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Food and Feeding Habits of *Parailia Pellucida* (Boulenger, 1901) (Schilbeidae) in the Freshwater Reaches of the Nun River of the Niger Delta, Nigeria

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Abstract The Food and Feeding habit of the glass schilbid *Parailia pellucida* was investigated with 654 stomachs using the numerical, frequency of occurrence and points methods such that 368 (56%) were empty while 286 (44%) contained food items. In the numerical method the number of different food items were counted and recorded. For the frequency of occurrence method, all stomachs containing particular food items were recorded while in the points method; points or marks were allocated to the food items based on their relative volumes in the stomachs. The degree of fullness was estimated by an arbitrary 80 points scale allotted as follows: empty stomach (o); trace (10); quarter-full (20); half-full (40); three-quarter full (60) and fully distended stomachs, 80 points. Observed food items were cladocera, copepoda, insecta, nematoda, ostracoda, rotifera, algae, fish remains/eggs, sand and unidentified organisms. By the numerical composition, cladocerans were predominant, (63.8%) algae (24.96%), rotifera (4.67%), copepoda (4.5%) and insecta (1.74%). By the frequency of occurrence method, cladocera was dominant in (39.8%), algae (21.8%), while insects, copepods and rotifers constituted 13.6%, 10.4% and 9.5% respectively. In the points method, *Cladocerans* scored the highest (81.6%), copepods (6.3%) while insects, rotifers and algae scored 4.22%, 3.86% and 3.18% respectively. The primary food items of *P. pellucida* with the index of food significance (IFS) ≥ 3% include cladocerans such as *diaphanosoma* (35.4%), *bosmina* (34.95%), *pseudosida* spp (17.71%) and copepod *cyclop* spp (5%). The major secondary food items (IFS ≥ 0.1 to < 3%) were *brachionus* (rotifer) (2.35%), *monoraphidium* (algae) (1.91%) and *bosminopsis* (cladoceran) of 1.56%. The diet breadth of *P. pellucida* based on the Shannon - Wiener function (H) computed from the IFS data H ($_{IFS}$) was -1.42. Food richness (i.e. food items with IFS ≥ 0.1%) observed, consisted of 12 food items with cladocera as dominant ranging from 17.17–35.4% and ceratopogon (Dipteran insect) of 0.1%, as the least. Feeding was most intense in April, (19%) closely followed by February (18.9%) and December (18.4%) while the least intensity of 0.2% was obtained in July. *P.pellucida* of all sizes fed most intensively on cladocerans with a steady increase in mean intensity from the smallest (4.1-5.0cm) size group (92.11) to a maximum (418.62) in the 8.1 – 9.0cm

SL size class and declined thereafter. A similar trend in feeding intensity also occurred with algae ranging from 38.87 in the 4.1 – 5.0cm SL class to 182.24 in the 8.1 – 9.0 cm SL class.
Keywords *Incidental, Occurrence, Primary, Secondary, Stomach*

1. Introduction

Food is one of the major factors that influence the dynamics of fish population in the aquatic environment. Information on the food habits of fishes such as the predator – prey relationships, is useful in order to assess the role fishes play in the ecosystem. Food habit studies in recent times include non-lethal methods of extracting the gut contents [1, 2] and fatty acid analysis [3] and are necessary for fish stock assessment and ecosystem modeling [4], habitat preference [5], predation [6] and evolution [7]. Such information is of great value and a key element in the protection of species and ecosystems [8]. Information on the dietary composition of fishes is vital for multi- species virtual population analysis [9, 10]. According to Braga et al. (2012), feeding ecology of a species is thoroughly linked to its population dynamics and contributes to the understanding of such subjects as resource partitioning, habitat preferences, prey selection, predation, evolution, competition and energy transfer between and within ecosystems. FAO (2010) [11] observed fish as an important food resource for human consumption of animal protein.

This study is aimed at providing comparative information on the diet of *P. pellucida* in the freshwater river system that may be useful for stock- and ecosystem- level analysis.

2. Materials and Methods

This study was carried out at the lower Nun River around Anyama-Ijaw Community Lat. 4^{o} 51' N and 4^{o} 54' N; Long 6^{o} 11' E, and 6^{o} 13' E, in Bayelsa State in the Niger Delta, Nigeria covering an area of about 2180 Km^{2}. The concave bank in the study area is moderately steep sloping with loamy bottom while the convex bank is relatively shallow and sandy. The average depth of water at the sampling locations, that is, the convex, central and concave sections of the river was 2.7m, 5.4m, and 7.8m respectively in the dry season and 6m, 8.75m and 11.20m, respectively during the rainy season.

The tidal influence is very mild during the dry season. However, a slightly reversed flow occurs during the rising tide at the peak of the dry season. During the flood period, there is a swift one-directional current in the study area. The flood sets in from about the end of May and recedes between the 4th week of October and 1st week of November. Flood heights range between 2.7m and 4.0m with a mean of 3.35m.

The stomachs of the preserved *P.pellucida* specimens were cut open after dissecting them. The stomach content was then examined with the stereo microscope to determine the food of the fish and the food analyzed by the numerical, frequency of occurrence and points methods [12]. In the numerical method the number of different food items were counted and recorded. In the frequency of occurrence method, all stomachs containing particular food items were recorded. In the points method, points or marks were allocated to the food items based on their relative volumes in the stomachs [13]. The degree of fullness was estimated by an arbitrary 80 points scale allotted as follows: empty stomach (0); trace (10); quarter-full (20); half-full (40); three-quarter full (60) and fully distended stomachs, 80 points.

