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Growth Performance and Activities of Some Liver Enzymes in *Clarias gariepinus* Burchell 1822 Juveniles Cultured in a Water Hyacinth (*Eichhornia crassipes* [Mart] Solms-Laubach) Infested Media

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Growth performance and activities of some liver enzymes in *Clarias gariepinus* Burchell 1822 juveniles cultured in a water hyacinth (*Eichhornia crassipes* [Mart] Solms-Laubach) infested media

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ABSTRACT

A 12-week exposure study was conducted to evaluate the growth performance and activities of some liver enzymes of African catfish (Clarias gariepinus) juveniles cultured in water hyacinth (Eichhornia crassipes) infested concrete and plastic culture media. 152 fish juveniles were introduced into two large concrete tanks (6.0 m × 5.0 m × 1.2 m) for the outdoor experiment. A tank was infested with E. crassipes plant at a density of 12.30 plants/m^2, while the other tank, which served as the control, contained no water hyacinth plant. For the laboratory experiment, 50 fish juveniles were stocked in duplicate into five circular plastic tanks (labelled CT, T1, T2, T3, and T4) containing water hyacinth plants cultured at densities of 0, 5, 10, 20, and 40 plants/tank respectively. The on-growing fish were fed (2 mm) fish feed (45% crude protein) at 5% body weight twice daily. The result showed that fish juveniles grown in the water hyacinth-infested culture media (both indoor and outdoor) had significantly ($p<0.05$) better growth performance indices than those grown in a water hyacinth-free medium. The hepatic enzyme analyses revealed that fish juveniles cultured in concrete tanks with water hyacinth had significantly ($p<0.05$) higher levels of DNA and glycogen activities. Fish cultured with the highest plant densities in a plastic medium had significant levels ($p<0.05$) of liver protein, DNA, glycogen, G6PDH, and LDH activities compared with the control. The study concluded that fish raised in water hyacinth-infested media at moderate plant density performed better than those grown in the control weed-free media.

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Introduction

Water hyacinth (Eichhornia crassipes), which is reported to be the most prevalent perennial weed that is deleterious to the aquatic ecosystem, especially in tropical water bodies [9,30,56] have become a scourge of waterways, creating a major impediment to the movement of boats; enabling rapid development of mosquitoes and other health hazards; impeding fishing activities and also harbouring waterborne pathogens, and dangerous animals like crocodiles (Guardian Newspaper, 2008; [17,42,52,60]).

The successful and rapid growth of water hyacinths in aquatic ecosystem is due to photosynthetic versatility ([85]; Hauser et al., 2014) and efficient utilization of raw materials in the water [38]. Water hyacinth removes large quantities of nutrients from the aquatic environment because its growth corresponds directly to the level of the nutrients in water ([83]; Britton et al., 2007). Infestation of aquatic ecosystem by this water weed, among other things, imparts negative effects on the physico-chemical parameters of water [30,49]; reduces euphotic zones by acidifying the water medium [51]; consumes major biogenic ions in water by mats of weeds [6]; hinders fish exploitation [26,55]; and considerably reduces economic activities [5,25,52]. The profound adverse effects exerted on the ecology of the water body by the presence of the weeds could affect the growth rate, feed efficiency, and physiology of fishes ([31,48,55,72]). Some factors that could contribute to the instability of water characteristics in the presence of the weeds include temperature, dissolved oxygen, pH, carbon dioxide, ammonia, and nitrite [43,53].

The aquaponics potential of water hyacinth to effectively remove nitrate, ammonium, or other nutrients in water body (as a result of fish excretion, high feed input, anthropogenic activities, or other sources) on fish wellbeing should not be discarded. However, a reduction in dissolved oxygen level and or little disturbance in the balance between the plant, filter, and fish can increase the stressor levels, which could result in fish kills, reduced growth, reduced feeding, higher energetics cost, reduction in the response of the immune system to viral and bacterial infections and many other physiological dysfunctions [43].

Various organs of fish have been employed in assessing fish wellbeing or health condition in response to changes in their environment. However, fish liver which has relative uniformity in the morphology of the cells, abundance of metabolizing enzymes activities, and ease of preparation, has been a preferred organ in several biochemical and histological studies. Biochemical indicators, such as enzymes, could be used (as biomarkers) to identify possible environmental contamination before the health of aquatic organisms is seriously affected [73]. Biochemical parameters reveal the underlying physiological conditions of any organs or tissues of an organism [44]. Enzymes which are biological catalysts, have a significant role in the metabolic processes in the fish body, and deficiency or surplus of enzymes indicates various diseases conditions ([22,57,84]; Adeyemi et al., 2015; [44]).

Lactate dehydrogenase (LDH), Glucose-6-phosphate dehydrogenase (G6PDH), and Malate dehydrogenase (MDH) are important marker enzymes in the tricarboxylic cycle (TCA) (Al-Attar, 2004). LDH is generally associated with cellular metabolic activity, which is inhibited or elevated under oxidative stress, especially after exposure to pollutants [20]. LDH has been used as an indicator of hypoxic conditions in organisms and plays an important role in glycolysis [20,66,76,81].

The main role of pentose phosphate pathway is NADPH production, and G6PDH catalyzes the first step of this pathway [76]. NADPHs, which are produced in this pathway, are generally used in the synthesis of fatty acids, steroids, some amino acids, reduced glutathione and DNA (Bonsignore et al., 1966; [14]). Malate dehydrogenase catalyzes the reversible oxidation of malate to oxalacetate requiring NAD+ as a cofactor. It is involved in gluconeogenesis and lipogenesis, and in the malate-aspartate shuttle during aerobic glycolysis [59]. The mitochondrial form (mMDH) acts in the Krebs cycle (Zink and Shaw, 1968).

