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The Effects of Dietary Levels of Protein and Greenhouse on Growth, Behaviour and Fecundity of Nile tilapia (*Oreochromis niloticus* L.) Broodstock

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Abstract: ***The aim of the study was to assess the main and interactive effects of greenhouse and dietary crude protein levels on growth, behavior and fecundity of Nile tilapia at Kegati aquaculture research station, Kisii, Kenya from July to August, 2012. Mixed sex tilapia broodtock (251.76±1.57g) were distributed at random among 12 hapa ($1m^3$), 6 under greenhouse and 6 in open pond, 6 fish in each hapa at the ratio of 1male: 2 females. With the exception of temperature, there was no significant ($p > 0.05$) variation in water quality parameters between greenhouse and open pond. Greenhouse was able to raise water temperature with an average of 7.82 ^0C above the open pond conditions. Dietary crude protein level and greenhouse had a strong interactive effect on WG (%), SGR, fecundity and spawning frequency of tilapia broodstock. The current study suggests that the recommended maximum level of dietary crude protein level to maintain the welfare and performance of Nile tilapia broodstock is between 25-30 % with a maximum advantage of greenhouse culture***

Keywords: greenhouse, *Oreochromis niloticus,* growth, temperature, protein

1. Introduction

Nile tilapia (*Oreochromis niloticus*) is one of the most important cultured fish species in many tropical and subtropical countries of the world [1, 10]. Its resistance to diseases, fast growth, efficient feed conversion, ease of spawning, and good consumer acceptance make it of commercial importance in aquaculture [16, 23, 24, 28]. Its growth rates are influenced by a variety of factors such as sex, supplemental feeding, stocking density and similarly, by the declining of water temperature which noticeably affects their growth rate. In areas outside their natural range, the major limiting factor determining the possibility for establishment of tilapia populations is the normal minimum temperature during the coldest period of the year [28].

Temperature is an important parameter for fish growth [11, 21, 35]. Water temperature is a major influence on aquaculture husbandry practices and it has a profound impact on metabolic activity [21]. The three important factors synergistically affecting the growth of fish are Protein level, water temperature, and fish size [21]. Low temperature causes sluggishness by retarding the digestion speeding of fish [3]. Some researchers have found that the digestion rate has been increased as the temperature increases [42]. Environmental temperature is one of the most important ecological factors which also influence the behavior and physiological process of aquatic animals [49]. All fish species are characterized by an ideal range of temperature in which they show their maximum growth [6, 39, 40]. Previous studies indicate a temperature range of 25-33 ºC with 28 ºC being the most recommended for tilapia culture. Improved growth performance at higher temperatures is due to the presence of suitable conditions that enhance enzymatic response of fish physiological functions [5, 15, 25].

During cold season, in many parts of Kenya the water temperature drops below 20 ^0C and in particular in Kisii it descends down to 12-15 ^0C. The water temperature in Kisii and the surrounding environments drops below the desirable range during the months of March-August. The year round growth of fish remains a challenge for many small scale farmers in Kisii.

Pond culture is the most widely used production system in Kenya and other parts of the world but ponds demand large amounts of water [28] making them inefficient and vulnerable to climate change leading to low productivity. This calls for more resilient alternative systems like tanks and greenhouse based pond systems. In an open-air pond, temperature variation of only a few degrees represents a proportionally large change for fish growth and survivability [12, 48]. In all fish species, growth optimization is of paramount importance for profitability of fish farming activity. Several authors have indicated that water temperature can be highly maintained in greenhouse fish pond system [29, 33, 50]. It appears that the adoption of greenhouse technology could help farmers and hatchery managers achieve year round growth of fish. However, in Kenya, this technology is still in experimental stage due to lack of knowledge.

Although emerging evidence suggests that increases in temperature and dietary protein can individually affect metabolic processes and behaviour, the potential interacting effects of these variables on the performance of cultured fish are currently unknown. Many studies have been carried out to determine protein requirements for fish, however, to the best of our understanding, broodstock nutrition is without doubt one of the most poorly understood and researched areas of finfish nutrition. Therefore, the aim of this study was to assess the main and interactive effects of greenhouse and dietary levels of protein on growth, behavior and fecundity of tilapia during cold season.

