ENVIRONMENTAL REQUIREMENTS FOR DIFFERENT PRODUCTION INTENSITIES OF NILE TILAPIA (*Oreochromis niloticus*) IN RECIRCULATING AQUACULTURE SYSTEMS (RAS) IN KENYA

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Abstract
Kenya has a high potential for aquaculture. Through Flow systems such as raceways and ponds are the main types of aquaculture majorly practiced in Kenya. Due to the ever dwindling land and water resources, flow through system are becoming unsustainable for aquaculture production. RAS offer a better option to increase aquaculture production with the limited land and water resources while minimizing water pollution. The biggest challenges in RAS is to maintain favorable water quality for the fish to thrive. The practice of RAS in the country is minimal with improper matching of RAS components and production densities leading to the system failure. This study aimed at evaluating environmental requirements for different production intensities of Nile tilapia in a RAS set in a greenhouse. In this experiment, both production intensities and water flow rates were varied and the water quality parameters (Dissolved Oxygen, ammonia, pH, EC and temperature) monitored. Tilapia stocking intensities were varied between 2kg/m^3 and 10kg/m^3 while flow rate was varied from around 2.0l/min and increased at intervals of 1l/min or 1.5l/min to the maximum attainable flow rate. Crushed pumice rock packed in a 1000L tank was used as the biofilter. The study showed that, ammonia removal reduced with increasing flow rate with removal rates ranging from 75% at low flows to 2% at high flows. pH and electrical conductivity increased with increasing flow rate with R^2 ranging from 0.4 to 0.9 for both. Dissolved oxygen seemed to increase with flow rate and ranged between 5.2$\pm$2.4mg/l. Fish appetite was highest between 25-30˚C. The optimal environmental requirements for Nile tilapia were found to be 3mg/l dissolved oxygen, 7.0 pH, and 27˚C of temperature and 0.03mg/l of ammonia at different flow rates for each stocking density. Similar studies to be carried out for other fish species such as African cat fish.

Key words: RAS, Biofilter, Nile tilapia, Environmental requirements, water quality.

LIST OF ACRONYMS
DO – Dissolved oxygen
EC – Electrical conductivity
pH – Potential hydrogen
RAS – Recirculating aquaculture system
SSA – Specific Surface Area
TAN – Total Ammonia Nitrogen

1.0 Introduction

Recirculating Aquaculture Systems (RAS) used for farming aquatic organisms employ the principle of reusing the outlet water from the production tanks instead of discarding it and getting new water for the system (Musta, Verdegem, & Wahab, 2008). As a result, the quantity of new water required is reduced, thus reducing pressure on water supply systems (Yoram, 2006). It's possible to recycle all the water from the production tanks such that the replacement of water will only be done to cater for evaporative needs or consumptive needs of the fish (Lekang, 2008). However, due to the high cost of treating and purifying the effluent from the production system, the possibility of 100 percent recycling is not usually possible (Badiola *et al.*, 2010). Moreover, aaccording to Helfrich and Libey (1991), tank aquaculture systems can be referred to as closed aquaculture systems. However, RAS is not a completely closed systems since some water replacement has to be done to compensate evaporative losses and water lost to flushing settle-able solids. This is because no filter material is 100% efficient.

RAS employs the principles of efficient water utilizations and conservation with an aim of maximizing production of the target organism while minimizing pollution and water costs (Lekang, 2008). This has been brought about by the increasing demand for white meat around the world, Kenya included, with the fish caught from the natural water bodies on a decreasing trend (FAO, 2014). On the other hand, the ever dwindling land for practicing large space consuming aquatic production systems such as ponds and lagoons especially in the peri-urban areas calls for efficient utilization of available land space. This can be achieved through systems such as RAS that consume less space and water to produce aquatic organisms (Avnimelech, 2005).

The quality and quantity of water leaving the production tanks differ from one system to another depending on the type of aquatic organisms being raised (Avnimelech, 2005; Kazmierczak & Caffey, 1996). Some organisms are able to tolerate higher levels of contaminants as compared to others, for example, tilapia are able to survive levels of dissolved oxygen (DO) below 2.3mg/L while trout requires oxygen concentrations of at least 4.0mg/L to survive (Ngugi *et al.*, 2007).

As a result, the levels of contaminant removal will be different for different species of aquatic organisms. RAS have been in existence as early as 1950's although their potential to grow fish on a commercial -scale has only been realized in the past few years (Badiola *et al.*, 2010).

Upon the realization of the potential of RAS, water quality technology, testing and monitoring instrumentation widely used in waste water treatment have been extended to RAS.

A Recirculating Aquaculture System (RAS) includes the production unit which houses the aquatic organisms, a pump to transport the water around the system and water treatment system to remove contaminants from the effluent water, a pipe network joining, the production tank, the pump and the treatment system and sometimes an aeration component to add oxygen to the water (Lekang, 2008). The pump for recirculating the water and the water filtration system for removing contaminants from the water are the items that make the RAS system distinct from traditional flow-through systems. Physical, chemical and biological processes are involved in the water treatment system to improve the water quality to levels which the farmed species can tolerate and remain productive (Van Rijn, 2013). According to Lekang (2008), due to the water conserving nature of RAS, aquaculture can be practiced in areas where water is a limiting factor. Moreover, production in established farms can be increased with the existing amounts of water. Figure 1.1 after Brinkop & Piedtrahita (1996) presents a typical layout of an intensive RAS with all system components.