Biochemical changes such as muscle RNA: DNA ratio and serum 3, 5, 3′-triiodothyronine (T3) hormone level have also been used to provide relatively simple, indirect means for estimating recent growth in fish ([49,70]; Peyghan et al., 2013; Qu et al., 2016; Foley et al., 2016, [63]).

However, with the various aforementioned economic importance of E. crassipes on the infected waterbodies, the impact of this plant on the biology or physiology of fish which is one of the important aquatic organisms, has not been well documented. Therefore, this study intends to culture African catfish (Clarias gariepinus) with E. crassipes to ascertain the plant's effect on the fish growth performance and some liver enzyme activities.

Materials and methods

The study, made up of two phases, was carried out in indoor and outdoor culture systems at the Aquaculture Centre and Fish Laboratory Culture, respectively, in the Department of Zoology, Obafemi Awolowo University, Ile-Ife, Nigeria. The outdoor component of the study was carried out in large concrete tanks (6.0 m × 5 m × 1.5 m). The second phase of the study was carried out indoor in five black circular plastic containers (radius = 23 cm, and height = 74 cm).

Plant material collection

Freshwater hyacinth (E. crassipes) plants were obtained from an abandoned fish pond at Oke Ogbo area, Ife East Local Government, Ile-Ife. The plants were screened, identified, and authenticated at the Herbarium, Department of Botany, Obafemi Awolowo University, Ile-Ife, Nigeria. In all, 369 fresh plants which were introduced per tank (or 12.30 plants/m^2)

had a mean stalk length of 40 +1.02 cm (mean + SE), mean petiole length of 11.5 +0.95 cm (mean + SE) and mean leaf breadth of 6.8 +0.90 cm (mean + SE). The plants were held in a large concrete tank (6.0m × 5.0m × 1.2m) at the Aquaculture Centre of the Department of Zoology, Obafemi Awolowo University, Ile-Ife, and were allowed to grow until fish were introduced.

Experimental design

Experimental fish

Four weeks old fingerlings (2.36 +0.65g) (mean + SE) of African catfish (Clarias gariepinus) obtained from Prime Aquaculture Ltd., Ikorodu, Lagos, and transported to the Aquaculture Centre were held in a concrete holding tank (4.5m × 1.5m × 1.2m), acclimatized for 14 days and then allowed to be grown into juveniles. The fingerlings were screened for parasites by a gross examination to check for the presence of larger parasites such as leeches, copepods, or trematodes. The skin and gills scrapes of 50 fish juveniles within the stock selected at random were screened under a light microscope at x40 and x100 magnification to check for the presence of ciliated protozoans.

The fingerlings were fed ad libitum daily with 1.2 mm Coppens feed (45% crude protein), and about 80% of water was replaced after 2 – 3 days. After four weeks of culture to juvenile stage, 304 fish juveniles with mean weight (w) of 11.00 +1.04g (mean + SE) and mean total length (TL) of 10.43 +0.30 cm (mean + SE) were divided into two groups of 152 samples each and were introduced into two large concrete tanks (6.0 m × 5.0 m × 1.2 m) for the outdoor experiment. A tank infested with E. crassipes plant at a density of 12.30 plants/m2 (the least plant density in the indoor/laboratory experiment), while the other tank, which served as the control, contained no water hyacinth plant. For the indoor/laboratory experiment, 50 fish juveniles (Av. wt. 16.87 + 1.01 g (mean + SE)) each were stocked in duplicate into five circular plastic tanks (labelled CT, T1, T2, T3, and T4) containing water hyacinth plants cultured at densities of 0, 5 10, 20 and 40 plants/tank respectively. The fish were fed coppens feed (45% crude protein) at 5% body weight twice a day [3,86], 6 days a week for 90 days. Thirty (30) fish specimens were randomly selected from each tank every fortnight to determine their body weights and lengths. The water qualities were monitored daily in each experimental tank with dissolved oxygen (Milkawauke D.O. meter), pH (Jenway Model 3020 pH meter), temperature (Mercury in glass thermometer), and conductivity (Jenway Model 4071 Conductivity meter).

Fish growth performance evaluation

Various growth performance indices were determined from the length-weight data as described by Sveier *et al.* [74] and itemized below:

Total feed intake (TFI)

TFI was derived from the formula:

TFI = Total weight of feed fed (g) / Fish population per tank

Daily feed intake (DFI)

DFI in fish was derived from the expression:

DFI (g/fish) = TFI / t where t = duration of experiment in days

TFI = total feed intake

Mean weight gained (MWG)

MWG (g) = W_f - W_i

Where W_f = mean final weight (g); W_i = mean initial weight (g)

Mean daily weight gained (MDWG)

MDWG (g) = M W G / t where MWG = mean daily weight gained; t = the number of days in the feeding period

Percentages body weight gain (PWG)

PWG (%) = (MWG / W_i) × 100 where MWG = mean daily weight gained; W_i = mean initial weight (g)

Relative growth rate (% body weight per day)

This was calculated as described by Pelletier [61] as:

RGR (%) = 100 [(W_f - W_i) / W_i] / t = PWG/t

Where W_f = mean final weight (g); W_i = mean initial weight (g); t = culture period (in days)

The specific growth rate (SGR)

SGR was estimated from the expression.

