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THE EFFECT OF STOCKING DENSITY IN HAPAS ON *Labeo victorianus* PRODUCTION IN PONDS WITH HIGH OR LOW C:N RATIO

Name: ESTHER WAIRIMU MAGONDU

Reg.nr. 820613540110

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AQUACULTURE AND FISHERIES GROUP

LEERSTOELGROEP AQUACULTUUR EN VISSERIJ

Departement of Animal Sciences
Aquaculture and Fisheries Group
Marijkeweg 40, Postbus 338
6700 AH Wageningen
The Netherlands
Tel: +31 (0) 317 483307
Fax: +31 (0) 317 483937

MASTER THESIS

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STUDENT: ESTHER WAIRIMU MAGONDU

REG NO: 820613540110

AFI-80424

SUPERVISORS: DR. MARC VERDEGEM

DR. HARRISON CHARO KARISA

DEDICATIONS

To my late dad Samuel Magondu, My loving mother Jecinta Njeri; your prayers mum, endless encouragement and push for tenacity have kept me strong. My brothers; David, John, Geoffrey and Paul for your love and care.

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Now thanks be to God who always leads us in triumph in Christ, and through us diffuses the fragrance of His knowledge in every place. 2 Corinthians 2:14 (NKJV).

ABSTRACT

Culture of *Labeo victorianus* in Kenya can be used as measure of improving food security and as a means of supplementing income for rural families. This study was carried out in 18 hapas suspended in six, 150 m^2 earthen ponds to investigate the effects of C/N ratios of 10 and 20 of feed inputs and stocking density on *Labeo victorianus* growth, water quality, total heterotrophic bacteria counts, sediment quality and pond productivity. *L. victorianus* juveniles were stocked in hapas at densities of 10, 15 and 25 fish m^{-2}. All treatments were carried out in triplicate during a time period of seventy two days. A locally formulated and prepared feed containing 30% crude protein with a C/N ratio of 10 was applied. Maize flour was used as the carbohydrate source for manipulating C/N ratio and applied to the water column separately from feed. Increasing C/N ratio from 10 to 20 reduced ($P<0.001$) the total ammonia nitrogen (TAN), nitrite-nitrogen (NO_2-N) and nitrate-nitrogen (NO_3-N) in the water column and total nitrogen in the sediment ($P<0.001$). It also raised sediment pH, organic matter and total phosphorus ($P<0.001$). Increasing C/N ratio also increased phytoplankton by 13% and zooplankton biovolume by 25% in the water column ($P<0.001$), which was not to be used by bottom feeder *Labeo victorianus*. Total benthic macro-invertebrates biovolumes were also 30% higher ($P < 0.05$) with a C/N ratio of 20 compared to 10. The lowest protein efficiency ratio (PER), specific growth rate (SGR) and the highest food conversion ratio for the feed were recorded with a C/N ratio of 10 ($P<0.05$). Raising the C/N ratio from 10 to 20 increased the net yield by 15% from 1534 (C/N 10) to 1821 (C/N 20) kg ha^{-1} 72 d^{-1}. Based on highest growth, survival, production and net benefits, C/N ratio of 20 and a stocking density of 15 fish m^{-2} is optimal. Therefore, carbohydrate addition in *L. victorianus* culture is a promising option for sustainable aquaculture. However the underutilization of pond communities exhibited in this study indicates possibility for inclusion a water column feeder to further improve the total production per unit surface area.

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1. INTRODUCTION

1.1background of the study

Stocking density is a major factor affecting fish survival, growth, behavior, health, water quality, feeding and production (Rui et *al.*, 2006, Backiel and LeCren 1978). For the development of rearing techniques appropriate stocking densities must be determined for each species passing through successive production stages to enable efficient management and maximize production benefits. Culture of fish in enclosures applying the right stocking density can effectively improve the yield per unit area. However, stocking density can also cause stress, lower the feed conversion efficiency, increase fish energy consumption and reduce feeding rate and digestibility (Wallat et *al*., 2004; Islam et *al*., 2006; Rowland et *al*., 2006; Gibtan et *al*., 2008).

Labeo victorianus (Boulenger, 1901), commonly known as Ningu, is a potamodromus fresh water fish native of Lake Victoria (Green Wood, 1966). The fish was once widely distributed in the Lake Victoria basin and supported the most important fishery of all the potamodromus species in the lake (Cadwalladr, 1965b). The introduction of gill nets, set at the river mouths during spawning migrations, has been reported to be responsible for the rapid decline of the species since the 1950s (Ogutu-Ohwayo, 1990; Seehausen, 1996; Rutaisire, 2003). An alternative to ameliorate this problem is captive propagation with brooders from the wild. Although *L. victorianus* has not been cultured widely, it has potential where besides production of rich protein food, it would consequently reduce fishing pressure on the wild stocks. Members of the same genus rohu, *L. rohita* and *Labeo fimbriatus,* are widely cultivated in India (Jhigran and Pullin, 1985; Halder*et al*., 1991; Mridula et *al*., 2003) and *Labeo calbasu* in Bangladesh (Wahab*et al*., 1999). In Malawi two indigenous species *L. mesops* and *L.cylindricus* have been tested for small-scale aquaculture (Hiroyuki *et al*., 1999).

As the aquaculture industry develops towards intensification, which comes hand in hand with increase in culture densities, its environmental impact increases. It is important to investigate the most appropriate stocking densities in intensive and semi intensive culture systems which will help in maintaining appropriate water quality balances which are vital for profitable pond harvest. In most cases the principal factor affecting water quality is the amount of feed supplied and subsequent release of metabolites in form of nutrients (Milstein, 1990). Good husbandry practices like stocking densities, nutrient ratios, aeration and water exchange are aimed at reducing the impacts of metabolites that would hamper water quality. Finding a right balance between nutrient ratios and carrying capacity in fish ponds while maintaining favorable culture conditions is a common problem in aquaculture pond management. Discharges from aquaculture deteriorate the receiving environment with fish serving as reservoirs of nutrients generated from the culture environment. Fish may also transport nutrients between compartments within an ecosystem or transfer nutrients to other ecosystems (Tanner et *al*., 2000). Different measures have been used to remove nitrogen from water: use of biological filters, addition of substrate for periphyton development, use of bio-floc technology and C/N ratio control (Avinmelech, 2007). This study employed C/N ratio control by addition of carbohydrates to assess the economic viability of *L. victorianus* culture at different stocking densities.

The C/N ratio has been widely used as an index of the rate at which organic matter will decompose (Alexander, 1961). Where if the organic matter is low in nitrogen content, some nitrogen is obtained from the water column to enhance microbial growth and eventually immobilized as microbial protein. (Boyd, 1996). Jimenez-Montealegre (2001) showed that the percentage of protein in aquaculture feeds can range

from 20% for omnivorous species up to 55 % for carnivorous species. Such nitrogen-rich inputs cause the C/N ratio to drop to between 9.5 and 6. Rittmann and McCarty (2001) pointed that heterotrophic aerobic bacteria have a C/N ratio of around 5, but also release CO_2 as metabolic waste. Further, Alexander (1999) concluded that a good C/N ratio allowing for complete decomposition of organic matter under aerobic conditions is around 10. Previous studies by Lancelot and Billen (1985), have also shown that immobilization of inorganic nitrogen only occurs when the C/N ratio of the organic matter is higher than 10. This therefore indicates that a thorough understanding of fish–nutrient–ecosystem interactions requires an accurate determination of nutrient ratios in relation to the culture environment to provide appropriate growth conditions to the cultured fish. In this context therefore, there is lack of quantitative data on the African cyprinid *L. victorianus* and how the nutrient ratios would contribute to pond carrying capacity. Additionally, there have been conflicting reports on the influence of habitat, stocking densities, season, age or size and carrying capacities on fish nutrient composition and nutrient ratios. This study therefore investigated on the combined effects of stocking density in hapas on *L.victorianus* production in ponds with high and low C/N ratio. This information will be helpful to design a healthy and efficient hapa culture method for *L. victorianus*.

1.2 Statement of the problem

Currently fish farming is being revamped in Kenya to satisfy the growing demand and also to compensate for the reduced capture from overexploited fisheries. This development could be hampered by challenges in knowledge of best aquaculture intensification methods, which come with higher stocking densities and greater use of water, feeds and fertilizers, leading to increased waste production (Beveridge et *al*., 1997). In this regard, vigorous efforts should be put towards adopting better technologies that will make aquaculture systems more resource-efficient thus allowing the desired sustainable growth. Manipulation of the carbon and nitrogen ratios with feeds and cheap carbohydrate sources has proved efficient in lowering the ammonia levels in water and maximizing overall nutrient retention (Azim and Little, 2006). Also studies by (Schneider et *al*., 2005) showed that if carbon and nitrogen are well balanced the nitrogenous waste generated by the cultivated organisms and especially ammonium will be converted into bacterial biomass. Avnimelech (1999) further suggested that if properly adjusted, added carbohydrates can potentially eliminate the problem of inorganic nitrogen accumulation and promote overall pond sustainability. Although optimization of stocking ratios by use of feeds has been done for cyprinids like rohu *(Labeo rohita)* and catla (*Catla catla)* (Azim et *al*.,2001*)*, there is no data available on the most appropriate stocking density in hapas for *L. victorianus*. Also the use of C/N ratio control which is still an upcoming technology needs to be investigated locally, before the it can be made available to farmers.

1.3 Justification of the study

Stocking density is an important parameter in fish culture operations, since it affects growth and survival and hence production. Considering the high cost of fish feeds, pond water quality management, rapidly rising human population coupled with the need for food security, aquaculture needs to grow rapidly and urgently. Making cheap protein sources for fish culture available is important to make aquaculture economically sustainable. Aquaculture feeds are expensive and constitute an appreciable portion of the total production cost, and fish farmers cannot afford any misuse of feeds. Farmers should aim at increasing feed efficiency, especially of dietary protein, the most expensive component in fish diets.

This study focuses on *L. victorianus*, potentially a new culture species for Kenya. Little information is still available on appropriate stocking densities of this species in hapa culture, and if in hapas a C/N ratio increases productivity and profitability.

1.4 Aim of the study

The broad objective of this study was to assess the best combination of C/N ratio and stocking density on the production of *L. victorianus* in hapas.

1.5 Specific objectives

- To find out the effect of different stocking densities in hapas in *L.victorianus* ponds focusing on growth, survival and production.
- To determine the effect of C/N nutrient ratios on total pond production.
- To evaluate possible interactions between C/N ratio and stocking density on the water quality and production of *L. victorianus.*
- To evaluate the effect of C/N ratio on sediment quality.

1.7 Hypotheses

Ha (alternative hypothesis)

- C/N ratio affects *L.victorianus* production in hapas
- A higher C/N ratio allows for a higher stocking density

Ho (null hypothesis)

- There is no relationship between C/N ratio in *L.victorianus* production hapas
- A higher C/N ratio does not allow higher stocking densities

2.0 LITERATURE REVIEW

2.1 Influence of nutrients on pond production

Science of freshwater pond fish-culture has made great strides in recent years and there is a fast advancing frontier of knowledge on every aspect of pond culture to improve the productivity and sustainability of pond production. Aquaculture intensification, however, comes with higher stocking densities and greater use of water, feeds and fertilizers, leading to increased waste production (Beveridge et *al*., 1997). Organic matter accumulation from feeds is a common problem in aquaculture ponds, the management of these systems will therefore need to incorporate measures that remove toxic inorganic nitrogenous species NH_4^+ and NO^-_2. Approaches like exchanging pond water to counter the problem have been used previously but they are limited in different ways. The C:N ratios play a significant role in reducing the accumulation of inorganic nitrogen in intensive fish ponds. Avnimelech (1999) found out that Nitrogen accumulation can be controlled by feeding bacteria on a carbohydrate source which leads to uptake of nitrogen from water. Recent studies by (Asaduzzaman et *al*., 2010) showed that In C/N-CP system, the added carbon source together with the waste nitrogen is converted into microbial bio-flocs, which in turn can be eaten by the cultured organisms. This technique provides an additional inexpensive protein source and improves the overall nutrient efficiency of the pond. Adjusting the C/N ratios in fish feeds is essential for controlling and adjusting the changes in pond water quality. Chamberlain and Hopkins (1994), suggested that the optimum C/N ratio for bacteria growth is about 15:1. Avnimelech (1999) studied the C/N ratios in tilapia feed as a control element in aquaculture systems and found that the addition of carbonaceous substrate in commercial scale fish ponds reduced inorganic nitrogen and increased the growth of the bacteria. The resultant bacteria were then taken up by the tilapia. Avnimelech's conclusion was that about 20-25 g of carbonaceous materials was needed to convert 1 g of ammonia nitrogen into microbial protein, which can reduce ammonia to desired levels within 1-3 days.

2.2 Carbohydrate addition and protein level adjustments

The addition of carbohydrates has been shown to be a potential means to reduce the concentration of inorganic nitrogen in intensive aquaculture systems (Avnimelech, 1999). Carbohydrates are key ingredients in many fish formulated diets. Cousin et *al.* (1996) found that native starches (i.e., potato, tapioca, maize and wheat) can be used as cheap sources but he suggested that since they form suspensions they have to be well mixed prior to use due to their poor water solubility. Asaduzzaman et *al*. (2009b) also recommended use of maize (*Zea mays*) flour as a potential carbohydrate source due to its low cost, easy availability and wide acceptance by the farmers as one of the potential feed ingredients, as compared with tapioca starch. Pond ecological and growth data from a study done by Asaduzzaman et *al*. (2010) further revealed that maize flour can be a good source of organic carbon to maintain a high C/N ratio in ponds. However, carbohydrate addition has some disadvantages in that continuous addition can result in an accelerated sedimentation of organic matter to the pond bottom Avnimelech, (1999) pointed out that when the microbial biomass is not utilised by the fish as a result of sedimentation it will increase the organic load in the pond. A more advanced approach in C/N ratio control is to adjust the protein level in the feed so as to avoid the build-up of inorganic nitrogen in the water, (Avnimelech, 1999). Avnimelech, (1999) suggested that carbonaceous substrate addition can act as emergency responses in instances of increased ammonia concentrations. This can be

done through manipulation of C/N ratios in the system where carbonaceous substrate leads to the recycling and increased utilization of the microbial proteins by heterotrophic bacteria. This approach has been tested and proven successful in intensive ponds that are continually mixed and aerated.