2.1. General Food Habits

The numbers, occurrence and points scored by the various food items were pooled for all the stomachs examined during the sampling period and the relative proportions were plotted to indicate the significance of each group of food organisms in the diet of *P. pellucida.* The importance of the various food items was determined with the Index of Food Significance (IFS) following a modified Ezenwaji and Offiah (2003) [14] method as follows:

$$IFS = \frac{\% \text{ Occurrence (FO)} \times \% \text{ Points (PT)}}{\sum (\% \text{ FO} \times \% \text{ PT})} \times 100$$

Food with IFS $\geq$ 3% are regarded as primary,
$\geq$ 0.1 to < 3 % as secondary, whereas
$\leq$ 0.1% are considered as incidental
The IFS data was further used to compute diet breadth based on Shannon-Weiner function (H) as follows:

$$H_{(IFS)} = - \sum (ni/N) \text{Log}_e (ni/N)$$

Where: ni = IFS of each food item
N = IFS of all food items
Food richness was also determined as the number of food items in the diet with IFS > 0.1%.

2.2. Seasonal Food Composition

The numbers, frequency of occurrence and points scored by the different food items were assessed monthly and the trends in the quality and quantity of the food groups were then determined by plots of the various percentages of the food items.

2.3. Food of Different Size Groups

The variation of food with size was studied using only the numerical and frequency of occurrence methods in analyzing the stomach contents of classified groupings of *Parailia pellucda* sampled.

2.4. Feeding Intensity

This was assessed as the mean number of food items in the stomach and was determined for seasons and size classes.

3. Results

3.1. General Food Composition

Out of 654 stomachs examined, 368 (56%) were empty while 286 (44%) contained food items. The food items observed in the stomachs were cladocera, copepoda, insecta, nematoda, ostracoda, rotifera, algae, fish remains/eggs, sand and unidentified organisms. Cladocera consisted of bosminidae (*Bosmina longirostris*, *B. coregoni*, *B fatalis* and *Bosminopsis deitersi* grouped as *Bosmina* spp. and *Bosminopsis* spp) respectively. Sididae consisted of diaphanosoma (*D. excisum and D. sarsi*) and Pseudosida (*P. bidenta*). Copepoda consisted of only *Cyclop* spp. Insecta was made up of mostly diptera (*Chironomus, Cceratopogonid* and *Simulium)*. Others include of *Coleoptera, Hemiptera* and *Ephemeroptera sp*. Five Rotifera and ten Algae spp were also found in the stomachs made up of Green-algae, Diatoms, Blue-green algae and Yellow-algae. The Rotifers

were *Brachionus, Keratella, Notholca, Trichocerca* and *Lecane* while Algae comprised *Monoraphidium, Closterium, Amphipleura, Frustulia, Nitzschia, Cyclotella, Microcystis, Aphanothece, Coelospharium* and *Goniochloris* species. Figures 1A, B & C show the percentage composition by taxa of the food items by the various analytical methods.

3.2. Numerical Analysis

By the numerical composition, cladocerans (63.8%) were predominant followed by Algae spp. (24.96%) Rotifera (4.67%) and Copepoda constituted 4.5% while Insecta made up 1.74%. Other items such as fish remains, fish eggs, sand particles and unidentifiable organisms / materials, constituted 0.33% of the food items (Figure 1A).

3.3. Frequency of Occurrence

By this method (Figure 1B) Cladocera was dominant (39.8%). Algae ranked 2nd (21.8%) while insects, copepods and rotifers constituted 13.6%, 10.4% and 9.5% respectively. Other minor food items and unidentified organisms formed 4.9%.

3.4. Points Method

The percentage points scored by the various food items are as shown in Figure 1C. *Cladocerans* (81.6%), Copepods (6.3%) while insects, rotifers and algae scored 4.22%, 3.86% and 3.18% respectively. Trace Organisms constituted 0.85%.

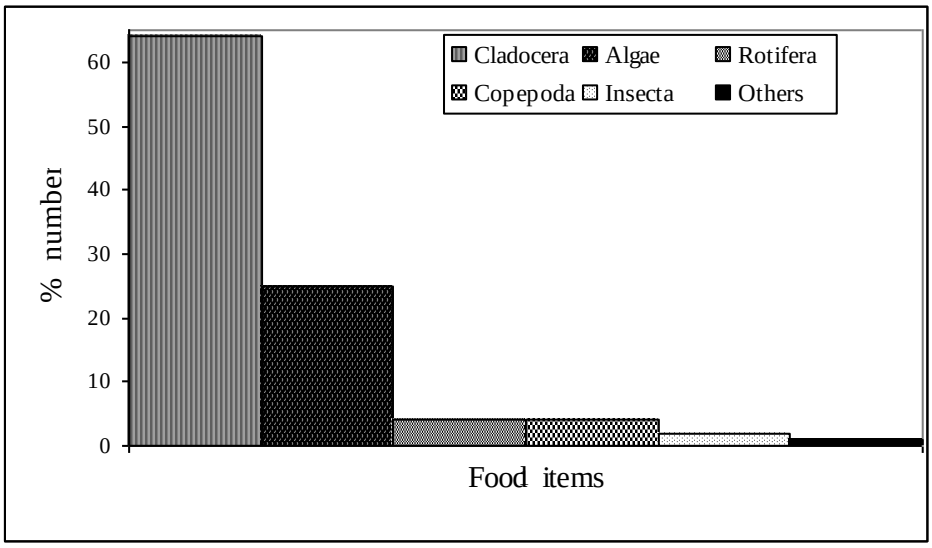

Figure 1A: *Numerical composition by different taxa of food items in the Stomachs of P. pellucida*

