

# **International Journal of Recirculating Aquaculture**

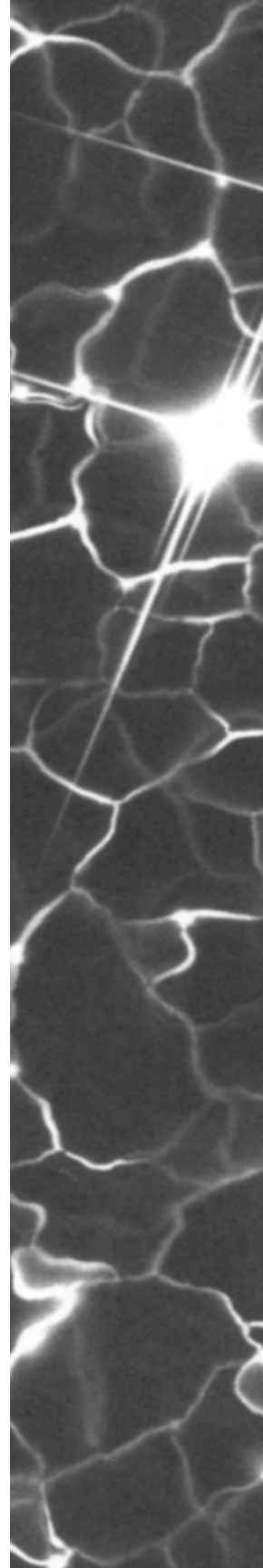
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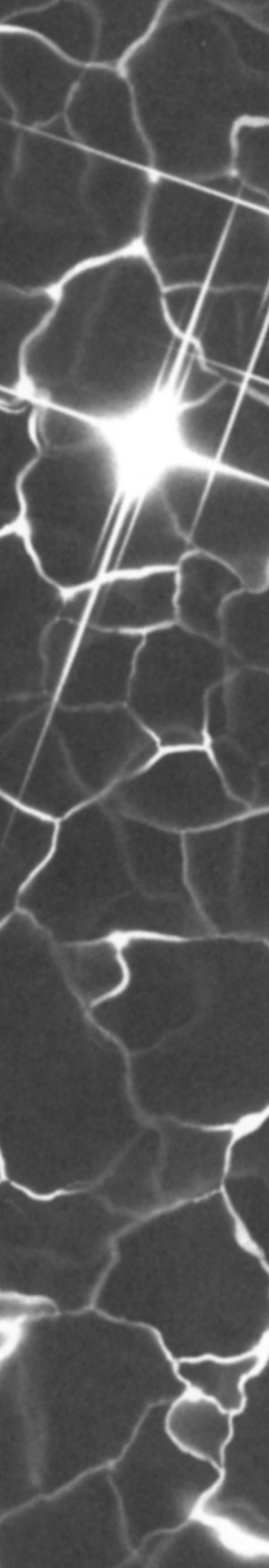
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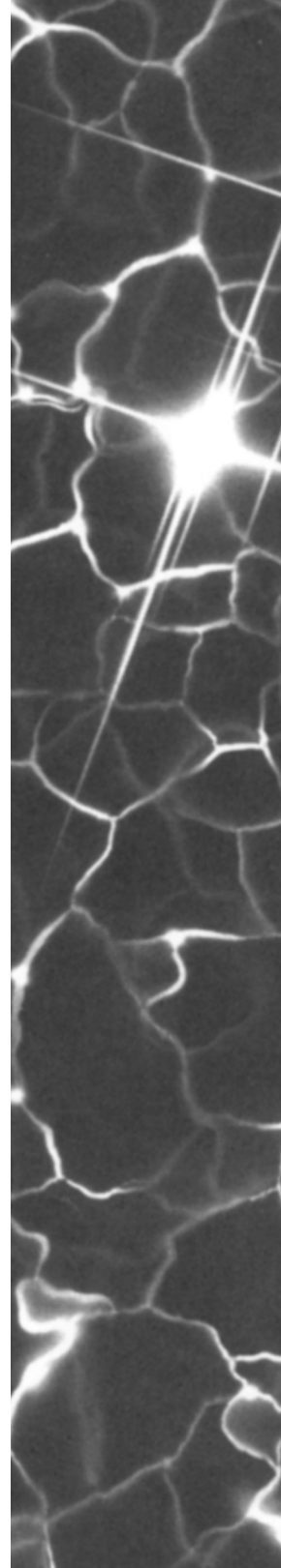


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# Select Hematological Values of the African Catfish (*Clarias gariepinus*) Raised in a Water Recirculating Aquaculture System

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Keywords: Hematology, recirculation, catfish aquaculture, Nigeria

## ABSTRACT

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Clinical evaluation of blood parameters is routinely used to assess the health of wild and domestic animals. The commercial catfish industry in Nigeria has undergone rapid expansion in recent years. An understanding of normal hematology values for healthy fish and the identification of predictors of the onset of health problems may enable fish health specialists to intervene before major losses occur. This paper reports values for selected hematological parameters of normal healthy African catfish (*Clarias gariepinus*) (n=120) raised in a recirculating aquaculture water recirculation system, including hemoglobin (Hb), red blood cells (RBC), packed cell volume (PCV), white blood cells (WBC), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), thrombocyte count, and leukocyte differential (lymphocytes, neutrophils and monocytes). Significant differences ( $p < 0.05$ ) were observed between males and females in the values of Hb, PCV, and thrombocytes. This work provides hematological baselines for selected values for *Clarias gariepinus* in recirculation systems, and is intended to enhance production performance through early detection and identification of infectious diseases.

## **INTRODUCTION**

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The African catfish, (*Clarias gariepinus*), belongs to the family Claridae, and is the most popular fish cultured in Nigeria, next to the tilapia fishes (FAO 1997, Adeogun *et al.* 2007). Aquaculture production in Nigeria was concentrated on tilapia culture before the clariids began to assert special importance in fish production in many parts of the country (AIFP 2004). According to De-Graaf and Janssen (1996), the reasons for preference of the clariids in tropical aquaculture includes hardiness to adverse environmental conditions, fast growth rates in captivity, easy procurement of fingerlings, adaptation to artificial feed, and high consumer preference. Additional attributes of this species, relevant to culture include high fecundity, potential for year-round induction of final oocyte maturation, remarkable nutrient conversion efficiency, and tolerance of high culture density (Legendre *et al.* 1992, Ayinla and Nwadukwe 2003).

With the recent expansion of the catfish industry in Nigeria, many farmers are now raising their fish using recirculating aquaculture systems. This is a clear departure from the traditional earthen pond culture system, which makes fish production seasonal and unreliable, to a more advanced, reliable, intensive, and results-oriented culture system (Akinrotimi *et al.* 2007a, Gabriel *et al.* 2009). With this level of intensive stocking density of fish, there is therefore the need to monitor the health status of cultured fish to prevent the outbreak of devastating diseases.

One of the difficulties in assessing the health of fish has been the scarcity of reliable references describing the normal condition. To achieve this goal, fish physiologists have employed hematology assessments to characterize the physiological status of fish (Kori-Siakpere *et al.* 2005). According to Wedemeyer *et al.* (1983), hematological studies are carried out in fish to ascertain the normal values in relation to age, sex, and culture system. Therefore the establishment of reference values with accepted limits is important for comparison of data obtained from a wild population with that of fish maintained in aquaculture conditions. With this information, significant changes in these values can be used to interpret the metabolic condition and overall health of fish (Gabriel *et al.* 2007a, Akinrotimi *et al.* 2007b).

Previous studies have determined blood parameters for this species in various culture systems such as reservoirs (Sowunmi 2003; Ezeri *et al.*

2004) and earthen ponds (Erondu *et al.* 1993, Omitoyin 2006, Akinrotimi 2008), but reports from recirculating aquaculture systems are lacking, thus necessitating the need for this work. The objective of this study was to report the hematological reference values for selected parameters of *Clarias gariepinus* reared in a recirculating system using sufficient numbers of fish to provide representative baseline values.

## **MATERIALS AND METHODS**

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Post-fingerlings of *Clarias gariepinus* were stocked in production recirculating aquaculture systems at Watershed Fish Farms, Nigeria Limited, Rumuodara, Port Harcourt, Rivers State, Nigeria and reared to market size (average 1,800.00g  $\pm$  1.01SD) over 5 months. The recirculating system in this farm measured 25m x 10m x 7m. The rate of water flow was 40,000 liters/day and the water exchange was continuous, with a stocking density of 150 fish/m<sup>2</sup>. For this study, 200 apparently healthy fish were carefully moved to other holding tanks to avoid stress from crowding and maintained for ten days. The fish were later sexed following the methods described by De-Graaf and Janssen (1996). The following water quality parameters were determined daily for a period of 10 days, and included temperature, pH, ammonia, nitrite, nitrate and dissolved oxygen using methods described by APHA (1998).

The fish were individually restrained manually, then blood samples (5.0 mL) were collected from the caudal vessels of male and female fish (60 each), using a heparinized plastic syringe fitted with a 21 gauge hypodermic needle, and immediately transferred to EDTA tubes. After collection of the blood samples, the fish were weighed (Sartorius model H112, Portugal). Measurement of each blood parameter was repeated for all 120 animals. Total RBC counts were obtained using a hemacytometer (Improved Neubauer, Model BS-713, Weber Scientific Limited, Middlesex, UK) using the method of Wintrobe (1934). Packed cell volume (PCV) was determined by filling heparinized hematocrit capillary tubes with blood, which was centrifuged for 5 minutes at 500  $\times$  g in a microhematocrit centrifuge (Model TDL60B, Hunan Xingke Scientific Instruments Co. Ltd, Hunan, China), following the methods of Serveid (1983). Hemoglobin was determined using the cyanmethemoglobin method (Blaxlall and Daisley 1973). The total WBC counts (WBC) were later enumerated in a hemacytometer (Improved Neubauer, Model BS-

713, Weber Scientific Limited, Middlesex, UK) using Shaw's diluting fluid (Miale 1982). Blood smears were stained with Grumwald-Giemsa for differential examination (Tavares Dias *et al.* 1999).

Statistical analysis was performed using SAS Software package (SAS Institute Inc., Cary, NC, USA). One-way analysis of variance (ANOVA) was applied to check for significant changes between male and female fish. Statistically significant differences were determined by Tukey's multiple comparison test. The reference values were calculated based on the minimum and maximum values of blood parameters (Zar 1996).

## RESULTS AND DISCUSSION

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The water quality parameters examined in this study indicated values characteristic of recirculation systems (Hrubec and Smith 2004). All parameters were within an acceptable range to enhance production performance of cultured catfish (Table 1).

In fish medicine, hematological profiles are one of the most frequently used methods to predict levels of disease and the impact of stressors in fish. Hematological characteristics of a number of cultured fish species have been studied, with the aim of establishing reference intervals useful in cases where significant deviations may indicate a disturbance in the physiological process (Raiza-Piava *et al.* 2000, Gabriel *et al.* 2004; Akinrotimi *et al.* 2007c). Many of these studies were attempted to determine if significant variations from normal values could be attributed to internal factors, or to factors external to the culture environment (Gabriel *et al.* 2007b).

Several factors have been reported to affect hematological parameters of teleost fish; these include species, sex, age, size, and environmental and culture conditions (Sowunmi 2003; Akinrotimi *et al.* 2009). In the assessment of the blood profile of black jaw tilapia, (*Sarotherodon melanotheron*), Akinrotimi *et al.* (2007d) observed that results from the female fish were consistently higher in all parameters examined, and suggested the need to separate blood component data on the basis of sex.

In this present work, significant differences ( $p < 0.05$ ) were found in female fish for Hb, PCV, and total thrombocyte count (Table 2). Similar findings were reported by Kori-Siakpere and Egor (1997) in *Clarias*

*buthapogun* and Kori-Siakpere (1985) in *C. isheriensis*. The gender differences may be due to the larger size of females (1900.00g  $\pm$  1.02SD) and higher hormonal interaction compared with the males (1700.00g  $\pm$  1.02SD) (Sowunmi 2003). The higher values of Hb observed in the female fish corroborate the reports of Akinrotimi *et al.* (2010) in *Tilapia guinnensis*. The higher values of blood parameters associated with oxygen transport suggest that under adverse environmental conditions that impact negatively on available oxygen, the females may be better equipped to handle such stressors than the males.

Values for hematological reference values determined in the 120 samples (Table 3) are comparable to those reported previously for hybrid striped bass (*Morone chrysops x Morone saxatilis*) raised in recirculating systems (Hrubec *et al.* 2004). The results were within the same range except in the value of Hb content. The reference interval of Hb in *Clarias gariepinus* (10.02-18.64 g/dL) was higher than that of hybrid striped bass (4.2-8.4 g/dL). The difference may be due to species-specific variation of the fish (Nikinmaa 2001). Hemoglobin may also show wide variability in sensitivity to effectors like organic phosphate, environmental conditions, and various fish management procedures in aquaculture (Angelids *et al.* 1987, Brauner and Randall 1999, Pelster 2001). However, the blood reference values obtained in this study contradict those reported for yellow perch reared in recirculating systems (Hrubec and Smith 2004). This difference may be due to species-specific hematological characteristics in teleost fish. Mauel *et al.* (2007) reported that species origin and breeding systems can influence hematological reference values in fish, as observed in *Tilapia* species maintained in recirculating systems. The hematological variables observed in this work were lower than those obtained previously for *C. gariepinus* cultured in freshwater tidal earthen ponds (Akinrotimi 2008). The higher values of *C. gariepinus* in tidal earthen ponds may be due to relatively high physical and metabolic activity in the fish raised in tidal systems, which are known to elicit a higher erythrocyte to plasma ratio in response to tidal shifts, which occur every six hours (Akinrotimi *et al.* 2010b).

Hence, the data reported in this study and those published previously indicate that these values can be a useful tool for veterinarians and aquaculturists in evaluating the health of cultured African catfish in recirculating aquaculture systems.

*Table 1. Water quality parameters in the recirculating aquaculture system for African catfish, (Clarias gariepinus), over 10 days.*

<b>Parameter</b>	<b>Mean <math>\pm</math> SD</b>	<b>Range Min-Max</b>
Temp ( $^{\circ}$ C)	28.66 $\pm$ 4.21	26.44 – 30.64
pH	7.64 $\pm$ 1.21	6.81 – 8.12
Dissolved oxygen (mg/L)	6.91 $\pm$ 1.02	5.81 – 7.99
Ammonia (mg/L)	0.016 $\pm$ 0.02	0.004 – 0.027
Nitrite (mg/L)	0.014 $\pm$ 0.01	0.006 – 0.024
Nitrate (mg/L)	3.64 $\pm$ 0.61	2.01 – 5.67

Table 2. Hematological parameters (Mean  $\pm$ SD) for male and female African catfish, (*Clarias gariepinus*), reared in recirculating aquaculture system.

Parameter	Male (n=60)	Male Reference	Female (n=60)	Female Reference
	(W=1700.00g $\pm$ 1.01SD)	Range	(W=1900.00g $\pm$ 1.02SD)	Reference Range
Hemoglobin (g 1D/dL)	14.86 $\pm$ 2.42 <sup>a</sup>	10.02 – 16.74	16.99 $\pm$ 3.26 <sup>b</sup>	11.22 – 18.64
Red blood cell (x10 <sup>12</sup> /L)	4.98 $\pm$ 0.81 <sup>a</sup>	3.05 – 6.99	7.38 $\pm$ 1.22 <sup>b</sup>	4.26 – 8.64
Packed cell volume (%)	36.21 $\pm$ 4.11 <sup>a</sup>	32.64 – 40.70	41.31 $\pm$ 1.21 <sup>b</sup>	36.71 – 45.74
White blood cell (x10 <sup>9</sup> /L)	21.68 $\pm$ 3.41 <sup>a</sup>	18.66 – 23.98	22.74 $\pm$ 3.66 <sup>a</sup>	19.10 – 25.61
Mean corpuscular volume (fl)	72.71 $\pm$ 10.32 <sup>a</sup>	72.11 – 78.66	82.95 $\pm$ 9.74 <sup>b</sup>	75.14 – 82.95
Mean corpuscular hemoglobin (pg)	33.92 $\pm$ 4.61 <sup>a</sup>	30.21 – 36.22	34.11 $\pm$ 5.22 <sup>a</sup>	34.18 – 46.74
Mean corpuscular hemoglobin concentration (g/dL)	41.63 $\pm$ 7.11 <sup>a</sup>	38.21 – 46.72	41.12 $\pm$ 6.34 <sup>a</sup>	38.28 – 46.68
Thrombocytes (x10 <sup>9</sup> /L)	102.64 $\pm$ 1.11 <sup>a</sup>	92.01 – 114.68	142.61 $\pm$ 7.11 <sup>b</sup>	110.34 – 158.74
Lymphocytes (%)	64.22 $\pm$ 6.71 <sup>a</sup>	51.14 – 70.10	64.64 $\pm$ 5.11 <sup>a</sup>	55.22 – 70.16
Neutrophils (%)	32.14 $\pm$ 3.66 <sup>a</sup>	28.21 – 40.14	31.58 $\pm$ 3.76 <sup>a</sup>	27.64 – 39.78
Monocytes (%)	3.64 $\pm$ 1.14 <sup>a</sup>	1.86 – 3.92	3.78 $\pm$ 1.31 <sup>a</sup>	1.99 – 4.01

Where W = average weight  
Means within the row are denoted with different superscripts where significant (P < 0.05)

Table 3. Hematological reference values of African catfish, *Clarias gariepinus*, reared in a recirculating aquaculture system (n = 120).