SGR (%) = 100(log W_f – log W_i) / t

Where W_f = mean final weight (g);W_i = mean initial weight (g) t = time (days) between weighing

Feed conversion ratio (FCR)

FCR was calculated as described by [32] as:

FCR = Weight of feed consumed (g) / Weight gained by fish (g)

Gross diet efficiency (GDE)

[29] was calculated as described by Olaleye [54] by the equation:

GDE = 100 (W_f – W_i) / Ft where F = mean dry weight of feed/fish = TFI t = duration of experiment

W_f = mean final weight (g)

W_i = mean initial weight (g)

The Fulton's condition factor (KF)

Condition factor in fish was determined according to Ricker [67] by the formula:

$K_F = 100W_f / L^3$ where L = mean total length of fish (cm); W_f = mean final weight (g)

Protein utilization in fish were as PI and PER calculated according to Gatling and Wilson (1976);

PI (g/fish) = Daily feed intake (g) × protein amount in the diet

PER = body weight gained (g) / protein intake

Hepatosomatic index (HIS)

The Hepato-somatic index in the fish was calculated according to the method of Keebiyehetty and Gatlin (1997) using following formula:

HSI (%) = 100 (liver weight (g)) / body weight (g)

Percentage (%) survival

The number of surviving fish population in each tank was used to estimate the percentage survival as:

% Survival = N_f / N_i where: N_f = final fish population at the end of the experiment

N_i = initial fish population at the beginning of the experiment

Estimation of biochemical parameters

Sampling and preparation of fish specimens

After 90 days of experiment, fish samples collected from the outdoor and indoor treatments were sacrificed for biochemical analysis. The fish were weighed, sacrificed (by decapitation) and their livers excised within one minute of death. The livers were rinsed in ice-cold 0.25M sucrose solution and quickly put in pre-cooled petri-dishes at 0°C.

Preparation of liver homogenates

The liver was divided into two portions. A portion weighing 1.5g stored in a Thermocool deep-freezer at -20°C was used for the determination of glycogen according to Dubois et al. (1956) and the method of Jermyn [41] as described by Oyedapo and Araba [58]. The other portion was also weighed and homogenized in ice-cold 0.25M sucrose solution using a motor-driven glass-Teflon Potter-Elvejhem (TRI-R STIR-R K43) homogenizer, running at 1000 rev/min, to give a final tissue suspension of 1:10 (w/v). The resulting crude homogenates were put in precooled centrifuge tubes and centrifuged at 5000 rpm for 5 minutes. The supernatant from the homogenized portion was used for the assay of the activities of lactate dehydrogenase according to the methods described by Reitman and Frankel [65], and glucose-6-phosphate dehydrogenase and malate dehydrogenase according to the method of von Worthington [82]. Protein concentration was estimated by the method of Lowry et al. [46] as described by Plummer [62], while DNA isolation and concentration estimation were done as described by George [28] and Schneider [68], respectively.

Statistical analysis

Data obtained were presented as means and standard errors of the mean (SEM). The statistical analyses were performed using MS Excel, Sigma plot® version 11.10 (Systat Inc., Jandel Scientific, Germany), Graphpad Instat 3 (Demo version), SPSS software version 12.0 (SPSS, Richmond, USA), and PASTR programmes for Windows. Comparisons of growth performance and other biochemical parameters across the treatments were made by Welch's corrected t-test and one-way analyses of variance (ANOVA) with a Tukey-Kramer's multiple comparisons post-hoc test at 0.05 probability levels [88].

Results

Analyses of water physico-chemical parameters

The ranges and means of the determined physico-chemical parameters of both the outdoor and indoor experiment is shown in Table 1. In the outdoor experiment, water hyacinth-free treatment had significantly higher ($p < 0.05$) mean temperature, pH, and dissolved oxygen compared to the water hyacinth-infested treatment. However, in the indoor culture media,

Table 1
Range and Mean (+ SE) of some culture water physico-chemical parameters during the experimental period.

Parameters		Treatment Groups						
		Concrete Tanks[1]			Plastic Tanks[2]			
		WH-infested (12 plts/m^2)	WH-free	CT	T_1	T_2	T_3	T_4
	Range	27.0 – 29.0	27.0 – 30.5	24.0 – 30.0	24.0 – 30.0	24.0 – 30.0	24.0 – 30.0	24.0 – 33.0
Temperature (°C)	**X + SE**	27.50 + 0.24^a	28.89 + 0.34^b	31.10 + 0.80^a	27.20 + 0.41^b	27.05 + 0.24^b	28.10 + 0.68^b	30.80 + 0.80^a
Dissolved Oxygen (mg/l)	**Range**	3.2 – 8.0	7.6 – 10.2	4.2 – 7.4	4.2 – 7.4	4.0 – 7.0	4.2 – 7.4	4.2 – 7.4
	X + SE	5.85 + 0.55^a	9.13 + 0.26^b	6.12 + 0.25^a	5.94 + 0.60^a	5.85 + 0.70^a	5.02 + 0.75^a	4.84 + 0.92^a
pH	**Range**	7.26 – 7.99	7.32 – 9.98	6.9 – 8.6	6.9 – 7.9	6.9 – 7.9	6.9 – 7.9	6.9 – 7.9
	X + SE	7.42 + 0.08^a	6.99 + 1.56^a	7.01 + 0.56^a	7.18 + 0.09^a	7.19 + 0.10^a	7.26 + 0.21^a	7.29 + 0.15^a
Conductivity (μS/cm)	**Range**	252.0 – 336.0	153.0 – 292.0	297.0 – 299.0	770.0 – 774.0	863.0 – 867.0	907.0 – 913.0	1449.0 – 1453.0
	X + SE	301.13 + 11.34^a	221.63 +18.19^b	299.0 + 1.16^a	772.0 + 1.16^b	865.0 + 1.16^c	910.0 + 1.16^d	1451.0 + 1.16^e