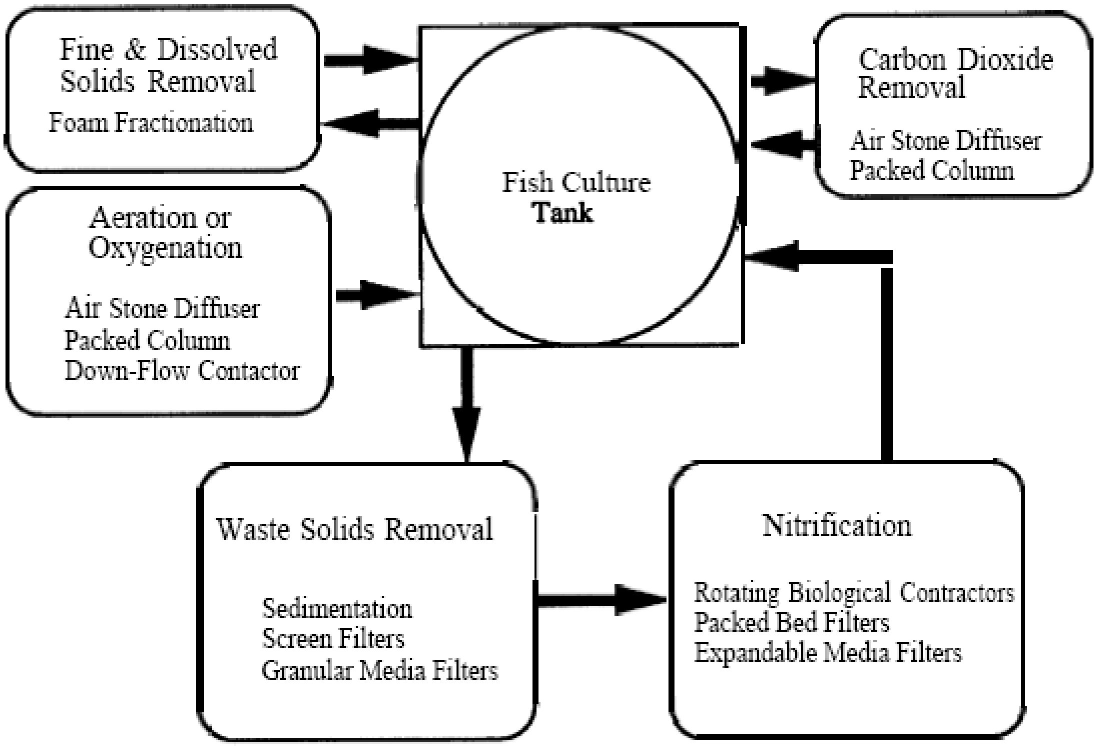

Figure 1.**Error! No text of specified style in document.**.1: A typical layout of an intensive RAS with all system components

Water reusing fish farming systems will be the most suitable in the current situation of diminishing fresh water resources and increasing competition for the same resources (Colt, 2006). With reuse systems, there are less energy requirements for heating where heating is required to keep the water at desirable temperature for the organisms. RAS have proved to be effective and efficient on usage of water and land. With RAS, production can be maximized in a small area of land with up to 80% reduction om space usage as compared to the through flow systems (Martins, *et al.*, 2010; Helfrich & Libey, 1991).

RAS systems are also easier to clean and have less costs associated with water treatment and water delivery especially if the water used in the production has to be cleaned prior to use or is pumped (Crab *et al.*, 2007). However, RAS despite their many pros have a number of limitations (Pillay & Kutty, 2005). This includes the initial costs of installation, the operation costs and costs of maintenance. The design of water reuse system requires a good understanding of the interactions between biology, chemistry, physics engineering and economics. As at present, there are no identical systems in RAS and therefore making it difficult to construct a good RAS using a particular example. A good choice of an effective biofilter whose purification efficiency is known may also prove to be very expensive to acquire.

With RAS, fish can be produced in the home backyards and the family white meat needs met at low costs and additionally, provide a source of income through the sale of the surplus. To achieve the efficiency and effectiveness of the RAS systems, one of the most limiting issues in all Aquacultural productions, water quality, must be addressed (Avnimelech, 2005; Pillay & Kutty, 2005). To address the issue of water quality in aquaculture, fish stocking intensities must be matched with the water recirculation rates for proper functioning of the system. The biofilter material which is the home to the nitrifying bacteria facilitates the conversion of Ammonia into the non-toxic forms of nitrogen before the water is pumped back into the production system (Badiola *et al.*, 2010; Gutierrez-Wing & Malone, 2006). Unfortunately, in Kenya there are no developed specifications and standard designs for various RAS stocking intensities.

Most farmers in Kenya practicing fish production using the RAS, use locally available inert materials such as sand and charcoal and sometimes combine them with plant material for the uptake of the nitrates upon conversion (aquaponics) (Obwanga, Lewo, & Bolman, 2017).

RAS is one type of the various modes of aquaculture practiced in Kenya (Munguti *et al.*, 2014). The biggest challenge in intensive fish production is to maintain favorable water quality for the fish to thrive and at the same time minimize on the cost of power required for pumping and aeration (Pillay & Kutty, 2005). Most of the farmers practicing inland fish farming are using the pond and raceway systems majorly due to availability of water to refill the system. With these old systems it is cheaper to get new water and release the polluted water instead of treating it and returning it into the system (Obwanga *et al.*, 2017). Never the less, water sources are decreasing day by day from competing needs while the amount of land available for setting up huge fish ponds with little fish stocking is diminishing due to land fragmentation. RAS provides an answer to the problems of reducing water and land requirements for fish production but not without involving some cost due to the need to treat and reuse the water in the system.

RAS provides a conducive environment for fish growth with little water requirements and possibility of high productivity as compared to pond and raceway systems (Pillay & Kutty, 2005). This is because, with RAS, the outlet water is cleaned and used again, which means that the amount of added new water can be reduced (Lekang, 2008). However the main challenge in RAS is to remove ammonia from water and create a conducive environment for the fish to thrive and keep the system profitable at the same time (Yoram, 2006). This research aimed at determining the environmental requirements of Nile Tilapia at different production intensities in a recirculating aquaculture system.