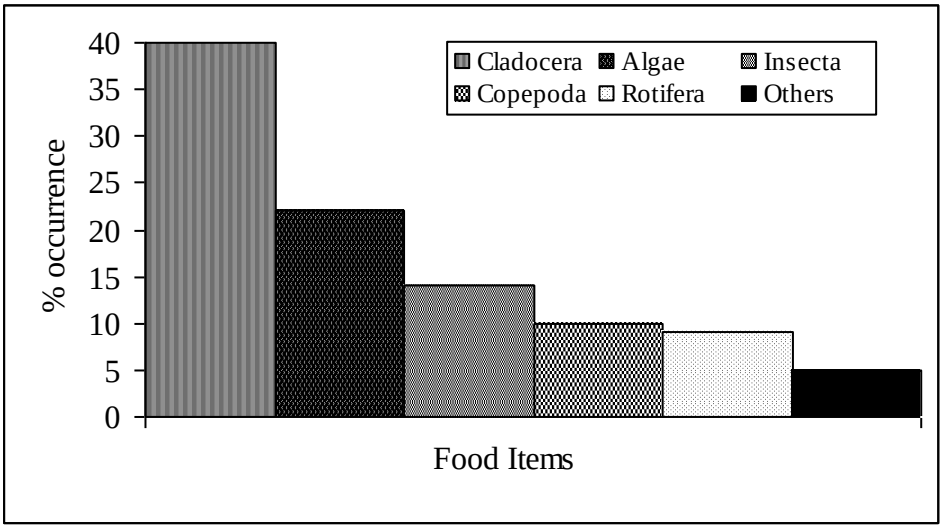

Figure 1B: *Percentage occurrence by different taxa of food items in the stomachs of P. pellucida*

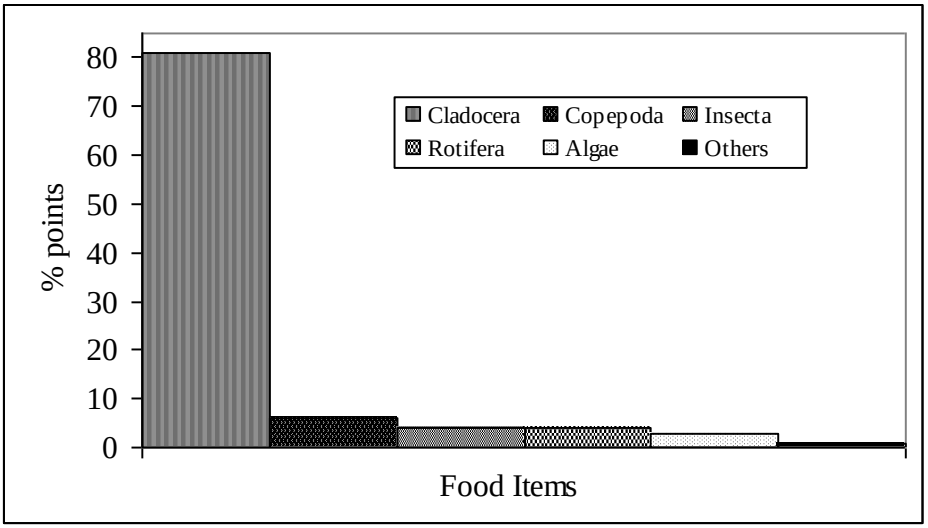

Figure 1C: *Percentage points scored by different taxa of food items in the stomachs of P. pellucida*

3.5. Classification of Food Items

The results of the index of food significance (IFS) and the diet breadth of *Parailia pellucida* as determined by the percentage frequency of occurrence (%FO) and percentage points (%PT) methods are shown in Table 1 while Figure 2 shows the relative abundance of the major food items by the 3 analytical methods.

The primary food items of *P. pellucida* with IFS ≥ 3% include cladocerans such as *Diaphanosoma* (35.4%), *Bosmina* (34.95%), *Pseudosida* spp (17.71%) and copepod *Cyclop* spp (5%). Secondary food items (IFS ≥ 0.1 to < 3%) were *Brachionus* (rotifer) (2.35%), *Monoraphidium* (algae) (1.91%) and *Bosminopsis* (Cladoceran) of 1.56%. These may be regarded as major secondary food items. Other secondary food items include unidentified Dipteran insecta (0.2%), *Chironomus* larva (0.18%) and unidentified copepods (0.13) *Ceratopogonid* larva and pupa (0.11 and 0.10%) respectively. Food with IFS < 0.1% consists, unidentified insecta (0.09%), Coleoptera (0.04%) unidentified zooplankton (0.03%), *Frustulia* (0.03%) *Ceratopogonid* nymph (0.02%), *Keratella* (0.02%) and Amphipleura

(0.02%). Others, included Hemiptera, *Microcystis,* fish remains/eggs, and unidentified organisms which had 0.01% each. The diet breadth of *P. pellucida* based on the Shannon - Wiener function (H) computed from the IFS data H ($_{IFS}$) was -1.42. Food richness (i.e. food items with IFS ≥ 0.1%) observed, consisted of 12 food items with Cladocera as dominant ranging from 17.17–35.4% and Ceratopogon (Dipteran insect) of 0.1%, as the least.