2. Materials and Methods

A 60 days dietary protein level and greenhouse experiment was carried out from July to August 2012 at Kegati aquaculture research station, Kisii, Kenya (Figure 1). Kegati station is located at 0042’ 50.44S and 0344 47’ 59.4 E’ and at an altitude of 1974m in Kisii County. The experimental fish were obtained from the Kegati fish farm. The fish were acclimatized to the hapas and conditions for one month under 15%, 25% and 30% dietary crude protein under open and greenhouse. At the start of the experiment, the fish were starved for two days before they were individually measured to obtain the initial weight. During the measurement, the fish were anaesthised using Tricaine methane sulfonate (MS-222) used in the ratio of 40-50 mg per litre of water. Mixed sex tilapia brooders (251.76±1.57g) were distributed at random among 12 hapas ($1m^3$), 6 under greenhouse and 6 in open pond, 6 fish in each hapa at the ratio of 1male: 2 females. A set of two ponds measuring 104 m^2; one open and the other under greenhouse structure were used. The experimental ponds were limed once at the beginning of the experiment at 2.5 tonnes ha^{-1} with agricultural lime. All the ponds were fertilized with 20 kg N $ha^{-1}wk^{-1}$ and 5 kg P ha^{-1} wk^{-1} using urea and Di-ammonium phosphate (DAP), respectively to stimulate natural food items

Three different experimental diets were formulated containing the same digestible energy. The diets contained 15%, 25% and 30% crude protein herein referred to as LCP15, MCP25 and HCP30 respectively. The crude protein was provided by fish meal. The brooders were fed *ad libitum* twice a day at 0900 and 1600hrs

Water (W_t), ambient air (A_t) and inside greenhouse air temperature (G_t) were recorded at hourly basis (60 minute interval) for 24 hours once in a week using calibrated alcohol-filled, glass-bulb thermometer (least count was 1^oC). Water samples of the entire period were recorded once in a week at 8.00 and 16.00 hours for pH, dissolve oxygen, and total alkalinity. The parameters were monitored *insitu* using a multiparameter metre (Hanna Instruments Ltd., Chicago, IL, USA). Ammonium nitrogen and nitrate nitrogen were also measured once in a month. Water samples were analyzed using standard methods [2]

Fish were sampled monthly using a scoop net, where all the eggs were removed from the females’ mouth, counted and mean fecundity for the female calculated. The stages of the eggs were noted and subsequently transferred to the laboratory for incubation. The female’s mouth was monitored again on monthly basis for subsequent spawning. All the fish in each hapa were counted and batch weighed to the nearest 0.01g using a bench scale (DS10) and mean weight was calculated. Their total lengths were then measured using a ruler to the nearest one-tenth of a centimeter. The total length was measured as the distance from snout to the tip of the caudal fin. Prior to measurements, the fish were starved for one day.

The specific growth rate (SGR) was calculated as % change in body weight per day according to Brett and Groves [7] as follows:

$$SGR (\%) = 100 * (\ln FBW - \ln IBW)/day$$

Where FBW and IBW are the final and initial mean body weights, respectively.

The data was analyzed using STATISTICA 8.0 computer package. Before statistical analysis, normality of the data was determined using Shapiro-Wilk test, while homogeneity of variance was ascertained using Levene’s test. All results were expressed as mean ± S.D. Comparison of mean values was made by one way analysis of variance (ANOVA) at a significance level of $p < 0.05$.