2.0 Materials and Methods

2.1 Materials

The materials, tools and equipment used for the study included; Nile Tilapia (*Oreochromis niloticus*) of 200±20g, 1″ pipes (PPR), 1000L grow tanks, 0.5hp pumps, water quality sensors, biofilter material (pumice), electricals, 500L water storage tanks, water meters, a greenhouse (8 by 15m), power source and laptops for recording and analysis of data and preparation of reports.

2.2 Methodology

In this study, the components constructed or assembled included; a fish tank, a biofilter, connection of pipes and their fittings, pumps and aerators as well as installation of a green house in which the complete set up was housed.

Each and every component was fabricated and installed in place and equipped with the relevant accessories. Production tanks were then filled with water and the system allowed to run before stocking to identify any leakages and correct any areas that might not be functioning as expected. The production tanks were fitted with flush out pipes at the side near the bottom to allow for the removal of any solids from fish waste and uneaten feeds to be drained out. About 10% of the water was drained to allow for the removal of these solids (Badiola *et al.*, 2010). This water was then replaced from a nearby fresh water source to restore the system capacity on a weekly basis.

Once the whole set up was found to be functioning properly, the fish tanks were stocked with grown fish (200± 20g) each so as to enable rapid generation of ammonia. The biofilter was also inoculated with raw water from a nearby fish pond to accelerate the growth of the nitrifying bacteria as opined by Helfrich & Libey (1991). The RAS was operated with low, medium and high stocking densities. The system was run for one to two weeks before the stocking density was increased to a next level.
Figure 2.1 Flow diagram showing the various components the RAS.

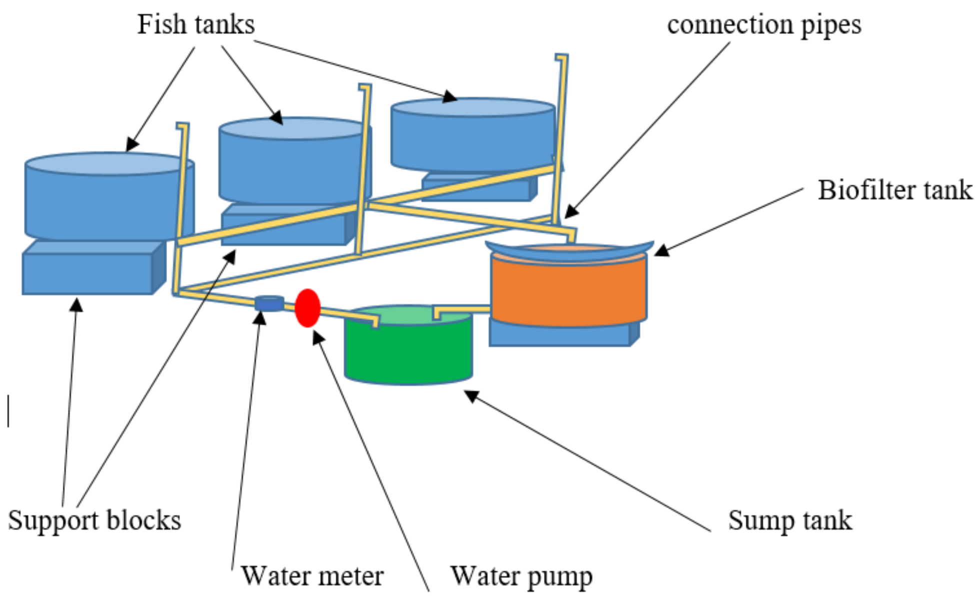

*Figure 2.**Error! No text of specified style in document.**.2: A layout of the RAS components for a single set up.*

Three 1000L production tanks were used and the stocking densities were varied from 2kg/m^3 (low) to 10kg/m^3 (high). Circular tanks, made of plastic were used. Stocking densities of up to 15kg/m^3 have been used successfully in cage systems and RAS studies (Gibtan, 2008; Ridha, 2006; Sri-uam, et al., 2016) Aquaculture System On the other hand, stocking densities below 3kg/m^3 have been used in pond systems successfully. This makes 3-10 kg/m^{3}stocking densities suitable for the RAS.

2.3 The Biofilter

The volume of the biofilter required was determined by dividing a biofilter's Specific Surface Area (SSA) by the value of the SSA of the biofilter available in a unit volume of the biofilter (m^2/m^3). Putting into consideration the highest stocking density to be used for this study, the biofilter material used was packed in a 500L tank and operated as a trickling filter. Porous lava rock (pumice) was the substrate of choice that was used as a biofilter material for this study. Pumice was selected as the choice for this study because it is light in weight, highly porous and provides sufficient porosity for movement of water as compared to sand, gravel and other substrates. The high density of pores in pumice provides a high Specific surface Area for growth of bacteria films. The biofilter's retention time was computed for the different flow rates for the different fish stocking densities. The design of the biofilter was aimed at keeping the ammonia levels below 0.05mg/l and nitrite levels below 0.5mg/l (Badiola *et al.*, 2010).

2.4 Pump and Aerator And Connection Pipes

0.5hp pump was used for pumping the water from the sump after purification back into the production tanks. A gate valve fitted just after the pump provided for the variation of flow rates. A water meter fitted immediately after the pump was provide for the measurement of flow rate. PPR pipes of 1inch internal diameter were used to connect the various tanks to allow for water recirculation. The choice of pipe diameter selected follows pump's designer's recommendation that, the used pipe diameter correspond to the pumps' intake and discharge diameters to avoid reducing the pumps working efficiency (Larralde & Ocampo, 2010). Aerators with aeration rates of 15L/min (900L/hr.) were used to pump air into the production tanks to boost oxygen availability. Moreover the recirculated water was released from a height of about 0.5m above the level of water in the production tanks so as to splash the water in the production tanks thus increasing infusion of oxygen into the water.

In all this cases, the corresponding measurements of water flow was done and recorded.