2.3 Stocking density

Fish stocking density is an important factor in regulating growth, production, survival, behaviour, health, water quality, feeding and bacterial population for sustainable fish farming. Lack of information regarding optimum stocking density in *L.victorianus* ponds and hapas and nutrient response is the main driving force to the present concern. The purpose of this study is to provide knowledge for a sustainable hapa cum pond based culture and efficient utilization of nutrients that can be used as a viable means of rural fish production for poverty alleviation and nutritional security. Furthermore to protect this valuable fish from extinction and enhance stocks for aquaculture and fisheries, it is essential to develop appropriate breeding and rearing techniques under controlled captive conditions. However different factors related to stocking densities have been shown to have an effect in fish pond culture. To start with, fish yield is affected by stocking density (Veerina et *al*., 1999). Kohinoor et *al*.,1994; Kohinoor et *al*., 1998 and Rahman et *al*., 2004 pointed out that environmental parameters and low availability of natural food can be a contributing factor to low growth rates in higher stocking densities. Qin *et al*., 1995b showed that stocking density is also a factor which influences the physico-chemical factors of pond water. A 5000 fingerlings/ha of Indian major carps resulted in maximum dissolved oxygen which decreased with the increase in stocking density (Jena et *al.,* 2002b). Culture potential with different cyprinids has been studied to assess the optimal stocking densities. Rahman et *al*. (2006) studying Indian major carp polyculture system with rohu, (*Labeo rohita*), Catla, (*Catla catla*), Mrigal, (*Chrrhinus mrigala*), Silver carp, (*Hypophthalmich thysmolitris*) and Thai sharpunti (*Barbodes gonionotus*) showed that stocking densities of different species plays a vital role on overall fish production in a polyculture system. This is because higher density of one species may affect the growth of other species and also lower density may reduce overall production. They found out that growth was proportionally related to stocking densities due to intraspecific competition and synergistic interactions between the carps. Finding suitable stocking densities is therefore of outmost importance to attain a good marketable size and avoid disease risks. Alternatively in a monoculture system studies have been done with rohu (*Labeo rohita*) a closely related genus with the species of interest in this study *(L.victorianus)* where one farmed and five wild stocks of rohu were tested in earthen monoculture and polyculture ponds, (Reddy et *al*.,2002). From their studies differences in growth were small in both systems only with a higher coefficient of variation for harvest body weight in polyculture than in monoculture. This indicated a stronger inter than intra-species competition for food among the individuals, and that Rohu is less competitive than Catla and Mrigal. In this experiment *L.victorianus* was cultured alone.

Environmental variables, farming conditions and food availability are the main factors that affect fish growth. In terms of the fish production in enclosures, stocking density, which is related to the volume of water or surface area per fish is an important factor. Increase in stocking density results in increasing stress, which leads to higher energy requirements, causing reduction in growth rate and food utilization. Contrarily, low stocking densities may lead to feed wastage and feeling uncomfortable to fish that form shoals. Consequently, identifying the optimum stocking density for a species is a critical factor not only for designing an efficient culture system (Leatherland and Cho, 1985), but also for optimum husbandry practices. A number of studies e.g Holm *et al.*, 1990; Haylor,1991 Bjørnsson,1994; Huang and Chiu, 1997; Irwin *et al*., 1999; Ma *et al*., 2006 showed that in many cultured fish species, growth is inversely related to stocking density. This is mainly attributed to social interactions through competition for food and/or space which can

negatively affect fish growth. The price of fish is determined by market demand and supply (size and production), that in turn depends on growth. Papst *et al.* (1992) suggested that in intensive aquaculture the stocking density is an important indicator that determines the economic viability of the production system.

2.4 Production Biomass

Production estimation, which are based on biomass estimates adjusted for mortality and corrected for growth, is the basis for estimating the economic revenue from fish culture operations (Abou et *al*., 2007). In most instances net yield and annual production increases with increasing stocking density. These positive relationships between stocking density and yield have been described in culture-based fisheries in reservoirs and hapas (Phan and De Silva, 2000; Sugunan and Katiha, 2004; Nguyen et *al*., 2005). The different studies have shown that culture of various species in highest stocking density led to the highest biomass. In this regard controlling the fish size and production are the two important tasks to meet the market demands, but increase in stocking density to produce more fish which increases fish intensification may not be the best way of dealing with problem of space shortage. Control measures on waste and ammonia levels should be put in consideration. However views from Diana, Lin and Schneeberger. (1991) studying Nile tilapia showed that there is an adequate protein level from natural food that can sustain growth, until a critical biomass of fish is reached. The current study adopted three different stocking densities that have been used for tilapia farming in hapas. This background formed the basis of answering the research questions of determining the influence of C:N ratio, optimum stocking density and biomass in *L.victorianus* hapas.

3.0 MATERIALS AND METHODS

3.1 Study area

3.1.1 Geographical Location and Area

The study was conducted at National Aquaculture Research Development and Training Centre (NARDTC), Sagana formerly Sagana fish farm (Fig 1). It is managed as an aquaculture farm experimenting with different species and production technologies to support aquaculture development in Kenya. NARDTC lies at a latitude of 0° 39'S, a longitude of 37° 12'E and an altitude of 1230 m above sea level. It is located about 105 km Northeast of Nairobi the capital city of Kenya, which is mainly a flat plain, lying within the meander of River Ragati, a tributary of Tana River that drains into the Indian ocean. The total area of the farm is about 50 ha of which 20 ha are used as fish ponds (Government of Kenya, FD, 2002).

3.1.2 Climate and vegetation

Sagana experiences a warm-humid climate in most parts of the year, with a mean temperature range between 17 to 26°C (Veverica and Bowman, 1999). Papyrus (*Cyprus papyrus*) and couch grass (*Digeteria scalarum*) are the dominant emergent weeds found mainly along the outlet channels. Dominant tree species include *Jacaranda mimosifolia* and *Manginifera indica.* There are two main distinct seasons: dry and rain seasons. Short rains are experienced between October and December, while long rains fall from March

through May, with a single month peak of 500 mm or more in April. The annual mean rainfall ranges from 1166 mm year $^{-1}$ to 1612 mm year $^{-1}$ (Government of Kenya, FD, 2002).

3.1.3 Topography, Geology and Soils

NARDTC Sagana lies on gently sloping plains at the southern slopes of Mt. Kenya from which River Ragati meanders before it joins the Tana River. The catchment covers Muranga district, Nyeri and Kirinyaga districts. Water flows to the ponds through gravity by a diversion canal approximately 1.5 km on the west of the farm. The soils of the area are mainly black cotton soils with a pH range of 5.4 to 7.5 (Veverica and Bowman, 1999). Nutrients into the ponds are mainly through organic and inorganic fertilizer application.

3.1.4 Historical background of the farm

According to the farm reports, (Government of Kenya, FD, 1992), the Sagana Fish Farm was established in 1948 by the British colonial Government with primary objectives of providing extension services and producing both market size and fish seeds for the local people and fish farmers. Currently, the farm falls under the Government of Kenya in the Ministry of Fisheries Development, Kenya Marine and Fisheries Research Institute, the research wing of the ministry is in charge of research while the Fisheries Department is in charge of extension work. Initially, four species were tested for culture: the African catfish (*Clarias gariepinus)*, Largemouth bass *(Mycropterus salmoides),* and two tilapia species; (*Tilapia zilii)* and (*Tilapia nigra)*. Currently African catfish (*Clarius gariepinus)* and Nile tilapia *(Oreochromis niloticus)* are produced to market size whereas the common gold fish (*Carassius auratus)* and Koi carp (*Cyprinus carpio carpio*) are produced as ornamental fish. Research on semi-artificial propagation and culture of common carp (*Cyprinus carpio*) *Labeo cylindricus* and *Labeo victorianus* is ongoing. The farm also contributes to the protection of indigenous species from Lake Victoria by conserving and propagating them in ponds. Presently, *Synodontis victoriae, Oreochromis esculentus, Labeo victorianus* and various Haplochromine and *Barbus* species are maintained. Integrated aquaculture is also practiced at the farm with chicken, sheep, cattle, food crops, agroforestry and vegetable production.

THE STUDY AREA

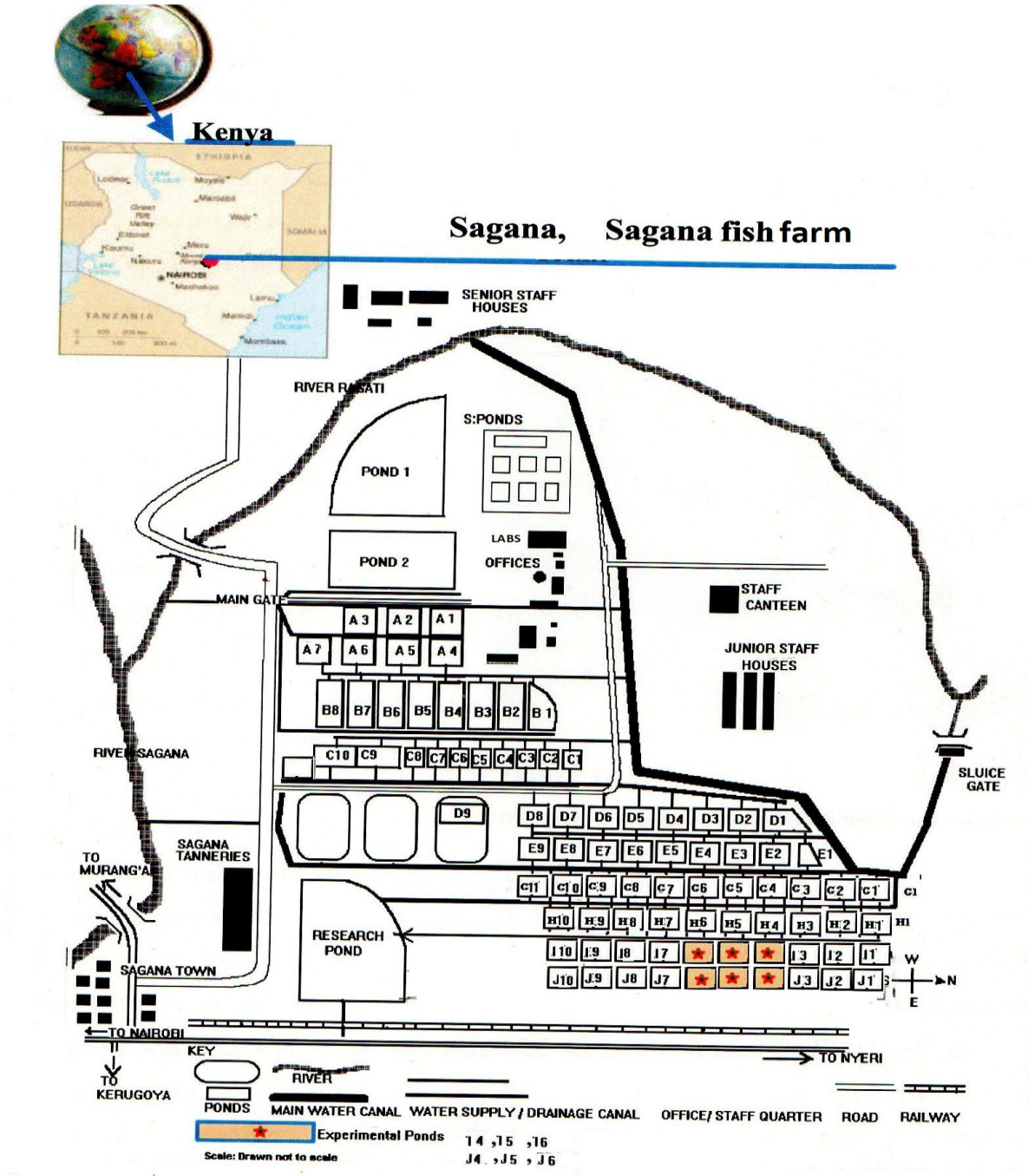


Figure 1. A map of Kenya showing the location of the National Aquaculture Research Development and Training Centre Sagana and a section of the farm layout.

3.2 Experimental fish

Juveniles of *L. victorianus* were collected by use of an electro fisher from Mara river (12˚ 57' 53'' S to 77˚29' 40'' E), Nyanza province, Kenya. After collection, juveniles were transferred into inert polyethylene containers with river water and transported to holding tanks (size 250 x 50 x 50) in the farm and acclimated for five days. During this period, juveniles were fed on dry fish feed pellets. Water was aerated continuously and changed once every two days. From this stock, juveniles ranging in total length from 5.0 to 8.5 cm were selected and were held at densities of 10, 15 and 25 fingerlings per m^2 (SD_{10}, SD_{15} and SD_{25}), in white, 100 µm mesh size (Photograph plate 1) hapas. A sub-sample of juveniles in the size range of experimental individuals were taken from the stock and weighed using an electronic digital balance (make: Orion; precision: 1 mg) to determine the initial live body weight which varied from 4.75 to 9.45 g. Differences in total length and body weight of these juveniles were analyzed with a non-parametric analysis of variance (Kruskal–Wallis test, 1952). No significant differences were found in the initial total length and body weight of the fish stocked in the different experimental units (Kruskal–Wallis; H = 0.62, P = 0.73).

3.3 Experimental design

The experiment was conducted in a 3x2 factorial design, with three levels of fish density in hapas as first factor and C/N ratios as second factor, with 2 levels: C/N ratio 10 and 20. Six, 150m^2 1 m deep earthen ponds were used in this study. Ponds were limed at 2000 kg ha^{-1} with agricultural lime prior to filling. Eighteen hapas made from 100 µm mesh cloth, and 1 m^3 volume (1x1x1 m) were attached to wooden poles in 6 ponds (3 hapas per pond) so that the hapa bottom was 10 cm above the pond bottom. The tops of the hapas were covered with hapa cloth to prevent predation and fish escape. Thanks to the 100 µm mesh feed spillage was minimal. To replace water loss due to seepage and evaporation, water was added to the ponds on a weekly basis. The complete experimental treatment set-up is shown in table 1, with all treatments executed in triplicate.