[1] Row means with the same superscript are not statistically different at $P < 0.05$ (following a Welch corrected t-test).
[2] Means across rows with different superscript differ at $P < 0.05$ (Means tested with Tukey-Kramer multiple range test following one way ANOVA) CT = Water hyacinth -free control tank; T_1 = 12 plants/m^2 (5 plants/tank); T_2 = 26 plants/m^2 (10 plants/tank); T_3, = 52 plants/m^2 (20 plants/tank); T_4 = 104 plants/m^2 (40 plants/tank).

Table 2
Measured growth parameters of cultured water hyacinth in the various culture media during the period of study.

Parameters	Concrete tank	T_1(12 plants/m^2)	T_2(26 plants/m^2)	T_3 (52 plants/m^2)	T_4 (104 plants/m^2)
Visual observation	Plant growth stunted followed by plant mortality. New shoot sprout from the root	Plant flourished with flushed green leaves	Plant flourished with flushed green leaves	Obvious plant stunting preceding subsequent plant mortality	Obvious plant stunting preceding subsequent plant mortality
Initial plant number stocked	369	5	10	20	40
Final plant number harvested	1130	11	17	27	43
Initial Stalk length (cm)	38.0 + 1.02	38.0 + 1.02	38.0 + 1.02	38.0 + 1.02	38.0 + 1.02
Final Stalk length (cm)	15.2 + 0.95	38.5 + 0.89	38.0 + 0.98	29.6 + 1.83	20.1 + 2.05
Initial Petiole length (cm)	11.5 + 0.95	11.5 + 0.95	11.5 + 0.95	11.5 + 0.95	11.5 + 0.95
Final Petiole length (cm)	5.7 + 0.92	11.5 + 0.60	11.4 + 0.51	8.5 + 1.05	7.9 + 0.86
Initial Leaf width (cm)	6.8 + 0.90	6.8 + 0.90	6.8 + 0.90	6.8 + 0.90	6.8 + 0.90
Final Leaf width (cm)	3.3 + 0.64	6.9 + 0.87	6.7 + 0.73	5.2 + 1.14	4.1 + 0.79

water hyacinth-free tank and tank T4 (with 40 plants/tank) had significantly higher ($p<0.05$) temperature values. Irrespective of the culture medium, the presence of water hyacinth plants increased the pH ($p>0.05$) and conductivity ($p<0.05$) of water in the culture media with increasing number of water hyacinth plants. The dissolved oxygen level was also observed to decrease ($p>0.05$) with increasing number of test plants (Table 1).

Growth of E. crassipes under culture

Although there was a significant increase in the water hyacinth plant population cultured in the outdoor experiment, it was observed that the plants were stunted with a final mean stalk length compared to the initial length. The initial petiole length was reduced by half at the end of the culture period. The initial leaf width was also reduced by the end of the experiment (Table 2). The culture medium was observed to become black with muddy odour.

In the indoor experiment, the plant population and growth rate increased rapidly in the culture tanks with between 5 and 10 plants/tank. The plants were found growing with leaves and leaf stalks increasing in size. However, in culture tanks with 20 plants/tank (T3) and 40 plants/tank (T4), the plants dried up before new shoots sprouted from their root base. It was also observed that in culture tank with 40 plants/tank (T4), the new shoots that grew from the withered plants were found to be stunted (Table 2).

Growth performance and nutrient utilization in fish

Growth performance

Fish juveniles grown in water hyacinth-infested tanks (both outdoor and indoor media) had significantly ($p<0.05$) better growth rates than those grown in water hyacinth-free tanks (Table 3). The study also showed that fish in the medium containing 104 plants/m^2 (T4) had significantly ($p<0.05$) better growth performance than fish in other treatments and control (Table 3). Lower mortality was recorded in cultured tanks with or without water hyacinth plants in the outdoor experiment, while the indoor experiment had a survival rate of 21.43% in the control medium with no mortality in water hyacinth-infested media (Table 3).

In the presence or absence of water hyacinth plants, the mean weights of the fish juveniles cultured in both outdoor and indoor treatments increase with the duration of the culture experiment (Figures 1 and 2).

Hepatosomatic index

Significantly higher ($p<0.05$) liver weight and the hepatosomatic index which correlate positively ($r^2 = 0.90$ and 0.57 respectively) with percentage body weight gained per day in the fish juveniles was observed in outdoor treatment with water hyacinth-infested medium (Table 3). However, the liver weights and the hepatosomatic index of fish juveniles grown in indoor culture media with and without water hyacinth plant were not significantly different ($p>0.05$) (Table 3).

Nutrient utilization indices in fish juveniles

In the outdoor experiment, gross diet efficiency (GDE) in *C. gariepinus* juveniles was the only feed utilization parameters that differs significantly ($p<0.05$) between the two treatments (Table 4). However, in the indoor treatments, among the hyacinth infested media, *C. gariepinus* juveniles in the treatment with highest density of water hyacinth showed significant ($p<0.05$) feed utilization indices which were not significant ($p>0.05$) compared to the control (Table 4).