2.5 Measurement of Flow Rate and Water Environmental Parameters

Once the system was fully functional, the water quality parameters -temperature, ammonia, DO, pH and EC were measured. The parameters were measured within 15min after sample collection on a daily basis (between 4:00-5:00pm) for the different stocking densities and water flow rates using the HACH^R equipment (probes and multi-meter). The procedures of measurement are as described in the HACH^R manuals (Manual, 2013). Flow rate was varied from around 2.0l/min and increased at intervals of 1l/min or 1.5l/min to the maximum attainable flow rate. At any time of water meter failure, the stop watch and bucket system was used to measure the flow rate. Once in a week, about a quarter of the water in the production tanks was replaced with clean water. All the collected data was recorded in excel sheets. The primary computation such as purification rates of biofilter at different flow rates were then computed for each set of flow rate for the various stocking densities. Both descriptive and inferential statistics were used in the analysis and presentation of the collected data. Line and bar graphs as well as means were generated from the collected data. Analysis of variance (ANOVA) of the environmental parameters were also conducted for the stocking densities and the corresponding flow rates. The ANOVAs were conducted based on the randomized block design format with the two primary factors being Flow rate and stocking density.

3.0 Results and Discussion

3.1 General Introduction

The laboratory setup of the recirculating aquaculture system was as shown in figures 3.1, 3.2 and 3.3 below. The RAS system made of a connection of PPR pipes and plastic tanks for the production tank, biofilter tanks and sump tanks are housed in an 8 by 15m greenhouse. The greenhouse structure helped in building up the appropriate ambient temperature and consequently the water temperatures for the fish to thrive well. Environmental parameters and power were measure and recorded for different flow rates and stocking densities in this experimental setup.

Figure 3.1: The RAS systems in the greenhouse.

Figure 3.2: The RAS showing the production tanks, biofilter tank and sup tank among other components

Figure 3.3: The production tanks showing return water pipes and aerator leads pumping air into the water.

3.2 Environmental Parameters for Different Production Densities and Water Flow Rates in Recirculation Aquaculture Systems

3.2.1 Ammonia

Figure 3.4 and 3.5 below shows the variation of ammonia with flow rate and stocking densities. In most of the scenarios, the ammonia concentration increases with increase with flow rate. This is a clear indication of reducing removal of ammonia with increasing flow rate. At low flow rates, the hydraulic detention time is longer as compared to that at higher flow rates. As a result, longer detention time at lower flow rates lead to better removal of ammonia at lower flow rates as compared to higher flow rates. The increase in ammonia concentration with flow rate tend to fit an exponential trend with the mean square value being as high as 0.7 at some stocking densities. All in all the ammonia concentration levels are substantially below the lethal levels for most flowrates in both the production tanks and the biofilter as opined by a number of researchers (Ngugi, et al., 2007; Sri-uam, et al., 2016). Feed intake did not decrease up to dissolved ammonia levels of 0.434mg/l (Verster, 2016). From the analysis of variance at 0.05 confidence level, variation of stocking density had a significant effect on the ammonia concentration as compared to the variations in flow rate.

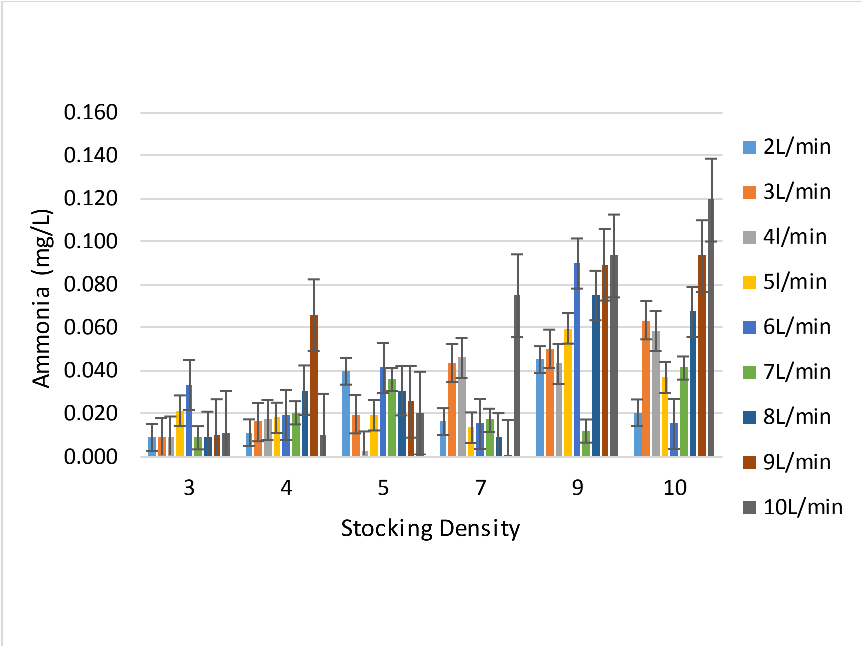

Figure 3.4: Variation of Ammonia with stocking densities at different flow rate.