Parameter	Mean ± SD	Reference range
Hemoglobin (g/dL)	15.93 ± 4.61	10.02 - 18.64
Red blood cell (x10 <sup>12</sup> /L)	4.68 ± 1.71	3.051 - 8.64
Packed cell volume (%)	38.76 ± 8.42	32.64 - 45.74
White blood cell (x10 <sup>9</sup> /L)	22.21 ± 6.46	18.66 - 25.61
Mean corpuscular volume (fl)	82.81 ± 9.66	72.11 - 91.34
Mean corpuscular hemoglobin (pg)	34.02 ± 6.01	30.21 - 46.74
Mean corpuscular hemoglobin concentration (g/dL)	41.38 ± 7.11	38.21 - 46.74
Thrombocytes (x10 <sup>9</sup> /L)	122.63 ± 12.61	92.01 - 158.74
Lymphocytes(%)	64.43 ± 9.64	51.14 - 70.16
Neutrophils (%)	31.86 ± 6.42	27.64 - 40.14
Monocytes (%)	3.71 ± 1.02	1.86 - 4.01
Hemoglobin (g/dL)	15.93 ± 4.61	10.02 – 18.64
Red blood cell (x10 <sup>12</sup> /L)	4.68 ± 1.71	3.051 – 8.64
Packed cell volume (%)	38.76 ± 8.42	32.64 – 45.74
White blood cell (x10 <sup>9</sup> /L)	22.21 ± 6.46	18.66 – 25.61
Mean corpuscular volume (fl)	82.81 ± 9.66	72.11 – 91.34
Mean corpuscular hemoglobin (pg)	34.02 ± 6.01	30.21 – 46.74
Mean corpuscular hemoglobin concentration (g/dL)	41.38 ± 7.11	38.21 – 46.74
Thrombocytes (x10 <sup>9</sup> /L)	122.63 ± 12.61	92.01 – 158.74
Lymphocytes (%)	64.43 ± 9.64	51.14 – 70.16
Neutrophils (%)	31.86 ± 6.42	27.64 – 40.14
Monocytes (%)	3.71 ± 1.02	1.86 – 4.01

Means within the row are denoted with different superscripts where significant (P < 0.05)

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# Water Quality and Nutrient Aspects in Recirculating Aquaponic Production of the Freshwater Prawn, *Macrobrachium rosenbergii* and the Lettuce, *Lactuca sativa*

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## ABSTRACT

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The purpose of this study was to investigate the effects of different nutrients and their ability to improve the production of *Macrobrachium rosenbergii* and *Lactuca sativa* in a prototype recirculating aquaponic (RA) system. Experimental units were set up with different amounts of supplemented organic and inorganic (complex minerals) nutrients to carry out the study. The results indicated that desirable growth of *M. rosenbergii* might be possible in RA systems when supplied sufficient levels of macro-micro nutrients. Analyses of nutrients in the prawn culture tanks demonstrated that ammonia and nitrate concentrations were critical in maintaining proper water quality during the culture period. Five-day biological oxygen demand (BOD<sub>5</sub>) increased significantly with the increased loading of organic supplement in the rearing tanks. A significant linear relationship of chlorophyll a and N:P ratio was observed among the treatments. The combination of complex minerals and organic chicken manure (CM15) displayed a higher N:P ratio, maximal total yield and did not show adverse effects of NH<sub>3</sub> concentrations and other important water quality parameters.

## INTRODUCTION

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In the last decade, there has been increased interest in integrated aquaculture systems in line with increased activities for sustainable agriculture in developing and developed countries (Langdon et al. 2004). A wide variety of organic and inorganic materials (raw or pure by-product) can be used as supplements in fish and prawn aquaculture (Green et al. 1989). Meanwhile, large volumes of discharged aquaculture waste can become a serious source of pollution with environmental risk (Pillay 1992; Brown et al. 1999; Troell et al. 2009; Endut et al. 2010).

The giant freshwater prawn (*Macrobrachium rosenbergii*) has received the most attention from researchers and farmers due to its nutritional value, taste and demand in the market (Schwantes et al. 2009). *Macrobrachium rosenbergii* production is economical and more environmentally sustainable compared to conventional intensive shrimp production. Information on stocking density and requirements of *M. rosenbergii* in monoculture systems is available (Marques et al. 2000). However, the development and production of freshwater prawn with high level efficiency in aqua/agriculture systems still requires the identification and evaluation of specific requirements (food and nutrient) of the different species cultivated in these systems.

Aquaculturists are continually looking for new ways to produce more aquatic animals with less water, land and pollution to minimize adverse environmental impacts. One source–waste reduction approach is the production of vegetables in the wastewater and effluents. Wastewater, effluents and sludge from semi-extensive or intensive aquaculture systems are potential sources of irrigation water, nutrients and media for vegetable crops (Adler et al. 2003). Accordingly, recirculating aquaponic technology acts as a small sewage treatment system to clean up the water and decrease nutrient concentrations. Aquaponic thin-film allows plants to selectively extract nutrients from water making dilute effluents a similar source of nutrients as more concentrated effluents.

Although integrated systems appear to show diversification and efficiency, they are not always successful and popular in some regions (tropical and subtropical for example). Undesirable results, lack of financial support and technical problems have led to a significant

decrease in the importance of integrated culture farming. A basic problem in such a system may arise from the discrepancy between productive compartments and un-optimized intensity of the plant and aquatic species in the system (Rakocy et al.1993; Khoda Bakhsh 2008).

In fact, very little information is available on the concentration limits of nutrient elements (especially microelements) at which deficiency or toxicity may occur in the recirculating aquaponic systems (Khoda Bakhsh et al. 2007). Poor quality of water, mineral toxicity and nutrient deficiency are still problematic in integrated fish/prawn production, especially in the early stages of the life cycle (fry and fingerling). Indeed, for widespread utilization of recirculating aquaponic systems and exploitation of their maximal potential, there is a need for more information on the types of inorganic nutrients, volumes of organic substances, proper stocking densities, feed conversion ratios (FCR) and water quality.

The objective of this study was to evaluate the beneficial effects of supplemented inorganic and organic substances on the production of *M. rosenbergii* and *L. sativa* in a prototype recirculating aquaponic system. The outputs and relevant expected information including nutrient dynamics, biological oxygen demand (BOD), primary productivity (chlorophyll a), and growth performance will serve as a basis for future studies and provide some recommendations for aquaculturists and farmers that might improve their chances of succeeding with new production technology.

## **MATERIALS AND METHODS**

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Twenty fiberglass tanks (1m<sup>3</sup>) were installed to evaluate different amounts of supplemental nutrients and new design in recirculated culture systems. Experimental units consisted of a rearing container (500 liters), aeration tank (300 liters) and hydroponic nutrient film technique (NFT) trays (110 L x 80 W x 5 cm H). Each NFT unit consisted of 45 lettuce seedlings (m<sup>2</sup>) and all plant troughs were located over the reservoir-aeration tanks. Rearing tanks were exposed to natural light conditions (12 hours/day) to mimic natural conditions for prawn growth (Figure 1).

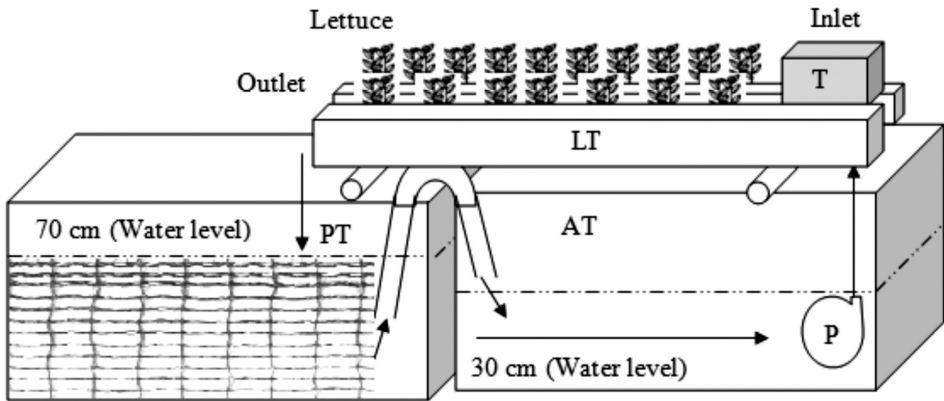


Figure 1: Arrangement of the prawn culture, aeration tank and aquaponic troughs in the recirculating aquaponic system (PT-Prawn Tank, AT-Aeration Tank, AS-Artificial Substrate, P-Pump, T-Trickling system and LT-Lettuce Trough).

The culture water effluent was transferred to the aeration tank continuously and passed through the vegetable troughs by using an electric pump (Aquanic Power Head 1500). *Macrobrachium rosenbergii* juveniles were stocked at 380/m<sup>3</sup> and all tanks were provided with artificial substrate (polyethylene net) to increase available surface area (50%). To acclimate prawns to the prototype system, the partial stock of *M. rosenbergii* (55 juveniles /day) were adjusted together with seedlings of lettuce during the first week of the study. This system was not provided a specific fluidized-sand biofilter to remove solid-suspended waste. The simple trickling system and shallow streams in plant trays provided a suitable compartment for trap and mineralization of suspended solids in recirculating water before returning to the prawn tanks. Juveniles of *M. rosenbergii* were fed a commercial prawn diet two times daily (9:00 and 17:00). The feeding rate was adjusted according to the average body weight of the prawns every week, and gradually reduced from 30% (starter) to 10% (grower) during the study.

The physical and chemical parameters of the water in the prawn tanks were monitored weekly. Water quality factors were measured using standard apparatus and all determinations were recorded between 12:00 and 13:00. Dissolved oxygen (DO), temperature (°C) and pH of the rearing water were determined using an YSI DO (550 DO) and pH meter (60-10 FT). The specific conductivity (mS/cm), salinity (ppt), and turbidity (NTU) were measured in the field by in situ measurement with an HYDROLAB DATASONDE® 4a. The chemical parameters, including ammonia (NH<sub>3</sub>) and nitrate (NO<sub>3</sub>), were measured by the salicylate method (HACH kit DR

2010). Available nitrogen (N) and phosphorus (P) were determined with an auto-analyzer (LACHAT instrument, 8000 Series) and atomic absorption spectrometry (Perkin Elmer 350). Five-day biological oxygen demand (BOD<sub>5</sub>) and chlorophyll a contents of benthic algae were measured by standard methods (APHA 1995). The chlorophyll a content in benthic algae was initially determined by measuring the absorbance of acetone extract at 750, 664, 647, and 630 nm with a spectrophotometer (Thermo Spectronic 4001/4).

Lettuce growth analysis included total yield, and fresh and dry weight (oven dried at 105°C) which were carried out using a digital balance (Sartorius, BP 310S) at the end of the experiment. The survival and specific growth rate (SGR), average daily growth (ADG), net yield and feed conversion ratio (FCR) of freshwater prawn were calculated at the end of the experiment. The available information on water quality and *M. rosenbergii* growth (SGR and ADG) of nearby prawn ponds was recorded for overall comparison of the different culture system.

Complex mineral and organic supplements were used in order to meet nutrient requirements of *L. sativa* and *M. rosenbergii* together. Minerals were prepared to adjust specific conductivity from 0.2 to 0.4 mS/cm as followed: calcium nitrate (68.80 mg/l), EDTA iron (3.50 mg/l), potassium dihydrogen phosphate (18.10 mg/l), potassium nitrate (21.90 mg/l), magnesium sulphate (41.40 mg/l), manganous sulphate (0.4 mg/l), boric acid (0.10 mg/l), copper sulphate (0.02 mg/l), ammonium molybdate (0.023 mg/l) and zinc sulphate (0.03 mg/l). The complex minerals were applied to the first treatment group (CM15) together with 15 g/m<sup>2</sup>/week of oven dried chicken manure. By increasing the rate of chicken manure (30-50 g/m<sup>2</sup>), the level of supplemented minerals was reduced by 50% in CM50 and 30% in CM30 treatment, respectively. Unfertilized freshwater (UFW) and culture system enriched with 70 g chicken manure (CM70) were operated as controls in this study. The fixed-equivalent portion of the nutrients was added to the reservoir-aeration tanks every week.

### **Statistical Analysis**

Experimental units were arranged in a randomized design with two replicates. Significant difference in the mean number of water quality and growth rate variables between control (no supplements) and enriched media were determined by one-way analysis of variance (ANOVA) followed by Duncan's New Multiple Range Test (P<0.05).

## RESULTS

### Water Quality Variables

Most water quality parameters were significantly higher in the recirculating aquaponic systems than in natural ponds except for temperature, turbidity and ammonia concentration (Table 1). A quadratic response of ammonia over time was observed for CM15 ( $y = 0.0103x^2 - 0.0548x + 0.2489$ ,  $R^2 = 0.60^*$ ,  $n = 8$ ), CM30 ( $y = 0.017x^2 - 0.1105x + 0.3631$ ,  $R^2 = 0.81^{**}$ ,  $n = 8$ ) and CM70 ( $y = 0.0084x^2 - 0.0335x + 0.271$ ,  $R^2 = 0.64^*$ ,  $n = 8$ ) treatments. A sharp increase in ammonia was evident in weeks 4 and 7 of the UFW treatment (Figure 2).

Table 1: Mean ( $\pm$ se) temperature (T), dissolved oxygen (DO), specific conductivity (SPC), salinity (Sal), turbidity (Tur), pH, total dissolved solid (TDS) and ammonia (NH<sub>3</sub>) concentration of different treatments in the recirculating aquaponic system.

Treatment	T (°C)	DO (mg/l)	SPC (mS/cm)	Sal (ppt)	Tur (NTU)	pH	TDS (g/l)	NH <sub>3</sub> (mg/l)
CM15	27.0 $\pm$ 0.3 <sup>a</sup>	7.09 $\pm$ 0.11 <sup>b</sup>	0.24 $\pm$ 0.04 <sup>b</sup>	0.12 $\pm$ 0.02 <sup>ab</sup>	1.53 $\pm$ 0.4 <sup>a</sup>	7.54 $\pm$ 0.17 <sup>b</sup>	0.16 $\pm$ 0.02 <sup>b</sup>	0.27 $\pm$ 0.04 <sup>a</sup>
CM30	26.7 $\pm$ 0.2 <sup>a</sup>	7.09 $\pm$ 0.16 <sup>b</sup>	0.23 $\pm$ 0.04 <sup>b</sup>	0.11 $\pm$ 0.02 <sup>ab</sup>	1.96 $\pm$ 0.6 <sup>a</sup>	7.47 $\pm$ 0.12 <sup>b</sup>	0.15 $\pm$ 0.02 <sup>b</sup>	0.31 $\pm$ 0.05 <sup>a</sup>
CM50	26.7 $\pm$ 0.3 <sup>a</sup>	7.06 $\pm$ 0.11 <sup>b</sup>	0.22 $\pm$ 0.03 <sup>b</sup>	0.19 $\pm$ 0.09 <sup>b</sup>	1.69 $\pm$ 0.6 <sup>a</sup>	7.41 $\pm$ 0.06 <sup>b</sup>	0.14 $\pm$ 0.02 <sup>b</sup>	0.30 $\pm$ 0.03 <sup>a</sup>
UFW	26.6 $\pm$ 0.3 <sup>a</sup>	7.01 $\pm$ 0.09 <sup>b</sup>	0.17 $\pm$ 0.02 <sup>b</sup>	0.08 $\pm$ 0.01 <sup>ab</sup>	0.95 $\pm$ 0.3 <sup>a</sup>	7.53 $\pm$ 0.04 <sup>b</sup>	0.11 $\pm$ 0.01 <sup>b</sup>	0.30 $\pm$ 0.04 <sup>a</sup>
CM70	26.7 $\pm$ 0.2 <sup>a</sup>	7.20 $\pm$ 0.09 <sup>b</sup>	0.21 $\pm$ 0.03 <sup>b</sup>	0.10 $\pm$ 0.02 <sup>ab</sup>	2.03 $\pm$ 0.5 <sup>a</sup>	7.67 $\pm$ 0.06 <sup>b</sup>	0.13 $\pm$ 0.02 <sup>b</sup>	0.36 $\pm$ 0.05 <sup>a</sup>
Pond	29.7 $\pm$ 0.8 <sup>b</sup>	4.90 $\pm$ 0.30 <sup>a</sup>	0.06 $\pm$ 0.01 <sup>a</sup>	0.02 $\pm$ 0.01 <sup>a</sup>	22.53 $\pm$ 1.5 <sup>b</sup>	6.87 $\pm$ 0.11 <sup>a</sup>	0.04 $\pm$ 0.01 <sup>a</sup>	0.41 $\pm$ 0.21 <sup>a</sup>

Means within a column followed by the same letter are not significantly different by determination of the Duncan's multiple-range test ( $P < 0.05$ ).