Table 1: *Trophic spectrum of the diet of Parailia pellucida in the lower Nun River Niger Delta, Nigeria*

Food Species/Groups	%FO	%PT	IFS (%)	H(IFS)
CLADOCERA				
Diaphanosoma	10.7	30.7	35.4	- 0.37
Bosmina	10.0	31.8	34.95	- 0.37
Pseudosida	9.9	16.6	17.71	- 0.31
Bosminopsis	6.6	2.2	1.56	- 0.06
Unidentified	2.6	0.3	0.08	0.005
	(39.8)	**(81.6)**		
COPEPODA				
Cyclops	8.0	5.8	5.0	- 0.15
Unidentified	2.4	0.5	0.13	- 0.009
Zooplankton unidentified	1.2	0.2		
	10.4	**6.3**	0.03	- 0.002
INSECTA: (Diptera)				
Chironomus larva	2.8	0.6	0.18	- 0.011
Chironomus pupa	0.2	0.05	0.001	
Ceratopogonid larva	2.0	0.5	0.11	- 0.007
Ceratopogonid pupa	1.8	0.5	0.1	- 0.017
Ceratopogonid nymph	0.3	0.6	0.02	- 0.007
Simulium larva	0.6	0.05	0.003	- 0.002.
Unidentified	2.3	0.8	0.02	- 0.012
	(10.0)	**(3.1)**		
Coleoptera	0.8	0.5	0.04	- 0.003
Hemiptera	0.5	0.2	0.1	
Ephemeroptera	0.2	0.02	0.0004	
Insecta Unidentified	2.1	0.4	0.09	
	(13.6)	**(4.22)**		
Nematode	1.3	0.06	0.008	-0.001
Ostracoda	0.5	0.03	0.002	
ROTIFERA*: Brachionus*	5.9	3.7	2.35	
Keratella	1.4	0.1	0.02	- 0.013
Notholca	1.1	0.01	0.001	- 0.002
Tricocherca	1.0	0.03	0.003	
Lecane	0.1	0.02	0.0002	
	(9.5)	**(3.86)**		
ALGA*E :* *Monoraphidium*	6.1	2.9	1.91	-0.075
Closterium	2.3	0.03	0.007	-0.001
Amphipleura	4.4	0.04	0.02	-0.002
Frustulia	4.3	0.06	0.03	-0.002
Nitzschia	1.9	0.04	0.008	-0.001
Cyclotella	0.2	0.006	0.001	
Microcystis	1.5	0.08	0.01	-0.001
Aphanothece	0.5	0.003	0.0002	
Coelosphaerium	0.3	0.005	0.0002	
Goniochloris	0.1	0.005	0.0001	
	(21.8)	**(3.17)**		
Fish/eggs	0.9	0.07	0.01	- 0.001
Sand	0.4	0.30	0.01	- 0.001
Organisms unidentified	0.6	0.90	0.01	- 0.001
	(100)	**(100)**	**(100)**	**(-1.420**)

IFS ≥ 3% = primary
≥ 0.1 to < 3% = secondary
≤ 0.1% = incidental

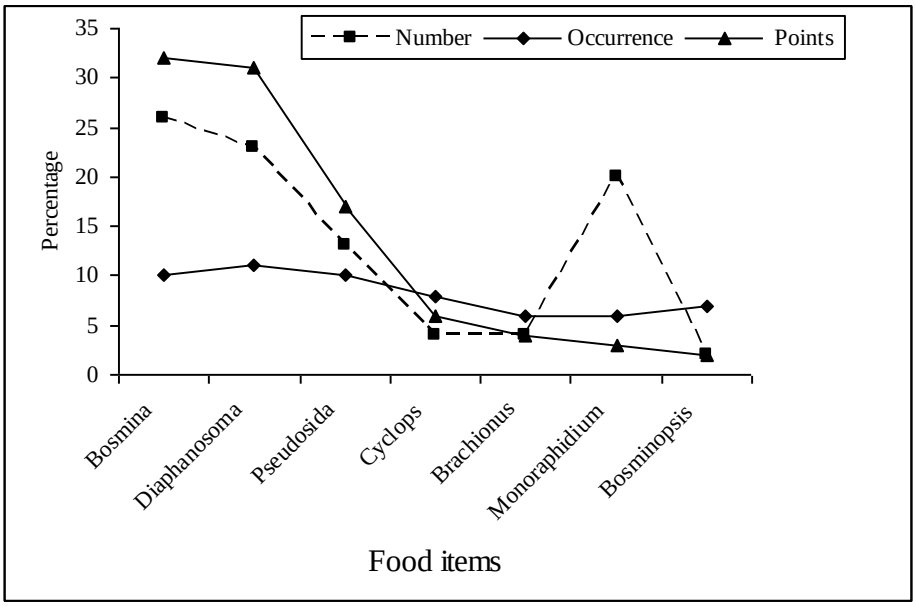

Figure 2: *Relative abundance of major food items of P. pellucida by the numerical, frequency of occurrence and points method*