Biochemical indices

Liver protein, DNA and glycogen concentration

Significant lower levels ($F = 4.96$, $df = 1$, $Sig. = 0.00018$, ($P < 0.05$) of protein, DNA and glycogen activities were observed in the liver tissues of the experimental fish in the WH infested culture compared to WH free outdoor experiment, however,

Table 3
Growth performance (mean + SE) of the African catfish reared in different culture tanks with different densities of water hyacinth plants.

Parameters	Treatment Groups						
	Concrete Tanks[1]			Plastic Tanks[2]			
	WH-free	WH-infested (12 plants/m^2)	Water Hyacinth-free	T_1(13 plants/m^2)	T_2(26 plants/m^2)	T_3(54 plants/m^2)	T_4(104 plants/m^2)
Initial mean weight (g)	11.00 + 1.04^a	11.00 + 1.04^a	16.87 + 1.01^a	16.87 + 1.01^a	16.87 + 1.01^a	16.87 + 1.01^a	16.87 + 1.01^a
Final mean weight (g)	185.46 + 8.89^b	237.64 + 30.30^a	36.84 + 0.41^{ab}	32.28 + 1.46^a	44.32 + 1.86^c	37.27 + 2.16^{ab}	44.88 + 3.45^c
Mean weight gain (g)	174.46 + 5.38^b	230.64 + 6.81^a	19.97 + 4.08^{ab}	15.41 + 1.00^a	27.45 + 1.96^c	20.40 + 1.76^{ab}	28.01 + 1.67^c
PBWG (%)	57.02 + 21.38^a	59.73 + 16.96^a	118.38 + 26.59^a	91.35 + 4.60^b	162.72 + 5.51^c	120.93 + 10.99^a	166.03 + 4.72^c
RGR (%d^{-1})	17.24 + 1.53^b	22.40 + 1.16^a	3.95 + 0.89^a	3.05 + 0.15^a	5.42 + 0.18^b	4.31 + 0.37^c	5.53 + 0.16^b
MDWG (g/d)	1.94 + 0.37^b	2.92 + 0.94^a	0.67 + 0.05^a	0.51 + 0.01^a	0.92 + 0.03^b	0.68 + 0.04^a	0.93 + 0.04^b
SGR (%d^{-1})	1.25 + 0.38^b	1.48 + 0.26^a	1.13 + 0.13^a	0.94 + 0.02^b	1.40 + 0.02^a	1.15 + 0.05^a	1.42 + 0.03^a
Condition factor (K)	1.85 + 0.89^a	1.98 + 0.92^a	1.60 + 0.52^a	1.61 + 0.09^a	1.49 + 0.11^a	1.54 + 0.24^a	1.51 + 1.01^a
Survival rate (%)	96.71 + 0.58^a	98.68 + 0.22^a	21.43^a	100^b	100^b	100^b	100^b
Liver mass (g)	2.64 + 0.38^b	1.65 + 0.23^a	0.46 + 0.03^{ab}	0.35 + 0.03^{ac}	0.52 + 0.06^{bd}	0.42 + 0.04^{ad}	0.83 + 0.03^e
Hepatosomatic Index	1.54 + 0.10^b	1.00 + 0.07^a	1.27 + 0.08^a	1.43 + 0.09^a	1.18 + 0.09^a	1.23 + 0.05^a	1.23 + 0.01^a

[1] Row means with the same superscript are not statistically different at $P < 0.05$ (following a Welch corrected t-test).
[2] Means across rows with different superscript differ at $P < 0.05$ (Means tested with Tukey-Kramer multiple range test following one way ANOVA) PWG = percentage body weight gain; RGR = relative growth rate; MDWG = mean daily weight gain; SGR = specific growth rate.

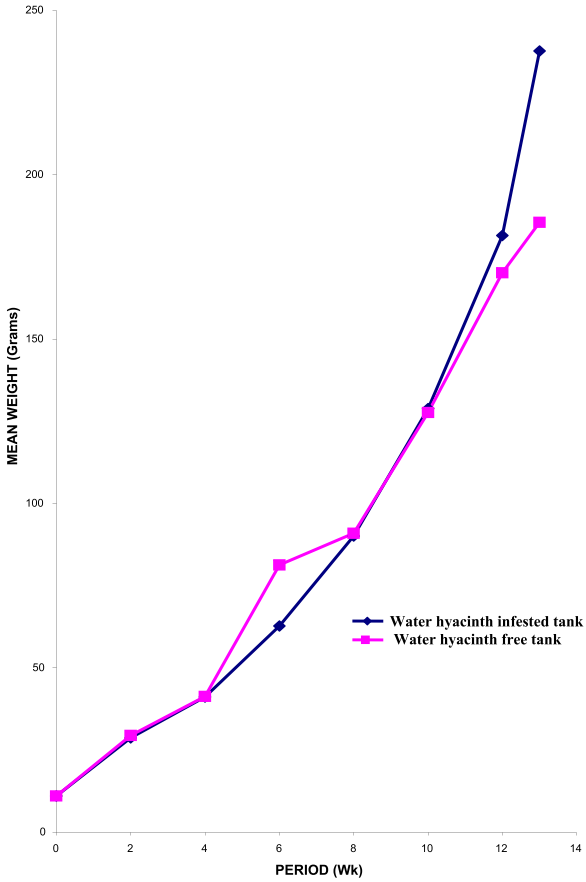

Fig. 1. Mean weights of C. gariepinus juveniles cultured in concrete media during the fortnightly weighing

Table 4
Nutrient Utilization in C. gariepinus juveniles reared in different culture tanks containing different water hyacinth plants densities.