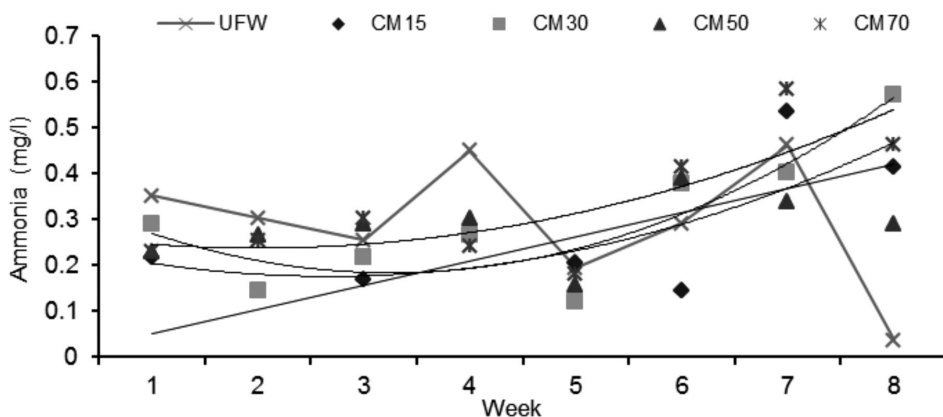


Figure 2: Changes of ammonia concentration in the recirculating aquaponic system.

### Five-day Biological Oxygen Demand (BOD<sub>5</sub>)

Five-day biological oxygen demand was significantly higher in all enriched treatments compared to the UFW media (Table 2). The BOD<sub>5</sub> increased significantly with increasing chicken manure loading rates in the rearing tank (Figure 3). The value of BOD<sub>5</sub> can be predicted from the amount of chicken manure used ( $x$ , g CM week<sup>-1</sup>) with the following equation:  $y = 0.0018x + 0.0813$ ,  $R^2 = 0.9096^{**}$ ,  $n = 10$ .

Table 2: Five-day biological oxygen demand (BOD<sub>5</sub>) in the recirculating aquaponic system (mean  $\pm$  se).

Treatment	BOD <sub>5</sub> mg/l
CM15	0.12 $\pm$ 0.01 <sup>b</sup>
CM30	0.15 $\pm$ 0.00 <sup>bc</sup>
CM50	0.17 $\pm$ 0.01 <sup>cd</sup>
UFW	0.07 $\pm$ 0.01 <sup>a</sup>
CM70	0.20 $\pm$ 0.02 <sup>d</sup>

Means within a column followed by the same letter are not significantly different by determination of the Duncan's multiple-range test ( $P < 0.05$ ).

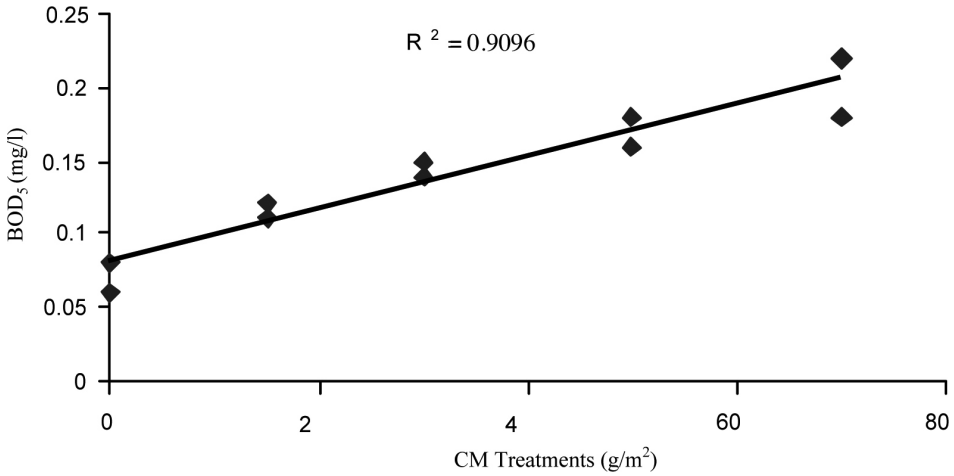


Figure 3: Linear relationship between five-day biological oxygen demand (BOD<sub>5</sub>) and treatments fertilized with chicken manure (CM).

### Chlorophyll a content and N:P Ratios

A significant linear relationship between the chlorophyll a content (periphyton and benthic algae) and N:P ratios was observed among the treatments (Figure 4). The lowest chlorophyll a content ( $P < 0.05$ ) was recorded in CM15, followed by the CM30 and CM50 treatments ( $y = 254.43x + 76.255$ ;  $R^2 = 0.8651^{**}$ ,  $n = 10$ ). The combination of complex minerals and chicken manure showed increasingly higher N:P ratios in CM15, CM30 and CM50, respectively ( $y = -1.698x + 14.94$ ;  $R^2 = 0.7803^{**}$ ,  $n = 10$ ). The UFW and CM70 media represented the lower range of nitrogen versus phosphorus during the culture period.

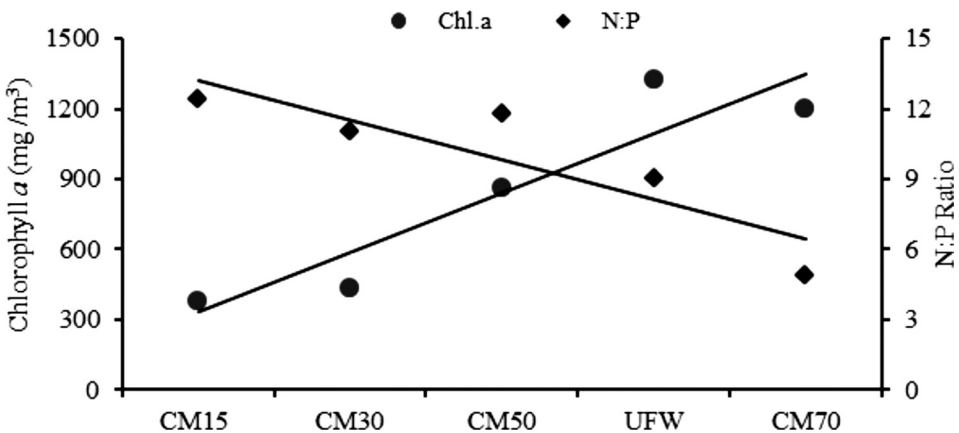


Figure 4: Concentration of chlorophyll a (benthic algae) and N: P ratio in *M. rosenbergii* culture tanks.

## Plant and Prawn Growth

The plant bioassay did not show any significant differences among enriched treatments. Plant growth was low in UFW media and displayed a significant difference in the yield, leaf and root weight (dry) when compared to the CM15 treatment at the end of the experiment (Table 3). No significant difference in root weight (wet) was observed among the treatments. The best performance of plant growth was recorded in the CM15 medium supplemented with minerals plus chicken manure (15 g/week).

*Table 3: Weight (g/plant) and total yield of Lactuca sativa at harvest in the recirculating aquaponic system (mean  $\pm$  se).*

Treatment	Leaf wet weight (g)	Leaf wet weight (g)	Root wet weight (g)	Root wet weight (g)	Yield (g/tank)
CM15	39.6 $\pm$ 7.22 <sup>b</sup>	1.7 $\pm$ 0.43 <sup>b</sup>	4.2 $\pm$ 1.11 <sup>a</sup>	0.22 $\pm$ 0.03 <sup>b</sup>	1783.4 $\pm$ 325 <sup>b</sup>
CM30	24.1 $\pm$ 9.12 <sup>ab</sup>	1.1 $\pm$ 0.23 <sup>ab</sup>	4.1 $\pm$ 2.72 <sup>a</sup>	0.12 $\pm$ 0.08 <sup>ab</sup>	1086.3 $\pm$ 410 <sup>ab</sup>
CM50	17.6 $\pm$ 2.01 <sup>ab</sup>	0.9 $\pm$ 0.01 <sup>ab</sup>	2.1 $\pm$ 0.28 <sup>a</sup>	0.10 $\pm$ 0.01 <sup>ab</sup>	791.6 $\pm$ 90 <sup>ab</sup>
UFW	1.9 $\pm$ 0.62 <sup>a</sup>	0.3 $\pm$ 0.05 <sup>a</sup>	0.3 $\pm$ 0.04 <sup>a</sup>	0.03 $\pm$ 0.01 <sup>a</sup>	84.2 $\pm$ 28 <sup>a</sup>
CM70	16.7 $\pm$ 10.33 <sup>ab</sup>	0.7 $\pm$ 0.33 <sup>ab</sup>	1.4 $\pm$ 0.87 <sup>a</sup>	0.06 $\pm$ 0.04 <sup>ab</sup>	753.1 $\pm$ 465 <sup>ab</sup>

Means within a column followed by the same letter are not significantly different by determination of the Duncan's multiple-range test ( $P < 0.05$ ).

For prawn, the CM15, UFW and CM70 treatments resulted in better SGR (%) than in natural ponds and the ADG was significantly higher in the CM15 treatment followed by the UFW and CM70 culture tanks. The highest prawn yield was observed in CM15, followed by CM50, CM30, CM70 and UFW. The minimum and maximum levels of FCR (0.42-1.18) were observed in the CM15 and UFW rearing tanks, respectively (Table 4).

Table 4: Survival rate (%), specific growth rate (SGR), average daily growth (ADG), net yield and feed conversion ratio (FCR) of *Macrobrachium rosenbergii* in the recirculating aquaponic system and prawn pond (mean  $\pm$  se).

Treatment	Survival (%)	SGR (%/d)	ADG (per day)	Yield g/tank	FCR
CM15	87.9 $\pm$ 0.8 <sup>c</sup>	6.02 $\pm$ 0.003 <sup>e</sup>	0.07 $\pm$ 0.001 <sup>e</sup>	1343.0 $\pm$ 11 <sup>d</sup>	0.42 $\pm$ 0.004 <sup>a</sup>
CM30	90.0 $\pm$ 0.7 <sup>c</sup>	5.17 $\pm$ 0.011 <sup>b</sup>	0.04 $\pm$ 0.001 <sup>b</sup>	840.1 $\pm$ 13 <sup>b</sup>	0.67 $\pm$ 0.010 <sup>b</sup>
CM50	93.8 $\pm$ 1.4 <sup>c</sup>	5.16 $\pm$ 0.002 <sup>b</sup>	0.04 $\pm$ 0.001 <sup>ab</sup>	869.5 $\pm$ 14 <sup>c</sup>	0.65 $\pm$ 0.010 <sup>b</sup>
UFW	41.0 $\pm$ 1.7 <sup>a</sup>	5.68 $\pm$ 0.027 <sup>d</sup>	0.05 $\pm$ 0.001 <sup>d</sup>	481.1 $\pm$ 15 <sup>a</sup>	1.18 $\pm$ 0.035 <sup>c</sup>
CM70	75.0 $\pm$ 3.5 <sup>b</sup>	5.47 $\pm$ 0.059 <sup>c</sup>	0.05 $\pm$ 0.002 <sup>c</sup>	820.5 $\pm$ 14 <sup>b</sup>	0.69 $\pm$ 0.012 <sup>b</sup>
Pond	-	5.03 $\pm$ 0.015 <sup>a</sup>	0.04 $\pm$ 0.001 <sup>a</sup>	-	-

Means within a column followed by the same letter are not significantly different by determination of the Duncan's multiple-range test ( $P < 0.05$ ).

## DISCUSSION

### Water Quality

In an aquatic ecosystem, fish and prawn are directly affected by several chemical and physical factors. The major water quality factors important in freshwater aquaculture were evaluated in this study. All pH, DO and measured water quality parameters were within acceptable limits for freshwater prawn culture, however, disparity and abnormal concentration of ammonia influenced the productivity of *M. rosenbergii* and *L. sativa* in the recirculating aquaponic systems. The DO concentration was higher in prawn culture tanks when compared to that in natural ponds (Table 1). Adequate DO is necessary for good water quality in intensive aquaculture systems (Stickney 1994; Alon et al. 2008). The DO results in the prawn-plant system illustrated the effectiveness of the second aeration tank to re-oxygenate water from *M. rosenbergii* rearing tanks. Temperature ranged from 26.6 to 27.0°C, typical of operating during the rainy season (November to January). Most of the available studies on temperature tolerances were conducted on *M. rosenbergii* production in earthen ponds

or larval stages in tanks (FAO 2002). Data on the quantitative relationship between water temperature and juvenile or adult production of prawns in indoor recirculating aquaponic systems are still rare. Generally, temperatures of 26-31°C are considered satisfactory for prawn growth (New 1995). There is an advantage of a lower range of temperatures (at the lower end of 25-32°C) for freshwater prawn growth because lower temperatures delay sexual maturity so more energy is used for muscle growth rather than sexual development. According to Tidwell et al. (1994), prawn cultured in ponds with average water temperatures of 25° showed higher production rates (11.5 kg/ha/day). These lower culture temperatures appeared to increase both total production and the percentage of market-size prawns.

The CM15 treatment with the higher level of total dissolved solids (TDS) showed lower turbidity than the other enriched treatments. In fact, high turbidity in CM30, CM50 and CM70 culture tanks was related to different application rates of chicken manure (brown color) rather than suspended solids. In water or wastewater, total solid (TS) includes both total suspended solids (TSS) and TDS and is related to both specific conductance and turbidity (APHA 1995). Changes in TDS concentrations (either too high or too low) can be harmful and may even cause death because their relative densities determine the flow of water into and out of an organism's cells (Murphy 2002). The increase in conductivity, TDS and TSS, based on accumulation of nutrients and solid waste, are important factors for design, waste and operating performance. In aquaponic systems, the conductivity may reach critical levels (2000 mg/l as TDS) by additions of approximately 10 kg feed/m<sup>3</sup> system volume (Rakocy et al. 1993). High concentrations of suspended solids should be avoided as they form an additional source of ammonia, which in its unionized form is highly toxic to fish and crustaceans. Furthermore, suspended solids may cause gill damage by fouling, resulting in stress and increased susceptibility to diseases (hyperplasia in gill tissue). Removal of small suspended solids can be accomplished by either chemical or biological oxidation. Rakocy (1999) stated that large amounts of TSS may accumulate on plant roots and produce a deleterious affect by creating anaerobic zones and blocking the flow of water and nutrients into the plant. The mineralization of organic matter (suspended solids) by microorganisms and aerobic bacteria may produce adequate nutrients for plant growth. In aquaponics, solids mineralization may occur in deeper

parts of media beds. With high fish density and more solid fish waste, the deeper tank. The water level beneath the rafts is anywhere from 250 to 500 mm deep and as a result the volume of water is approximately four times greater than in other systems. This higher volume of water results in lower nutrient concentrations hence, higher feeding ratios are recommended for the release of soluble nutrients and improved plant growth (Rakocy et al. 2006). By increasing feed, the total solids and suspended waste will be increased to accelerate mineralization. This trend seems unsustainable and costly, because feeds are the only available source to produce minerals through long physical-biological pathways. Thus, a separate biofilter is needed to remove excess solid wastes and provide suitable surface-medium for bacterial activities. parts of the bed can actually turn into an anaerobic zone (lack of oxygen) where anaerobic mineralization can occur. This anaerobic zone can release gases and chemicals which may be toxic to the organisms living in the system, whether fish, plants or aerobic bacteria. Therefore, commercial models of aquaponic systems are calibrated to provide required bed surface area to aerobically mineralize the solid fish wastes using a separate biofilter or 30 cm gravel-sand deep media in floating raft system and flood–drain system (Rakocy et al. 2006).

These aquaponic models do not require the addition of synthetic, chemical fertilizer as the fish waste from the rearing tank and mineralization of bacteria provides sufficient amounts of ammonia, nitrate, nitrite, phosphorus, potassium and micronutrients (Diver 2006; Spade 2009; Connolly and Trebic 2010). Complex macro-micronutrients would be required in shallow media beds to recover low mineralization and sequence nutrient deficiency in hydroponic plants.