The experimental hapas were stocked with *L. victorianus* juveniles averaging 5-10 g. All hapas received a 30% crude protein diet with a C/N ratio of 10:1 fed at 3% body weight per day. Feed was administered twice daily at 09:00 and 03:00 o'clock. The C/N 20 ponds received additionally locally purchased maize (*Zea mays*) flour, at 1.14 kg per kg 30% protein feed. The maize flour was fed besides the pelleted feed. Combined, the pelleted feed and maize flour had a C:N ratio of 20. The pre-weighed maize flour starch was mixed in a beaker with hapa water and uniformly distributed over the hapa surface directly after the feed application at 09:00 h. The diet proximate composition is detailed in Table 2. The daily feed quantity was adjusted biweekly after sampling.

Table 1: Experimental design following a 2x3 factorial design with stocking density and C/N ratio as factors resulting in 6 treatments.

C/N ratio	Stocking density (ind.m^{-2})		
	10	15	25
10	SD10-C/N10	SD15-C/N10	SD25-C/N10
20	SD10-C/N20	SD15-C/N20	SD25-C/N20

3.4 Fish sampling and Growth analysis

Fish from hapas were sampled on a biweekly basis. The weights of 5 fish was measured using a weighing bench scale, TCM,TChibo GmbH 221 144, Hamburg model, to the nearest 0.1 g. The average fish weight per hapa at the end of the experiment was determined by counting and bulk weighing all *L.victorianus* harvested. The fish were removed from the hapa using a scoop net. To reduce stress, the fish were handled under a shade in plastic sampling basins halfway filled with pond water. Further caution in fish handling was ensured by aerating the water using a 12 Volt aquarium aerator to ensure adequate dissolved oxygen concentrations. To ensure continuous fresh water supply to the fish during handling, water was replaced in the basins after each hapa handling.

3.4.1 Growth analysis for *L.victorianus*

Fish growth calculations involved computation of mean weight (g) and their standard deviations (± SD) for fish samples from each treatment and stocking density at each sampling occasion. Graphical plots of mean weights against time were used to visualize growth. At the end of the experiment all fish were harvested and weighed up to the nearest 0.1 g. Specific growth rate (% body weight day^{-1}) was calculated using the formula, $SGR\ (\ln WT_F - \ln WT_I) * 100/T$ where WT_F=average final fish weight (g), WT_I=average initial fish weight (g), T=duration of the experiment (days).

3.4.2 Geometric mean body weight (Wg)

Geometric mean body weight was calculated to determine the estimate for the body weight of the fish at the middle of the experiment period.

$$Wg = e^{((\ln WT_F + \ln WT_I)/2)}$$

3.4.3 Metabolic growth (RGR_m)

Metabolic growth was calculated to determine the growth achieved by the fish after utilizing the available food to generate energy for metabolism during the experiment period. This formula was used

$$RGR_m = (WT_F - WT_I)\, W_g^{0.8}/T$$

3.4.4.Survival

In hapas, survival was estimated by checking the hapas daily for dead fish and recording the number of dead fish and removing them. Survival was calculated by subtracting the number of dead fish from the initial number stocked. Survival percentage = Final number of fish/ initial number x 100

3.4.5 Gross fish yield (GFY)

This is the total biomass of fish at harvesting given by the formula: Final number of fish x final average weight (g).

3.4.6 Net fish yield (NFY)

To obtain NFY, the biomass at stocking was subtracted from the gross fish yield. The final yield was then converted to Kg unit ha^{-1}.

3.4.7 Apparent feed conversion ratio (Apparent FCR)

Feed conversion ratio is the ratio of the quantity of food distributed (g) to the weight gain of fish (g), over the culture period. This was used to judge the efficiency of feed utilization by fish for both diets. It was calculated by dividing the total amount of feed used (dry matter basis) and then dividing by the weight gain of the fish.

3.4.8 Protein efficiency ratio

Protein efficiency for both diets was calculated by subtracting the initial weight from the final weight and dividing this by the fraction of protein in the feed.

$PER=(WT_F-WT_I)/(g\ feed \times P_f)$

3.5 Proximate analysis for the feeds used.

Sun-dried and ground feed samples were purchased from local suppliers and taken to the farm laboratory for analysis where they were further ground into finer particles using an electric grinder fitted with a 1 mm *sieve (Thomas-Wiley intermediate mill, 3348-L10 series,* USA) and dried in an oven to a constant weight at 60 °C. For the carbohydrate source, maize flour was purchased locally and analyses followed as explained below. Analyses of crude protein, crude fibre, ether extracts, ash and moisture content were done in triplicates, generally following the procedure by AOAC (1995). Crude protein was quantified by the standard micro-Kjeldahl Nitrogen method, using a sample size of 0.5 g, a Behroset InKje M digestion apparatus and a Behr S 1 steam distillation apparatus (both: Labor-Technik GmbH, Düsseldorf, Germany). The distillate containing ammonia was trapped in 4 % boric acid solution prior to titration with 0.1N HCl. Crude protein was estimated by multiplying the nitrogen content with a factor of 6.25. Ether extracts were analyzed using a sample size of 2 g in a soxhlet extractor with petroleum ether (boiling point 40–60 °C). Crude fiber (CF) was determined by boiling 2 g of sample in a standard solution of 3.13 % H_2SO4 for 10 minutes. The remaining sample was rinsed with hot water followed by boiling in 3.13 % NaOH for another 10 minutes. Thereafter the remaining sample was rinsed repeatedly with hot water followed by acetone. The residue was oven dried at 60° C for 4 hours, cooled in a desiccator and weighed. The residue was ashed at 550 °C in a muffle furnace overnight. CF was quantified by expressing the loss in weight after ashing as a percentage of the original weight of the sample. Dry matter (DM) was determined by drying 5 grams of sample in an oven for six hours to constant weight at 105 °C. Nitrogen Free Extracts were estimated by difference (DM-CP-EE-CF-Ash). To come up with a 30 % crude protein diet to be used for the experiment, Pearson's square

method of feed formulation was used and the above procedure repeated to get the proximate composition of the prepared feed.

Table 2: Proximate composition of the prepared feed and maize flour. The percentages are given on a wet weight basis.

Proximate composition %	Feed ingridients			Treatment diets	
Overall composition %	Rice bran	Cotton seed cake	Fresh water shrimps	Prepared feed	Maize floor
Crude protein	7	35	63.3	29.5	7.71
Crude lipid	4.2	10.5	1.3	7.2	4.42
Crude fibre	30.9	25	5	5.1	5.4
Ash content	22.9	63	22.8	13.2	1.52
Dry matter	92.3	89.4	87.7	87.4	88.3
Nitrogen free extacts	35	19.2	6.7	32.4	69.6

3.6 Water quality analysis

Samples for pond water quality were taken biweekly using a 112 cm intergrated water column sampler. Water samples were taken between 0700 to 0800 hrs from hapas and pond and taken for water quality analysis to the NARDTC Sagana laboratory. Temperature (Celsius thermometer), dissolved oxygen (HI 9142 DO Meter Hanna instruments), pH range (HI 9024 Hanna Instruments) and transparency (20 cm diameter Secchi disk) were measured in situ at depth intervals of 0, 20 and 50 cm at 0700hrs-0800hrs and 1500hrs-1600hrs. Water samples from the hapa were filtered through microfiber glass filter paper (Whatman GF/C 45 mm diameter), using a vacuum pressure air pump. The filtered water was used for the analysis of dissolved nutrients: ammonium-nitrogen (NH_4-N), nitrate nitrogen (NO_3-N), nitrite nitrogen (NO_2-N) and soluble reactive phosphorus (PO_4-P). The filter paper was kept in a test tube containing 10ml of 90% acetone, ground with a glass rod and preserved in a refrigerator for 24hrs. Later chlorophyll-*a* was determined spectrophotometrically (Milton Roy Spectronic, model 1001 plus) at 750 and 633 nm wavelength following Boyd (2000). Hundred ml of the unfiltered samples was used for total alkalinity determination. The procedures followed were according to standard methods described in American Public Health Association (APHA, 1995) cross-referenced to Boyd and Tucker (1992). To avoid contamination, all the glassware was acid washed and rinsed with distilled water before use.

3.6.1 Total alkalinity

Total alkalinity was determined titrimetrically through acid titration of the unfiltered sample with 0.02 N sulfuric acid and using methyl orange indicator from Merck Indicators (APHA, 1995) to end pH of 4.6. Hundred ml of the unfiltered sample was placed in an Erlenmeyer flask. Three to four drops of mixed methyl orange indicator was added to the same sample and the titration continued until a faint orange colour was formed marking the end point (pH 4.6). The total alkalinity was then calculated as:

$$\text{Total alkalinity (meq } l^{-1}) = \frac{\text{volume of acid used (ml)} * \text{Normality of the acid} * 10^3}{\text{Volume of the sample (ml)}}$$

The principle of this titration method is that, the hydroxyl ions present in the water sample as a result of dissociation or hydrolysis of the solutes reacts with additions of the standard acid (H_2SO_4). The alkalinity thus depends on the end-point pH used.

3.6.2 Nitrate-Nitrogen (NO_3)

Nitrate-nitrogen was determined using the sodium-salycilate method according to APHA, (1995). Fresh sodium salycilate solution was always used in each determination. A standard calibration curve was made by dissolving 1.805 g of sodium nitrate ($NaNO_3$) in 250 ml of distilled water to make a stock solution of 1000 mg l^{-1}. A working solution of concentration 5 mg l^{-1} NO_3-N was then made from the stock solution by dissolving 5 ml into 1 litre of distilled water. From this working solution, a standard series with different concentrations was made. Duplicate samples were used for each concentration to make a calibration curve. Nitrate-nitrogen determination in the hapa filtered water samples was done using the following reagents:

- Sodium salycilate-solution which is stable for one day only; where 0.5 g Na- salycilate was dissolved in 100ml of distilled water.
- Concentrated sulfuric acid.
- Potassium-sodium tartarate (Seignette salt) solution; where 400 g NaOH and 50 g K-Na- tartarate were dissolved in 1 litre distilled water.

The following procedure was then used for the analysis. Twenty five ml of the filtered water sample was placed in an evaporation bottle as the blank and working volumes and 1 ml of freshly prepared sodium salycilate solution was added. The bottles were oven dried at 95°C and the resulting residue dissolved quantitavely in 1 ml of conc. H_2SO_4. The bottles were carefully swirled while still warm and after 25 ml of distilled water was added and mixed. Finally 7 ml of potassium- sodium hydroxide-tartarate solution was added, mixed and the absorbance at 420 nm wavelength was determined.

3.6.3 Nitrite-nitrogen (NO_2-N)

Nitrite-nitrogen was determined using the diazotizing method. This method is based on the reaction between sulfanilamide and N-Naphthyl-(1)-ethylendiamin-dihydrochlorid which gives a pink colour with nitrate. This works on the principle that, nitrite-nitrogen reacts with diazotizing reagents in acidic solution to form diazonium salts (Boyd and Tucker,1992). The salts coupled with amino or hydroxyl groups of aromatic compounds to form pink coloured Azo compounds. The colour intensity corresponds to the amount of nitrites present. A standard calibration curve was made using NaNO2 salt of 69 g molar weight. A stock solution was made with a concentration of 100 mg l^{-1} NO_2-N by dissolving 4.925 g of $NaNO_2$ salt in 1 litre distilled water. A working solution of 1 mg l^{-1} NO_2-N was made diluting 1ml of the stock solution with 100 ml distilled water. To 50 ml of the filtered water sample, blank and standards 1 ml of the following reagents were added:

- Diazotizing reagent: where 5 g Sulphanilamide and 50 ml of concentrated hydrochloric acid were added to 500 ml distilled water.
- Coupling reagent: where 0.5 g of N- (1-naphtyl) ethylenediamine dihydrochrolide were dissolved in distilled water.

The solution was left standing for 10 minutes and thereafter at a spectrophotometer wavelength of 543 nm the absorbance was read.

3.6.4 Ammonium-nitrogen (NH_4-N)

Ammonium-Nitrogen was determined using the Indophenol / phenate method. This method is based on the reaction between sodium salycilate and hypochlorid solution. A standard calibration curve was made by using NH_4CL solids with a molecular weight of 53.492 g in which the nitrogen proportion of NH_4-N is 1:14 of the weight. A stock solution with a concentration of 1 g l^{-1} NH_4-N was made by dissolving 0.955 g of NH_4CL into 250 ml flask. An intermediate solution with a concentration of 250 ugl^{-1} was then made by taking 10 ml of the stock solution and diluting it further to 1 litre with distilled water. Finally, a working solution was made with concentration 10 mgl^{-1} by taking 25ml of the intermediate solution and diluting it up to 1 litre. The standard calibration curve was made with 25ml as the working volume. The following reagents were used:

A. Sodium salycilate solution: where 130 g of sodium salycilate and 130 g of trisodium-citrate dehydrate was mixed in 800 ml of distilled water. 0.97 g of sodium nitroprusside was then added to this solution. The solution was filled up to 1 litre with distilled water and had a bench life of up to two months.
B. Hypochlorid solution: where 0.1 g of sodium dichlorosicynurate was dissolved in 50ml NaOH solution.

Ammonium-nitrogen in the water samples was analyzed by taking 25 ml of the filtered sample. To this, 2.5 ml of reagent A was added followed immediately by the addition of 2.5 ml reagent B. the sample was then placed in a 25°C water bath in the dark for 90 minutes. Absorbance was then determined at 655 nm wavelength.

3.6.5. Soluble reactive phosphorus (SRP)

Soluble reactive phosphorus was determined using ascorbic acid method. The method involves formation of phosphomolybdic acid and the subsequent reduction of this acid to intensely coloured molybdenum blue. The blue colour intensity is proportional to the amount of dissolved phosphorous present. The standard calibration curve was first made by dissolving 0.2195 g of potassium dihydrogen phosphate (KH_2PO_4) in 1000ml distilled water. A standard working solution was made by diluting 50 ml of the stock solution with 500 ml distilled water to make 5mgl-1 PO_4-P solution. The following reagents were used:

A. Sulphuric acid solution,5 N: where 70ml concentrated sulphuric acid was diluted with distilled water in a 500 ml volumetric flask, allowed to cool to room temperature and then diluted to volume.
B. Potassium antimonyl tartrate solution: 1.3715 g of potassium antimonyl tartrate was dissolved in 500 ml distilled water.
C. Ammonium molybdate solution: 20 g of ammonium molybdate was dissolved in 500 ml distilled water and stored at 4°C.
D. D.Ascorbic acid solution: 1.76 g ascorbic acid was dissolved in 100 ml distilled water and stored at 4°C.