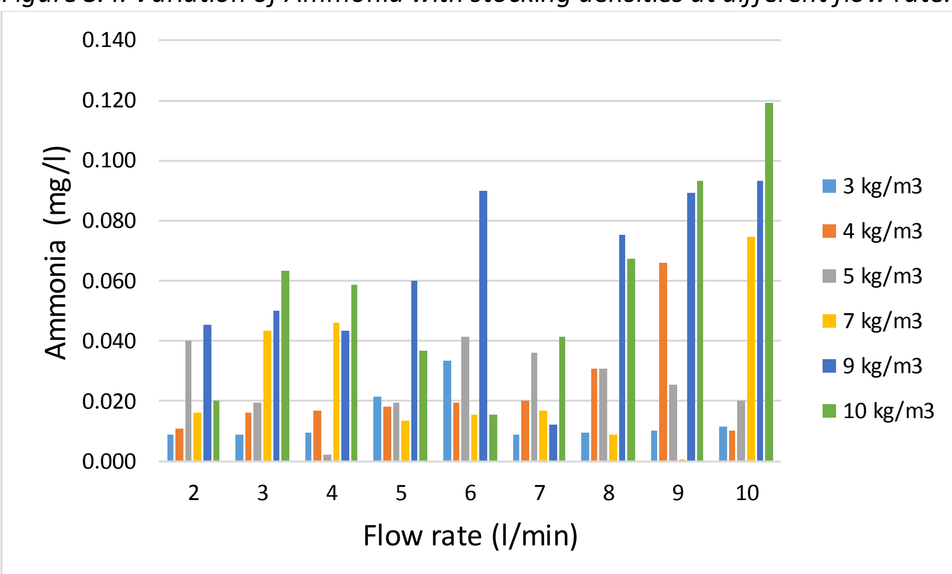

Figure 3.5: Variation of Ammonia with flow rate at different stocking densities

3.2.2 Dissolved Oxygen

Dissolved oxygen seemed to increase gradually with increasing flow rate as shown in figure 3.6 and 3.7 below. This was attributed to the increased oxygen dissolution with increasing flow rate. The aerator which operated at a pumping rate of 15l/min maintained the dissolved oxygen levels above 2.3mg/l for most stoking densities. However, there was an observed decrease in oxygen concentration with increasing stocking density especially at stocking densities above 7kg/m^3. According to Ngugi et al. (2008) and Sri-um et al. (2016), the Nile Tilapia are a bit hardy and can survive low oxygen concentrations to levels below 3mg/l.

As a result, the RAS maintained favorable oxygen levels for the fish to grow and thrive. The tendency of the dissolved oxygen to increase with flow rate can be attributed to the mixing of the waters in the production tank as the water is recirculated back into the tank. Moreover, the oxyge n concentration in the production tanks was relatively higher than that in the sump tank after the water goes through the biofilter as shown in figure 4.9 at 3.46kg/m^3. This is because the nitrification process is an aerobic process in which oxygen is consumed to facilitate for the conversion of ammonia into nitrites and then into the less harmful nitrates. Both variations in flow rate and stocking density had a significant effect on the oxygen concentrations in the RAS water.

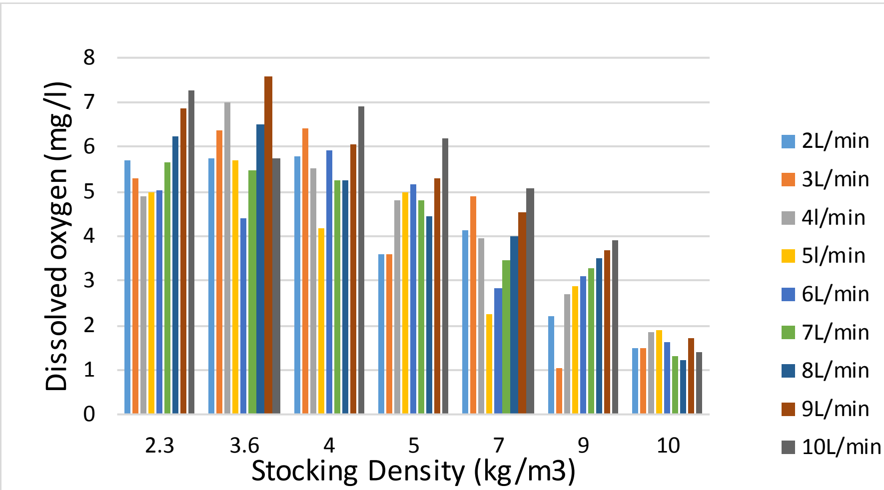

Figure 3.6: Variation of dissolved oxygen with stocking density in the biofilter.

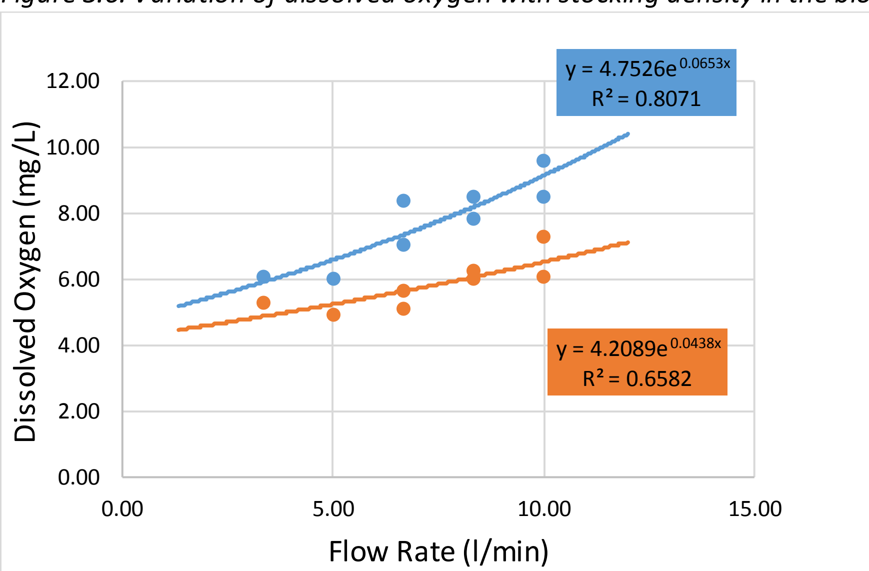

Figure 3.7: Variation of dissolved Oxygen with flow rate at 2.3kg/m^3 stocking density

3.2.3 Electrical Conductivity

The levels of electrical conductivity seemed to increase with increasing flow rate and stocking density with R^2 of over 0.8 - 0.95 shown in figures 3.8 and 3.9. This may be attributed to the decreasing conversion of ammonia into nitrites and nitrates with increasing flow rates. The levels of EC did not seem to be different both before and after the biofilter. However a slight increase in EC in the water after passing the biofilter was visible at higher flow rates as compared to lower flow rates.