The major difference between the commercial raft systems and our recirculating aquaponic system is the amount of water used (depth of media) and reservoir-aeration

### **Five-day Biological Oxygen Demand (BOD5)**

The density of organic matter in aquaculture ponds is related to feed losses, aquatic animal feces and other organic wastes produced by culture activities. A 5-day biological oxygen demand is the measure of organic matter in the waste over a five day time period. This study indicated a significant relationship of BOD5 and loading of CM ( $R^2=0.91^{**}$ ) in rearing tanks (Figure 3). Maclean et al. (1994) reported that mean concentration of BOD0.5 increased from 1.8 to 2.0 mg O<sub>2</sub>/l/12h

by decreasing the frequency of CM application in prawn ponds. They concluded that higher oxygen requirement, due to algal and bacterial respiration (through organic decomposition), influenced the trend of BOD<sub>0.5</sub> in the treatments.

Organic fertilizer has been used to stimulate the development of heterotrophs (bacteria), autotrophs (alga) and other food organisms in the aquatic ecosystem. When biodegradable organic matter is released into the water, microorganisms, especially bacteria, feed on the waste and break it down into simpler organic and inorganic substances. This decomposition takes place in aerobic conditions (Polprasert 1996). The total amount of oxygen required for biodegradation is an important measure of organic loading and bacteria activity, while stabilizing decomposable organic matter under aerobic condition (Boyd 1990). In our recirculating aquaponic system, the installation of a second aeration tank assisted all biodegradation phenomena with exposure to adequate dissolved oxygen. Dissolved oxygen affects water chemically by the oxidation of minerals and physically through the stripping of organic volatiles that are generated in prawn rearing tanks.

### **Organic Fertilizer, Primary Productivity and N:P Ratio**

From our study, the application of 70g/m<sup>3</sup> CM (alone) promoted the growth of benthic and filamentous algae. The relative advantages of organic and inorganic fertilizers and their effectiveness in fish and prawn production have been previously demonstrated (Obasa et al. 2009). Chicken manure is valuable in aquaculture systems because of its effectiveness in promoting natural food, growth of second level food chain organisms, and ease in handling and application (Qin et al. 1995; Yi et al. 2003; Anakalo et al. 2009).

A suitable N:P ratio of nutrient or fertilizer will enhance consumable phytoplankton and zooplankton growth while at the same time controlling the blue-green algal blooms (Reyssac and Pletikotic 1990). Phytoplankton communities are an essential component of most pond systems. Primary production by phytoplankton is the base of the food chain in a pond ecosystem that depends on natural or artificial feed to support fish or prawn production.

Table 5 shows estimated nitrogen and phosphorus contents of organic fertilizers commonly used in agriculture and aquaculture. Manure

from chickens displayed the lowest N:P ratio (0.6:1) compared to other fertilizers. Accordingly, the N:P ratio was low in the CM70 and UFW treatments (Figure 4). One of the limits of N is that it encourages blooms of blue-green and periphyton algae or drives the total productivity down to the bottom where N is more available (Levings and Schindler 1999). These algae are also well adapted to high and low light conditions. The CM settled at the bottom of the culture tanks and slowly released nutrients near the bottom and, therefore, may have established good conditions for the growth of benthic algae. The results of this experiment strengthen the notion that nutrient ratio is an important determinant of species composition in natural phytoplankton communities (Tilman et al. 1986; Qin et al. 1995; Khoda Bakhsh et al. 2010). Different algal species have different specific requirements for N:P ratio inputs.

Table 5: Concentration of nitrogen (N) and phosphorus (P) in different sources used for pond fertilization regime (% dry weight).

Manure	N (%)	P (%)	N:P	
			ratio	Reference
Chicken	1.4	2.2	0.6: 1	Knud-Hansen et al. (1991)
Cow	1.5	0.6	2.5: 1	Green et al. (1989)
Duck	4.4	1.1	4.0: 1	Asian Institute of Technology (1986)
Buffalo	1.4	0.2	7.0: 1	Asian Institute of Technology (1986)

### Growth Rate Parameters

The best yields of *L. sativa* and *M. rosenbergii* were obtained in the CM15 treatment. Moreover, survival (87.9%), SGR (6.02%/d), ADG (0.07/d), yield (1343 g/tank) and FCR; (0.42) obtained with this treatment confirmed the potential of prepared formulation and complex nutrients for high density production of *M. rosenbergii* in recirculating aquaponic systems. Organic and mineral elements are important in many aspects of fish and prawn metabolism. These elements are chemically combined in the organism's body to form complex molecules and allow the conversion of food to energy or to build organic molecules and provide strength and rigidity to bones in fish and exoskeleton in crustaceans.

The CM15 media was enriched with complex mineral and organic supplements. The recommended value of dry CM at 15 g/week was equal to 1000-1200 birds/hectare (Little and Muir 1987) in a natural integrated

poultry-fish production system. In a rural integrated fish farming system, CM has been claimed to be better and more popular than other manures such as cattle and pig manure. Some fish farmers build chicken coops over fish ponds; manure and uneaten chicken feed can then be washed into the pond. Pens may also be built with flooring that allows waste to fall or be swept directly into the pond. In recirculating systems, to maintain water quality, CM should be applied at regular intervals. Maclean and Ang (1994) showed that the highest ( $P<0.05$ ) prawn mean weight (2.0 g) and growth rate (0.06 g day<sup>-1</sup>) was achieved when organic CM partially replaced pelleted feed. The maximal survival of prawn was 66% in their report which is lower than in our study (93%).

The lowest yields of *L. sativa* and *M. rosenbergii* were observed in the UFW culture tank ( $P<0.05$ ). However, this treatment led to significantly higher SGR and ADG than with fertilized treatments, except in the case of CM15. Increased individual weight of *M. rosenbergii* was related to a lower survival rate and decreased prawn population (41.0%) in UFW culture tanks during the 45 day culture period. Previous research on prawn-fish integrated culture with poultry manure indicated that the growth and survival of fish and prawns are independent, and that prawns were influenced only by their stocking density, which correlates positively with yield but negatively with survival and individual growth. Wohlfarth et al. (1985) showed that the mean weight of prawns decreased (from 40 g to 24 g) as stocking density increased and the proportion of prawns with marketable weight decreased. Similar results have been observed in monocultures of *M. rosenbergii* (Willis and Berrigan 1977; Brody et al. 1980).

The concentration of ammonia varied among the treatments during the production cycle (0.27 to 0.36 mg/l). The poor results of *M. rosenbergii* survival and growth rate in the UFW treatment could be related to toxicity of ammonia and nitrate concentrations. In contrast to the low survival rate of freshwater prawn, New (1995) stated that the survival rate was more closely related to DO levels than to any other water quality parameter. The recirculating aquaponic tanks showed a acceptable range of DO from 6.4 to 7.8 mg/l during the production cycle. It seems that the factors leading to poor survival in this study are more related to toxic and lethal chemical parameters. Unlike physical parameters (i.e. turbidity), the chemical changes in the rearing water of *M. rosenbergii* can occur with no visible

signs. These changes would be due to the metabolic waste produced by the organisms or by the degradation of excess feed. Some of these sudden changes can be extremely harmful to aquatic organisms. For instance in tropical systems, one of the most serious phenomenon is the increasing non-ionized form of ammonia, as this can be associated with high water pH. Details of ammonia concentrations in the UFW treatment showed a sudden peak in weeks 4 and 7 (Figure 2). *Macrobrachium rosenbergii* is highly sensitive to abnormal environmental conditions and stress, and sudden changes of water quality parameters may have adverse, even lethal effects on prawn survival and growth (FAO, 2002).

In the natural environment, turbidity is composed of organic, inorganic and bio constituents; however, in prawn rearing tanks, turbidity is influenced by algae and phytoplankton population (free of clay and suspended sediments). Phytoplankton production is enhanced when nutrient concentrations increase in the system. The concentration of NO<sub>3</sub> showed a significant increased during weeks 3 to 6 in the UFW culture tanks (Figure 5). However, turbidity originating from phytoplankton growth did not show any significant response and was relatively constant during these weeks of the experiment. It seems that the phytoplankton community and plants with insufficient population and poor growth were not effective enough to absorb soluble nitrogenous compounds (NO<sub>3</sub> and NH<sub>3</sub>) from metabolism activity and decomposition of waste in the system. These phenomena caused harmful conditions in the UFW rearing tanks, as well as a detrimental influence on prawn survival and growth rate.

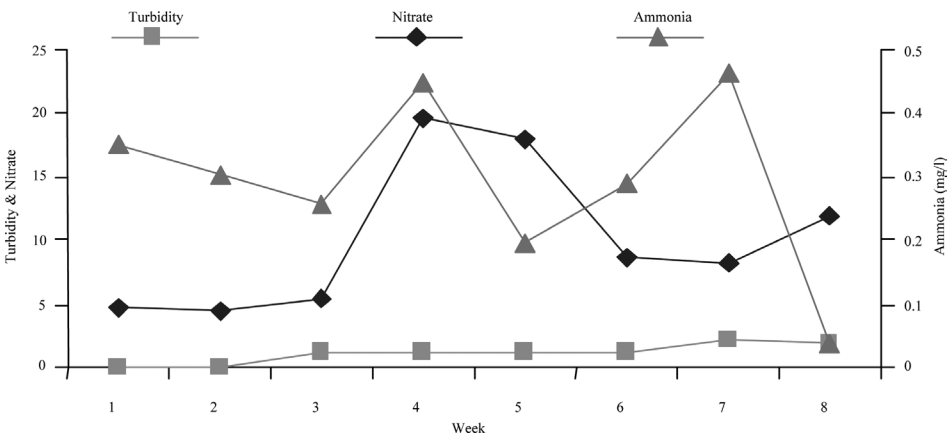


Figure 5: Fluctuation of turbidity (NTU), nitrate and ammonia (mg/l) concentrations in freshwater (FW) culture tanks.

This study illustrated that an optimal dosage of minerals and CM is essential to obtain the best results in recirculating aquaponic systems. The results also showed obvious reduction in plant and prawn yields with CM70, which was only enriched with high density CM. In natural earthen ponds the appropriate fertilization regime (organic by-product) can promote the growth of both autotrophic and heterotrophic organisms which will be directly consumed by aquatic animals along the ecological pathway. The choice of the appropriate nutrient model should consider mineral stimulation and tolerance of aquatic organisms, feeding habit of the cultured species, effect on the desired natural food organisms, cost and abundance and proximity of the source to the vertebrate and invertebrate production farms. In recirculating aquaponic systems, application of organic-inorganic complexes would be an ideal approach to improve production techniques during short periods. Organic-inorganic complexes not only provide minerals for aquaponic plants, but also organic substrates vital for enhancing primary productivity within aquatic environments.

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# **Effect of a Parabolic Screen Filter on Water Quality and Production of Nile Tilapia (*Oreochromis niloticus*) and Water Spinach (*Ipomoea aquatica*) in a Recirculating Raft Aquaponic System**

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Keywords: Aquaponics, water quality, parabolic screen filter, Nile tilapia, water spinach

## **ABSTRACT**

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**Aquaponics** is an integrated fish and plant recirculating production system. Solid fish waste must be removed from the production system to maintain optimal **water quality** parameters for fish and plant health. The University of the Virgin Islands (UVI) raft aquaponic system's primary treatment device for solids removal is a cylindro-conical clarifier; however, alternative mechanical filtration devices such as a **parabolic screen filter** (PSF) may offer advantages. The objectives of the eleven-week experiment were to compare water quality parameters, **Nile tilapia** (*Oreochromis niloticus*) production and **water spinach** (*Ipomoea aquatica*) production in a raft aquaponic system using either a cylindro-conical clarifier or parabolic screen filter for primary treatment of solids in the waste stream.

## *Effect of Screen Filter on Production of Tilapia and Spinach*

The water quality results showed no significant differences ( $P > 0.05$ ) between treatments for temperature, oxygen, pH, alkalinity, EC, TAN,  $\text{NO}_2\text{-N}$  and  $\text{NO}_3\text{-N}$ , macronutrients and micronutrients concentrations, with the exception of copper and zinc. There was no significant difference ( $P > 0.05$ ) between treatments for the total suspended solids (TSS) concentration entering either primary filtration device; however, there was a significant difference ( $P \leq 0.05$ ) between treatments for TSS concentrations exiting the primary filtration device. The PSF treatment had a significantly higher ( $P \leq 0.05$ ) TSS concentration exiting the unit and a significantly higher ( $P \leq 0.05$ ) TSS concentration in the secondary treatment device (net tank) compared to the clarifier.

There were no significant differences ( $P > 0.05$ ) between treatments for Nile tilapia production, average weight, survival, or feed conversion ratio. There were no significant differences ( $P > 0.05$ ) in water spinach production or plant tissue analysis between treatments. In conclusion, the PSF used in this experiment performed less effectively in removing TSS compared to the clarifier, would require more labor to clean and would not be recommended for use in a larger raft aquaponic system. In addition, water spinach assimilated dissolved fish wastes well and grew vigorously in the raft aquaponic system.

## **INTRODUCTION**

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Aquaponics is the combined culture of fish and plants in a recirculating, aquaculture system and has received considerable attention as a result of the system's capability to raise fish at high density, sustain water quality, minimize water exchange, and produce a marketable vegetable crop (Rakocy 1997; Adler et al. 2000; Al-Hafedh et al. 2008; Graber and Junge 2009). The vegetable crop is responsible for the direct assimilation of dissolved fish wastes and products of microbial breakdown in the recirculating aquaponic system. However, methods to remove solids from the production system are still necessary to prevent sub-optimal water quality parameters, such as high un-ionized ammonia, nitrite and low dissolved oxygen, (Cripps and Bergheim 2000; Piedrahita 2003) in order to sustain fish and plant health.

Primary methods used to remove solids from aquaculture effluent are settling and sieving. The principal method for solids removal in the University of the Virgin Islands (UVI) raft aquaponic system uses

settling via a cylindro-conical clarifier (Rakocy 1997). The clarifier uses the simple method of gravity separation to remove solids from the waste stream. Solids settle and concentrate to a cone bottom for daily discharge. The clarifier requires little energy input resulting in inexpensive operational costs; however, disadvantages of the clarifier are its large size and arduous labor required to excavate soil for installation. In addition, the water turnover rate for the fish production unit is limited by the 20 - 30 minute retention time (Rakocy 2003) required to settle solids in the clarifier that comes after the fish production unit. Alternative components for solids removal could replace the clarifier and still provide good water quality conditions for fish and vegetable production in a raft aquaponic system.

Screen filters are typically used as a primary treatment technology to remove solids from aquaculture effluent (Cripps and Bergheim 2000). Removal of solids occurs by straining the water with a specific mesh size and particles larger than the mesh size are removed from the waste stream (Mäkinen et al. 1988). Mesh screen pore sizes of 60–200  $\mu\text{m}$  are commonly used for in-land, intensive fish farms (Mäkinen et al. 1988; Cripps and Bergheim 2000) and solids removal of 30 – 80% can be achieved with screen sizes of 40 -100  $\mu\text{m}$  (Timmons et al. 2001). One type of screen filter is a parabolic screen filter (PSF). The PSF utilizes an angled, stationary screen to sieve solids from the waste stream using the Coanda effect. The advantage of a PSF compared to other variations of screen filters is its ease of operation, relatively low expense and it contains no mechanical parts which could breakdown (Timmons et al. 2001). Similarly to the clarifier, a PSF can operate with little energy input, but foreseen advantages of a PSF are its compact size, installation at ground level and increased flow rates leaving the fish production tanks. Nonetheless, a potential disadvantage of the PSF could be an increase in the number of cleaning intervals to remove solids trapped on the stationary screen. Rinsing the sieved wastes from the screen maintains the desired hydraulic capacity of the PSF. Our literature search found no research articles utilizing a PSF in a raft aquaponic system.

The objectives of this experiment were to compare water quality parameters, Nile tilapia (*Oreochromis niloticus*) production and water spinach (*Ipomoea aquatica*) production in a raft aquaponic system using either a cylindro-conical clarifier or PSF for primary treatment of solids in the waste stream.