E. Phenolphthalein solution: 0.5 g phenolphthalein was dissolved in 50 ml of 95% ethyl alcohol and 50 ml distilled water. 0.02 N sodium hydroxide was added drop wise till appearance of a faint pink colour. The combined reagent mix was 50 ml sulphuric acid, 5 ml potassium antimonyl trartrate solution, 15 ml ammonium molybdate solution and 30 ml ascorbic acid solution.

The following procedure was then used for analysis. 25 ml of the filtered sample was put into a 125ml Erlenmeyer flask. 4 ml of combined reagent mix was added and given 30 minutes for colour development. Absorbance was then measured at 880 nm.

3.6.6 Chlorophyll – *a*

Chlorophyll-*a* was used to estimate of the algal biomass in the culture units. This was done using the chlorophyll-*a* method (Wetzel and Likens, 1991; Boyd and Tucker, 1992).

3.7 Sediment quality

Sediment samples were collected from three locations of each pond using PVC pipes 4 cm diameter and 10 cm sampling depth. They were collected on biweekly basis at 1000h. Samples were dried, ground and sieved with a 2 mm sieve. Soil pH was determined by directly reading a pH meter with soil water ratio 1:2.5 (McLean., 1982). Organic matter of sediment was determined by ignition method (Page et *al*., 1989). Total nitrogen of sediment was determined by Micro-Kjedahl digestion method following Page et *al*. (1989). Total phosphorus of sediment samples were determined by acid digestion method (Jones and Case, 1990; Watson and Issac, 1990).

3.8 Assessment of plankton in water column

Plankton samples were collected monthly by pooling 10 liters of water from different locations in each hapa and passing them through a 45 µm mesh plankton net. The concentrated samples were preserved in small plastic bottles with 5% buffered formalin, and subsequently filled up to 100 ml with distilled water. Qualitative and quantitative estimations of plankton were done using a Sedgewick-Rafter (S-R) cell containing 1000 1-mm^3 cells. A 1 ml sample was put in the (S-R) cell and left for 15 minutes undisturbed to allow plankton to settle. The plankton in 10 randomly selected cells were identified up to genus level and counted under a compound microscope (Swift, M-4000). Planktons were identified using keys by Belcher and Swale (1976) and Bellinger (1992). Plankton abundance was calculated using the following formula:

$N = (PxCx100)/L$

Where N=the number of planktonic organisms per liter of original pond water; P=the number of planktonic organisms counted in ten fields; C=the volume of the plastic bottle holding the sample (100 ml); L=the volume of the pond water sample (10 l).

3.9 Assessment of benthic macro-invertebrates

The benthic macro invertebrate samples were collected monthly with an Ekman grab (area 225 cm^2). In each pond, bottom mud samples were collected from 3 different locations, which were then combined into a composite sample. Benthic macro invertebrates were then collected after filtering sediments through a 250 µm mesh sieve and preserved in a plastic vial containing 10 % buffered formalin. Identification keys were used from Brinkhurst (1971) and Pinder and Reiss (1983). Benthic macro invertebrate density was calculated using the formula:

N=Yx10000/3A

Where N= the number of benthic organisms per m^{-2}, Y= total number of benthic organisms counted in 3 samples; A = area of the dredge in cm^2 .

The bio volumes of plankton and benthic macro invertebrates were calculated using literature values according to Rahman et *al*. (2006). Zooplankton bio volumes were calculated using length in their respective length/volume formula according to Bottrell et *al*. (1976) and McCauley (1984). In some cases, bio volume approximations were made using the values of species of similar shape. For benthic macro invertebrates, the bio volumes were calculated according to Riera et *al*. (1991).

4.0 Total heterotrophic bacterial load in water and sediment

Water samples were collected from different sites of each hapa, pooled into one and used aseptically for microbial examination at a fixed hour of the day (10.00 h) biweekly. Aliquots of tenfold dilution 10^{-1} to 10^{-4} of collected water were made in sterile distilled water. 5.0 g of sediment samples were weighed and transferred to a sterile conical flask and made up to 50ml with phosphate buffered saline (PBS) and the contents mixed thoroughly to prepare a stock solution. Serial dilution of up to 10^{-8} were prepared with PBS. Conventional spread plate technique under aerobic conditions was used to enumerate viable counts of aerobic heterotrophic bacteria (HB). Volumes (0.1 ml) of each dilution were spread over the surface of triplicate plates of tryptone soya agar and incubated at temperature of 35°C for three days. Arithmic means of the three petri plates were used in the present study.

4.1 Data analysis and statistical tests

The bio volumes of plankton and benthic macro-invertebrates were calculated using literature values (Table 3) according to Rahman et *al*., 2006. Zooplankton bio volumes were calculated using length in their respective length / volume formula. They were further analyzed by repeated measures ANOVA with stocking density and C/N ratio as the main factors and time as sub factor. Sediment quality, water quality and total heterotrophic bacteria count were compared by repeated measures ANOVA with stocking density and C/N ratio as the main factors and time as sub factor. Growth and yield parameters (growth, yield, FCR, SGR, PER and survival) were analyzed by two way ANOVA with stocking density and C/N ratio as the main factors. Prior to analysis, the data was checked for normality and percentage data were arcsine transformed. Data were expressed as mean ± S.E. or ± S.D and statistically analyzed by two way ANOVA (Gomez and Gomez, 1984). All ANOVA were performed using SPSS (Statistical Package for Social Science) version 18. If the main effect was found significant, the ANOVA was followed by a Tukey ‘s Test at $P<0.05$ level of significance.

Table 3: Phytoplankton and Zooplankton species individual biovolume as used to convert numbers into total biovolume

Group	Genus	Organism volume (μm^3)	References	Assumption
Bacillariophyceae	Achanthes	600	-	Cyclotella
	Actinella	600	-	Cyclotella
	Cocconeis	600	-	Cyclotella
	Cymbella	600	-	Cyclotella
	Cyclotella	600	Berman and Pollingher (1974)	-
	Fragilaria	810	Peerapornpisal (1996)	-
	Frustrularia	810	-	Fragilaria
	Gomphonema	600	-	Cyclotella
	Melosira	910	Berman and Pollingher (1974)	-
	Navicula	850	Berman and Pollingher (1974)	-
	Nitzschia	1240	Peerapornpisal (1996)	-
	Surirela	2630	-	Phacus
	Synedra	1240	-	Nitzschia
	Tabellaria	1240	-	-
Chloropyceae	Ankistrodesmus	52	Beveridge at al. (1993)	-
	Actinustrum	52	-	Ankistrodesmus
	Bothriococcus	6190	Peerapornpisal (1996)	-
	Chaetophora	1326	-	Anabena
	Chlorella	30	Berman and Pollingher (1974)	-
	Chrysococcus	6190	-	Bothriococcus
	Closteridium	335	-	Closterium
	Cloestrum	1675	Peerapornpisal (1996)	-
	Coelastrum	1208	Peerapornpisal (1996)	-
	Cosinodiscus	1208	-	Coelastrum
	Crucigenia	220	Peerapornpisal (1996)	-
	Elakatothrix	15	Peerapornpisal (1996)	-
	Geminella	1326	-	Anabena
	Gonatozygon	1326	-	Anabena
	Haematococcus	6190	-	Bothriococcus
	Merismopedia	896	-	Pediastrum
	Microspora	896	-	Pediastrum
	Oedogonium	896	-	Pediastrum
	Oocystis	250	Berman and Pollingher (1974)	-
	Pediastrum	896	Peerapornpisal (1996)	-
	Pleurococcus	6190	-	Bothriococcus
	Scenedesmus	115	Berman and Pollingher (1974)	-

Group	Genus	Organism volume (μm^3)	References	Assumption
	Sprogyra	1326	-	Anabena
	Sphaerocystis	896	-	Pediastrum
	Tetraedron	85	Berman and Pollingher (1974)	-
	Tetraspora	90	-	Tetraedron
	Ulothrix	1326	-	Anabaena
	Volvox	30	-	Chlorella
	Zignema	1326	-	Anabaena
Cyanophyceae	Anacystis	1326	-	Anabaena
	Anabaena	1326	Beveridge at al. (1993)	
	Coelospharium	1208	-	Coelastrum
	Gleocapsa	1208	-	Coelastrum
	Microcystis	11300	Peerapornpisal (1996)	-
	Lyngbya	1326	-	Anabaena
	Aphanizomenon	1231	Peerapornpisal (1996)	-
	Aphanocapsa	1231	-	Aphanizomenon
	Aphanotheca	1231	-	Aphanizomenon
	Chroococcus	280	Berman and Pollingher (1974)	-
	Gompospheria	1208	-	Coelastrum
	Oscillatoria	1326	-	Anabaena
Euglenophyceae	Euglena	1956	Peerapornpisal (1996)	-
	Phacus	2630	Peerapornpisal (1996)	-
	Trachelomonas	5089	Peerapornpisal (1996)	-
Rotifera	Asplancha	483840	-	Polyarthra
	Branchionus	483840	-	Polyarthra
	Filinia	483840	-	Polyarthra
	Polyarthra	483840	McCauley (1984) and Bottrell et al. (1976)	Length (L)=120 µm volume=0.28 L^3
	Keratella	944023	McCauley (1984) and Bottrell et al. (1976)	Length (L)=162.5 µm volume=0.22 L^3
	Trichocera	483840	-	Polyarthra
Cladocera	Daphnia	483840	-	Polyarthra
	Diaphanosoma	483840	-	Polyarthra
	Moina	483840	-	Polyarthra
Copepeda	Diaptomus	483840	-	Polyarthra
	Nauplius	944,023	-	Polyarthra
	Monstyla	483,840	-	Polyarthra
	Cyclops	944,023	-	Keratella
Oligochaeta		1.237 (mm^3)	Riera et al. (1991)	Volume=$\prod LD^2/4$, with D =average diameter (0.4 mm), L=length (10.13 mm), L= -1.408+28.835 D
Chironomidae		1.237 (mm^3)	-	Oligochaeta

Genus name in the assumption column indicates assuming similar biovolume of the genus given in the same row. All assumptions were made on the basis of average size under microscopic observation. Source : Rahman et *al*., 2006