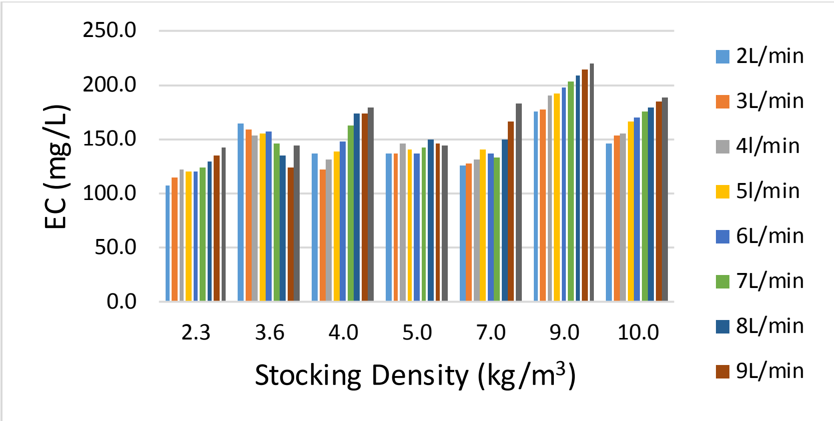

Figure 3.8: Variation of EC with stocking density in the production tank

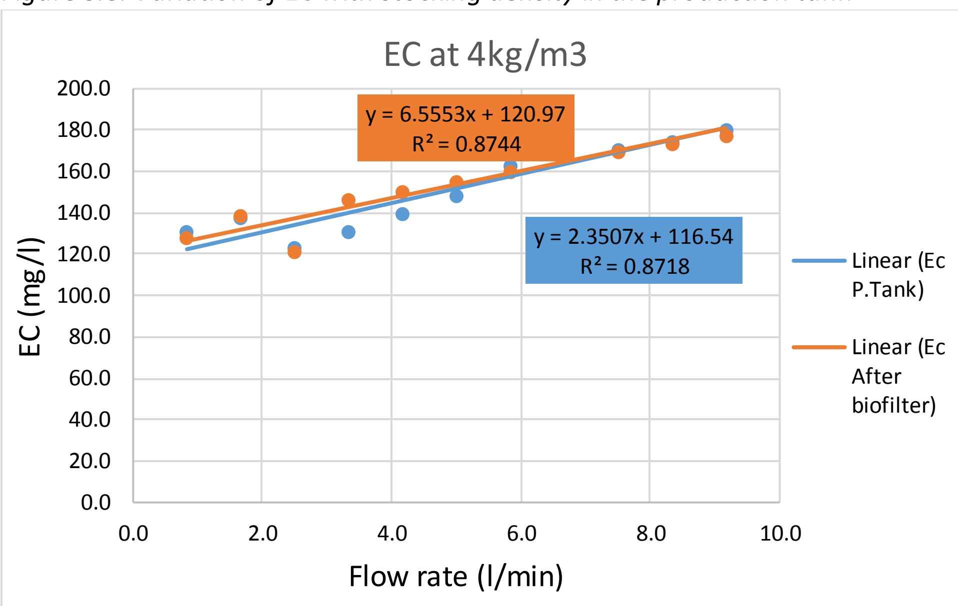

Figure 3.9: Variation of electrical conductivity with flow rate at 4kg/m3 stocking density.

3.2.4 pH

pH increased gradually with increasing flow rate both before and after biofilter. At a stocking density of 3.5, it can be seen that the pH levels are higher in the production tank and lower in the sump tank after the biofilter as shown in figures 3.10 and 3.11 below. This is an indication of ammonia removal by the biofilter. Ammonia varies proportionately with temperature and pH. At lower Ammonia levels, the pH is also lower and at higher ammonia levels the pH is also higher. The pH levels in the production tank and after the biofilter were within the acceptable range (6.9-9) for the fish and the nitrifying bacteria to thrive. Like ammonia, the pH levels were affected by both the flowrates and stocking density variations.