Parameters	Treatment Groups						
	Concrete Tanks[1]			Plastic Tanks[2]			
	WH-free	WH-infested (12 plts/m^2)	Water Hyacinth-free	T_1(13 plants/m^2)	T_2(26 plants/m^2)	T_3(54 plants/m^2)	T_4(104 plants/m^2)
Daily Feed Intake (g/day)	0.29^a	0.29^a	1.02^a	0.27^b	0.50^b	0.33^b	1.12^a
Gross Diet Efficiency (g/day)	9.18^a	7.06^b	3.26^a	6.47^b	6.27^b	6.88^b	3.01^a
Feed Conversion Ratio	$0.20 + 0.06^a$	$0.15 + 0.02^a$	$0.92 + 0.14^a$	$0.55 + 0.05^b$	$0.55 + 0.03^b$	$0.53 + 0.05^b$	$1.16 + 0.08^a$
Protein Intake (g/day)	11.96^a	11.96^a	45.90^a	12.15^b	22.50^c	14.85^b	50.40^a
Protein Efficiency Ratio	$2.76 + 0.57^a$	$2.07 + 0.45^a$	$0.52 + 0.09^a$	$1.27 + 0.08^b$	$1.24 + 0.06^b$	$1.38 + 0.12^b$	$0.60 + 0.03^a$

[1] Means across rows with different superscript differ at $P < 0.05$ (following a Welch corrected t-test).
[2] Means across rows with different superscript differ at $P < 0.05$ (Means tested with Tukey-Kramer multiple range test following one way ANOVA).

juvenile fish cultured in indoor, had significantly higher levels ($F = 2.93$, $df = 4$, Sig. $= 0.00062$ ($P < 0.05$) of activities of hepatic protein, DNA and glycogen in water hyacinth-infested compared to the control experiment (Table 5). The result of the study also showed that the glycogen level in this treatment increased with increasing water-hyacinth densities (Table 5).

Hepatic marker enzymes (G6PDH, MDH and LDH) activities

The activities of the assayed hepatic marker enzymes in C. gariepinus juveniles reared in different water hyacinth-infested media, as shown in Table 5, revealed that G6PDH, LDH, and MDH activities in the liver of the fish juveniles in the outdoor experiment were not significantly different ($p>0.05$). However, in the indoor experiment, the level of G6PDH activity in the fish liver decreased as the density of the water hyacinth plant increased and was significant ($p<0.05$) in the medium containing a plant density of 104 plants/m^2. The activity of MDH in the liver of juveniles C. gariepinus revealed that the fish juveniles in T2 (26 plts/m^2) treatment had significantly higher ($P<0.05$) MDH activity compared with the control. The LDH

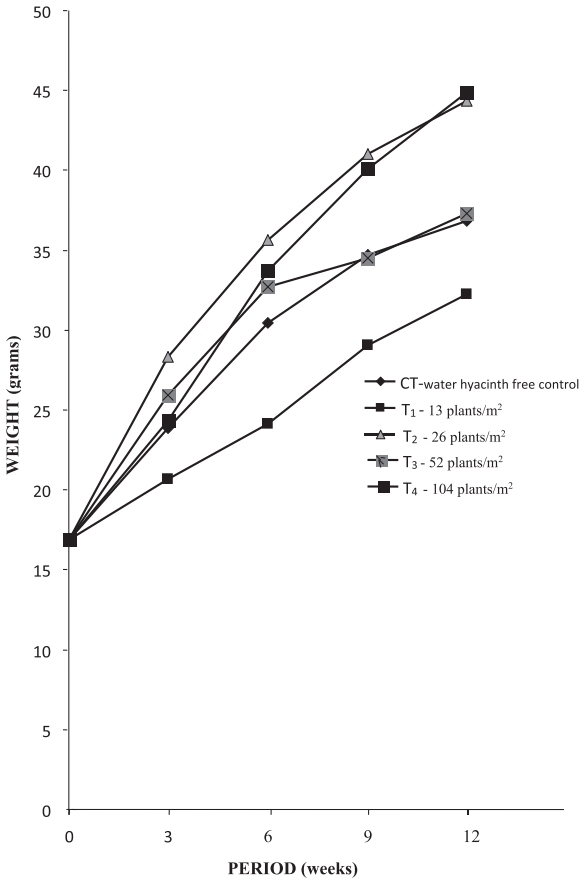

Fig. 2. Mean weights of *C. gariepinus* juveniles cultured in media with different water hyacinth plant densities during the period of study

Table 5
Hepatic biochemical activities in C. gariepinus juveniles in different culture systems.

Hepatic Biochemical Indices	Treatment Groups						
	Concrete Tanks[1]			Plastic Tanks[2]			
	WH-free	WH-infested (12 plts/m^2)	Water Hyacinth-free	T_1 (13 plts/m^2)	T_2(26 plts/m^2)	T_3(54 plts/m^2)	T_4(104 plts/m^2)
Protein (mg/g)	125.15 + 19.46^a	91.90 + 4.24^b	50.35 + 3.54^a	45.17 + 2.59^a	45.85 + 2.50^a	42.20 + 2.26^a	57.48 + 2.63^b
DNA (μg/g)	1.25 + 0.29^a	1.97 + 0.18^b	0.37 + 0.05^a	0.35 + 0.06^a	0.34 + 0.14^a	0.55 + 0.05^a	0.94 + 0.17^b
Glycogen (mg/g)	9.11 + 1.28^a	24.59 + 3.37^b	8.65 + 0.36^a	10.46 + 0.30^b	10.80 + 0.22^b	15.60 + 0.22^c	19.45 + 0.34^d
G6PDH (mU/ml)	0.68 + 0.07^a	0.70 + 0.10^a	1.52 + 0.14^a	1.47 + 0.13^a	1.39 + 0.07^a	1.40 + 0.16^a	0.84 + 0.18^b
MDH (U/mg)	3.02 + 0.26^a	3.17 + 0.32^a	3.32 + 0.17^a	3.69 + 0.11^{ab}	3.99 + 0.13^b	3.52 + 0.16^{ab}	3.73 + 0.08^{ab}
LDH (*U/I)	9.00 + 0.92^a	6.99 + 1.56^a	8.38 + 0.97^a	10.29 + 0.39^b	10.35 + 0.53^b	11.22 + 0.97^b	12.03 + 0.96^b