PHOTOGRAPH PLATES

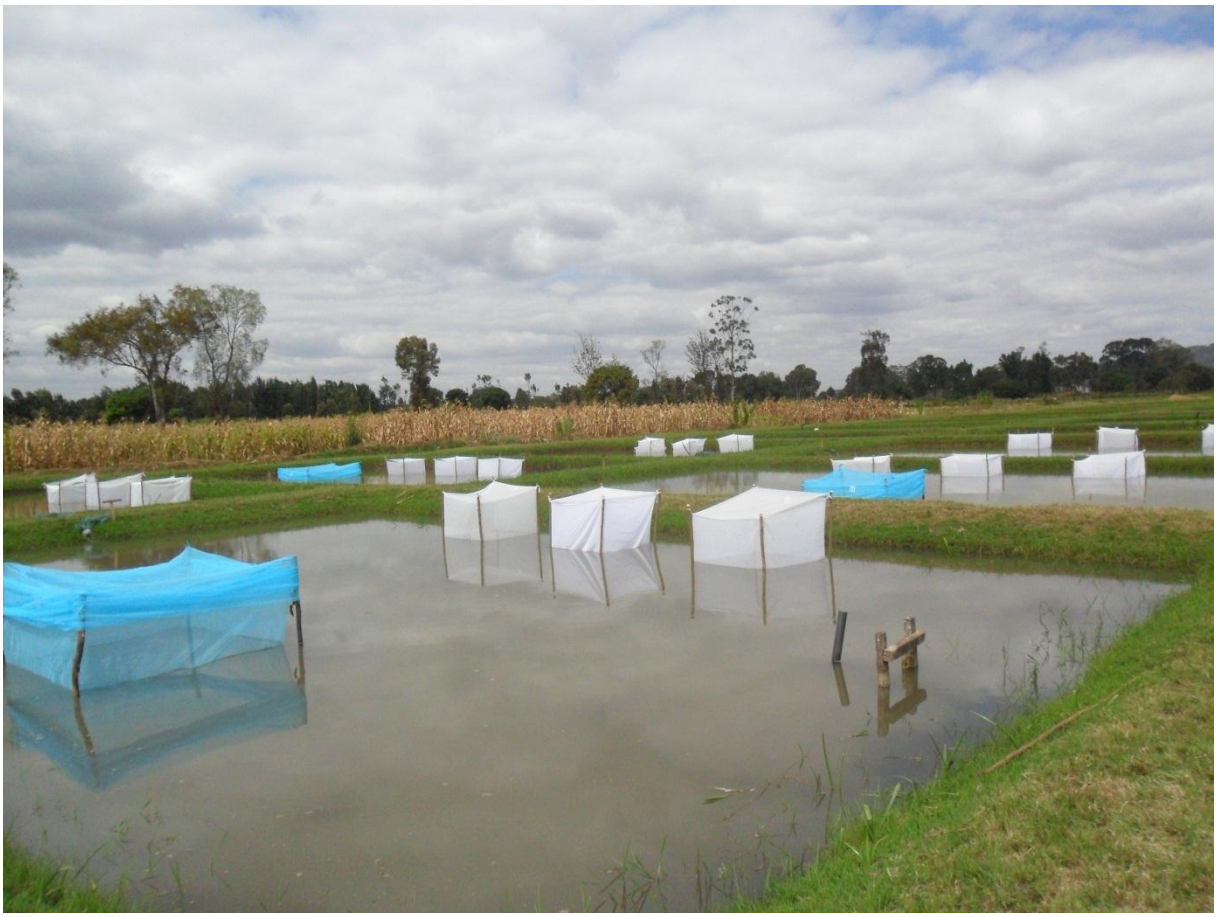


Plate 1. Experimental ponds

Plate 2a determination of total length

Plate 2b. Labeo fish at sampling

Plate 3a. Fish sampling exercise

Plate 3b. bacteria culture

plate 4a. Water quality determination

Plate 4b. Nutrients analysis

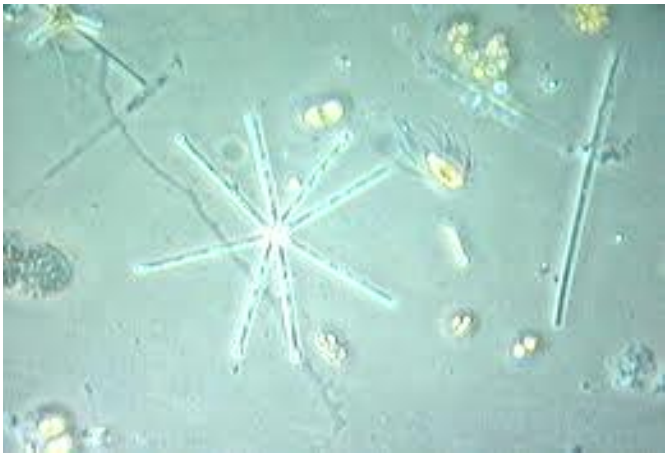


Plate 5a. Plankton in the water column

Plate 5b. Benthos in sediment

4.0 RESULTS

4.1 Water quality parameters

Mean values of water quality parameters and outcomes of ANOVA are presented in Table 4. Increasing C/N ratio from 10 to 20 increased the dissolved oxygen, temperature, pH, chlorophyll-*a* and total alkalinity in water ($P<0.05$). It also reduced the water transparency. The stocking density of *L. victorianus* influenced the water quality parameters differently. Dissolved oxygen, temperature, pH and transparency were higher in open pond water as compared to hapas but not significantly different among the stocking densities ($P>0.05$) except for dissolved oxygen which was relatively low in the medium and high density. Higher stocking density of *L.victorianus* concurred with low chlorophyll-*a* concentrations inside the hapas. Transparency and total alkalinity were not influenced by stocking density, but changed with C/N ratio. The ANOVA results showed that increasing C/N ratio reduced nitrate-N, TAN and nitrite-N in the water column, while phosphorus availability increased as shown by soluble reactive phosphorous (SRP). Levels of nitrate-N, TAN, nitrite-N and SRP did not change between hapas. Nevertheless, the concentration of TAN was lower in the open pond water than in the hapas, while the opposite was the case for SRP. C/N ratio and stocking density did not have a significant interaction effect apart from nitrate–nitrogen at ($P=0.04$). There was a significant interaction between C/N ratio and sampling time for all the parameters apart from temperature and pH. For the *in situ* parameters dissolved oxygen, temperature and pH there was a significant difference between the time of the day that the sampling was done ($P<0.05$) with highest mean for all the parameters recorded in the afternoon. For the same parameters there was no significant interaction between sampling time and density.

Table 4: Effects of C/N ratio, stocking density and sampling time on different water quality parameters based on two-way ANOVA

Water quality parameters	Means Tukey test															
	C/N ratio (C/N)		**Density (fish/m2) (D)**				**Sample time**					**M & A**		**P interaction**		
	10	20	10	15	25	pond	18-08-11	01-09-11	15-09-11	29-09-11	13-10-11	Morning	Afternoon	C/N x D	C/N xST	ST x D
Dissolved oxygen (mg L^{-1})	6.8 ^{b}	7.2 ^{a}	7 ^{b}	6.8 ^{bc}	6.7 ^{c}	7.6 ^{a}	6.6 ^{b}	6.9 ^{c}	7.1 ^{ab}	7 ^{ab}	7.5 ^{a}	5.2 ^{b}	8.9 ^{a}	NS	**	NS
Temperature (^{o}C)	23.1 ^{b}	23.3 ^{a}	23.2 ^{ab}	23.1 ^{b}	23 ^{b}	23.3 ^{a}	21.7 ^{b}	24.6 ^{ab}	21.3 ^{c}	23.2 ^{ab}	24.7 ^{ab}	20.9 ^{b}	25.3 ^{a}	NS	NS	NS
pH	7.9 ^{b}	8.3 ^{a}	8 ^{b}	8.1 ^{ab}	8.1 ^{ab}	8.3 ^{a}	8.3 ^{a}	7.9 ^{b}	8 ^{ab}	8.2 ^{ab}	8.1 ^{ab}	7.8 ^{b}	8.5 ^{a}	NS	NS	NS
Transparency (cm)	28.4 ^{a}	26.2 ^{b}	27.5	27.3	27	227.4	35.8 ^{a}	26.5 ^{b}	24.9 ^{ab}	25.2 ^{c}	24.1 ^{ab}			NS	**	
Chlorophyl a (µg L^{-1})	138.4 ^{b}	188.6 ^{a}	165.1 ^{ab}	163.3 ^{ab}	160.3 ^{b}	165.3 ^{a}	133.1 ^{e}	150.3 ^{d}	162.7 ^{c}	179.1 ^{b}	192.7 ^{a}			NS	***	
Total Alkalinity (mg L^{-1})	72.6 ^{b}	81.3 ^{a}	76.3	77	77.3	77.3	81.6 ^{a}	81.4 ^{ab}	69.6 ^{c}	74.9 ^{b}	77.9 ^{b}			NS	***	
SRP (mg L^{-1})	0.99 ^{b}	0.105 ^{a}	0.102 ^{ab}	0.097 ^{b}	0.099 ^{ab}	0.109 ^{a}	0.039 ^{d}	0.132 ^{c}	0.021 ^{e}	0.153 ^{b}	0.164 ^{a}			NS	***	
NO3-N (mg L^{-1})	0.034 ^{a}	0.015 ^{b}	0.022 ^{ab}	0.028 ^{ab}	0.031 ^{a}	0.016 ^{b}	0.004 ^{c}	0.028 ^{b}	0.037 ^{a}	0.026 ^{ab}	0.027 ^{ab}			*	***	
TAN (mg L^{-1})	0.191 ^{a}	0.104 ^{b}	0.146 ^{b}	0.15 ^{b}	0.186 ^{a}	0.108 ^{c}	0.1 ^{c}	0.173 ^{b}	0.257 ^{a}	0.162 ^{ab}	0.135 ^{ab}			NS	***	
NO2-N (mg L^{-1})	0.178 ^{a}	0.1 ^{b}	0.14 ^{b}	0.123 ^{b}	0.171 ^{a}	0.122 ^{b}	0.221 ^{a}	0.125 ^{ab}	0.129 ^{ab}	0.177 ^{b}	0.042 ^{c}			NS	***	

C/N ratio=Carbon/Nitrogen ratio; 10=treatments with C/N ratio 10; 20=treatment with C/N ratio 20; SD_{10},Labeo stocking density of 10, SD_{15}, Labeo stocking density of 15, SD_{25}, Labeo stocking density of 25. CN×D=Interaction of C/N ratio and stocking density. CNxST= Interaction of C/N ratio and sampling time. STxD=Interaction of sampling time and density. The mean values followed by the different superscript letter within factor indicate significant difference at (P<0.05). If the effects were significant, ANOVA was followed by Tukey test. *P<0.05;**P<0.01; ***P<0.001; NS, Not significant.

4.2 Sediment quality

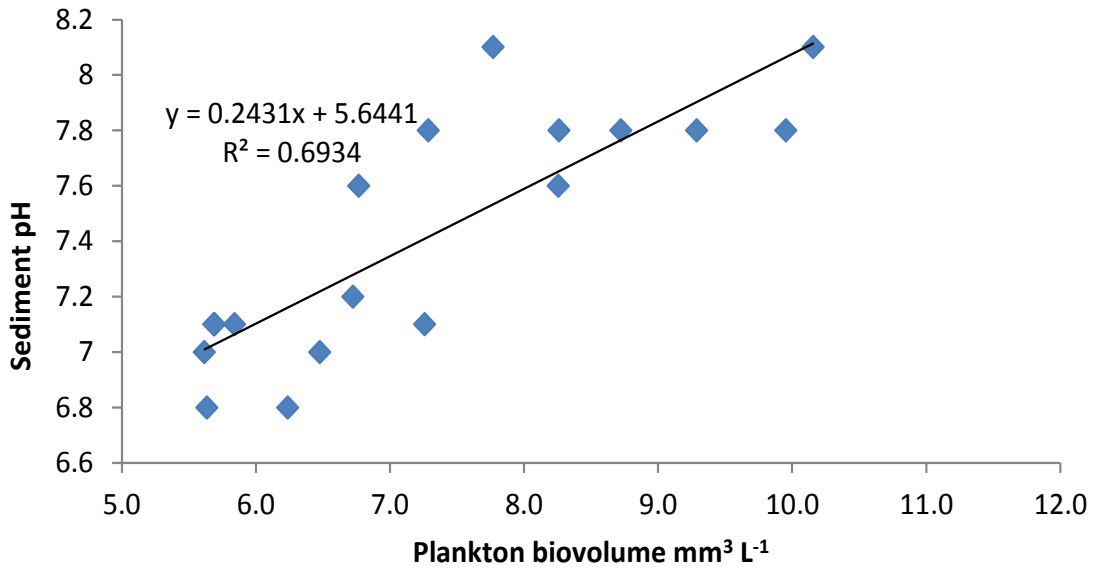
The sediment quality parameters are summarized in Table 5. The addition of carbohydrates for increasing C/N ratio increased the organic matter content in the sediment, pH and total phosphorus. Total nitrogen concentration in the sediment was reduced by increasing C/N ratio. The ANOVA results showed that sampling time influenced the means of all the parameters with the highest values recorded at the end of the experiment. There was also significant interaction between C/N and sampling time. The Pearson correlation analysis showed that there was a significant positive relationship among sediment pH, plankton biovolume and total heterotrophic bacteria count (P<0.05) (Fig 2).

Table 5: Effects of C/N ratio on different sediment quality parameters based on two-way ANOVA

Variable	Means Tukey test							
	C/N Ratio (CN)		Sample time (T)					interaction
	10	20	19-08-11	02-09-11	16-09-11	30-09-11	14-10-11	CN X T
pH	7.01 ^{b}	7.82 ^{a}	7.2 ^{c}	7.43 ^{b}	7.41 ^{bc}	7.29 ^{bc}	7.74 ^{a}	***
Organic matter (%)	2.3 ^{b}	2.8 ^{a}	2.5 ^{b}	2.4 ^{bc}	2.6 ^{a}	2.6 ^{d}	2.6 ^{a}	***
Total nitrogen (%)	19.8 ^{a}	13.6 ^{b}	15.8 ^{c}	15.3 ^{c}	17.2 ^{b}	16.9 ^{d}	18.4 ^{a}	***
Total phosphorus (mg L^{-1})	14.6 ^{b}	15.1 ^{a}	15.8 ^{cd}	15.2 ^{d}	16.4 ^{bc}	16.8 ^{b}	17.8 ^{a}	***

C/N ratio=Carbon/Nitrogen ratio; 10=treatments with C/N ratio 10; 20=treatment with C/N ratio 20. CNxT=Interaction of C/N ratio and sampling time. Within factor, the mean values followed by the different superscript letter in each factor indicate significant difference at (P<0.05). If the effects were significant, ANOVA was followed by Tukey test. *P<0.05;**P<0.01; ***P<0.001; NS, Not significant.

A



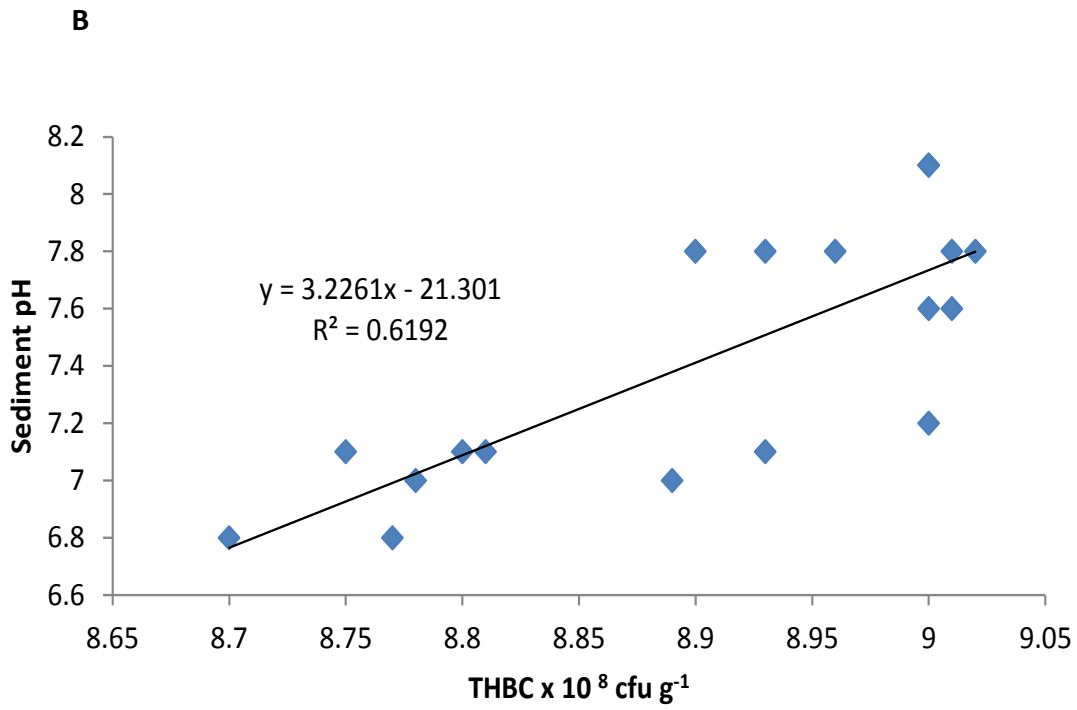


Figure 2: Relationship between (A) sediment pH and plankton biovolume and (B) sediment pH and heterotrophic bacteria count.

4.3 Effects on total heterotrophic bacterial load in water and sediment

The mean total heterotrophic bacterial load of water and sediment are summarized in Table 6. The results of ANOVA showed that a high C/N ratio promoted growth of heterotrophic bacteria population in water column and sediment. There was a significant difference $P<0.05$ in the sample location in the pond with highest THB count being recorded in the sediment and lowest in the open pond (outside hapas). There were no significant differences among the three densities ($P>0.05$). The THB count increased significantly with time and showed a significant interaction with C/N ratio (Fig 4). Stocking density and sample location did not show any significant interaction. The Pearson correlation analysis showed that there was a significant negative correlation between THB count and nitrate-N in the water column and between TAN in the water column and total plankton concentration (Fig 3).