## **MATERIALS AND METHODS**

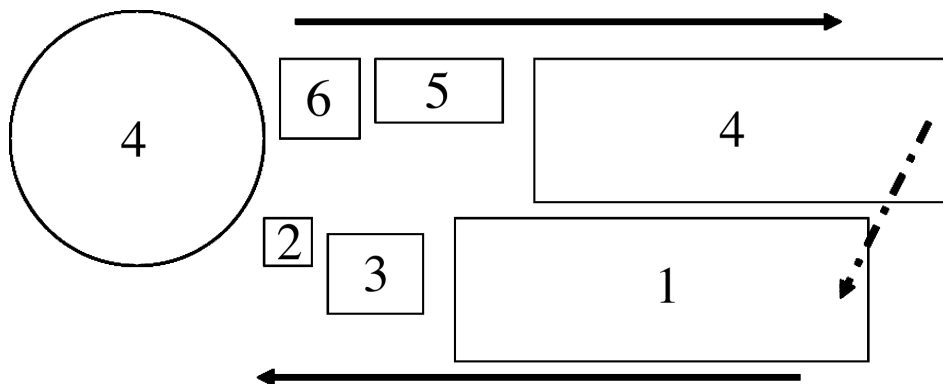
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### **Experimental System**

The experiment was carried out in six outdoor aquaponic systems located at the Agricultural Experiment Station, University of the Virgin Islands, St. Croix, United States Virgin Islands. The experiment consisted of two treatments with three replicates each. The Control used a 1.2 m diameter fiberglass, cylindro-conical clarifier (total volume = 1.7-m<sup>3</sup>) containing a baffled wall perpendicular to the waste stream flow to dissipate the incoming current and facilitate solids settlement. The cone bottom had a 60° slope. Treatment two used a stainless steel PSF (Aquasonic, LTD, Wauchope, Australia) equipped with a 200-micron, wedged-wire removable screen. The PSF had a volume of 0.13-m<sup>3</sup> and a screen surface area of 1,440-cm<sup>2</sup> for solids filtration. According to the manufacturer, the filter could accept a 265 L/min flow rate which equates to a hydraulic loading rate of 2,650 m<sup>3</sup>/m<sup>2</sup>/day of parabolic screen area.

To prevent sun exposure and algal growth the fish culture tank for each treatment replicate was constructed under a cold frame and shaded with a 100% high density polyethylene cloth. Each experimental system (Figure 1) consisted of a 3 m x 1.1 m fish culture tank (volume for fish production = 7.8 m<sup>3</sup>), the primary solids filtration component tested, a net tank (0.7 m<sup>3</sup>) with 15 m of orchard netting (1.2 cm square mesh) which acted as a secondary solids filtration component, two hydroponic raceways (area 6.1×1.2×0.3 m each; total volume 4.4 m<sup>3</sup>) and a sump (0.6 m<sup>3</sup>). Although water flowed from the fish tank to the sump via gravity, a 1/6 Hp Sweetwater® centrifugal pump (Aquatic Ecosystems, Apopka, FL, USA) was used to return water from the sump to the fish culture tank at a flow rate of 57 L/minute. Thus, the hydraulic loading rate on the PSF was 570 m<sup>3</sup>/m<sup>2</sup>/day of parabolic screen area and the surface loading rate on the clarifier was 73 m<sup>3</sup>/m<sup>2</sup>/day of plan area. Water loss due to daily waste removal, evaporation and plant transpiration was replaced with rainwater at the sump and controlled with a float valve. The quantity of rainwater was recorded with a water meter installed at each system. Hydroponic raceways were lined with a 20-mil white, food-grade liner (In-Line Plastics, Inc, Houston, TX, USA). The six experimental units were aerated by one, 1.5 Hp Sweetwater® regenerative blower (Aquatic Ecosystems, Apopka, FL, USA). Each fish tank had twelve, 8.0×4.0 cm silica airstones spaced 0.75 m apart around the tank perimeter and each hydroponic trough

had four, 8.0×2.5 cm silica airstones placed in the middle of each trough and spaced every 1.2 meters.



*Figure 1. Layout of aquaponic system. System components were: fish tank (1), solids removal device being tested (2), net tank (3), hydroponic raceway (4), sump (5), pump (6). Water recirculates in the direction of the arrows by gravity until an electrical pump returns water from the sump to the fish tank. Rainwater used to make-up water lost to waste removal, evaporation and plant transpiration was added at the sump.*

## **Water Quality**

Dissolved oxygen (DO), temperature and electrical conductivity (EC) were monitored directly from each aquaponic system every two weeks. The DO and temperature were monitored in the fish culture tank using a YSI Model 550A meter (Yellow Springs Instruments, Yellow Springs, Ohio, USA) and a Commercial Truncheon pen (NZ Hydroponics International Ltd, Tauranga, New Zealand) was used to record EC at the end of the second hydroponic raceway. The pH was monitored at the end of the second hydroponic raceway three times per week using a pH Testr 10 (Oakton Instruments, Vernon Hills, IL, USA) to maintain a desired pH of 7.0. The raft aquaponic system maintains a pH of 7.0 to accommodate the needs of fish, plants and nitrifying bacteria. The addition of 300 – 500 grams of calcium-hydroxide [Ca(OH)<sub>2</sub>] or potassium-hydroxide (KOH) was added on an alternate basis when pH fell below 7.0 to neutralize pH and supplement calcium and potassium concentrations. An 11% DTPA iron chelate (Akzo Nobel, Lima, Ohio, USA) was added initially and periodically thereafter to maintain an iron concentration of 2 mg/L to prevent plant nutrient deficiency. One, 250-mL grab sample was taken every two weeks from the end of the second hydroponic raceway

in each system to measure water quality parameters in a laboratory at the Agricultural Experiment Station.

A HACH DR/2000 spectrophotometer (Hach Company, Loveland, Colorado, USA) was used to measure total ammonia-nitrogen (TAN), nitrite-nitrogen ( $\text{NO}_2\text{-N}$ ), and nitrate-nitrogen ( $\text{NO}_3\text{-N}$ ). Alkalinity was measured using the method described in Boyd and Tucker (1992). An additional 250-mL grab sample was taken every two weeks from the end of the second hydroponic raceway and sent to a lab (MicroMacro International, Inc., Athens, GA, USA) for macronutrient and micronutrient analysis. Samples were prepared at MicroMacro International (MMI) using US EPA method 6010a (USEPA 1986) and measured via inductively coupled plasma spectroscopy.

Total-suspended solids (TSS) entering and exiting the clarifier and PSF along with TSS exiting the net tank were sampled every two weeks one-hour after the morning feeding. A 2.5-cm PVC sampling port was installed just before and after each filter for sampling purposes. At each sampling event the sample port was flushed and a 4-L sample was taken from which one, 250-mL aliquot was collected. The TSS concentration was quantified according to the method described in Boyd and Tucker (1992).

Wastes were discharged twice daily (0900 and 1600 h) from the clarifier and PSF. Effluent was discharged from the clarifier based on the concept of hydrostatic pressure. A 5 cm ball-valve was opened to allow settled solids in the cone bottom to discharge and closed immediately when the effluent went from a dark brown appearance to clear in color. For the PSF, solids that did not move into the waste trough as a result of the Coanda effect were carefully washed down into the trough with influent water entering the PSF. This method was slow, but resulted in little water unintentionally entering the waste trough. If the PSF screen clogged, its design allowed water to bypass the screen and flow into the net tank. In this circumstance aquaculture staff carefully scrubbed the screen to allow water to pass through the wedge-wire screen again. Then remaining solids were hand washed into the trough as described previously. After every discharge event, the PSF screen was removed and sprayed with a garden hose to clear the screen openings. Screen removal and replacement during the rinsing process took approximately 60 – 90 seconds. The minute amount of particulate matter that was rinsed from the screen during this rinsing process was not quantified as part of the effluent discharged.

The volume of effluent discharged was quantified at least twice weekly. Additionally, the TSS concentration of discharged effluent was measured every two weeks from one, 250-mL aliquot taken from the combined morning and afternoon discharged effluent. An additional 250-mL sample was collected and sent to MMI for macronutrient and micronutrient concentration. Samples were prepared at MMI using US EPA method 3050b (USEPA 1986) and measured via inductively coupled plasma spectroscopy. At the end of the experiment the orchard netting in each experimental unit's net tank was cleaned of solids via gentle shaking. The slurry in the net tank was manually stirred to suspend solids and two, 250-mL aliquots were taken to quantify TSS concentration.

### **Tilapia**

On 4 November 2009, sex-reversed male Nile tilapia ( $231.8 \pm 21.7$  g) were counted into groups of 40 fish then weighed and stocked in rotation until each experimental unit was stocked with 360 fish ( $46 \text{ fish/m}^3$ ). Nile tilapia were fed an extruded diet (6.3 mm pellet) containing 32% protein (PMI Nutrition International, Mulberry, FL, USA) twice daily (0900 and 1600 h) based on the recommended feeding rate of 60 – 100 grams of tilapia diet/ $\text{m}^2$  of hydroponic plant growing area/day (Rakocy 2003). The culture period for tilapia was 79 days and Nile tilapia were harvested on 22 January 2010. A final count was conducted to determine survival and bulk weight was recorded for each tank to determine final production, average weight, and feed conversion ratio (FCR). Feed conversion ratio (FCR) was calculated as:  $\text{FCR} = \text{feed fed}/\text{weight gain}$  (Tidwell et al. 1999).

### **Water Spinach**

Cuttings of water spinach were allowed to root for a two-week period in a commercial-scale aquaponic system. On 31 October 2009 a total fresh weight of  $3.3 \pm 0.1$  kg of water spinach was transplanted into the hydroponic raceways of each experimental system. Spinach was placed on-top of 2.5 cm thick polystyrene floating boards and the roots were able to contact the water through a series of 4.8-cm diameter circular cutouts. For the duration of the experiment, spinach stems and leaves were harvested from these initial transplants every 3 weeks. Spinach was sprayed twice weekly with DiPel® PRO DF (Valent USA Corporation, Walnut Creek, CA, USA) biological insecticide to control caterpillar pests. The spinach was grown for 81 days and on 20 January 2010 all spinach was removed from each experimental unit and total wet weight

of spinach production was calculated. Total spinach production did not include roots, only the marketable leaf and stem biomass harvested from the top of the polystyrene sheets.

On 20 January, cuttings of water spinach were taken, immediately weighed, and put into paper bags. The bags were placed into a forced air oven and dried at 80°C for 72 hours to determine percent moisture content. In addition, samples of leaf and stem were sent to MMI for plant tissue analysis. At MMI, plant tissue samples were oven dried and ashed according to AOAC test method 922.02 and 900.02b, respectively (AOAC International 2007). Then, samples were analyzed for nutrient content using US EPA method 6010a (USEPA 1986) and measured via inductively coupled plasma spectroscopy.

A two-sample t-test was used to compare water quality parameters, tilapia production and spinach production between treatments for significant ( $P \leq 0.05$ ) differences. Data was analyzed in Microsoft® Excel 2007 (Microsoft® Corporation, Redmond, Washington, USA). If required, percent data was transformed to arc sin values prior to analysis (Bhujel 2008); however, data are presented in the untransformed form to facilitate interpretation.

## **RESULTS AND DISCUSSION**

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The water quality results showed no significant differences ( $P > 0.05$ ) between treatments for temperature, oxygen, pH, alkalinity, EC, TAN, NO<sub>2</sub>-N and NO<sub>3</sub>-N (Table 1). All aforementioned parameters were within optimal ranges for a raft aquaponic system producing tilapia (Rakocy 2003; Al-Hafedh et al. 2008). There was no significant difference ( $P > 0.05$ ) between treatments for TSS concentration entering either primary filtration device; however, there was a significant difference ( $P \leq 0.05$ ) between treatments for TSS concentrations exiting the primary filtration device (Table 1). The TSS concentration was significantly higher ( $P \leq 0.05$ ) exiting the PSF (11.3 mg/L) compared to the clarifier (7.4 mg/L). The PSF was only able to remove 5.8% of the solids entering it compared to a 30.8% removal efficiency for the clarifier. Chen et al. (1993) and Kelly et al. (1997) found 80 - 95% of the solids in their recirculating systems were less than 30 µm in size. Although particle size distribution was not calculated in the present experiment it is suspected solids passed through the 200-µm screen in the PSF because there was a significant

difference ( $P \leq 0.05$ ) between treatments for TSS retained in the net tank. The purpose of the net tank is to retain small particulate matter that escapes the clarifier (Rakocy 1997; Rakocy et al. 2003).

The TSS concentration in the net tank was significantly higher ( $P \leq 0.05$ ) in the PSF treatment (4,300 mg/L) than the clarifier treatment (3,560 mg/L) (Table 1). The net tank component in the PSF treatment acted as a storage reservoir for solids over the 11-week experiment and was able to handle an increased solids loading rate as a result of solids passing through the PSF wedged-wire screen. Furthermore, the wedge-wire

*Table 1. Treatment mean ( $\pm$  standard deviation) of water quality parameters sampled during the eleven-week aquaponic experiment. Treatment means within a row and followed by a different letter are significantly different ( $P \leq 0.05$ ) using a two-sample t-test.*

Parameter	Treatment	
	Clarifier	Parabolic Screen Filter
Temperature (°C)	26.3 $\pm$ 0.1 <sup>a</sup>	26.1 $\pm$ 0.1 <sup>a</sup>
Oxygen (mg/L)	6.1 $\pm$ 0.1 <sup>a</sup>	6.1 $\pm$ 0.2 <sup>a</sup>
pH	7.1 $\pm$ 0.1 <sup>a</sup>	7.1 $\pm$ 0.1 <sup>a</sup>
Alkalinity (mg/L)	54.8 $\pm$ 9.9 <sup>a</sup>	62.4 $\pm$ 4.6 <sup>a</sup>
Electrical Conductivity ( $\mu$ S/cm)	0.3 $\pm$ 0.0 <sup>a</sup>	0.3 $\pm$ 0.0 <sup>a</sup>
Total Ammonia-Nitrogen (mg/L)	0.5 $\pm$ 0.0 <sup>a</sup>	0.5 $\pm$ 0.0 <sup>a</sup>
Nitrite-Nitrogen (mg/L)	0.6 $\pm$ 0.3 <sup>a</sup>	0.6 $\pm$ 0.3 <sup>a</sup>
Nitrate-Nitrogen (mg/L)	6.9 $\pm$ 0.5 <sup>a</sup>	6.4 $\pm$ 1.3 <sup>a</sup>
Total Suspended Solids (mg/L)		
Entering filter	10.7 $\pm$ 2.3 <sup>a</sup>	12.0 $\pm$ 1.5 <sup>a</sup>
Exiting filter	7.4 $\pm$ 1.2 <sup>b</sup>	11.3 $\pm$ 1.8 <sup>a</sup>
Retained in net tank	3,560 $\pm$ 483 <sup>b</sup>	4,300 $\pm$ 592 <sup>a</sup>
Exiting net tank	6.8 $\pm$ 0.7 <sup>a</sup>	5.7 $\pm$ 0.6 <sup>a</sup>
In discharged effluent	8,100 $\pm$ 2,208 <sup>a</sup>	5,364 $\pm$ 3,011 <sup>a</sup>
Daily effluent discharged (L)	7.6 $\pm$ 0.3 <sup>a</sup>	7.3 $\pm$ 0.4 <sup>a</sup>

screen frequently clogged allowing solids to bypass the PSF and enter the net tank. Most of the time the PSF clogged between the previous afternoon cleaning at 1600 hr and the subsequent morning cleaning at 0900 hr. Occasionally, the PSF would clog with solids between the morning and afternoon cleaning on the same day resulting in the waste stream bypassing the screen and entering directly into the net tank. In addition, the hand cleaning of solids to allow water to flow through the PSF when it was found clogged may have resulted in some solids getting squeezed through the wire screen. However, the authors feel the time elapsed between the afternoon and subsequent morning cleaning resulted in the majority of solids entering the net tank.

Clogging of stationary screen filters is problematic in aquaculture (Mäkinen et al. 1988) and more frequent cleaning would be required to ensure the PSF functioned properly. The authors recommend the PSF used in this experiment be cleaned in six hour intervals if used in a similar sized raft aquaponic system with a flow rate of 57 L/min and maximum daily feeding of 80 grams/m<sup>2</sup> of hydroponic growing area/day. However, additional cleaning would result in increased daily management of the raft aquaponic system compared to a system utilizing a clarifier. Alternatively, installing a PSF with an increased screen surface area may result in less frequent clogging by supplying a larger area to filter solids. The PSF used in this experiment was rated for a maximum flow rate of 270 L/min; yet, the PSF could not handle the aquaculture waste at a maximum feeding rate of 80 grams/m<sup>2</sup> of hydroponic growing area/day (1,120 g feed/system/day) and one-fifth its maximum flow rate. The soft organic matter and fecal waste clogged the screen without difficulty. As a result, the feeding rate never exceeded 80 grams/m<sup>2</sup> of hydroponic growing area/day.

Although the PSF treatment was shown to have an increased TSS concentration (11.3 vs 7.4 mg/L) exiting the filter, there was no significant difference ( $P > 0.05$ ) between treatments in TSS concentration exiting the net tank (Table 1). Overall the TSS concentration exiting the net tank was 6.3 mg/L. The 1.2 cm, square mesh orchard netting placed in the net tank was able to capture the additional solids in the PSF treatment and prevent their escape. The net tank for the PSF and clarifier treatments were able to retain approximately 50 and 8 %, respectively, of the solids that entered. These solids remained in the aquaponic system, specifically the net tank, but no adverse effects on water quality were

observed, except for the increased copper and zinc concentrations. This finding demonstrates the importance of the net tank for capturing remaining solids that may escape when the primary solids removal device does not perform optimally.