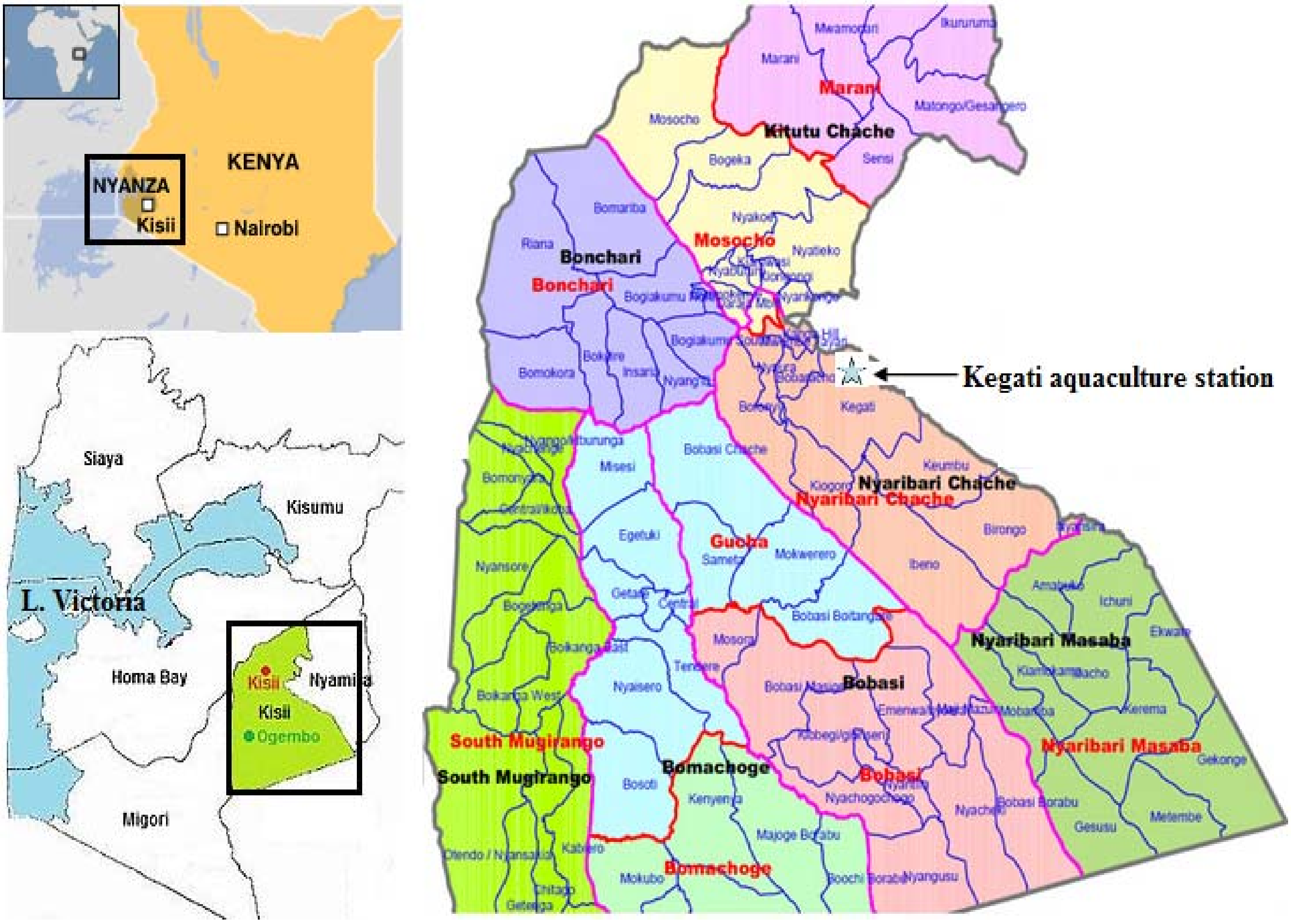

Figure 1: Map of the location of the experimental set up at Kenya Marine and Fisheries Research Institute (KMFRI) Kegati aquaculture station, Kisii, Kenya

3. Results

3.1 Water Quality Parameters

Table 1 shows the main effects of greenhouse and dietary crude protein on water quality parameters during the experimental period. Water temperature was significantly higher ($P<0.01$) in greenhouse than in open pond condition. Results indicated that greenhouse was able to raise water temperature with an average of 7.82 ^{0}C above the open pond conditions. With the exception of temperature, there was no significant ($p > 0.05$) variation in water quality parameters between greenhouse and open pond. Notably, there was no significant variation ($p>0.05$) in water quality parameters between the three dietary treatments. Dissolved oxygen level was significantly higher ($p < 0.03$) in the afternoon than in the morning in both open and greenhouse ponds. In the morning, high dissolved oxygen was recorded in open pond and the lowest in greenhouse condition but it was not significantly ($p> 0.05$) different from that in the greenhouse. In the afternoon, high dissolved oxygen was recorded in greenhouse and lowest in open pond condition but it was not significantly ($p>0.05$) different from that in the open pond. Mean pH in all the treatments ranged from 7.2 ± 0.04 - 7.84 ± 2.22. There was no significant difference in morning and afternoon pH values ($p > 0.05$) in the open pond and greenhouse pond. No significant differences ($p>0.05$) were found for mean NH_4^+-N, NO_3-N, and alkalinity among the treatments. There were no significant interactive effects of greenhouse and dietary protein on water quality parameters ($p>0.05$).