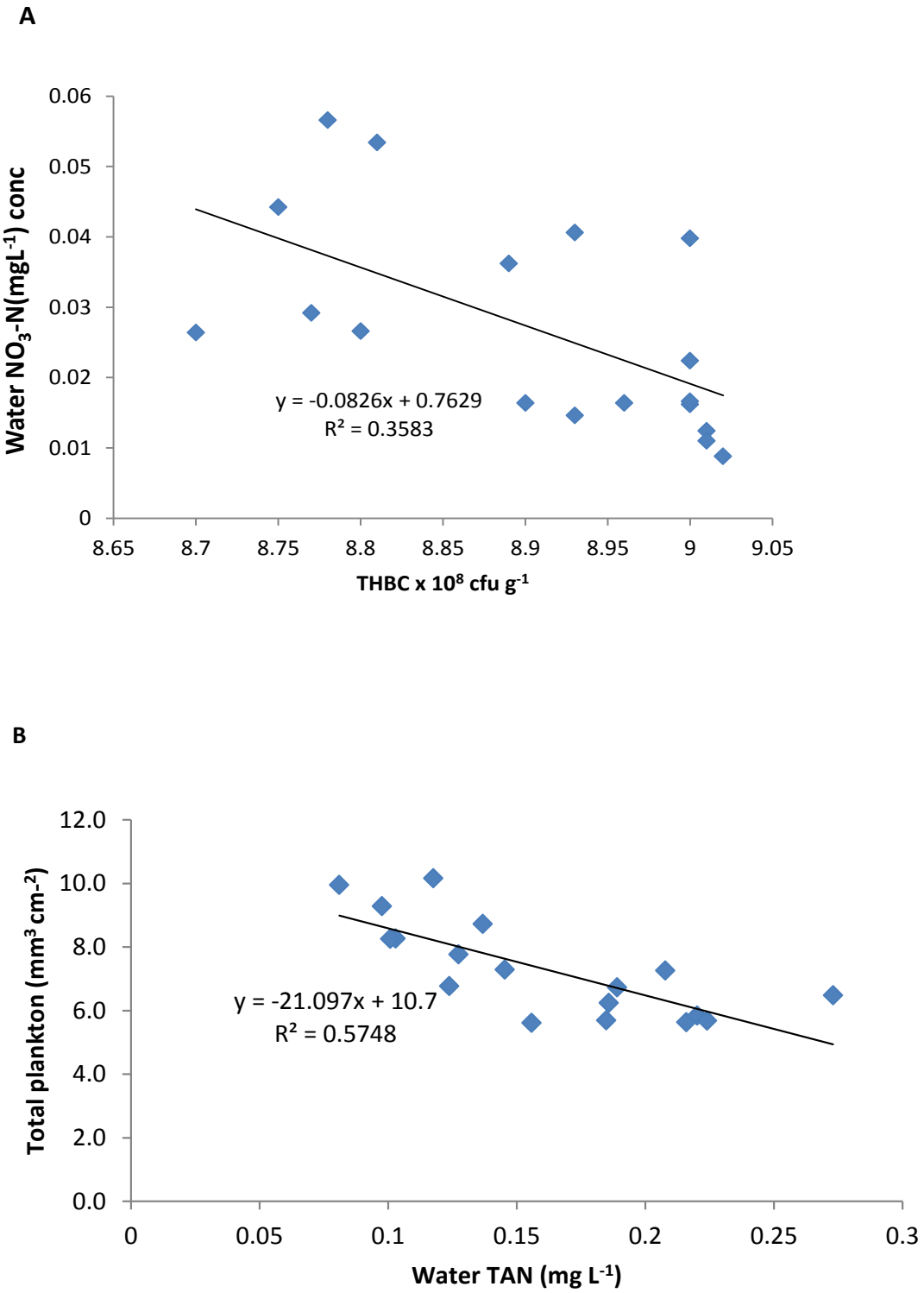


Figure 3: Relationship among water NO_3-N and total bacteria count (A) and total plankton biovolume and water TAN (B)

Table 6: Effects of C/N ratio and stocking density on total heterotrophic bacteria (THB) load of water and sediment based on two- way ANOVA

Variable	C/N ratio (CN)		Sample location in pond (SL)				
	10	20	Hapa density 10	Hapa density 15	Hapa density 25	Outside hapa	Sediment
THBC (x 10^8 cfu g^{-1})	5.58 ^{b}	7.88 ^{a}	6.4 ^{bc}	6.7 ^{b}	6.94 ^{ab}	5.49 ^{c}	8.07 ^{a}
Variable	Time (T)				Interaction		
	02-09-11	16-09-11	30-09-11	14-10-11	C/N x SL	C/N x T	C/N x D
THBC (x 10^8 cfu g^{-1})	3.95 ^{d}	6.89 ^{c}	7.73 ^{b}	8.31 ^{a}	NS	***	NS

C/N ratio=Carbon/Nitrogen ratio; 10=treatments with C/N ratio 10; 20=treatment with C/N ratio 20; C/N×D=Interaction of C/N ratio and stocking density, C/NxSL=Interaction of C/N ratio and sampling location, C/NxT=Interaction of different C/N ratio and sampling time. The mean values followed by the different superscript letter in each factor indicate significant difference at (P<0.05). If the effects were significant, ANOVA was followed by Tukey test. *P<0.05;**P<0.01; ***P<0.001; NS, Not significant.

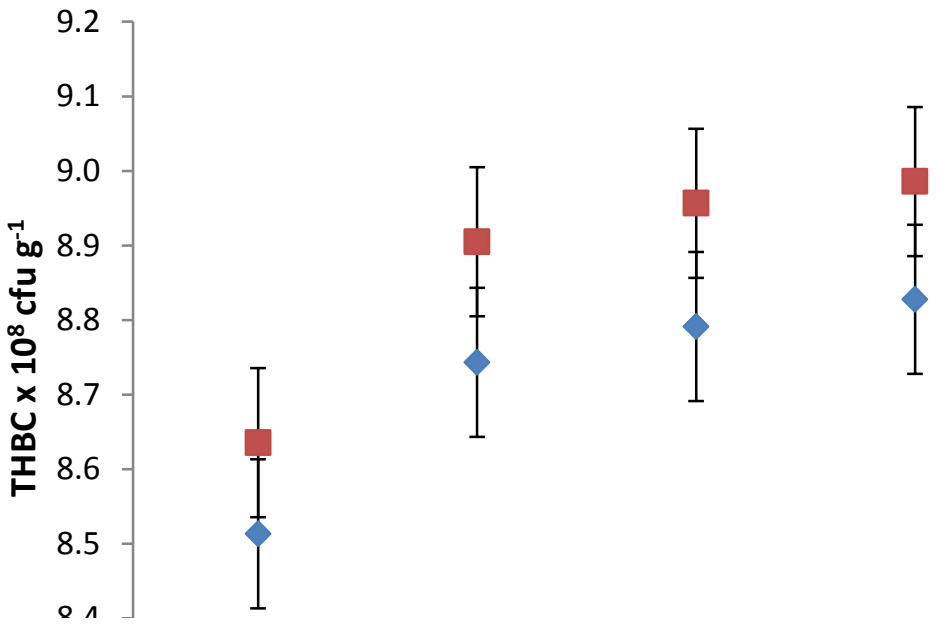


Figure 4: Interaction effects of CN ratio and time on the total heterotrophic bacteria count.

4.4 Effects on abundance of plankton and benthic macro-invertebrates

4.4.1 Effect on plankton biovolume

The plankton communities in pond water consisted of three groups of phytoplankton and two groups of zooplankton in all the treatments. Twenty two genera of phytoplankton belonging to Chlorophyceae - green algae (9 genera), Bacillariophyceae - diatoms (9 genera) and Cyanophyceae - blue green algae (4 genera) were found (Table 7). Twelve genera of zooplankton, including 6 genera of rotifera and 6 genera of crustaceans were also identified. In all the treatments the same genera of plankton were found with high abundance in high C/N ratio treatment. Among phytoplankton; *Actinastrum, Bothriococcus*, *Chelophora, Chlorella, Chryssococcus, Coelastrum, Oocystis, Spirogyra* and *Volvox* (Chlorophyceae), *Actinella, Cyclotella, Diatoma, Fragillaria, Melosira, Navicula, Nitzshia, Synedra* and *Tabellaria* (Bacillariophyceae), *Anabena, Anacystis, Microcystis* and *Aphanocapsa* (Cyanophyceae) and among zooplankton *Asplancha, Brachionus, Filinia, keratella, Trichocerca* and *polyyathra* (Rotifera), Crustaceans included *Daphnia, Daphanosoma* and *Moina* (Cladocera) *Daptomus, Nauplius* and *Cyclops* (Copepoda) were the dominating genera. The results of the ANOVA on the biovolume of major groups of plankton are shown in Table 8. C/N ratio control influenced the biovolume of all the plankton groups. The mean total biomass was higher in the high C/N ratio treatment. Increasing C/N ratio from 10 to 20 increased total phytoplankton biovolume by 13 % and zooplankton by 25 % ($P<0.05$). Stocking density influenced the plankton biovolume differently with the open pond and low stocking density hapa having higher amount of phytoplankton as compared to the medium and high density. Zooplankton biovolume was high in the low density hapa and low in the other densities and open pond. There was a significant interaction effect between C/N ratio and stocking density among the total zooplankton and total plankton but not with the phytoplankton (Fig 5). All plankton biovolumes increased significantly with time during the three months experiment period ($P<0.05$). Sampling time had a significant interaction effect with C/N ratio among all the plankton groups (Table 8).

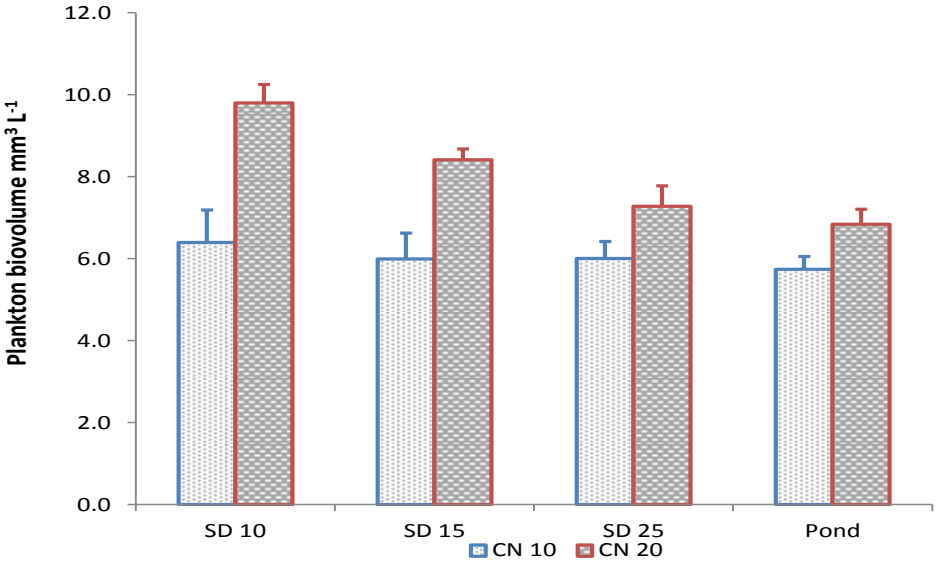


Figure 5: Interaction effect of C/N ratio and stocking density on total plankton biovolume.

Table 7: List of plankton genera recorded from experimental hapas

Group	Genus	Plankton
Bacillariophyceae (diatoms)	*Actinella*	x
	Cyclotella	x
	Diatoma	x
	Fragillaria	xx
	Melosira	x
	Navicula	xx
	Nitzchia	xx
	Synedra	x
	Tabellaria	xx
Chlorophyceae (Green algae)	*Actinastrum*	x
	Botryococcus	x
	Chaetophora	x
	Chlorella	xx
	Chrysococcus	x
	Coelastrum	x
	Oocystis	x
	Spirogyra	xx
	Volvox	xx
Cyanopyceae (Blue green algae)	*Anabena*	xx
	Anacystis	x
	Microcystis	x
	Aphanocapsa	x
Rotifera	*Asplanchna*	xx
	Branchionus	x
	Filinia	x
	Keratella	x
	Trichocera	xx
	Polyarthra	x
Crustaceans		
A. Cladocera	*Daphnia*	xx
	Diaphanosoma	x
	Moina	x
B. Copepoda	*Daptomus*	x
	Nauplius	x
	Cyclops	xx

' x ' indicates presence; 'xx' indicates dominating genera

Table 8: Effects of C/N ratio and stocking density on the abundance (based on total volume, $mm^3 L^{-1}$) of different groups of plankton in ponds based on two-way repeated measures ANOVA.

Variable	Means Tukey test										
	C/N Ratio (CN)		Density (D)				Sampling time (ST)			interaction	
	10	20	10	15	25	Pond	02-09-11	02-10-11	02-11-11	CN x D	CN x ST
Bacillariophyceae	0.017 ^{b}	0.02 ^{a}	0.02 ^{a}	0.018 ^{b}	0.015 ^{c}	0.021 ^{a}	0.015 ^{b}	0.02 ^{a}	0.021 ^{a}	NS	*
Chlorophyceae	0.03 ^{b}	0.04 ^{a}	0.035 ^{ba}	0.03 ^{ba}	0.022 ^{c}	0.043 ^{a}	0.02 ^{c}	0.034 ^{b}	0.044 ^{a}	NS	*
Cyanophyceae	0.129 ^{b}	0.144 ^{a}	0.139 ^{a}	0.137 ^{a}	0.128 ^{b}	0.143 ^{a}	0.135 ^{ab}	0.134 ^{b}	0.141 ^{a}	NS	**
Total pytoplankton	0.176 ^{b}	0.204 ^{a}	0.194 ^{b}	0.185 ^{b}	0.165 ^{c}	0.207 ^{a}				NS	
Crustacea	2.94 ^{b}	4.08 ^{a}	3.83 ^{a}	3.65 ^{a}	3.38 ^{ab}	3.18 ^{b}	2.707 ^{c}	3.67 ^{b}	4.16 ^{a}	***	***
Rotifera	2.92 ^{b}	3.79 ^{a}	4.07 ^{a}	3.36 ^{b}	3.09 ^{b}	2.89 ^{b}	2.56 ^{c}	3.21 ^{b}	4.28 ^{a}	***	***
Total Zooplankton	5.86 ^{b}	7.87 ^{a}	7.9 ^{a}	7.01 ^{b}	6.47 ^{ab}	6.07 ^{c}				***	
Total plankton	6.036 ^{b}	8.074 ^{a}	8.094 ^{a}	7.195 ^{b}	6.635 ^{ab}	6.277 ^{c}				***	

C/N ratio=Carbon/Nitrogen ratio; 10=treatments with C/N ratio 10; 20=treatment with C/N ratio 20; SD_{10},Labeo stocking density of 10, SD_{15}, Labeo stocking density of 15, SD_{25}, Labeo stocking density of 25. CN×D=Interaction of C/N ratio and stocking density, CNxST=Interaction of C/N and sampling time. The mean values followed by the different superscript letter in each factor indicate significant difference at (P<0.05). If the effects were significant, ANOVA was followed by Tukey test. *P<0.05;**P<0.01; ***P<0.001; NS, Not significant.