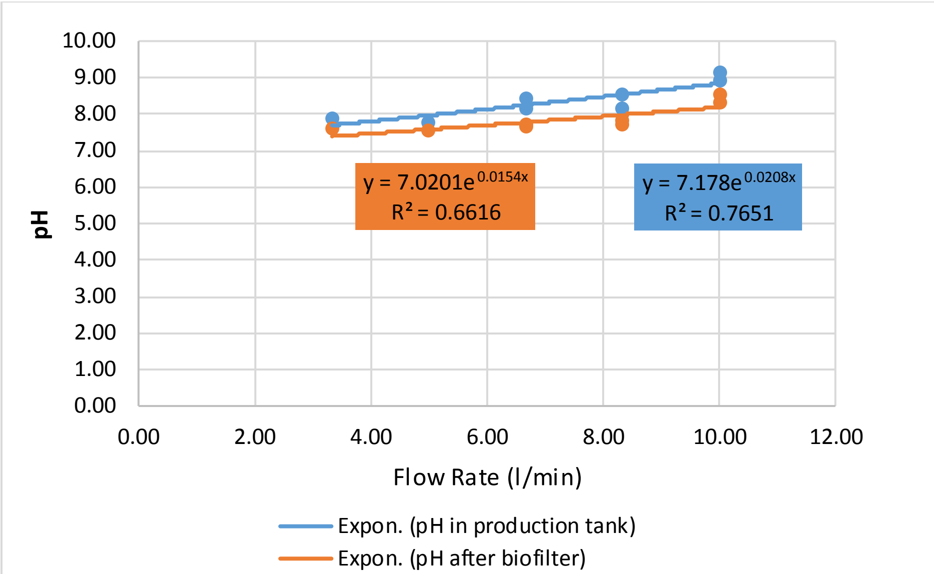

Figure 3.10: Variation of pH with flow rate at 2.3kg/m3 stocking density

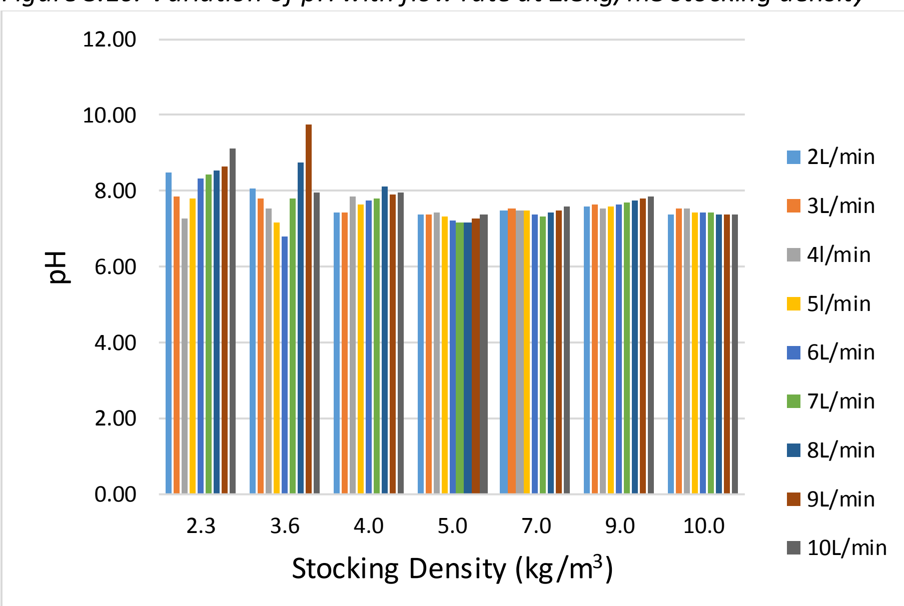

Figure 3.11: Variation of pH with stocking density in the production tank.

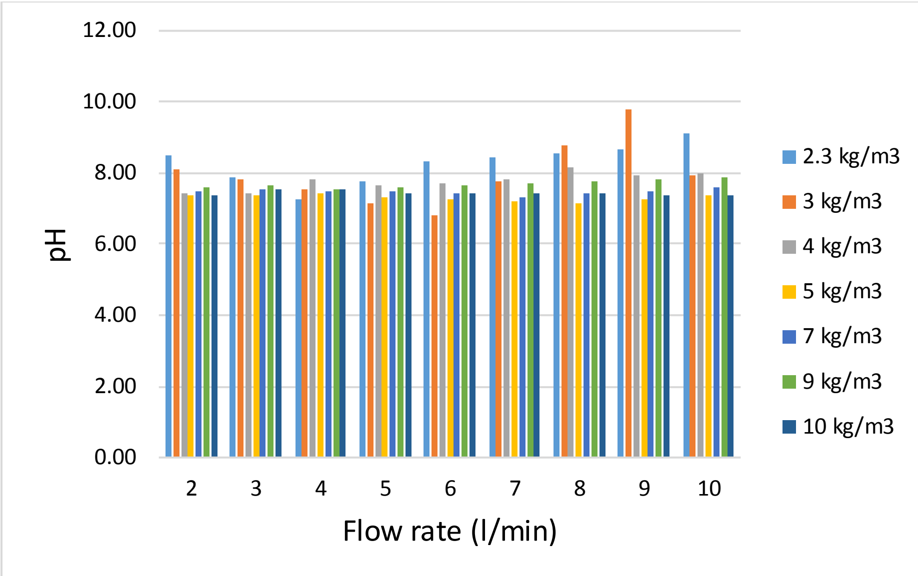

Figure 3.12: Variation of pH with stocking density in the production tank.

3.2.5 Temperature

There was no heating provided for the water in the production tanks. The water temperatures in the RAS at different flow rates was as shown in table 4.1 below. However, the greenhouse cover in place helped to keep the temperatures within acceptable range for the Nile tilapia to survive. The most favorable temperatures for tilapia survival are within a range of 25-33˚C according to Ngugi, (2007). The average temperature of the water in this study is 26.4 ± 3.1 ˚C which makes it suitable for both the fish and the nitrifying bacteria. Variation of stocking density and flow rate had no influence on the RAS water temperature.

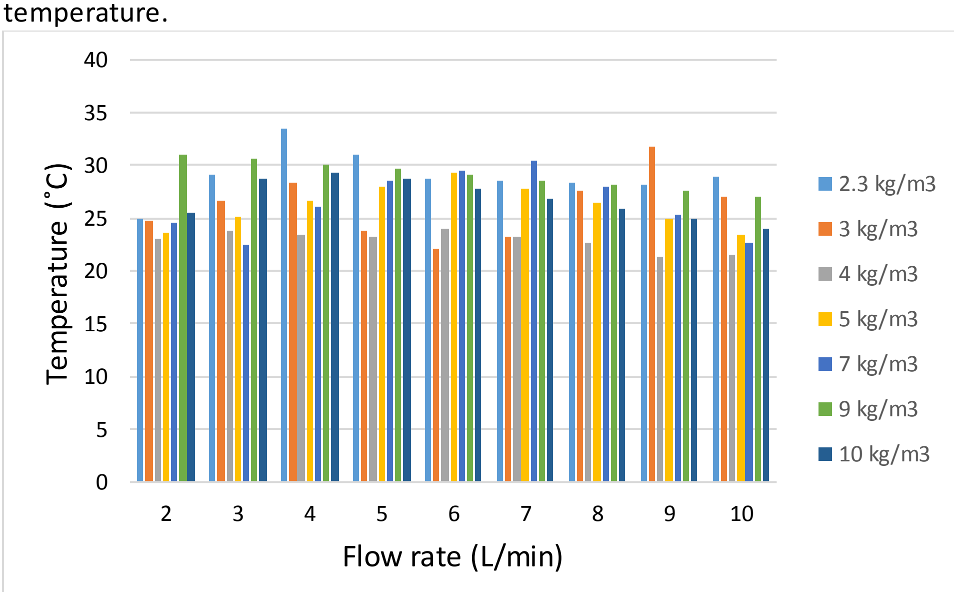

Figure 3.13: Variation of temperature with stocking density in the production tank

3.2.6 Purification Efficiency

The purification efficiency seemed to decrease sharply with increasing flow rate as shown in figure 3.14 from the study it was observed that, ammonia removal reduced with increasing flow rate with a R^2 ranging from 0.3 to 0.7 for most stocking intensities. It was also observed that purification efficiencies decreased with increasing stocking density at a given flow rate. ANOVA for purification efficiency showed that, both variations in flow rate and stocking density had a significant influence on the purification efficiency.