Table 1: The main effects of dietary crude protein levels and greenhouse on water quality parameters at the end of 60 days (mean ± S.D.)

Treatments	Time	Temperature	pH	Do (mg/l)	Total alkalinity (mg/l)	NH_4^+-N (mg/l)	NO_3-N
CP (%)							
L15	Morning	12.22±0.22^{a}	7.6±0.28^{a}	5.23±0.09^{a}	364.36±39.44^{b}	0.02±0.1^{a}	0.21±0.08^{a}
	Afternoon	22.42±2.09^{c}	7.78±0.09^{a}	7.16±1.27^{a}	381.07±42.57^{b}		
M25	Morning	12.32±3.42^{b}	7.77±0.29^{a}	5.64±0.35^{a}	363.57±43.30^{b}	0.04±0.01^{a}	0.24±0.03^{a}
	Afternoon	20.20±0.04^{d}	8.10±0.10^{a}	6.48±2.24^{a}	387.29±26.74^{b}		
H30	Morning	16.42±4.22^{a}	7.6±0.28^{a}	5.33±0.19^{a}	364.36±39.44^{b}	0.02±0.01^{a}	0.19±0.03^{a}
	Afternoon	25.22±1.09^{c}	7.78±0.09^{a}	6.22±1.37^{a}	381.07±42.57^{b}		
Cover							
GH	Morning	18.22±1.22^{a}	7.6±0.28^{a}	5.53±0.19^{a}	364.36±39.44^{b}	0.02±0.1^{a}	0.21±0.08^{a}
	Afternoon	28.02±1.09^{c}	7.78±0.09^{a}	8.06±0.57^{a}	381.07±42.57^{b}		
OP	Morning	12.32±3.22^{b}	7.77±0.29^{a}	5.77±0.35^{a}	363.57±43.30^{b}	0.03±0.01^{a}	0.19±0.03^{a}
	Afternoon	20.20±0.04^{d}	8.10±0.10^{a}	7.48±0.44^{a}	387.29±26.74^{b}		
Interactions							
L15 x GH		ns	ns	ns	ns	ns	ns
L15 x OP		ns	ns	ns	ns	ns	ns
M25 x GH		ns	ns	ns	ns	ns	ns
M25 x OP		ns	ns	ns	ns	ns	ns
H30 x GH		ns	ns	ns	ns	ns	ns
H30 x OP		ns	ns	ns	ns	ns	ns

Values are means ± SD; values within the same column without a common superscript are significantly different ($p < 0.05$*).* OP= open pond, GH=greenhouse, CP= crude protein

Hourly variations of water, ambient and inside greenhouse air temperatures for a typical week are shown in Figure 2. The results indicated that the greenhouse pond retained heat for a longer period hence uniform water temperature throughout the day and night. However, open pond water easily lost heat to environment hence showed higher fluctuations of water temperature. The temperature inside greenhouse air was significantly different ($p<0.05$) from the open condition.

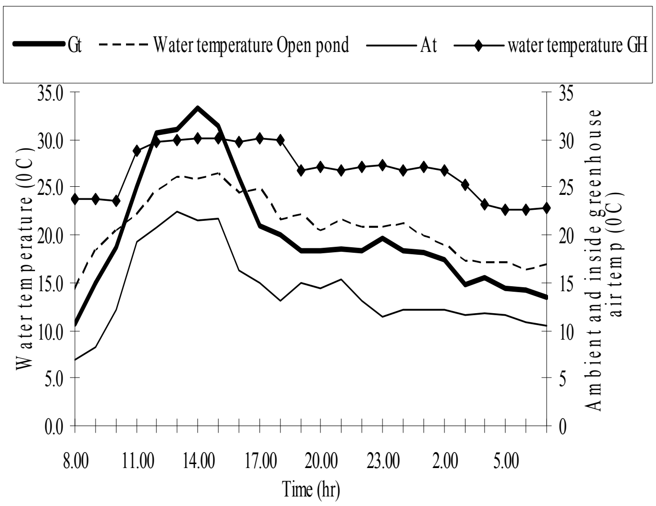

Figure 2: Hourly fluctuations of water, ambient and inside greenhouse air temperature in open and greenhouse condition for a typical week.

3.2 Behavioral observation

Generally elevated food consumption and swimming activity was observed, although not quantified, in greenhouse than in open pond. Notably, feeding rate was elevated in high dietary crude protein as compared to low dietary crude protein. In the morning, the fish in greenhouse ponds had increased ventilation, although not quantified, as compared to open pond. The greenhouse ponds experienced algal blooms frequently as compared to open pond necessitating dilution of the waters more frequently.