4.4.2 Effect on benthic macro invertebrate bio volume

The results of the ANOVA of major groups of benthic macro invertebrate biovolume are shown in Table 9. The benthic macro invertebrates were divided into Chironimidae, Oligochaeta, Mollusca and unidentified groups. Chironomidae was the most dominant group among the benthos contributing 67 % to 73 % to the total biomass followed by Oligochaeta. C/N ratio control influenced the biovolumes of all the groups of benthic macro invertebrates. Increasing C/N ratio from 10 to 20 increased the biovolume of total benthic macro invertebrates by 30 % (P<0.05). Benthos biovolume increased significantly with time and showed a significant C/N ratio and sampling time interaction in all the major groups apart from Oligochaetae. Pearson correlation analysis showed a significant correlation between sediment organic matter content and total benthos (Fig 6).

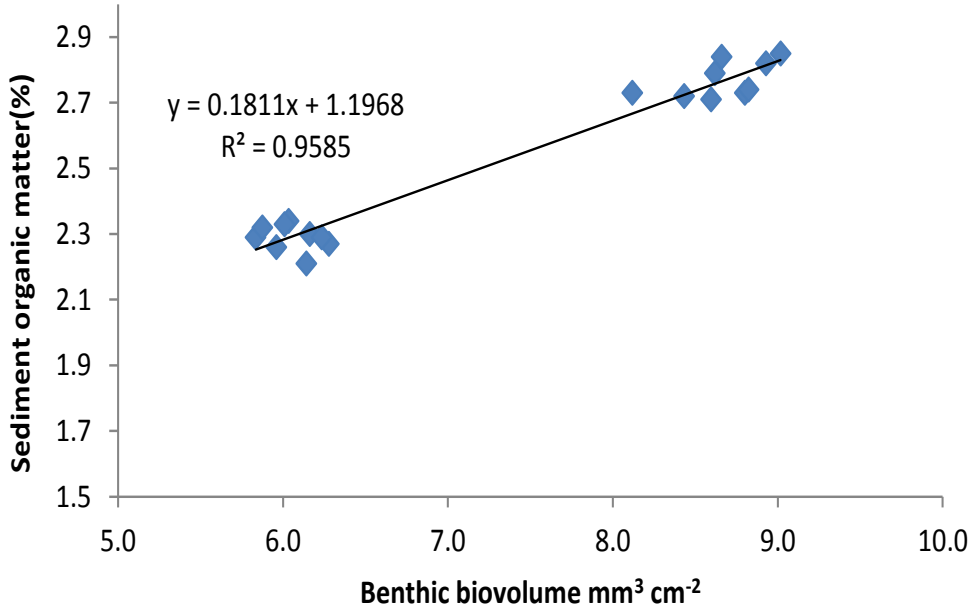


Figure 6: Relationship between benthic macro invertebrates biovolume and sediment organic matter.

Table 9: Effects of C/N ratio on the abundance (based on total volume, cm^3 m^{-2}) of different groups of benthic macro invertebrates in ponds based on two- way repeated measures ANOVA

Variable	Means Tukey test					
	C/N Ratio (CN)		**Sample time (T)**			**Interaction**
	10	20	1	2	3	CN xT
Chironomidae	4.07 ^{b}	6.38 ^{a}	4.76 ^{c}	5.21 ^{b}	5.71 ^{a}	*
Oligochaetae	1.19 ^{b}	1.41 ^{a}	1.26 ^{c}	1.31 ^{b}	1.34 ^{a}	NS
Mollusca	0.18 ^{b}	0.23 ^{a}	0.17 ^{b}	0.229 ^{a}	0.225 ^{a}	**
Un- indentified group	0.604 ^{b}	0.64 ^{a}	0.576 ^{c}	0.623 ^{b}	0.665 ^{a}	**
Total benthos	6.044 ^{b}	8.66 ^{a}				

C/N ratio=Carbon/Nitrogen ratio; 10=treatments with C/N ratio 10; 20=treatment with C/N ratio 20. C/NxT=Interaction of C/N ratio and sampling time. The mean values followed by the different superscript letter in each factor indicate significant difference at (P< 0.05). If the effects were significant, ANOVA was followed by Tukey test. *P<0.05;**P<0.01; ***P<0.001; NS, Not significant

4.5 Effects on growth, survival and yield parameters of *Labeo victorianus*

Growth and yield parameters of *L. victorianus* and their combined performances under different treatments are shown in Table 10. The ANOVA results showed that increasing C/N ratio increased the individual *L. victorianus* weight at harvest (P<0.05) (Fig 7). Considering the formulated diet, the FCR decreased by increasing C/N ratio. Protein efficiency ratio increased with increasing C/N ratio. Individual weight gain, survival percentage and specific growth rate were higher in the high C/N ratio treatment. Geometric mean body weight, metabolic growth, metabolic feed rate, gross and net yield were higher with the high C/N ratio treatment. Stocking density influenced the growth and yield parameters differently. The highest average individual weight at harvest and the highest individual weight gain were obtained with the intermediate stocking density (Fig 8). FCR for both diets was lower in the medium density as compared to the low and high densities. PER, individual weight gain, SGR, geometric mean body weight, metabolic growth and FCR were highest with a stocking density of 15 fish per hapa. With increasing stocking density from 10 to 25 fish per m^2 the combined gross and net yield of *L. victorianus* increased by 62% and 58% respectively. The interaction of C/N ratio and stocking density was not significant for all the growth parameters.

Table 10: Effects of C/N ratio and stocking density on growth and yield parameters of *Labeo victorianus* based on two-way ANOVA

Growth and yield parameters	Means Turkey test										
	C/N ratio (CN)			Density (fish/m^2) (D)						P interaction	
	10		20	SD_{10}		SD_{15}		SD_{25}		C/N x D	
Individual stocking weight (g)	6.7		7.0	6.95		6.97		6.59		NS	
Individual harvest weight (g)	17.7	b	19.8	a	17.2	b	20.8	a	18.2	b	NS
Food conversion ratio diet 1	3.8		3.1	3.95		2.83		3.6		NS	
Food conversion ratio diet 1and 2	3.8	b	4.7	a	4.8	a	3.5	b	4.5	ab	NS
Protein efficiency ratio diet 1	0.8		1.0	0.8	b	1.1	a	0.9	ab	NS	
Protein efficiency ratio diet 1 and 2	0.8		0.9	0.7	b	1.0	a	0.8	ab	NS	
Individual weight gain (g)	11.0	b	12.7	a	10.23	b	13.8	a	11.58	b	NS
Survival (%)	90		91.7	95		92.1		86		NS	
Specific growth rate (%bw d^{-1})	1.3		1.4	1.25		1.51		1.41		NS	
Geometric mean body weight (g)	10.8	b	11.7	a	10.9	b	12	a	10.91	b	NS
Metabolic growth (gbw/d/kg^0.8)	5.7		6.2	5.28	b	6.58	a	5.98	ab	NS	
Metabolic feeding rate (gfeed/day/kg^0.8)	23	b	33.4	a	28.4	ab	26.4	b	30.4	a	NS
Conversion Efficiency (%)	25	a	18.6	b	19.05	b	25.6	a	20.1	b	NS
Gross yield (Kg ha $^{-1}$ 72 d^{-1})	2633.1		2982.1	1638.8	c	2867.3	b	3916.6	a	NS	
Net yield (Kg ha^{-1} 72 d^{-1})	1534.4		1821.6	943.6	b	1821.3	a	2269.1	a	NS	

C/N ratio=Carbon/Nitrogen ratio; 10=treatments with C/N ratio 10; 20=treatment with C/N ratio 20; SD_{10}, Labeo stocking density of 10, SD_{15}, Labeo stocking density of 15, SD_{25}, Labeo stocking density of 25. CN×D=Interaction of C/N ratio and stocking density. The mean values followed by the different superscript letter in each factor indicate significant difference (P<0.05). If the effects were significant, ANOVA was followed by Tukey test. *P<0.05;**P<0.01; ***P<0.001; NS, Not significant.

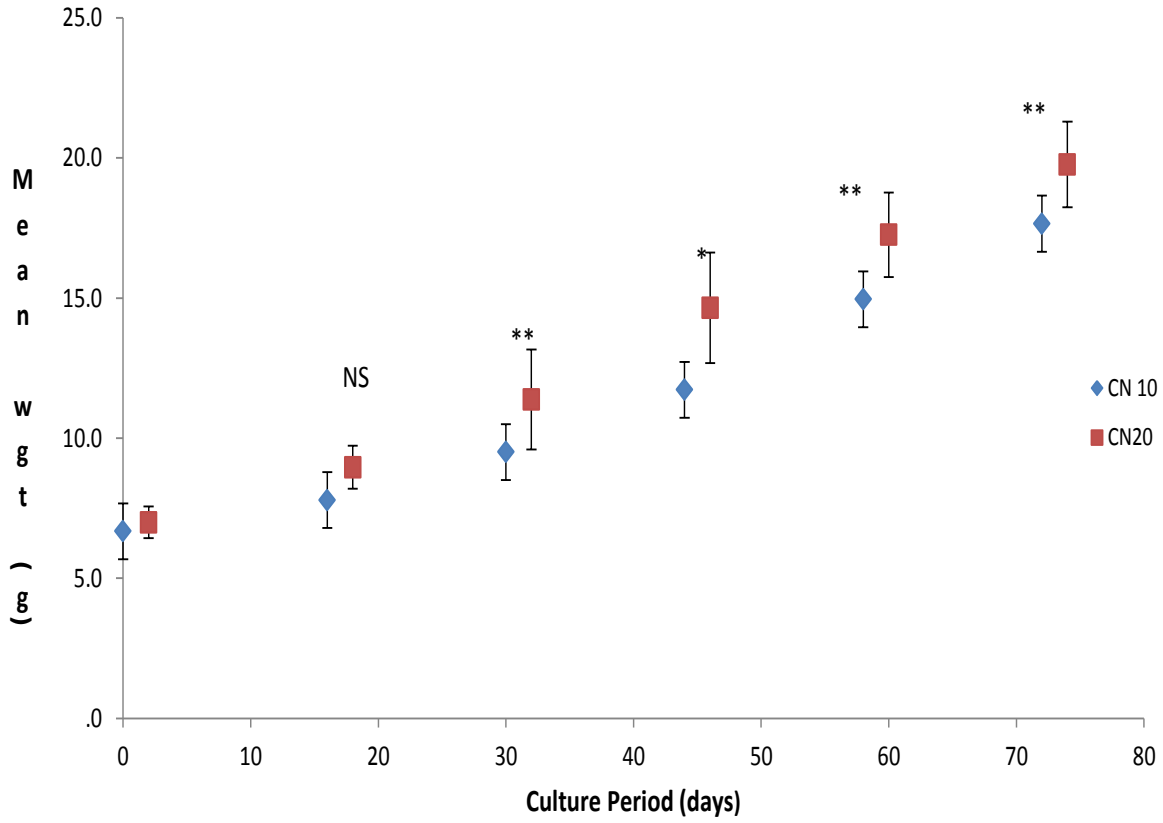


Figure 7: Mean weight of *Labeo victorianus* for different C/N ratios during the experimental period. Values are means (±S.D) of nine hapas in each treatment per sampling date. C/N 10 and C/N 20 are the C/N ratios in the different treatments.

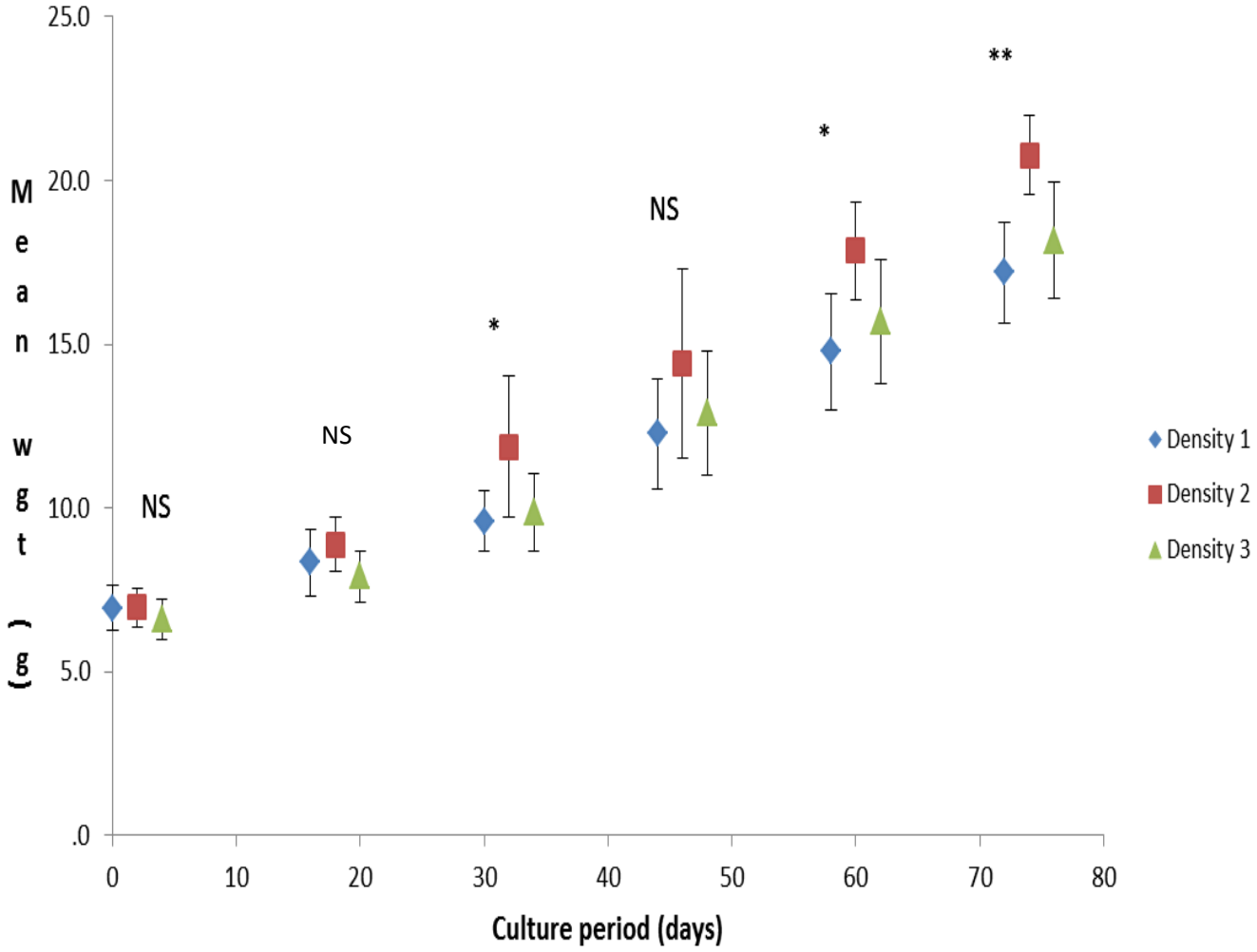


Figure 8: Mean weight of *Labeo victorianus* in the different stocking densities during the experimental period. Values are means (± S.D) of three replicates in each stocking density per sampling date.

