the water and consequently reduced the fish feeding response as what was the case in Avnimelech (2007). The last reason was likely to occur in this experiment as visual observation showed that the feeding response of fish in BFT treatments was lower than the control. However, as reproduction process alters the energy for growth, the biomass gain of BFT treatments was lower than the control groups. Hence, even though feed input in BFT treatment was lower, the feed efficiency was not significantly different from control.

In conclusion, fish density as well as BFT application appears to have some influences on water quality and fish production performances. Our data confirms other studies on fish stocking density that higher fish density resulted in higher production but lower fish survival and growth. The application of BFT in red tilapia culture may improve the water quality and fish survival as well as reduce external feed requirement. The uncontrolled reproduction process however interrupted fish growth, and eventually other production parameters of red tilapia in BFT treatments. Therefore, another research using monosex species is required to closely study the effect of BFT application in red tilapia culture in stagnant water. Nonetheless, the higher number of offspring collected from BFT treatments indicates that bioflocs may have an effect on fish reproduction and it is therefore of interest to be further explored.

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