3.3 Growth performance

The main effects of crude protein and greenhouse pond on growth parameters at the end of 60 days are shown in Table 2. At the end of 60 days study period, no mortality was observed in any of the treatments. Higher WG% and SGR was observed in HCP30 treatment and it was significantly different ($p<0.001$) from LCP15 treatment. However, it was not significantly different ($p>0.05$) from MCP25. Notably, higher WG%, and SGR was observed in GH treatment and it was significantly different ($p<0.01$) from OP treatments. Dietary protein and greenhouse had a strong interactive effect on WG (%) and SGR.

Table 2: The main effects of dietary crude protein levels and greenhouse on growth parameters at the end of 60 days

Treatments	Initial W (g)	WG (%)	SGR (%day-1)
CP (%)			
L15	251.76±0.22^{a}	572.1 ± 26.2 ^{c}	0.06 ± 0.05 ^{b}
M25	251.76±1.02^{a}	798.8 ± 29.9 ^{b}	1.59 ± 0.04 ^{a}
H30	250.76±0.12^{a}	818.3 ± 34.4 ^{b}	1.61 ± 0.04 ^{a}
Cover			
GH	250.76±0.25^{a}	799.7 ± 31.4 ^{a}	1.16 ± 2 ^{b}
OP	250.76±0.23^{a}	647.1 ± 27.9 ^{b}	0.06 ± 2 ^{a}
Interactions			
L15 x GH	-	*	*
L15 x OP	-	ns	ns
M25 x GH	-	**	**
M25 x OP	-	ns	ns
H30 x GH	-	**	**
H30 x OP	-	ns	ns

Values are means ± SD; values within the same column without a common superscript are significantly different ($p< 0.05$*). Significant level of differences: ns,* $p > 0.05$*; *,* $p \le 0.05$*; **,* $p \le 0.01$. OP= open pond, GH=greenhouse, CP= crude protein

Mean individual weight of tilapia subjected to open and greenhouse ponds increased over the 60 days of the experiment (Figure 3). Fish growth was similar within the first 30 days after which there was differential growth pattern among the treatments. On the 60th day, there was significant difference ($p<0.02$) in the mean size of the groups with the fish under open pond being significantly smaller than in greenhouse. There were no significant differences ($p > 0.05$) between *hapas* within treatments. Adding time as a factor into the ANOVA to compare the growth trajectories of the different groups showed significant effects of time ($p<0.0001$), greenhouse ($p<0.0004$) and interaction between the two factors ($p<0.0001$).

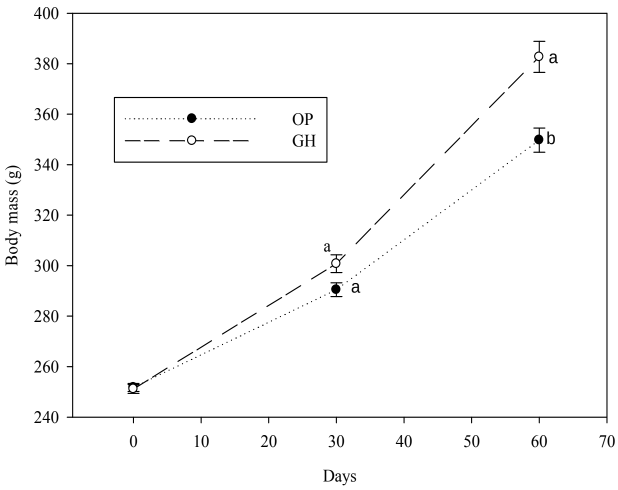

Figure 3: Mean body mass (±S.D) of fish reared under open and inside greenhouse ponds. Significant differences are indicated with superscripted letters (Tukey's test, $p < 0.05$). OP= open pond, GH=greenhouse.

Mean individual weight of tilapia subjected to different levels of crude protein increased over the 60 days of the experiment (Figure 4). At the termination of the experiment, fish under LCP15 treatment recorded significantly ($p<0.05$) smaller weights as compared to HCP30. However, there was no significant difference ($p > 0.05$) in the mean body mass of the groups exposed to MCP25 and HCP30.