There was no significant difference ( $P > 0.05$ ) in the TSS concentration of effluent discharged daily (Table 1). The authors acknowledge the reported concentration of solids discharged from the PSF treatment is not as precise as the clarifier treatment due to the cleaning process. Nonetheless, each treatment discharged an average daily TSS concentration of 6,732 mg/L and 7.4 L of effluent, overall. This resulted in an overall average daily discharge of 50.3 g of solids/day and represented approximately 4.5% of the daily feed fed on dry matter basis. It was initially thought the PSF would have created a more concentrated effluent compared to the clarifier because it would strain the solids; however, over time water from the waste stream naturally settled in the PSF waste trough. This water that entered the trough was also discharged and resulted in dilution of the screened solids. Water loss due to daily waste removal, evaporation, plant transpiration and fish splashing during feeding was equivalent to 1.6% of the system volume. This demonstrates the recirculating aquaponic system conserves freshwater resources in the production of fish and water spinach.

There was no significant difference ( $P > 0.05$ ) between treatments for macronutrient concentration in the culture water (Table 2). However, there was a significant difference ( $P \leq 0.05$ ) between treatments for two micronutrients in the culture water (Table 2). The PSF had a significantly higher ( $P \leq 0.05$ ) copper (0.06 mg/L) and zinc (0.38 mg/L) concentration compared to the copper (0.03 mg/L) and zinc (0.29 mg/L) concentration in the clarifier treatment. This may have resulted from the increased solids concentration within the net tank of the PSF treatment and the opportunity for micronutrient leaching; however, this did not have a negative impact on Nile tilapia or water spinach production. Macronutrient and micronutrient concentrations were similar to previous studies examining floating raft aquaponics (Rakocy 1997; Rakocy et al. 2003) and was lower than concentrations reported in low exchange recirculating systems used for rainbow trout (*Oncorhynchus mykiss*) culture (Davidson et al. 2011).

Table 2. Treatment mean ( $\pm$  standard deviation) of macronutrient and micronutrient concentration in culture water during the eleven- week aquaponic experiment. Treatment means within a row and followed by a different letter are significantly different ( $P \leq 0.05$ ) using a two-sample *t*-test.

Parameter	Treatment	
	Clarifier	Parabolic Screen Filter
Macronutrients (mg/L)		
Phosphorus	1.7 $\pm$ 0.1 <sup>a</sup>	1.9 $\pm$ 0.2 <sup>a</sup>
Potassium	24.3 $\pm$ 3.9 <sup>a</sup>	27.1 $\pm$ 5.1 <sup>a</sup>
Calcium	34.7 $\pm$ 0.7 <sup>a</sup>	35.6 $\pm$ 4.5 <sup>a</sup>
Magnesium	3.9 $\pm$ 0.3 <sup>a</sup>	4.4 $\pm$ 0.7 <sup>a</sup>
Micronutrients (mg/L)		
Iron	1.86 $\pm$ 0.08 <sup>a</sup>	2.00 $\pm$ 0.29 <sup>a</sup>
Manganese	0.01 $\pm$ 0.00 <sup>a</sup>	0.00 $\pm$ 0.00 <sup>a</sup>
Boron	0.05 $\pm$ 0.01 <sup>a</sup>	0.05 $\pm$ 0.00 <sup>a</sup>
Copper	0.03 $\pm$ 0.01 <sup>b</sup>	0.06 $\pm$ 0.01 <sup>a</sup>
Zinc	0.29 $\pm$ 0.03 <sup>b</sup>	0.38 $\pm$ 0.02 <sup>a</sup>
Molybdenum	0.01 $\pm$ 0.01 <sup>a</sup>	0.01 $\pm$ 0.01 <sup>a</sup>
Sodium	7.62 $\pm$ 0.75 <sup>a</sup>	8.46 $\pm$ 0.36 <sup>a</sup>

There was no significant difference ( $P > 0.05$ ) in water spinach production between the clarifier (212.4 kg) and the PSF (192.6 kg) treatment (Table 3). Overall, total water spinach production in the aquaponic system was 202.5 kg, which equates to 14.5 kg/m<sup>2</sup> of hydroponic growing area or 1.3 kg/m<sup>2</sup>/week. The water spinach grew vigorously in the aquaponic system and produced dense masses of foliage within a few weeks of transplanting and between successive harvests. Water spinach has no relation to ordinary spinach (*Spinacia oleracea*), but is closely related to sweet potato (*Ipomoea batatas*) and is in the family Convolvulaceae.

We found few papers regarding the production of this Asian vegetable. Eddie and Ho (1969) and Snyder et al. (1981) suggest 70-100 mt/ha or 7-10 kg/m<sup>2</sup> annually is possible in traditional field production of water

*Table 3. Total fresh weight of water spinach harvested, total spinach production per unit surface area and weekly spinach production per unit surface area grown in the raft aquaponic systems during the eleven-week experiment. Treatment means within a row and followed by a different letter are significantly different ( $P \leq 0.05$ ) using a two-sample t-test.*

<b>Parameter</b>	<b>Treatment</b>	
	<b>Clarifier</b>	<b>Parabolic Screen Filter</b>
Total fresh weight harvested (kg)	212.4 ± 15.1 <sup>a</sup>	192.6 ± 6.2 <sup>a</sup>
Total production per unit area (kg/m <sup>2</sup> )	15.2 ± 1.1 <sup>a</sup>	13.8 ± 0.4 <sup>a</sup>
Weekly production per unit area (kg/m <sup>2</sup> /wk)	1.4 ± 0.1 <sup>a</sup>	1.3 ± 0.0 <sup>a</sup>

spinach. Savidov (2005) evaluated water spinach production in a large raft aquaponic system modeled after UVI and reported the water spinach had the highest annual yield (58.3 kg/m<sup>2</sup>/year) compared to other vegetable crops cultured. In the present aquaponic experiment both treatments could produce 7 times the biomass per unit area annually reported by Eddie and Ho (1969) and Snyder et al. (1981). Also, this experiment yielded an additional 17% water spinach biomass per unit area compared to Savidov (2005). The system Savidov (2005) used was enclosed in a climate controlled greenhouse in a northern Canada. It was not stated what time of year production occurred but day length may have become limiting for water spinach production.

This experiment's findings coincide with Endut et al. (2009) that water spinach produced in an aquaponic system showed a positive response to tilapia effluent in terms of growth and production. This leafy green has potential as a marketable crop in the mainland United States and United States Virgin Islands with an increasing ethnic population and a broader proportion of the residents starting to consume it (Palada and Crossman 1999); in addition, Prasad (2008) found water spinach had medicinal value which could help in marketing to consumers. Unfortunately, water spinach remains on the United States federal invasive plant species list and production may be prohibited in the mainland United States, especially southern states like Florida (Gordon 1998) where frost exposure is negligible.

### *Effect of Screen Filter on Production of Tilapia and Spinach*

The solids removal device did not significantly affect ( $P > 0.05$ ) the percent moisture content (90.5% overall) of the water spinach. This species of water spinach prefers a wet environment to flourish (Eddie and Ho 1969) and water was not limiting in the raft aquaponic system. There was no significant difference ( $P > 0.05$ ) in plant tissue analysis between treatments (Table 4). Nitrogen concentration (6.7% overall) in plant tissue was well above recommended levels (Mills and Jones 1996) for both the clarifier and PSF treatment, which may reveal water spinach quickly uptakes forms of inorganic nitrogen present in the treated fish effluent. No signs of nutrient deficiency were observed although plant tissue analysis revealed calcium and magnesium were below recommended ranges. Nitrogen concentrations can affect the level of calcium and magnesium uptake in plants (Mills and Jones 1996), but it depends on the form the plant is uptaking. Future studies may need to address this concern for raft aquaponic systems producing water spinach if signs of plant nutrient deficiencies occur. Results of this experiment demonstrate an average daily feeding rate of 70 grams of tilapia diet/m<sup>2</sup> of hydroponic growing area/day was sufficient for water spinach growth.

There were no significant differences ( $P > 0.05$ ) between treatments for Nile tilapia production. Overall, the Nile tilapia production, average weight, survival, and FCR were 16.7 kg/m<sup>3</sup>, 372.3 g, 97.5 %, and 1.6, respectively (Table 5). Both treatments resulted in Nile tilapia survival and FCR typical for raft aquaponics (Rakocy et al. 2003, 2006). The fish to plant production ratio is an important concept for aquaponics and a proper ratio creates a balanced production system through nutrient uptake and assimilation into plant biomass. Wilson (2005) discovered 1 kg of fish production resulted in 7 kg of vegetable biomass. Graber and Junge (2009) found 1 kg of fish production resulted in 4 kg of tomato production. In the present experiment the nutrients in the wastewater from the net production of 1 kg of Nile tilapia resulted in the net production of 4 kg of water spinach. In essence, aquaponic systems emphasize plant culture and nutrients in the fish waste are a valuable resource for vegetable crop production. When the total harvestable biomass (Nile tilapia + water spinach) was calculated the FCR fell to 0.32 and reveals the importance of integrated systems in maximizing nutrient utilization. This is especially important with the increasing cost of commercial fish diets.

Table 4. Percent moisture, macronutrient levels, micronutrient levels and recommended nutrient levels for water spinach plant tissue at final harvest of aquaponic experiment. Treatment means within a row and followed by a different letter are significantly different ( $P \leq 0.05$ ) using a two-sample *t*-test.

Parameter	Treatment		Recommended <sup>1</sup>
	Clarifier	Parabolic Screen Filter	
Percent Moisture (%)	90.4 ± 0.4 <sup>a</sup>	90.5 ± 0.5 <sup>a</sup>	
Macronutrients (%)			
Nitrogen	6.8 ± 0.0 <sup>a</sup>	6.7 ± 0.0 <sup>a</sup>	3.3 – 4.5
Phosphorus	0.5 ± 0.0 <sup>a</sup>	0.5 ± 0.0 <sup>a</sup>	0.2 – 0.5
Potassium	3.6 ± 0.0 <sup>a</sup>	3.3 ± 0.0 <sup>a</sup>	3.1 – 4.5
Calcium	0.5 ± 0.0 <sup>a</sup>	0.5 ± 0.0 <sup>a</sup>	0.7 – 1.2
Magnesium	0.1 ± 0.0 <sup>a</sup>	0.1 ± 0.0 <sup>a</sup>	0.4 – 1.0
Sulfur	0.3 ± 0.0 <sup>a</sup>	0.3 ± 0.0 <sup>a</sup>	0.3 – 0.5
Micronutrients (mg/L)			
Iron	61.5 ± 12.1 <sup>a</sup>	64.1 ± 23.7 <sup>a</sup>	40 – 100
Manganese	117.1 ± 50.5 <sup>a</sup>	76.3 ± 19.5 <sup>a</sup>	40 – 250
Boron	25.8 ± 3.9 <sup>a</sup>	24.2 ± 1.1 <sup>a</sup>	25 – 75
Copper	6.2 ± 0.7 <sup>a</sup>	6.4 ± 1.5 <sup>a</sup>	4 – 10
Zinc	60.2 ± 22.3 <sup>a</sup>	44.8 ± 9.9 <sup>a</sup>	20 – 50
Molybdenum	1.1 ± 0.1 <sup>a</sup>	1.1 ± 0.2 <sup>a</sup>	0.1 – 0.4

<sup>1</sup> Based on recommended levels for sweet potato (*Ipomoea batatas*) by Mills and Jones (1996).

*Table 5. Final production, individual harvest weight, survival and food conversion ratio (FCR) of tilapia grown in the aquaponic system. Treatment means within a row and followed by a different letter are significantly different ( $P \leq 0.05$ ) using a two-sample t-test.*

<b>Parameter</b>	<b>Treatment</b>	
	<b>Clarifier</b>	<b>Parabolic Screen Filter</b>
Final Production (kg/m <sup>3</sup> )	16.4 ± 2.7 <sup>a</sup>	16.9 ± 2.0 <sup>a</sup>
Individual harvest weight (g)	373.7 ± 18.0 <sup>a</sup>	370.8 ± 10.9 <sup>a</sup>
Survival (%)	95.7 ± 5.2 <sup>a</sup>	99.2 ± 0.5 <sup>a</sup>
FCR	1.7 ± 0.0 <sup>a</sup>	1.6 ± 0.1 <sup>a</sup>

In conclusion, using a PSF in the UVI raft aquaponic system did not negatively affect water quality, Nile tilapia production or water spinach production compared to the traditional cylindro-conical clarifier. However, the stationary screen of the PSF frequently clogged while straining solids from the waste stream and the required cleaning events were often times unpredictable. The PSF would require increased cleaning intervals compared to the clarifier. The authors would not recommend the PSF used in this experiment as the primary solids treatment method in a commercial-scale raft aquaponic system having a higher waste load and flow rate. Future studies could address the use of a PSF with similar mesh size, but with more frequent cleaning intervals or a PSF with a larger surface area for straining solids could be evaluated. In addition, an alternative solids removal device like a swirl separator should be evaluated as the primary solids removal device in the raft aquaponic system.

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## **The Effect of Solids Removal on Water Quality, Growth and Survival of *Litopenaeus vannamei* in a Biofloc Technology Culture System**

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suspended solids, clarification

### **ABSTRACT**

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Biofloc technology culture systems can increase the productivity of shrimp culture. Through the use of minimal or zero exchange, biofloc technology culture systems can also reduce the use of water. Diet enhancement through the addition of feed increases the amount of excreta. Together with unconsumed feed, the additional excreta increases the amount of suspended solids and reduces the concentration of dissolved oxygen. In addition, the excess of suspended solids can harm the culture by reducing light penetration. In turn, the lower light levels reduce the abundance of photosynthetic organisms (microalgae) that are also important for water quality and shrimp nutrition. The objective of this study was to evaluate the removal of suspended solids from the water of the culture system by a clarification process (i.e. particle settling). Two treatments were applied: with clarification and no clarification. Six tanks, each 35 m<sup>3</sup>, were used in the study. In the clarification treatment, 35 m<sup>3</sup> of water with bioflocs was pumped from the experimental unit for 6 hours. The water passed through a settling tank (1,000 L) and was returned to the culture unit through gravity. The clarification treatment reduced total suspended solids (24.5%), turbidity (27%) and chlorophyll

*a* (27.8%). The availability of dissolved oxygen and pH values were also greater in the clarification treatment. Growth, feed conversion ratio, survival and productivity were significantly higher ( $p < 0.05$ ) with the removal of suspended solids. Control of the concentration of suspended solids contributed to the improvement of water quality and the growth performance of the shrimp *L. vannamei* in the superintensive biofloc technology culture.

## **INTRODUCTION**

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Recent culture systems that involve low rates of water renewal may be cited as examples of sustainability in cultured penaeid shrimp. These systems minimize the disposal of water and increase production through the cultivation of heterotrophic organisms and the discharge of nitrogen compounds (Wasielesky et al. 2006a). Addition of even small amounts of water contributes to an increase in the diet availability by the natural productivity in ponds (Burford et al. 2003). This approach results in high yields (Browdy et al. 2001; Hopkins et al. 1995) from this type of system. Currently these systems are named biofloc technology systems (BFT). The increased stocking density and the large amount of uneaten feed and fecal matter (Wasielesky et al. 2006a) in a BFT may affect the production system adversely (McMillan et al. 2003). These conditions also cause the precipitation of solids, and sludge formation is common (Hopkins et al. 1994). Studies of this problem should focus on the rapid removal of relatively large-sized particles. The smaller particles are difficult to remove (McMillan et al. 2003) owing to their slow sedimentation. This process may be important for enhancing solubility and nutrient release (Patterson et al. 1999).

According to Thakur and Lin (2003), one of the biggest problems affecting water quality in closed systems is the rapid eutrophication of tanks and ponds. This problem is the result of increased concentrations of nutrients and organic matter during culture. Ebeling et al. (2006) have outlined the main problems affecting water quality in heterotrophic systems. They have indicated that the elevated production of bacterial biomass, compared with the biomass of phytoplankton produced in autotrophic culture, leads to an increase in suspended solids. However, Brune et al. (2003) and Cohen et al. (2005) have emphasized the importance of phytoplankton and nitrifying bacteria in this context.

These organisms exhibit a high capacity to absorb inorganic nitrogen and are consequently able to control the level of ammonia in the culture systems. The removal of suspended solids can improve light penetration, whereas the reduction of light may reduce primary production (Kirk, 1994). The photosynthetic microalgae added to maintain water quality are also a source of nutrition for shrimp (Burford et al. 2003). In closed culture systems for shrimp production at high stocking densities, the level of dissolved oxygen is reduced (Cohen et al. 2005) and nitrogen compounds, like ammonia and nitrite (Avnimelech 1999; Cohen et al. 2005), are produced from organic solids.