DNA - deoxyribonucleic acid; G6PDH = glucose-6-phosphate dehydrogenase; MDH = malate dehydrogenase; LDH = lactate dehydrogenase
WH = water hyacinth
* 1 unit of enzyme activity (U) = amount of enzyme required to convert 1.0 μ mol substrate to product in 1 minute at 25^O C.#plts/m^2 – plants/.m^2**Values across with a different common superscript differ at $P < 0.05$ (Means tested with Welch corrected t-test).

activities between the various water hyacinth-infested media were significantly different ($p<0.05$) compared to the control (Table 5).

Discussion

Physico-chemical parameter of water quality

The monitored physico-chemical parameters of water were observed to be within the range recommended (Temperature: 26 - 32°C; DO: 3 – 10 mg/L; pH: 5.5 – 10, conductivity: 30 – 5,000 μS/cm) for culturing the giant African catfish, *C. gariepinus* [10,13,37,75]. The variations recorded in temperature, dissolved oxygen content, and pH may be due to the

period of experiment. In tropical systems, marked variations in temperature and rainfall between seasons have been shown to influence the physico-chemical characteristics of water-bodies [37,45]. Irrespective of the presence or absence of water hyacinth plant, the water temperature in the culture media declined progressively until the end of the experiment, a fact probably attributable to the prevalent climatic condition during the period of study rather than on the shady effect of the water hyacinth plants on the culture media.

The pH of water in the culture media was in the alkaline range (6.90 – 9.98). With the duration of culture period, however, lower pH values in the culture media infested with water-hyacinth confirmed the findings of Dar *et al.* [19] and Ugya and Imam [78], about the potentials of water hyacinth plant to alter the acidity or alkalinity of the water to neutral level. However, the reduction in pH may be due to absorption of nutrients or by simultaneous release of H+ ions with the uptake of metal ions ([47]; Saeed and Al-nagaawy, 2013; [79]).

The lowering of the DO in water hyacinth infested tanks could be attributable to increased bacteria respiration of decomposing of dead plants (Saeed and Al-nagaawy, 2013). Scott *et al.* (1979) and Abbasi [1] observed similar decline in DO which they described as the most significant ecological effect during chemical control of water hyacinth. Troutman *et al.* [77] however observed that the amount of DO depleted from water will depend on the density of decaying plants, water condition and the amount of plant decay occurring within the water itself. This observation of the authors was corroborated by the results obtained in the indoor culture media where increase in water hyacinth plant density brought about a decrease in the DO levels. Reduction of euphotic zone which affected algal photosynthetic activity ([80]; Mayor, 2012), attenuated activities of the plant roots (Saeed and Al-nagaawy, 2013) coupled with the corresponding respiratory activities of the roots (Akinyemiju, 1993; Gupta *et al.,* 2012) were probably other factors responsible for the depressed DO condition recorded in the hyacinth infested media.

Conductivity of water was the only parameter which increased significantly irrespective of the culture media. The recorded increase in conductivity could also be said to be plant-density dependent in the plastic tanks as it increases with the density of plant in the culture tanks. Conductivity has a direct correlation with total dissolved solids, salinity (total salt content), mineralization and nutrient status of an aquatic ecosystem (Uka and Chukwuka, 2007; [4]). Water hyacinth and other organic matter can sink, decompose and in the process result in carbonate and bicarbonate ion resulting in an increase in electrical conductivity (Uka and Chukwuka, 2007). These could probably cause the observed increase in water conductivity in the water hyacinth infested media.

Growth performance and nutrient utilization indices of C. gariepinus

All the monitored indices showed that growth performance was better in water hyacinth-infested treatments. Since the same diet was used to feed the fish, it could be assumed that water hyacinth is nutritional and beneficial to the fish in the tank containing the aquatic weed. Besides, water hyacinths habour some insects that might be beneficial to fish as food [19]. The observation was the same in the indoor experiment where water hyacinth at different densities was integrated with C. gariepinus culture. This was probably because, at these densities, water hyacinth purifies the culture system by mopping up some undesirable nutrients in the culture system and thus improves the fish growth performance when compared to the fish grown in media with a higher density of water hyacinth and water hyacinth free control. Also, it could probably be due to the water hyacinth plants, with their characteristic behaviours, shading the aquatic environment by limiting the amount of insolation from the sun and thus making the environment look like the early hours of dawn and late hours of dusk. The fish being crepuscular (most active at dawn and dusk), utilized this darkness opportunity provided by the water hyacinth in the infested media to feed almost all the time (Britz and Pienaar, 1992; [34]). Ndimele et al. [51] also submitted that fish and fisheries generally benefit from availability of moderate levels of plant cover; thus, absence of aquatic vegetation can be as harmful as excess.