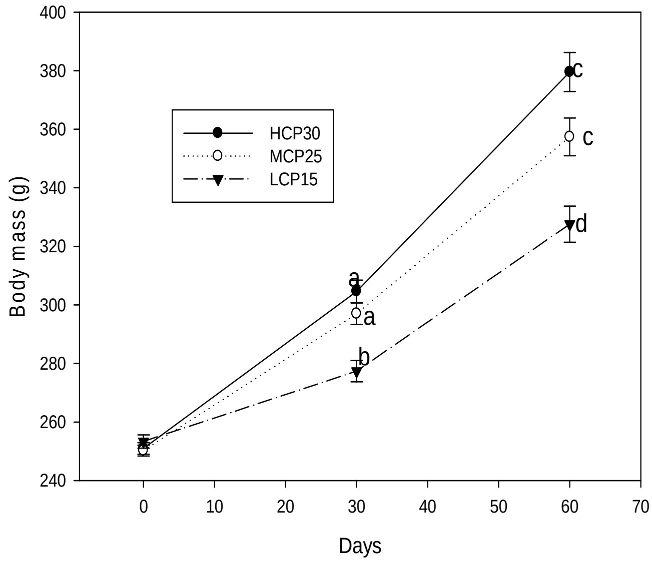

Figure 4: Mean body mass (±S.D) of fish reared under different levels of crude protein. Significant differences are indicated with superscripted letters (Tukey's test, $p < 0.05$).

3.4 Fecundity

Table 3 indicates the main effects of dietary crude protein and greenhouse on spawning frequency during the sampling period. Greenhouse had significantly higher ($p < 0.002$) number of eggs than open pond. Notably, HCP30 gave significantly higher ($p < 0.001$) number of eggs as compared to LCP15. However, HCP30 was not significantly different ($p>0.05$) from MCP25 in terms of number of eggs produced. The results of the present study indicated that in greenhouse, majority of the eggs were either in stage 2 or stage 3 of development while in open pond, they were all at stage 1 of development (Table 3). Spawning frequency was shorter for greenhouse (30 days) than the open pond (60 days). Notably, spawning frequency was shorter for HCP30 as compared to LCP15. Greenhouse and dietary crude protein did not interactively or independently affect the stage of development. However, dietary crude protein and greenhouse had a strong interactive effect of on fecundity and spawning frequency of Nile tilapia.

Table 3: The main effects of crude protein and greenhouse on fecundity of Nile tilapia.

Treatments	*Fecundity/ Female*	*Stage of development*	*Spawning frequency (days)*
CP (%)			
L15	$405.76{\pm}4.12^a$	1	60
M25	$1029.76{\pm}1.22^b$	2	30
H30	$1036.84{\pm}3.12^b$	3	30
Cover			
GH	$1198.59{\pm}0.15^a$	3	30
OP	$618.66{\pm}1.23^b$	1	60
Interactions			
L15 x GH	*	ns	*
L15 x OP	ns	ns	ns
M25 x GH	**	ns	**
M25 x OP	ns	ns	ns
H30 x GH	**	ns	**
H30 x OP	ns	ns	ns

Values are means ± SD; values within the same column without a common superscript are significantly different ($p < 0.05$*). Significant level of differences: ns,* $p > 0.05$*; *,* $p \leq 0.05$*; **,* $p \leq 0.01$*.* OP= open pond, GH=greenhouse, CP= crude protein

4. Discussion

Greenhouse water showed uniform water temperature through out the day and night. Consistent with other studies [16, 20, 27] this could probably be due to transmission of solar radiation by greenhouse and absorption by water. The *hapas* in open condition showed lower water temperatures due to continuous heat losses from the *hapas* to the ambient through conduction, convection, evaporation and radiation [43-44]. Generally fish do not like any kind of changes in their environment. Any changes add stress to the fish and the larger and faster the changes, the greater the stress. So the maintenance of temperature in pond culture by use of greenhouse becomes very essential for fish culturist/hatchery operators for getting maximum yield and fingerlings

The greenhouse treatment showed increased water temperature of average 7.82 ^{0}C above the open pond condition, suggesting that water temperature can be increased significantly in ponds by use of greenhouse in a tropical climatic condition similar to those of the study area. The results of the present study concur with those of several other studies, which indicate that greenhouse could increase the water temperature in fish culture [4, 20, 27, 30, 36, 41, 45, 50]. Crude protein did not have any significant effect on water quality. Notably, there were no significant interactive effects of crude protein and greenhouse on water quality parameters suggesting that the effect of different levels of dietary crude protein on water quality is independent of the temperature (as a result of greenhouse) of the rearing water.