3.6. Variation of Food Items with Fish Size and Season in *P. pellucida*

3.6.1. Numerical Method

A. *Food Selection with Size of Fish* Though zooplankton such as *cyclop* copepod, cladoceran species (*Bosmina, Bosminopsis, Diaphanosoma, Pseudosida*), Algae (*Monoraphidium, Frustulia*) and *Brachionus* (rotifer) appeared in the food of all size classes, food selection was observed in *Parailia pellucida* in the lower Nun River. In the diet of the smallest size class (4.1 – 5.0cm SL), the following: Food items that were absent *Ceratopogon* pupa and nymph, hemiptera, coleoptera, ephemeroptera, and algae such as *Aphanothece and Microcystis. Chironomous* pupa was absent in the 5.1-6.0cm SL class. *Ceratopogon* nymph and hemiptera did not also occur in the 8.1-9.0 cm classes.

In the largest size class (10.1-11.0cm S.L) *Chironmous* pupa, *Ceretapogon* pupa, Coleoptera, Hemiptera and nematode were absent. Others include the following algae: *Nitzchia, Aphanothece, Closterium, Microcystis and Amphipleura* .Generally, the mean consumption of food items such as copepoda, cladocera and insecta increased with fish size up to the 8.1-9.0 cm SL class which however was not significantly different (P>0.05).

Trichocerca (rotifer) was significantly higher (P<0.05) in the 5.1- 6.0cm size class with a mean of 5.5 ± 0.5; *Goniochloris* (algae) in the 7.1-8.0cm size class (3.0 ± 0.6) while *Aphanothece* and *Microcystis* had significantly higher means (1.5 and 12.2) respectively in the 8.1-9.0cm SL size class. Fish and fish egg showed significantly higher mean variation (30 ± 10) in the 9.1– 10.0cm SL class.

B. *Variation with Season* The mean variation in the food of *P. pellucida* with season by the Numerical method was *Cyclop* (74.2±26) *Ceratopogon* pupa (57.7±31.8) and *Ceratopogon* nymph (24.7±11.0) were significantly more abundant (P<0.05) in the dry season than the wet season (24.7±6.7; 6.2±2.2 and 6.5 ±5.5) respectively while Coleoptera (21.4±7.8; insecta) and *Brachionus* (95.9±25.6; rotifer) were significantly higher during the wet season than the dry season (23.4±4 and 6.0±1.0) respectively. Significant seasonal variation (P<0.05) was also observed in *Trichocerca* (rotifer) with a mean of 3.1±0.9 in the dry season and 1.1±0.1 in the wet season. Algae such as *Goniochloris* (3.0±0.93) and *Aphanothece* (1.2±0.2) were significantly higher in the wet season than the dry season of 2.0±0.0 and 1.0±0.0 rerspectively while *Mircocystis* was significantly higher (P<0.05) in the dry season (15.0±8.18) than the wet season (6.0±1.0).

No significant seasonal variation (P>0.05) in the mean occurrence of the various food items generally occurred between the wet and dry season except *Brachionus* and *Notholca* (rotifers) that were significantly different.

C. *Feeding Intensity* Feeding intensity defined as the mean number of food items in the stomach [15] was determined for seasons and fish size classes. The monthly mean variation of feeding intensity on the various food groups is shown in Table 2. Feeding was most intense in April, (19%) closely followed by February (18.9%) and December (18.4%) while the least intensity of 0.2% was obtained in July. *Parailia pellucida* fed virtually on all food items throughout except December where *rotifera, algae* and *ostracoda* were not observed. *Ostracoda* was the least food item consumed as it occurred in only 3 months (September, October and March).

Copepods and insects were most intensely consumed in February forming 11.0% and 3.4% respectively and were lowest in August forming 3.3% and 2.6% respectively. Maximum feeding intensity in the cladocerans occurred in December (91.3%), rotifers (14.8%) in March, algae (29.7%) in April, ostracods (2.7%) in October and nematodes (6.9%) in August (Table 2)

Feeding intensity of the various food groups for different size classes of *Parailia pellucida* is shown in Table 3. *P. pellucida* of all sizes fed most intensively on cladocerans with a steady increase in mean intensity from the smallest (4.1- 5.0cm) size group (92.11) to a maximum (418.62) in the 8.1 – 9.0cm SL size class and declined thereafter. A similar trend in feeding intensity also occurred with algae ranging from 38.87 in the 4.1 – 5.0cm SL class to 182.24 in the 8.1 – 9.0 cm SL class. The feeding intensity for other size classes on other food groups did not show any consistent pattern.