Clarification is a practical method for removing solids. Gravity is allowed to bring particles to the bottom of the water column through sedimentation or settling. Johnson and Chen (2006) considered this method an efficient method of clarification for the removal of suspended solids in the culture of rainbow trout. For cultured tilapia, Azim and Little (2008) proposed an intermediate system based on the principles of sedimentation and separation of flocs. Ray et al. (2010) have evaluated the role of clarification in improving shrimp production. Their study used a mesocosm and two different diets where turbidity was used as an indicator to monitor the process. In a superintensive shrimp culture system, a clarification process can successfully reduce the depletion of oxygen and the accumulation of suspended solids, thereby improving the water quality for the crop.

The aim of this study was to evaluate the effect of clarification on water quality in the superintensive culture of the shrimp *L. vannamei* in a BFT system. In this study, the concentration of suspended solids was kept at a value of 500 mg/l, the maximum value specified by Samocha et al. (2007).

## **MATERIALS AND METHODS**

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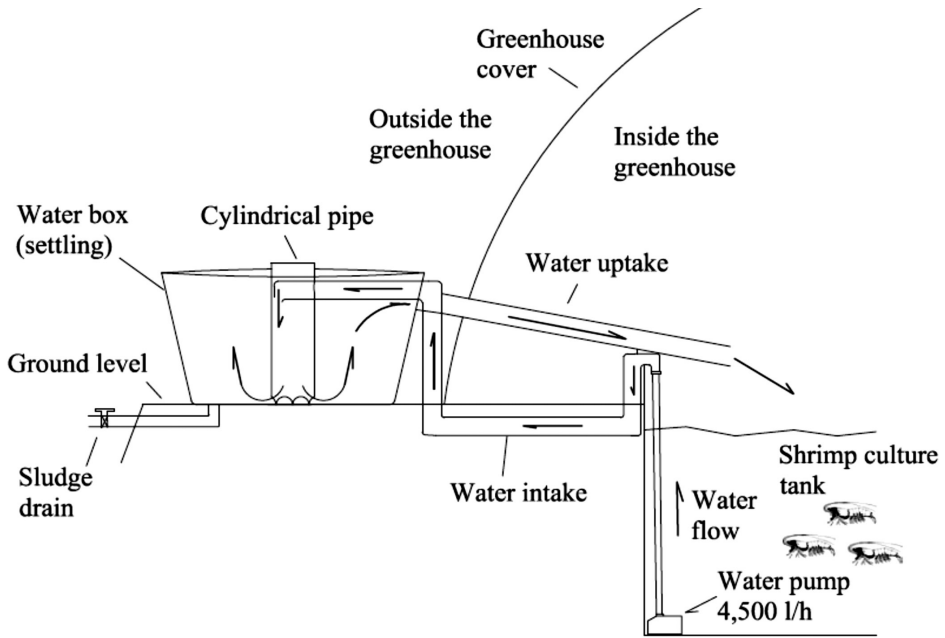
### **Experimental design**

The study was conducted over the winter from May 7 to August 28, 2010 in a greenhouse with six rectangular tanks (5.0 m X 7.0 m). Each lined tank had a usable volume of 35 cubic meters, 1.0 water column. The experiment was conducted using two treatments, namely clarification (C) and no clarification (NC), with three replicates per treatment in a fully randomized design. In both treatments, vertical structures were used

as substrates for the development of a natural biota. This natural biota represented an additional source of food for the shrimp (Ballester et al. 2007). Tanks were filled 90% with sea water initially treated with 10 ppm of chlorine. Prior to the beginning of the experiment, the shrimp were kept in a 70 m<sup>3</sup> nursery tank. Bioflocs from that nursery were supplied by providing an inoculum at a ratio of 10% of the volume of the tanks with water for microbial aggregates. The organic fertilization method used was based on Avnimelech (1999) and Ebeling et al. (2006). This method allows the conversion of nitrogen into bacterial biomass. A total of 6 g of carbon was added for each g of total ammonia nitrogen. Dextrose was used as the carbon source. The carbon content of the compound was considered to achieve the correct concentration of carbon. Necessary corrections to the pH were made by adding 700 g of hydrated lime, Ca(OH)<sub>2</sub>, to maintain pH values above seven. Corrections were made simultaneously in all tanks to not mask the effect of clarifying. A 7-hp blower was used to maintain aeration by allowing air to diffuse from air stones at the bottom of the tank. The density of the stones in the tanks was 1 stone per m<sup>2</sup>. Shrimp with an average weight of 2.65 ± 0.69 g were stocked in the six experimental units at a density of 250 individuals/m<sup>2</sup>. During the culture period, the shrimp were fed three times daily with a species-specific commercial feed containing 38% crude protein and 8% lipid (Guabi™). Feeding trays were used for the visual detection of possible unconsumed diet and for adjusting the amount of feed if necessary.

### **Clarifier**

The clarifier was developed at the Aquaculture Marine Station (FURG) of the Institute of Oceanography, Federal University of Rio Grande, located at Cassino Beach in Rio Grande City, Rio Grande do Sul State, Brazil. The methodology for developing the clarifier was adapted from the study by Johnson and Chen (2006). When the concentration of the suspended solids reached 500 mg/l, the maximum value specified by Samocha et al. (2007) for superintensive systems, the clarification process was applied. Each application lasted for 6 continuous hours. The clarifier was housed in a plastic water box of 1,000 liters capacity. A cylindrical pipe, 300 mm in diameter and 700 mm high, was located at the center of the box. During treatment, a submerged pump was used to supply water at a flow rate of 4,500 l/h to the top of the cylindrical pipe through an intake tube connected with the culture tank. This design was



*Figure 1. Schematic diagram of the clarifier used in this study. The clarifier was installed on the outside of the greenhouse. The arrows indicate the flow of water through the clarification system. The system was driven by a submerged pump at a flow rate of 4,500 l/h.*

used to reduce turbulence. Water flowed out of the box and into the water uptake tube of the shrimp water tank (Figure 1). A column of water was formed in the box, and gravitational action produced sedimentation of particulate organic matter in the clarifier.

### **Water quality**

Temperature, pH, salinity and dissolved oxygen were monitored daily using a multiparameter instrument (YSI® 556, YSI Inc., Yellow Springs, OH, USA). Water quality was monitored based on measurements of the levels of total ammonia nitrogen (TAN), nitrite-nitrogen ( $\text{NO}_2\text{-N}$ ) and total suspended solids (TSS) taken every two days and on measurements of the levels of nitrate-nitrogen ( $\text{NO}_3\text{-N}$ ), phosphate ( $\text{PO}_4\text{-P}$ ) and alkalinity taken every seven days. Analyses of TAN followed the methodology described in UNESCO (1983). The methodology of Bendschneider and Robinson (1952) was used for the analyses of  $\text{NO}_2\text{-N}$ , and the methodology of Aminot and Chaussepied

(1983) was used for the analyses of  $\text{NO}_3\text{-N}$  and  $\text{PO}_4\text{-P}$ . Alkalinity was determined following the methodology described in APHA (1998). The water turbidity was determined by a turbidimeter (Hach® 2100P, Hach Company, Colo, USA). The volume of settleable flocs was quantified using an Imhoff cone according to the methodology of Eaton et al. (1995) adapted by Avnimelech (2007). The volume of flocs on the bottom of the cone was measured after 15 minutes of sedimentation. However, the Imhoff cone method could not be used to compare treatments because some samples lacked sedimentation tanks. Water was collected for the analysis of suspended matter (particles larger than 45  $\mu\text{m}$ ) according to the method of Strickland and Parsons (1972). The weight of suspended solids was determined gravimetrically from the filtration rates with up to 20 ml of culture water and glass fiber Whatman GF/F filters. The filters were dried for approximately 24 hours at 60°C and then weighed on an analytical balance  $\pm 0.0001$  g (Sartorius MC1AC 210 S, Sartorius AG, Göttingen, Germany) to determine the final weight (AOAC, 2000). The analysis of chlorophyll *a* was carried out weekly on 20 ml of water. This volume was filtered in a dark room, and the material was stored in 90% acetone in dark bottles at -12°C. After 24 hours, the concentration of chlorophyll *a* was determined with a Turner TD700 (Turner Design, Inc., CA, USA) fluorometer (Welschmeyer 1994).

### **Shrimp monitoring**

Every 14 days, 50 shrimp were arbitrarily sampled from each tank. Wet weight was individually measured using a digital scale accurate to 0.01 g. The weekly growth rate (WGR) was determined by the following calculation:  $\text{WGR} = (\text{final weight} / \text{number of weeks of culture})$ . The feed conversion ratio (FCR) was calculated as  $\text{FCR} = \text{offered feed} / \text{biomass increment}$ . Survival was calculated as  $\text{S\%} = [(\text{final biomass} / \text{average individual weight}) / \text{number of individuals stocked}] \times 100$ . The survival data were transformed ( $\arcsin x^{0.5}$ ) before analysis. Productivity was calculated as  $\text{Prod} = (\text{final biomass} / \text{tank volume})$ .

### **Statistical analysis**

The homoscedasticity of variances and normality of the data obtained were verified by Levene's test. The Student *t*-test was used to detect possible differences ( $p < 0.05$ ) between treatments (Sokal and Rohlf, 1969).

## RESULTS

The variation in the water and shrimp parameters measured during the 16 week experiment in the treatments with NC and with C are shown in Figures 2 and 3. From the eighth week onward, no significant differences ( $p>0.05$ ) in the concentration of total suspended solids were observed. The concentration of total suspended solids was lower in the C treatment (Figure 2). The turbidity values measured during the clarification period were significantly ( $p<0.05$ ) lower in the C treatment than in the NC treatment (Figure 2). The average concentration of chlorophyll *a* was not significantly lower ( $p>0.05$ ) in the C treatment. Variations of dissolved oxygen (Figure 2) demonstrated no significant differences ( $p>0.05$ ) from the tenth week onward. The oxygen levels observed in the C treatment were better than those observed in the NC treatment (Figure 2). The pH in both treatments was maintained above 7. However, the value of pH was not significantly different ( $p>0.05$ ) among the treatments (Figure 2).

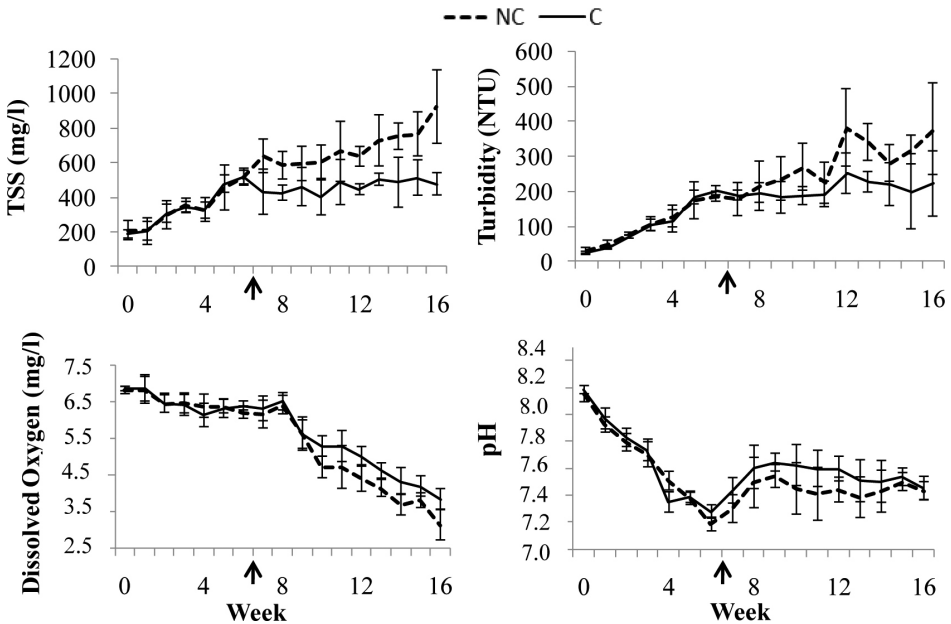


Figure 2. The variations in concentrations of total suspended solids (TSS), turbidity, concentrations of dissolved oxygen and pH values observed during the 16 week experiment in the treatments with no clarification (NC) and with clarification (C). The vertical bars indicate the standard deviation. The time at which the clarification process began is indicated by the arrow on the time axis.

The growth and survival of the shrimp differed quite significantly between treatments ( $p < 0.05$ ) during the clarification period (Figure 3, Table 2). No significant differences ( $p > 0.05$ ) between treatments were observed during the experiment for the water parameters measured (Table 1).

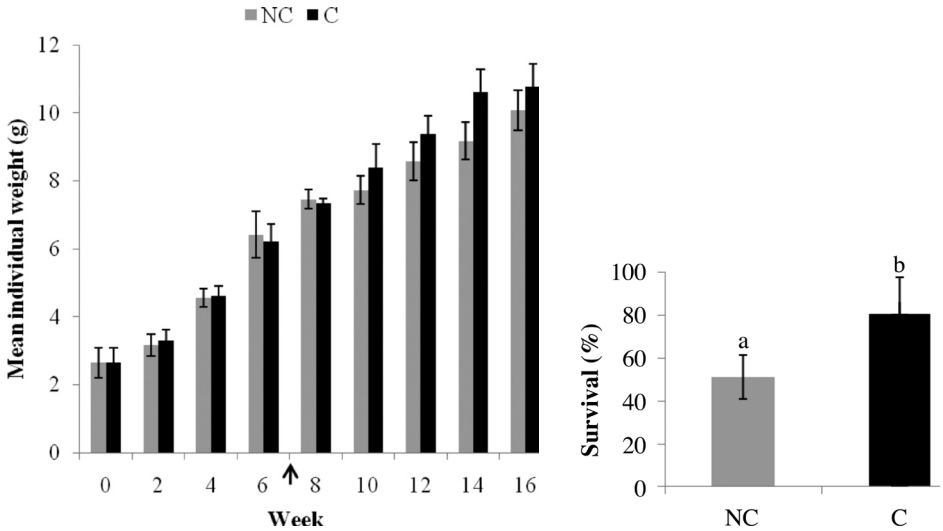


Figure 3. The growth (individual weight) and survival of *L. vannamei* in treatments with no clarification (NC) and with clarification (C) over the 16 week experimental period. The vertical bars indicate the standard deviation. The beginning of the clarification period is indicated by the arrow on the time axis.

*Table 1. The values of water parameters monitored for the period during which clarification was applied. The table shows the mean  $\pm$  standard deviation of the parameters in the treatments with no clarification (NC) and with clarification (C). The minimum and maximum values observed appear in parentheses.*

<b>Parameter</b>	<b>Treatments</b>	
	<b>NC</b>	<b>C</b>
Temperature ( $^{\circ}$ C)	22.03 $\pm$ 1.62 (19.20-25.50)	22.02 $\pm$ 1.61 (19.10-25.60)
Salinity	34.45 $\pm$ 0.59 (33.83-35.40)	34.46 $\pm$ 0.58 (33.83-35.40)
Alkalinity (mg/l)	114.06 $\pm$ 26.65 (73.75-146.25)	127.34 $\pm$ 21.26 (80.00-151.25)
Ammonia (mg/l)	0.12 $\pm$ 0.11 (0.04-0.56)	0.12 $\pm$ 0.14 (0.03-0.73)
Nitrite (mg/l)	0.12 $\pm$ 0.04 (0.03-0.20)	0.16 $\pm$ 0.11 (0.04-0.48)
Nitrate (mg/l)	39.35 $\pm$ 8.88 (3.58-55.25)	34.81 $\pm$ 8.04 (3.01-48.77)
Phosphate (mg/l)	5.34 $\pm$ 1.86 (2.40-7.60)	4.83 $\pm$ 1.76 (2.20-7.84)
TSS (mg/l)	649.53 $\pm$ 112.82 (518-923)	453.91 $\pm$ 95.07 (425-515)
Turbidity (NTU)	270.01 $\pm$ 96.15 (177-381)	197.01 $\pm$ 49.64 (187-251)
Chlorophyll <i>a</i> ( $\mu$ g/l)	378.42 $\pm$ 131.67 (240-485)	282.38 $\pm$ 97.45 (179-363)
Dissolved Oxygen (mg/l)	4.66 $\pm$ 1.18 (3.48-6.73)	5.07 $\pm$ 0.06 (3.32-6.83)
pH	7.45 $\pm$ 0.16 (7.33-7.76)	7.56 $\pm$ 0.15 (7.30-7.84)

Table 2. Comparison of the growth of *L. vannamei* in treatments with no clarification (NC) and with clarification (C). Different letters appearing on the same line indicate significant differences ( $p < 0.05$ ).