We observed that fish under greenhouse had elevated consumption and activity. This could possibly be as a result of elevated water temperature as a result of greenhouse effect. Many researchers have associated elevated food ingestion with temperature [9, 22, 38, 47]. This could be due to additional energy required to offset higher metabolic cost associated with higher temperature in order to maintain desired growth rates [6, 26]. This could have resulted into high ingestion rate of fish under greenhouse condition. Low oxygen in the morning in greenhouse resulted to increased ventilation. Consistent with other studies [13, 19], this could be a reaction to low oxygen by increasing the rate of water flow through the gills.

In this research, with regards to the dietary crude protein level, fish fed with high dietary protein gained significantly higher WG % and SGR while those fed with low dietary protein got low WG % and SGR. This could be because protein intake was high at high dietary protein level and the main component in diet which influences growth is protein. However, the present results are different from the results of El-Saidy and Gaber [17] who found no significant increase on growth performance at high dietary protein level (30%) compared to the low one (25%). It could be due to the size of the fish they used as it is known that the protein requirement varies with size and stage of development [46].

Several studies have established that at higher temperature, growth performance increases due to the presence of suitable conditions that enhance enzymatic response of fish physiological functions [5, 15, 25, 35]. Studies on several fish species have prevailed that with increase in water temperature, the growth rate increases at higher levels and decreases at lower levels [12, 48]. In this study, prevailing higher water temperature inside greenhouse showed higher growth rate in comparison with open condition possibly due to increased metabolism [34-35]. Similar findings have been reported by Josiah et al., [27] and Mohapatra [37] who found better growth of *Clarias gariepinus* and *Labeo rohita* in greenhouse. In open pond condition, growth rate was markedly dropped due to poor ingestion

There was a strong interactive effect of crude protein and greenhouse on growth of Nile tilapia, suggesting that the effect of crude protein on growth performance of Nile tilapia under culture conditions strongly depends on the environmental temperatures. The overall specific growth rate (SGR) of the fish in the present experiment (1.16 **%** day^{-1}) for fish reared under greenhouse condition was good in comparison to what is reported in other studies on tilapia. It was higher than the 0.4%day^{-1} reported by Liti et al [31] for slightly larger tilapia (500 g) and slightly less than the 1.3 % day^{-1} reported by Liti et al. [32] for slightly smaller fish (25 g). However, all these studies had recorded poor growth rate values during the first period (from day 1 to day 30). It is not clear why this case occurs. However, it is possible that the fish had not fully acclimated to the *hapas* before the experiment commenced. In contrast, the growth rate subsequent growth periods were very good and therefore, the overall growth performance was good under greenhouse condition as compared to open pond.

There was a strong interactive effect of greenhouse and dietary crude protein on fecundity and spawning frequency of Nile tilapia. Numerous studies have demonstrated that reproductive performance and egg quality are influenced by nutrients and environmental factors like temperature [14, 18]. Proteins and lipids are the main components of egg yolk and play an important role in fish reproduction. Furthermore, proteins are a major source of amino acids and are a reservoir of materials used for early stages of embryogenesis. Therefore, formulated feed for broodfish should contain sufficient amounts of crude protein. Because the metabolism of fish is affected by temperature and other environmental factors [21], it is often assumed that environmental conditions e.g. temperature may have a significant effect on feeding activity. In the present study, interactive influence of temperature and dietary crude protein could have enhanced feeding frequency of fish resulting in higher protein intake in the higher dietary protein level resulting to high production of eggs and spawning frequency

5. Conclusion and Recommendations

In conclusion, this study demonstrates that there is a strong interactive effect of greenhouse and dietary crude protein on growth performance, fecundity and spawning frequency of Nile tilapia. However greenhouse and dietary crude protein does not have a strong independent or interactive effect on the stage of development of the egg. Greenhouse can raise water temperature with an average of 7.82 ^{0}C above the open pond conditions in a tropical climatic condition similar to those of the study area. The current study suggests that the recommended maximum level of dietary crude protein level to maintain the welfare and maximum growth of Nile tilapia is between 25-30 % with a maximum advantage of greenhouse culture. However farmers need to watch out for excessive algal bloom under greenhouse possibly by not fertilizing ponds under greenhouse.

6. Acknowledgments

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