Table 2: *Monthly mean variation in feeding intensity of Parailia pellucida on major food groups in the lower Nun River, Niger Delta*

FOOD GROUPS	MONTHS												
	Aug.	Sept.	Oct.	Nov.	Dec.	Jan.	Feb.	Mar.	Apr.	May	Jun.	Jul.	Total
Copepoda No.	0.80	1.80	1.3	3.33	43.00	0	61.31	4.42	15.07	17.88	9.33	1.00	159.24
%	3.3	2.5	2.1	21.4	7.9	0	11.0	1.5	2.7	3.6	3.2	15.1	
Cladocera No.	17.40	43.25	23.5	10.55	494.50	12.38	347.28	156.55	371.80	340.43	182.18	0.00	1,999.82
%	71.2	60.3	37.8	67.9	91.3	69.2	62.5	51.9	66.4	68.1	63.4	0	
Insecta No.	0.64	9.20	9.00	1.00	3.00	0.30	18.69	3.24	2.04	5.75	1.73	5.11	59.70
%	2.6	12.8	14.5	6.4	0.5	1.7	3.4	1.1	0.4	1.1	0.6	77.3	
Rotifera No.	2.60	11.07	24.30	0.33	0	2.67	7.66	44.71	4.11	5.43	6.56	0	109.44
%	10.6	15.4	39.1	2.1	0	14.9	1.4	14.8	0.7	1.1	2.3	0	
Algae No.	1.30	5.00	1.67	0.33	0	1.55	120.25	92.50	166.30	129.59	87.09	0	605.57
%	5.3	7.0	2.7	2.1	0	8.6	21.6	30.6	29.7	25.9	30.3	0	
Ostracoda No.	0	1.00	1.67	0	0	0	0	0.18	0	0	0	0	2.85
%	0	1.4	2.7	0	0	0	0	0.06	0	0	0	0	
Nematodes No.	1.70	0.40	0.66	0	1.00	1.00	0.36	0.14	0.18	0.64	0.25	0.50	6.83
%	6.9	0.8	1.1	0	0.2	5.6	0.06	0.05	0.03	0.1	0.09	7.6	
OVERALL	24.44	71.72	62.1	15.54	541.5	17.9	555.55	301.74	559.5	499.71	287.14	6.61	2,943.45
Rel. %	0.8	2.4	2.1	0.5	18.4	0.6	18.9	10.2	19.0	17.0	9.7	0.2	

Table 3: *Feeding intensity by different sizes of Parailia pellucida with size on major food groups in the lower Nun River, Niger Delta*

SIZE CLASS SL (CM)	FOOD TYPE													
	COPEPODA		CLADOCERA		INSECTA		ROTIFERA		ALGAE		OSTRACODA		NEMATODA	
	N	Mean	N	Mean	N	Mean	N	Mean	N	Mean	N	Mean	N	Mean
4.1-5.0	12	20.67	12	92.11	2	5.50	7	2.71	8	38.89	-	1	1	1.00
5.1-6.0	49	5.61	56	121.92	32	6.94	39	28.77	49	48.98	14	0.21	26	0.23
6.1-7.0	43	29.32	58	151.41	45	3.58	56	14.0	58	55.76	-	-	32	0.41
7.1-8.0	77	16.32	81	220.74	64	7.76	76	13.04	79	63.58	8	0.12	42	0.31
8.1-90	53	14.13	56	418.62	49	6.86	55	13.69	45	182.24	4	0.25	11	0.45
9.1-10	8	7.62	8	172.62	8	14.37	5	13.8	7	56.71	-	-	5	0.60
10.1-11	1	10	1	130	-	-	-	-	1	67	-	-	-	-

N = Number of stomachs examined

4. Discussion

Feeding ecology studies of fishes is aimed at understanding the natural history of a species and its role in the trophic ecology of aquatic ecosystems (Braga et al. (2012). The food items of *P. pellucida* of the lower Nun River (copepods, cladocerans, diptera, insects, ostracods, nematodes, rotifers, algae and fish/eggs) are very similar with Olatunde (1979) [16] in the Kainji Lake. He observed invertebrates and phytoplankton such that small crustaceans constituted 87% and immature insects, 13%. Reynolds (1970) [17] also reported its food as consisting of small crustaceans, dipteran larvae/pupae and *Povilla adusta* nymphs (Ephemeroptera). According to Jordan et al, (2006) [18], Myers et al. (2007) [19] and Navia et al. (2010) [20], the knowledge of fish feeding ecology is fundamental to our ability to understand trophic, material and energy dynamics, to model precise outcomes for each ecosystem.

Sand particles found in the stomachs were not considered as food. They were probably sucked up accidentally while picking other food items from the river bed. They occurred mostly in stomachs of specimens that were caught at the bottom. Olatunde (1978) [21] also considered sand and small pebbles as non-food items. Schilbeids are reported as feeding on small crustaceans, insecta, fish, algae, rotifers and shrimp [22, 21, 16]. The wide food spectrum observed in the Lower Nun River population is indicative of a generalist food habit (utilizing a broad range of prey items) by *P. pellucida*. A similar observation was made by Bachok *et al.* (2004) [4] for marine fishes in Malaysia. The results are in line with Wilson and Wolkovich (2011) [23] theoretical ecosystem approach aimed at achieving community structure. Areas of dissimilarity were also observed in the food which may be location specific. Food items such as *Chaoborus* larvae, Caridina and *Conchostraca* reported in Kainji Lake were not observed in the diet of the lower Nun River population. Changes in fish diet with locations had been reported [24, 25].

The 80 points scale used in this study was to adequately analyze minute food items. The choice of a particular point scale depends on food type. Different workers have used points scale of their choice on stomach fullness state which include 4 points (Gramitto, 1999) [25]; 10 points (Ikomi and Sikoki, 2003) [26]; and 20 points by Ezenwaji and Offiah (2003) [14]. The choice of methodology is in line with Aguiar and Valentin (2010).