5.0 DISCUSSION

5.1 Effects on growth, survival and yield parameters of *Labeo victorianus*

Yield parameters of *L. victorianus* varied with stocking density. C/N ratio also had a significant effect on growth and yield parameters. The treatment with a C/N ratio of 20 and 15 fish /hapa had the highest growth of all treatments ($P<0.05$). The net weight gain of an individual fish with a C/N ratio of 20 was higher (12.7 g) than with a C/N ratio of 10 (11.0 g) agreeing with the hypotheses that C/N ratio has an effect on *L.victorianus* production in hapas. The intermediate density of 15 fish/hapa resulted in the highest individual mean weight gain at harvest (13.8 g) followed by stocking density 25 fish/hapa (11.58 g) and 10 fish/hapa (10.2 g). The present results concide with findings of Kawamoto et *al*. (1957) and Haque et *al*. (1984) who observed best growth at lower stocking densities. Results of Zhu et *al*. (2011) on cage fish farming with sturgeon for 75 days showed that stocking density of 15 fish /m^2 though not achieving the highest production, economic analysis revealed that it provided the best benefit cost ratio. The lowest stocking density provided more space, food and less competition as reported by Haque et *al*. (1984) and Narejo et *al*. (2005). The survival percentage was not significantly different in the different treatments and stocking densities indicating that C/N ratio control did not influence the survival of the fish. Survival was also not influenced by the stocking density of the fish (Table 9) only that the highest density had a bit lower mean survival which might have been a result of high competition for food and space among fishes. The highest net and gross yield of *L. victorianus* were recorded in the high C:N ratio treatment. The net yield increased by 15% when increasing C/N ratio from 10 to 20. The highest net production was obtained stocking 25 individuals per hapa (2269.1 kg ha^{-1} 72 d^{-1}). The results agree with findings of Barua (1990) and Narejo et *al.* (2005) and further show that the hypotheses a higher C/N ratio allows for higher stocking density was actually achieved. PER was higher and SGR was a little bit higher with a C/N ratio of 20. The use of wild fish caught juveniles, who were not used to a formulated diet, might have caused the observed low growth achieved in this experiment. However metabolic growth and geometric mean body weight was higher with a C/N ratio of 20. It can therefore be considered that maize flour can benefit *L. victorianus* fish farming through reducing toxic inorganic nitrogenous content, increasing heterotrophic bacteria and algal abundance, improving productivity and enhancing overall sustainability. In previous studies, different carbohydrate sources like tapioca starch and molasses (Hari et *al*., 2004, Burford et *al*., 2004) were used in shrimp and fish farming to improve water quality and productivity of ponds. Assaduzzaman et *al*., 2010 reported that pond ecological and growth data revealed that maize flour can be a good source of organic carbon to maintain high C/N ratio in ponds. In that study realistic economic analysis indicated that use of maize flour in C/N system reduced carbohydrate cost thereby improving economic benefits. Economic analyses were not done for the present study considering the short culture period leaving the fish no time to reach market size.

5.2 Water quality parameters

Water quality management is of imperative importance in aquaculture. It is usually strongly influenced by stocking densities, culture species combinations, quality and quantity of nutrient inputs and the culture system to be used (Diana et *al*., 1997). In the present study water temperature, dissolved oxygen and pH were within the suitable range for tropical fish culture (Boyd, 2002). Low temperatures at the start of the

experiment were due to the weather condition during the season of the year when the research was conducted. This might have negatively impacted on the growth of *L. victorianus*, which does best at optimal temperature conditions of about 24-26 ˚C (Rutaisire, 2003). The observed decreasing trend of dissolved oxygen concentration, Chlorophyll-*a* and transparency among the stocking densities (Table 4) might have been as a result of increase in fish biomass that increased pond turbidity reducing photosynthesis and hence low primary production. Chlorophyll-*a* concentration was however observed to increase with time which was as a result of carbohydrate addition that stimulated phytoplankton proliferation. Total alkalinity was within a suitable range of 70-150 mg L^{-1} recommended for tropical fish culture (Boyd, 2002). This provided a well buffered environment suitable for growth of the fish and pond primary productivity. Soluble reactive phosphorus was the same in the three different stocking densities but significantly different during the sampling periods and high in the high C/N ratio treatment. This could be attributed to the high bacterial production and decomposition with addition of carbohydrates. The relatively high SRP levels during the culture period could be the result of fertilizer application in addition to carbohydrates. Both soluble organic phosphorus and soluble reactive phosphorus are the main end-products of bacterial activity on organic matter (Elnady et *al.*, 2010). Among the inorganic nitrogen species concentrations, manipulation of C/N ratio by carbohydrate addition maintained good water quality conditions and significantly reduced inorganic nitrogen concentration in the water column. The findings are in agreement with Avnimelech (1999) and Hari et *al*. (2004) who reported that the addition of carbohydrate to the production systems will reduce the TAN concentration through immobilization by bacterial biomass. The very low NO_3-N, TAN and NO_2-N in the treatments compared to other studies using cyprinids (Azim et *al*., 2001) could be attributed to carbohydrate addition to maintain a high C/N ratio during the experimental period. Among the three stocking densities, concentration of the nitrogenous compounds was high in the high stocking density hapas as a result of high fish biomass. Acosta-Nassar et *al*. (1994), Gross et *al*. (2000) and Davenport et *al*. (2003) reported that fish in a pond assimilate only 15-30% of the nitrogen added in the feed. The remainder is lost to the system as ammonia and organic nitrogen in feces and feed residue, which undergoes decomposition and eventually produces ammonia. The results of this study show that higher dietary protein levels resulted in significantly higher TAN and NO_2-N concentrations in the water column. Li and Lovell (1992) reported that the ammonia concentration increased with increasing dietary protein concentration and protein feeding rate.

5.3 Sediment quality

In this experiment there was no direct contact of fish with the sediment since they were grown in hapas. Hence, exogenous factors contributed to the sediment quality of the experimental ponds. Nevertheless all the analyzed sediment quality parameters were within the acceptable pH range (6.5 to 7.5), organic carbon (0.5 to 2.5 %), total nitrogen (15 to 20 %) and total phosphorus (15-18 mg L^{-1}) Benerjea (1967). Manipulation of C/N ratio by carbohydrate addition in the high C/N ratio ponds significantly reduced total nitrogen in sediment. The increase in total heterotrophic bacteria count in the sediment during the culture period (Table 6) was mainly as a result of feed and carbohydrate application due to the increased biomass of fish over time. The bacterial load again led to higher decomposition rates releasing inorganic nutrients that in turn stimulated further bacterial development (Avinmelech et *al.*, 1989). The significant increase in pH and availability of total organic matter and total phosphorous with a high C/N ratio contributed to total pond productivity (Fig 2). Boyd and Musig (1981) reported that phytoplankton uptake and nitrification are considered the principle sinks of ammonia while as pond soils can be

considered a source of sink for phosphorous and other biologically important materials such as carbon, nitrogen and sulphur.

5.4 Effects on total heterotrophic bacterial load in water and sediment

In the present study, maize flour was used to increase the C/N ratio of the experimental diet resulting in a significant increase in the total heterotrophic bacteria count and lower concentration of NO_3-N in water (Fig 3). It also caused significant reductions in nitrogenous compounds which could be attributed the addition of carbonaceous substrates that led to an increased microbial biomass, which immobilized TAN for the synthesis of new bacterial cells (Hari et *al*., 2004) and uptake of the nitrogenous compounds by phytoplankton. The heterotrophic bacterial population utilizes the inorganic nitrogen to synthesize bacterial protein and new cells (single cell protein) and it may be utilized as a food source by carp, tilapia (Schroeder, 1987; Beveridge et *al*., 1989; Rahmatulla and Beveridge, 1993) or shrimp (Burford et *al*., 2004), thus lowering the demand for supplemental feed protein (Avnimelech, 1999). The observed higher total heterotrophic bacteria count in the water column and sediment in treatment C/N 20 revealed that heterotrophic bacteria utilized the added carbon source resulting in higher productivity (Hari et *al*., 2004). The reported increase in THB count in the experimental hapas and sediment (Table 6) was mainly because of increased amount of feed and carbohydrate application due to increase in fish biomass over time. Avnimelech et *al.* (1989) reported that increased bacterial loads leads to higher decomposition rates releasing inorganic nutrients that in turn further stimulate bacterial development. Avnimelech (1999) further showed that under aerobic condition, microbial breakdown of organic matter leads to production of new bacterial cells amounting to 40-60% of the metabolized organic matter. This therefore suggests that high bacteria loads function both as bioreactors controlling water quality and protein food source for *L. victorianus* which is a bottom grazer on detritus and algae.

5.5 Effects on abundance of plankton and benthic macro-invertebrates

5.5.1 Effects on plankton

The observed 25% increase in the biovolume of plankton with a high C/N ratio in the present study might be due to the higher amount of added organic matter in the ponds. The hapas with a C/N ratio of 20 received additional 1.14 kg maize flour starch for each kg of formulated feed in contrast to the hapas with a C/N of 10. Milstein (1993) and Diana et *al*. (1997) reported that the abundance of plankton in a culture system is influenced by stocking density, nutrient input quality and quantity as the most important factors. There was a comparatively lower abundance of phytoplankton in hapas as compared to the ponds with lowest means in the high density hapa which could have been an effect of Labeo feeding on them. There was no significant difference among the zooplankton in the medium and high densities and in the pond which might have indicated that *L. victorianus* as a bottom feeder had less preference for zooplankton. Rahman (2006) showed that Rohu (*Labeo rohita*) a column feeder, of the same genus with *L. victorianus* had positive electivity indices for zooplankton and negative for phytoplankton which might have been as a result of the mode of feeding. There was a steady significant increase in the abundance of phytoplankton over time, with the highest biomass being recorded during the last month of the experiment. This could have been attributed to the increased phosphorus

concentration in the pond water. Azim and Little (2006) showed that formation of autotrophic organisms in aquaculture ponds can be supplemented by addition of organic matter. Further the organic matter indirectly supplies inorganic nutrients through decomposition by bacteria (Milstein, 1992). In turn, the increased nutrient availability in a high C/N ratio treatment resulted in an increased phytoplankton biovolume (Asaduzzaman et *al*., 2008). Stimulatory effects between autotrophic and heterotrophic organisms as shown by Asaduzzaman et *al*. (2010) could have been a cause for the increased plankton abundance. Cole, 1982 showed that along with the added carbohydrate, algal detritus are a major source of organic substrate for heterotrophic bacterial growth whereas living algae provide oxygen for decomposition. In return, bacteria regenerate inorganic nutrients and vitamins that stimulate algal growth and productivity (Cole, 1982). Eventually, a higher biomass of phytoplankton and heterotrophic bacteria will immobilize ammonia and nitrate from the water column resulting in a significant lower concentration in high C/N ratio ponds (Asaduzzaman et *al.*, 2008).

5.5.2 Effects on benthic macro-invertebrates

The higher planktonic biomass and lower concentrations of toxic nitrogenous compounds influenced the amount of benthic macro-invertebrates, resulting in a 30% higher biomass with a C/N ratio of 20 compared to a C/N ratio of 10. There was an observed increase in biovolume of total benthos during the culture period. Absence of predation most likely is the primary cause. Different studies showed that macro benthos contribute to enhanced influx of ammonia and other materials across the sediment water interface through their burrowing activity (McCall et *al*., 1979). The material exchange via fluid advection, solute diffusion and excretion of metabolites creates local hot spots for bacterial activity (McCall et *al*., 1979 and Henrikisen et *al*., 1983). Eventually, this contributes to better water quality and pond productivity.

6.0. CONCLUSION AND RECOMMEDATIONS

The findings of this study demonstrated that increasing the C/N ratio by addition of carbohydrate benefited *L. victorianus* farming at a medium stocking density of 15 fish/m^2. The C/N ratio control improved water quality through reducing toxic inorganic nitrogen contents like ammonia and nitrite, improving nutrient utilization efficiency, reducing nutrient discharge, increasing THB population that converts inorganic nitrogen into single cell protein, promoting pond productivity and enhancing overall sustainability. Maize flour is already locally produced and used as an animal feed ingredient by farmers and therefore can be used as on farm carbohydrate source to culture *L. victorianus.* Nevertheless, maize flour in Kenya is also human food, hence potential human-animal user conflicts should be taken into account. This opens a scope for further improvement of economic sustainability of this technology by comparing the potential of other cheap carbohydrate sources such as sugarcane wastes, molasses and native starch like potato. More so sensitizing farmers and training them on adoption of this technology at the farm levels through direct participation would be of great benefit due to the high cost of protein rich feeds. In this research *Labeo victorianus* was used in relatively low stocking densities which might have underutilized the available pond communities. Therefore further research with much higher densities and in combination with a column and bottom grazing fish species like tilapia might enhance production further.

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APPENDENDICES

Appendix 1. Photograph pates