Hepatic DNA, glycogen, protein concentrations and some liver enzymes activities in C. gariepinus *juveniles*

Glycogen is the readily mobilized storage form of glucose and the blood glucose level is controlled primarily by the action of the liver. In the liver, glycogen synthesis and degradation are regulated to maintain blood-glucose levels to meet the requirement of the fish as a whole [23,69]. The liver glycogen levels have been shown to decrease in fish stocked at high densities in ponds [76] and due to intensive exercise caused by stress related to migrations (Izvenkova and Izvenkov, 2013). The concentrations of glycogen which increased significantly ($p<0.05$) in the water hyacinth infested medium to about 63% level over those of the fish in the control medium in both indoor and outdoor experiment could be attributed to the restriction of movement of the fish in their enclosure due to the plant densities and also the fish acclimatization to the environment and not due to stocking density stress [7].

The quantitative determination of the total protein in the liver of the fish reflected the liver capacity for protein synthesis and denoted the osmolarity of the blood [71]. The total protein concentration recorded in the liver of the fish was probably influenced by the nutritional and health status of the fish [39,50]. Proteins function as an essential element in contractile and mobile systems. The liver protein concentration in *C. gariepinus* juveniles cultured in outdoor experiment in water hyacinth-infested tanks showed a 26.57 % reduction of level compared to liver protein concentration in water hyacinth-free tank. The reduction in liver protein obtained however, did not correlate with the observed significant ($p<0.05$) increase in both liver mass and the hepatosomatic indices. The decrease in the total liver protein was found to be similar to those

of Yang and Somero, [87] who observed a decrease in total protein in the white muscle of both shallow living spotted scorpionfish (*Scorpaena guttata*) and deep-living short-pine thorny head (*Sebastolobus alascanus*). However, the increase in the total protein in the fish cultured indoor in the treatment with the highest plant density could probably be as a result of the death and decomposition of water hyacinth in the highest plant medium which possibly acted as a source of stress to the fish. Relative higher level of hepatic total protein in fish has been reported to be due to higher demands for repairing damage tissues or to boost the immunity response [40]. Generally, the observed high levels of protein in both outdoor and indoor tanks agreed to a report of Houlihan *et al.* [35], Ram *et al.* (2003) and Fafioye *et al.* [24] that the liver is an organ of high protein synthesis in well-nourished fish.

During the study, the Liver DNA reduced by 1.36% in the fish reared in water hyacinth-infested media to those in water hyacinth-free control media. Nucleic acids play a major role in growth and development [8]. DNA:RNA ratio is an eco-physiological index that gives a measure of the synthetic capacity of the cell and usually correlates with nutritional status [15,49]. This index is based on the assumption that the amount of DNA, the primary carrier of genetic information and also as chromosomal nucleoprotein in higher animals, is stable under changing environmental situations within the somatic cells of a species [33], whereas the amount of RNA, directly involved in protein synthesis, is known to vary with age, life-stage, organism size, disease state and changing environmental conditions [16,27]. The DNA, contrary to the assumption, decreased in the water hyacinth-infested media against the water hyacinth-free controls. However, Meyer *et al.* [49] related a decreasing trend in DNA concentration in larva coregonid fish to a switch from a higher proportion of hyperplasia in small fish to a higher proportion of hypertrophy in larger fish during development while Ching *et al.* [18] also attributed the decline in DNA concentration to development. It can therefore be assumed in this experiment that the decline in DNA concentrations in the water hyacinth infested media was due to the environmental impact of the water hyacinth that likely made the fish differ in their developmental stages.

G6PDH is an enzyme that is involved in catalyzing the first reaction of pentose phosphate pathways that leads to the production of NADPH which is used in a variety of biosynthetic pathways like the synthesis of fatty acids, steroids and other macromolecules that are essential for animal growth ([36]; Mukherjee and Ahmad, 2016). The increase in liver G6PDH activity of *C. gariepinus* in the outdoor water hyacinth infested medium indicated that the fish grew better and faster than those in the water hyacinth-free medium as indicated in their growth performance and nutrient utilization indices. However, the observed reductions in activities of liver G6PDH in the indoor water hyacinth infested media when compared to the water hyacinth-free control medium could be attributed to metabolic, hormonal and nutritional states of animals [2,11,12,21].

A similar pattern in the activities of MDH and LDH in the components of this study is that at a comparable plant density (13 plants/m^2 for the plastic tanks and 12 plants/m^2 for the concrete tanks), the increase in MDH and decline in LDH activities were not significant and could be attributed to the enzymes making use of anaerobic pathways to achieve their goals ([64], Martinez *et al.,* 2011; Pradeepkiran and Bhaskar 2016). However, at higher plant density, MDH activity increased significantly, whereas LDH activity showed no significant decrease. The decrease in the activities of LDH probably indicate a good state of health in the fish, since any sharp increase in activities of the enzyme could indicate tissue injury or organ dysfunction (Martinez *et al.,* 2011; Choudhurya *et al.,* 2017).

Conclusion

The evaluation of growth performance indices of the *Clarias gariepinus* juveniles established that the fish raised in water hyacinth infested media at moderate plant density performed better than those grown in the weed-free media. The result of the studies on the hepatic protein, DNA and glycogen levels and some glycolytic metabolic enzymes in the liver of fish also revealed that water hyacinth at higher density with limited space could affect the physiology of the fish.

Declaration of Competing Interest

The authors declare no conflict of interest.

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