Cladocerans and copepods constituted the main food of *P. pellucida* using the various analytical methods with the bulk from cladocera. A similar finding was made by Otobo (1995) [27] in *Sierrathrissa leonensis* in the Nun River. The presence of insects (especially chironomids, ceratopogons ephemeropterans, hemipterans and coleopterans), as well as fish, suggests some level of cannibalism in the feeding habit of this fish. The food spectrum futher suggests that *P.pellucida* feeds at the bottom, mid water and at the surface. Ikomi and Sikoki (2001) [28] observed a similar foraging tendency in *Brycinus nurse.* This trend is also reported in *Limnothrissa miodon* inhabiting Lake Kariba, Zimbabwe (Chifamba, 1993) [29], in *P. leonensis* of the Anambra River (Ezenwaji and Offiah, 2003) [14]; and in *P. leonensis* and *S. leonensis* in the Kainji Lake (Otobo and Imevbore, 1979) [30].

There was no qualitative seasonal variation observed in the food of *P. pellucida* as the fish fed basically on the primary diets during both seasons. However quantitatively, peak consumption of cladocerans occurred in January, cyclops in February while insects, had two peaks; a smaller one in September and the major in February. Rotifers also exhibited two peaks- a minor in October and the main in March while that of algae was in April.

The results of the monthly variations of these food items showed that they were more dominant in the dry season than the wet season with a percentage composition of 55%, 82.9%, 88% and 70.2% respectively except algae which constituted 58% in the wet season. Olatunde (1979) [16] also

observed seasonal variation in the diet of *P. pelluicda* in Lake Kainji. Otobo (1995) [27] reported high availability of insect larva in the food of *S. leonensis* in October and low number of food organisms in August. According to Olatunde (1978) [21] the food items selected at any time of the year seems to depend on abundance and availability to the predator. Ezenwaji and Offiah (2003) [14] observed that Ephemeroptera nymph, Chironomids and fish were significantly higher in the rainy season than the dry season while the converse was true for the crustaceans, water bugs, plant detritus and sand. Seasonal variation of calanoid copepods is recorded in the continental shelf of Nigeria with higher concentration during the rainy season between July and September [31, 32] and scarce from February to May [33].

Food items with high frequency of occurrence and regularity in this study which appeared in very small quantities may not have nutritional importance to the predator. Though the occurrence of the various food items showed a general increase up to the 8.1 – 9.0cm SL size class, it was not significantly different contrary to Olatunde (1979) [16] who observed size variation in the percentage frequency of occurrence of food items selected by the different length groups of *P.pellucida*.

Seasonal variation in the feeding of *P. pellucida*, being most intense in April (19%), February (18.9%) and December (18.4%) is in concert with Olatunde (1979) [16] who also noted a feeding intensity pattern with more than 50% of the fish feeding in most months. Inyang and Nwani (2004) [34] observed significantly higher seasonal variation in the feeding pattern of *Distichodus* species in Anambra River Basin with significantly higher feeding intensity in the wet season than the dry season. Feeding intensity varied with length groups in this study with an increased mean intensity of 92.11 from the small sized fishes (4.1 – 5.0cm) to a maximum (418.62) in the 8.1 – 9.0cm length group with a decline in the larger group. Other similar findings include King and Akpan (2002) [35] in *Nanaethiops unitaeniatus* and Olatunde (1978) [21] in *S. mystus*, *P. pellucida* and *E. niloticus*.

The high incidence of empty stomachs (56%) without a particular trend in this study is within the range (25-65%) recorded by Olatunde (1978) [21] and 35–75% of Sagua (1980) [36]. Thus it was not an index of starvation. Such high incidence could occur when sampling with passive gear. Other probable causes include intermittent feeding habit or high rate of digestion. Joyce *et. al.* (2002) [25] observed that quick digestion can complicate dietary analysis because prey organisms can become quickly eroded making identification difficult consequently reducing the proportion of such organisms that can be identified as well as reducing stomachs fullness. Regurgitation of stomach contents after capture is also advanced as the probable cause of high percentage of empty stomachs [37]. Lagler (1956) [38] also made a similar observation ascribing the phenomenon to be induced by the striated muscles extending from the esophagus to the stomachs. He further noted that carnivores generally digest their food faster than the herbivores.

5. Conclusion

The numerical, frequency of occurrence and point methods used to investigate the food and feeding habit of *P. pellucida.* This study has shown that cladocera, algae, rotifera, copepoda and insecta are the basic food items among others. However, cladocerans and copepods are the main food items being more abundant in the dry season than the wet season.

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Reef Fish Biodiversity and Complexity in the North Bay Reef of Andaman and Nicobar Islands, India