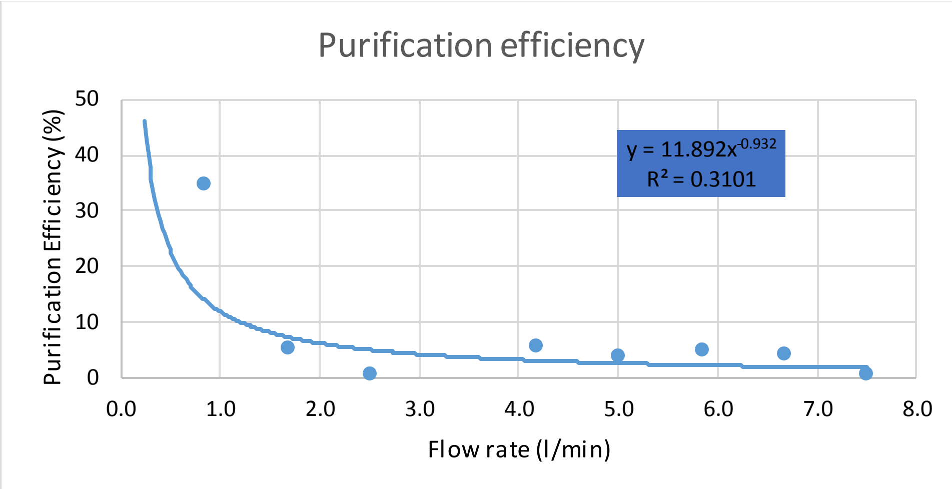

Figure 3.14: Variation of purification efficiency with flow rate at 4kg/m3 stocking density

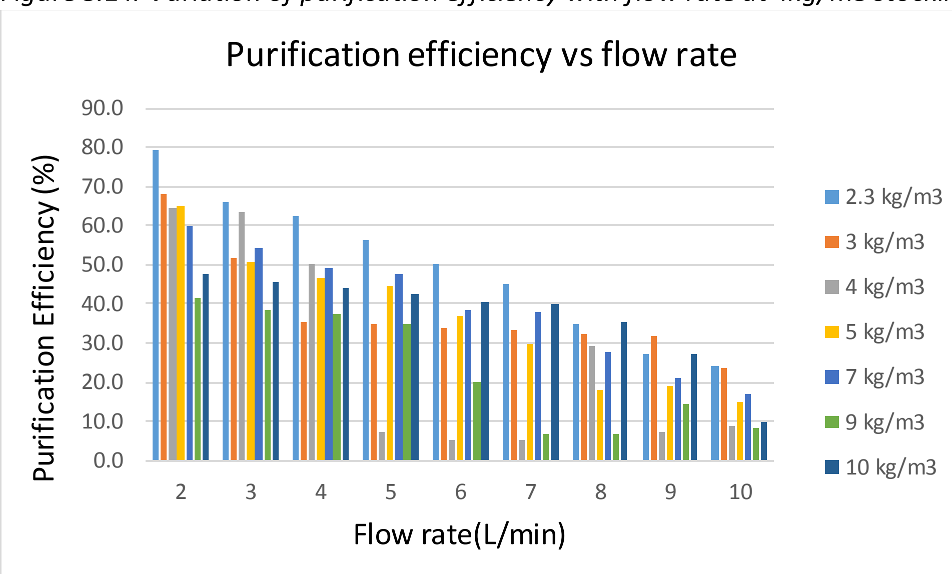

Figure 3.15: Variation of purification efficiency with stocking density at different flow rates.

3.2.7 RAS turnover and Biofilter retention time

The water flow rate influenced the system's turnover and the biofilter's retention time. According the table 4.2 below, the turnover duration decreases with increasing flow rate leading to a decrease in detention time with flow rate.

Table 3.1: Flow rate and corresponding turnover rates.

Flow rate (l/min)	**Turnover Rate (hrs)**	**No. of Turnovers**
3.3	15.0	1.6
5.0	10.0	2.4
6.7	7.5	3.2
6.7	7.5	3.2
8.3	6.0	4
8.3	6.0	4
10.0	5.0	4.8
10.0	5.0	4.8
12.0	4.2	5.76

4.0 Conclusion

For each stocking density, high flow rates led to poor environmental conditions than at lower flow rates. Nile tilapia suffered stress at oxygen concentrations below 2mg/l and pH below 6. However at oxygen concentrations above 3mg/l and pH above 6.9, Nile tilapia thrived well and fed without limitations. The pumice rock showed a good capacity to remove ammonia from RAS water at different flow rates and stocking densities. The purification efficiencies ranged from as low as 4% at low flow rates to as high as 70% at low flow rates. The optimal environmental requirements for Nile tilapia were found to be 4mg/l dissolved oxygen, 7.0 pH, and 27˚C of temperature and 0.03mg/l of ammonia at different flow rates for each stocking density. Both stocking density and flow-rate had a significant effect on the RAS water quality.

5.0 Recommendation

For best results on water quality, farmers practicing RAS should use the right combination of stocking density and water flow-rate in order to achieve the best water quality for the fish to thrive. Similar studies to be carried out for other fish species such as African cat fish as well as with other biofilter media other than pumice.

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