Growth Performance	Treatments	
	NC	C
Survival (%)	51.22 ± 10.32 <sup>a</sup>	80.70 ± 16.97 <sup>b</sup>
Initial weight (g)	2.65 ± 0.69	2.65 ± 0.69
Final weight (g)	10.07 ± 1.20 <sup>a</sup>	10.76 ± 0.67 <sup>b</sup>
WGR* (g/s)	0.46 ± 0.07 <sup>a</sup>	0.51 ± 0.05 <sup>a</sup>
Productivity (kg/m <sup>3</sup> )	1.34 ± 0.23 <sup>a</sup>	2.15 ± 0.24 <sup>b</sup>
FCR**	2.39 ± 0.43 <sup>a</sup>	1.47 ± 0.08 <sup>b</sup>

\*Weekly Growth Rate  
\*\*Apparent Feed Conversion Rate

## DISCUSSION

The shrimp *L. vannamei* can tolerate a temperature range between 15 and 35°C. The ideal temperature range for this species of shrimp is 28 to 32°C (Van Wyk and Scarpa, 1999). These temperatures determine the most favorable conditions for metabolism, oxygen consumption, growth and survival. Wyban et al. (1995) and Peixoto et al. (2003) have found that temperatures below 23°C represent suboptimal conditions for the growth of *L. vannamei*. Temperatures in this range affect growth negatively because the shrimp consume less food. The average temperature in the culture during the study was 22°C. Even though this experiment occurred during the winter, the greenhouse had the capacity to maintain the water temperature higher than the temperature outside the greenhouse (Krummenauer et al. 2011). This value falls within the tolerance range, but it also falls within the temperature range within which food intake is reduced. In this study, temperatures below the ideal value affected the observed weekly weight gains. These weight gains were less than the values reported by Wasielesky et al. (2006a) and Vinatea et al. (2010). These previously reported values reflect

temperatures within the optimal range for this species. The salinity in both treatments was within the tolerance range of the species (Van Wyk and Scarpa, 1999).

Nitrogenous compounds in the experiment followed a route determined by the nitrification system used in BFT (Azim and Little, 2008; Schryver et al. 2008). In heterotrophic systems, ammonia can be immobilized by heterotrophic bacteria. It is successively converted to nitrite and nitrate. The nitrogen assimilated by the bacteria during this process is converted into bacterial biomass (Ebeling et al. 2006; Hargreaves, 2006). The nitrification method used in this study was identical to that previously cited. According to Preston et al. (2000), the particulate fraction of nitrogen is reduced by the continuous flow of water and sediment, but the dissolved fraction may be higher and may remain in the water of the culture. Ray et al. (2010) suggest that sedimentation can remove feces and uneaten feed that would otherwise be available for the formation of total ammonia. Sedimentation can thereby reduce the concentration of nitrate to be converted by nitrification. In this experiment, particulate organic matter retained by decanting may have concentrated the degradation activity within the sedimentation box. Ammonia subsequently released into the water would then have maintained the nitrification process. This process was accentuated owing to the use of an inoculum of 10% of the volume of the tanks when the experiment with bioflocs was initiated. The amounts of nitrogenous compounds found in this study remained within the tolerance values for the species (Van Wyk and Scarpa, 1999).

The decomposition of unconsumed food and the excretion products of the organisms cultured are the main source of phosphorus in the culture system (Barak et al. 2003). Teichert-Coddington et al. (1999) have analyzed the sedimentation of particles through the hydraulic retention of the effluent from the intensive shrimp culture. These authors have found that phosphorus was associated with soil minerals, probably by adsorption on soil particles or as a precipitate because the concentration decreased concomitantly with the decrease of suspended solids, promoting a 14% reduction in the concentration of phosphorus. Jackson et al. (2003) achieved a 35% removal of phosphorus by using sedimentation. Ray et al. (2010) used a settling chamber for the sedimentation of particles and found that the removal of solids produced a 61% reduction of phosphate. In the present experiment,

the buildup of phosphate that occurred was typical of that occurring in superintensive systems with the reduction of phosphate occurring during the clarification. The concentrations of phosphate were lower than those found in the treatment with no clarification, but this difference was not statistically significant.

The amount of total suspended solids is an effective parameter for evaluating the efficiency of methods used for settling particles. Ray et al. (2010) achieved a 45% reduction of total suspended solids with a clarification system representing 3.2% of the volume of the culture tank of *L. vannamei* and settling chambers were operated when the turbidity found to be above 30 NTU. Johnson and Chen (2006) used a clarifier for the removal of particles greater than 104  $\mu\text{m}$ . Their system represented 3.10% of the volume of the culture of rainbow trout and achieved a TSS reduction of 82%. The clarifier used in this study represented 2.28% of the tank volume of the culture and removed 24.5% of TSS, conditional on the maintenance of suspended solids at 500 mg/l. These studies on the use of different clarifier systems involved different criteria for evaluation. Different parameters were compared with each other to evaluate the objectives proposed for each study. These parameters included turbidity, particle size and the concentration of suspended solids. Our system demonstrated a good capacity to retain suspended solids by sedimentation while maintaining the level of total suspended solids at 500 mg/l.

Turbidity has a direct relationship with suspended solids and regulates light penetration (Vinatea et al. 2010). These authors have confirmed that high levels of particulate matter reduce light penetration and therefore generate negative net photosynthetic rates, i.e., an oxygen deficit. Ramos et al. (2009) have used hydraulic retention for sedimentation and have found that turbidity decreased 18%. Ray et al. (2010) have achieved a 57% reduction in turbidity, which is the parameter they used to evaluate the performance of the clarification method in their study. They also reported an apparent increase in photosynthesis. In the present study, clarification resulted in a 27% reduction in turbidity relative to the value for the NC treatment, and the concentration of suspended solids was successfully maintained at 500 mg/l.

Microalgae play an important role in recycling nutrients from shrimp feed and feces and thereby help maintain the water quality of shrimp

culture. Control of phytoplankton biomass becomes important if respiration by microalgae in the absence of light can reduce the concentration of dissolved oxygen. A related problem is that the respiration of microorganisms resulting from the decomposition of the dead cells of microalgae can cause risks to the culture. In this study, the clarification achieved a reduction of 27.8% in chlorophyll *a*. A similar result was obtained by Jones et al. (2001), who used a static process for settling particles and achieved a chlorophyll *a* reduction of 27.7%, whereas Ramos et al. (2009) found a decrease of 45.4%.

In the bioflocs system, the concentration of dissolved oxygen in the culture water is directly related to the consumption of oxygen by shrimp, by aerobic microbes and by the decomposition of organic matter (Avnimelech, 2009). The aerobic metabolism of microbes in cultures with bioflocs can decrease the levels of dissolved oxygen (Schryver et al. 2008). In this study, the amount of oxygen consumed by the decomposition of particulate organic matter by aerobic microbes may have decreased as a result of the removal of suspended solids by sedimentation. Effects of this sort have been observed by Teichert-Coddington et al. (1999), Ramos et al. (2009) and Ray et al. (2010). Oxygen is directly associated with the conditions required for the growth and survival of shrimp. The recommended level of dissolved oxygen for shrimp is 5 mg/l (Cheng et al. 2003), and concentrations below 2.8 mg/l are considered hypoxic conditions (Mugnier and Soyez, 2005). In the present study, both treatments showed a constant decrease in the mean concentration of dissolved oxygen. The level of dissolved oxygen was below the recommended level during the last 28 days in the clarification condition and during the last 49 days in the no-clarification condition. The removal of suspended solids in the C treatment was reflected in the improved growth performance of the shrimp.

According to Ebeling et al. (2006), the alkalinity in the bioflocs system should be maintained between 100-150 mg CaCO<sub>3</sub>/l. Otherwise, a drop in pH may occur and thereby compromise the growth of the cultured organisms. These authors have also emphasized the consumption of alkalinity during the oxidation of ammonia to nitrate. Another important process is the release of CO<sub>2</sub> by respiration in the water column. The carbon dioxide dissociates to form carbonate (CO<sub>3</sub><sup>2-</sup>) and bicarbonate (HCO<sub>3</sub><sup>-</sup>) ions. This process causes the release of H<sup>+</sup> and produces a lower pH and alkalinity. Ray et al. (2010) have suggested that the removal

of suspended solids results in increased photosynthesis by the algal community during the day and causes increases in pH and in alkalinity. In this study, clarification did not affect the alkalinity in the treatment with solid removal, with the only increase in alkalinity being associated with the removal of particulate organic matter. During the experiment, the correction of pH using lime was the only application used to affect alkalinity. This method was probably sufficient to maintain the required level of alkalinity. In both treatments, the values of average alkalinity were within the acceptable range for *L. vannamei*.

During the period in which clarification was applied, the pH was significantly higher. Vinatea et al. (2010) have found that high levels of particulate matter provided a substrate for bacteria and other microorganisms. This finding was confirmed by the direct relationship between respiration in the water column and turbidity. Wasielesky et al. (2006a) have found that a lower pH resulted from the respiration of heterotrophic microorganisms. In their study, this process was found to produce increased CO<sub>2</sub> in the water of the culture. The removal of suspended solids may have reduced the oxygen consumption associated with the process of decomposition of organic matter by microorganisms in the tanks. This change would have reduced the rate of CO<sub>2</sub> production by respiration in the water column. The pH range for the optimal growth of marine shrimp is between 7 and 9 (Van Wyk & Scarpa, 1999). In BFT systems, Wasielesky et al. (2006b) have found a reduction in growth rates and feed conversion at pH values below 7. In this study, the average pH was maintained above 7 and within the recommended range for the species.

Among the physical and chemical parameters discussed, temperature, oxygen and total suspended solids were the main factors that reached values that may have affected the growth performance of *L. vannamei*. In this study, the growth found in both treatments may have been affected by the average temperature of 22°C. This species is ectothermic, and its metabolic rate is a function of the ambient temperature (Zhang et al. 2006). The highest concentrations of dissolved oxygen and the lowest amount of total suspended solids coincided with the best growth of shrimp in association with the removal of suspended solids. Similarly, the weekly growth rate was significantly higher in the C treatment in association with the reduction of suspended solids. This finding agrees with the results of Ray et al. (2010). These authors found an average survival of 71% in all their experimental tanks, but they reported no

differences between treatments. In contrast, the present study found significant differences between treatments. The highest survival rate, 81%, occurred in the C treatment. In the NC treatment, suspended solids were not removed, and the survival rate was 51%. Furthermore, the concentrations of total suspended solids in the NC treatment exceeded 500 mg/l. This excess represents a stressful condition for the culture of organisms. These high levels of total suspended solids can increase the biochemical oxygen demand (BOD) and cause occlusion of the gills of cultivated species (Hargreaves, 2006; McMillan et al. 2003). In the BFT system, the removal of suspended solids is very important because survival depends on the availability of dissolved oxygen and on the amount of suspended solids in the water column (Hopkins et al. 1995, 1996). According to Ray et al. (2010), the removal of solids can decrease BOD by reducing the stress levels of the shrimp. This process leads to an increase in production. These authors achieved a feed conversion ratio of 2.15 for a treatment that involved the removal of suspended solids. The present study found an even better rate of 1.47. This superior performance probably resulted from the fact that the clarification improved the water quality. This suggestion can be confirmed by the results of the study conducted by Vinatea et al. (2010) on the interactions of water quality variables with the growth of *L. vannamei*. These authors have found that the feed conversion rate decreased with increasing concentrations of volatile suspended solids.

## CONCLUSION

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The biofloc technology culture system used for clarification was effective for the maintenance of total suspended solids at 500 mg/l. The removal of suspended particulate organic matter increased the availability of oxygen. The removal of particulate organic matter improved water quality, and resulted in the better growth performance of *L. vannamei*. Thus, the water clarification system used in this study seems to be feasible and applicable. It successfully removed excess organic matter from the water used in the superintensive culture of shrimp and thereby maintained water quality. However, further studies of the use of clarification should be made to investigate the optimal concentration of total suspended solids and to understand the dynamics of suspended particles during the removal process.

## ACKNOWLEDGEMENTS

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## **BOOK REVIEW:**

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### **Recirculating Aquaculture, 2nd Ed.**

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The field of recirculating aquaculture has grown tremendously over the last twenty years, and this book, developed during those years, reflects not only the current state of the art but a bit of the history and reasoning for recirculating aquaculture systems (RAS) in general. The first edition of the book has been seen by many producers, industrial and academic professionals as “the book” in this area. The second edition has updated many areas of interest and clarified a number of issues. While the authors are well respected academic and research scientists (Timmons is at Cornell University, Ithaca NY; and Ebeling is with Aquaculture Systems Technologies, LLC, New Orleans, LA), the book is quite accessible for the practitioner. Several other specialists have also contributed to the book by writing or editing chapters and sections. These include Summerfelt and Vinci at Freshwater Institute; Liltved and van Rijn, with European and Mediterranean perspectives; Rakocy, perhaps the world’s premier aquaponics expert; and a number of experts in pathology, veterinary medicine, physiology, nutrition and related areas. This enhances the value and seriousness of the book.

The book is organized in such a way that preliminary comments on markets, economics, business and other practical aspects lead the way, after which basics such as water quality and various unit operations approaches for maintaining water quality integrate with basics of biology relevant to many species cultured in RAS. Physical and chemical aspects such as fluid mechanics and gas transfer each receive chapters, and process control for water and buildings receive attention in later chapters. Finally, the last few chapters deal with management, health, nutrition and

unique applications such as aquaponics. The appendix may be as useful as the text, with numerous tables and charts for basic design of piping, water quality parameters, unit operations and related parameters. Overall the book provides an excellent source of information on many subjects and is well organized.

Chapter 1 is an introduction to RAS and includes technical information on advantages; market and economic aspects; business aspects and other references. Chapter 2 gets directly to the most important aspects for species raised in an aquatic environment: water quality, and includes parametric standards, measurement techniques and related issues. Chapter 3 introduces the biological aspect of the systems: the fish and their water quality requirements, growth rates and some design examples. These examples continue through the text, giving specifics of how to size systems for optimal production.

Chapter 4 focuses on culture units, namely tanks and raceways, noting that when the percentage of recirculated water falls to zero, the analysis returns to a flow-through system. This means the mathematics and design techniques can apply to a variety of systems. Among the practical aspects in this chapter are comments on how to minimize solids (e.g. the teacup or Cornell Dual-Drain design) and how to remove dead fish, both critically important in real systems.

Chapter 5 focuses on solids capture and Chapter 6 on waste management and disposal. A variety of technologies are discussed and the solids issue reappears in Chapters 7, 8 and 9 which focus on biofiltration (e.g. nitrogen management) and design of biofilters. Many aquaculturists no longer custom design their own biofilters, but the background is helpful to appreciate how much the technologies have advanced. Where Chapter 7 focused on nitrification, Chapter 9 on denitrification and completes the series on nitrogen management in RAS. Chapter 10 explains the fundamentals of oxygen transfer into RAS and carbon dioxide transfer out of loaded systems. Chapter 11 focuses on ozonation and UV-irradiation to reduce pathogens and improve water quality. In some sense this chapter links with Chapter 16 on fish health.

Chapter 12 gets to the basics of fluid mechanics and pumps, including airlift pumps, which have the advantage of aerating and degassing as well as moving water. Chapter 13 focuses on monitoring and control,

mostly automated electronic systems these days. Specific water quality parameters and more general alarm and monitoring systems are included. Chapter 14 continues the control theme, focusing on how to maintain building environmental control, including air conditioning. Chapter 15 moves into even more general systems management such as site selection, backup power, laboratory and quarantine facilities, labor and related issues.

Chapter 16 focuses on fish health and includes discussion of biosecurity, health maintenance (via good water quality management), diagnosis, treatment and suggestions for diagnostic services. The chapter included a healthy focus on prevention of disease and recognizes that serious disease outbreaks can be difficult not only biologically but economically, which brings us to Chapter 17: Economic Realities and Management Issues. Discussion of economics, scale, risk, labor, price volatility of products and even comparison of fish to poultry provide useful advice – and fair warning – for potential investors. Despite the honesty, the advice is well taken and can be very useful to individuals or companies considering whether to start or expand an aquaculture business.

Chapter 18 focuses on fish nutrition and feeds, from physical to chemical to vitamin and mineral content. Discussion of types of feed and considerations for particular species are included. Additional references are included, as in each chapter, that allow the reader to access more information about specific areas of interest. Chapter 19 is an excellent chapter on aquaponics, which includes fish and plants in combination. The fish excrete waste and carbon dioxide which the plants use to grow. In this way, crops of fish and vegetables can be grown simultaneously, reducing wastes and improving economics. This chapter is placed last, perhaps because of thoughts that this adds another level of complexity and possible difficulties. Still, this type of system offers some of the best hope for a sustainable and productive future.

As noted, the appendix includes valuable information from conversion factors to information on many water quality parameters to fish health and mechanical and electrical sizing charts. In short, this book is an excellent text and reference that belongs in the library of any serious aquaculturist and is worth the read for anyone considering starting or expanding an aquaculture business.