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Abstract Under water visual census using traditional belt transect method (30m x 5m) was conducted to survey the community structure of reef fish assemblages in the North Bay reef of A & N Islands over a period of 3 months on May, June and July of 2008. The Bay is a 1 km^2 fringing reef area lies between $11^0$42'12.1″N latitude and $92^0$45'11.0″E longitude in the vicinity of the East Coast of main city Port Blair. Five sampling areas were used for this study and the depth ranged from 2.2 m to 5.7 m. The total abundance (TA) was 4130 individuals, with 159 species from 31 families. The average TA of the different sampling stations from North to South in entire reef was 826 ± 118.00, diversity index (H') 1.57, evenness index (J') 0.3097 and species richness index (d) 18.98. H' indicated less heterogeneity of species and individuals were unevenly distributed. J' indicated variation on communities between the species. The coral fish diversity index (CFDI) for the reef was 69 and the appropriate regression formula (4.234 X 69-114.446) predicted an appropriate total of 178 species, indicating that at least 19 more species could be expected. Monthly mean of TA increased from 593.5 in May to 869 in June to 1039 in July, indicated progressive addition of new species with rainy season due to influx of nutrient rich run-off from surrounding hill areas. Pomacentridae dominated the entire reef with a TA of 1677 (40.61%) individuals followed by Phempheridae, 369 (8.94 %) individuals. Species richness of Pomacentridae was highest with 28 species followed by Serranidae with 17 species. Highest single species abundance in the Bay was 448 (10.85%) individuals by *Chromis nigrura* (Pomacentridae) followed by *Phempheris vanicolensis* (Phempheridae) with 369 (8.94%) individuals, indicated settlement of these species from nearby mangrove and sea bed areas where bigger sized predatory fishes seldom visit because of shallower depth and anthropogenic disturbances mainly tourist activities as the site is open-access to them. Spatial and temporal variations are less prominent in the reef.

Keywords *Coral, Reef Fishes, Community Structure, UVS, North Bay, A & N Islands*

1. Introduction

Coral reef ranks as the most biologically productive and diverse of all natural ecosystems [1]. Coral reefs have globally important implications for marine biodiversity [2]. The global area covered by tropical reefs estimated to be around 617,000 km^2 at less than 30 m depth [3]. Around 30% of the world's marine fish species can be found on coral reefs [4].

Coral reefs in India are distributed along the east and west coasts at restricted places. Fringing and barrier reefs exist in A & N Islands (813.2 km^2) and atoll reefs are found in Lakshadweep. The Andaman reefs contain about 80% of the coral diversity in comparison to other reef of the world making the richest coral reefs in the Indian Ocean and an area of global significance [5, 6]. A total of about 135 species of corals belonging to 59 genera have been recorded from the Andaman Islands [7]. Vousden and Turner et al. [6, 5] have reported 197 species of coral within 58 genera while Kulkarni et al. [8] identified 115 species from the Mahatma Gandhi Marine National Park (MGMNP), Wandoor. The coral reefs of these Islands abound in a number of associated biota *viz*; the giant clams, top shells, Turban shell etc. including the coral fishes of ornamental and commercial importance [9]. Talwar [10] presented a comprehensive list of the fishes occurring in the A & N Islands, comprising 724 species under 406 genera and 145 families. In addition to this list, later on Devi [11] presented a supplementary list of fishes comprising 71 species under 57 genera and 41 families to make the information complete. Dorairaj and Soundararajan [12] reported from various Islands of MGMNP that 5 species of Pomacentridae out of 55 fish species recorded in the park area dominated the group in all the Islands. About 43 species of groupers and more than 200 species of marine ornamental fishes are known to occur in these Islands [13].

Coral reefs contribute 25% of all the fisheries harvested and 75% of animal protein consumed supporting not only the livelihood, but also contribute to the social welfare of Island communities [14]. Tropical reef fisheries provide employment and sustenance for millions of coastal dwellers [15]. The rich diversity of fin and ornamental fishes at reefs has great demand as aquarium stock and still there is immense scope for their live export.

2. Materials and Methods

2.1. Study Area

The study area North Bay Islands, a famous tourist site, lies between 11°42'12.1″ N latitude and 92°45'11.0″ E longitude and is situated at the vicinity of the main city Port Blair along the east coast of South Andaman Islands of A & N Islands (Figure 1). The reef area is about 1 km^2 receiving water from the open sea on the south east of the Bay. The North-West-East side is covered by hills (Mount Herriet) which receives heavy rainfall during monsoon season and the other side is open. Reefs are of fringing type extending about 300 m along the shore. The physiography of the study area is rolling to undulating land dotted with sporadic Hillocks and Mounts [16].

2.2. Sampling Procedure

All field observations were undertaken in five stations (A, B, C, D and E) from North to South keeping a gap of fifty meter between successive stations along the reef (Figure 1). Station A is the area very close to muddy and mangrove area from where reef starts. Station B is the boat anchorage area. Under water visual survey (UVS) technique [17] using traditional belt transect method (Brock, 1954) [18] was conducted to survey the community structure of reef fish assemblages in the North Bay reef of A & N Islands over a period of 3 months on May, June and July of 2008.

Modifications of existing methods have been used to suit the reef as reported by Vijay-Anand and Pillai (2002) [19]. Belt transects of 30 m lengths were used to record all visible fish species and numbers from 2.5 m on either sides of a pre laid line. Transect width was estimated visually and laid randomly positioning with their long axes parallel to the shoreline. Due to shallow depth and absence of SCUBA facility, the fishes were observed using a mask and snorkel apparatus. The observer swam along the transect rope up to length of 30 m. Thus a census covering an area of 30m X 5m was conducted. The observer took photography, counted and identified the fish assemblage visually within the area of 2.5 m either side of the transect lines during day light hours. Likewise 6 transects were conducted in each site. Census was done by viewing ahead and counting target species in an area of the transect contained well within the bounds of visibility. During the first scan of the section the most mobile target species were counted and recorded, with progressively less mobile species was recorded in consecutive counts.

2.3. Data Analysis

Data collected were grouped by species and transformed in to a data matrix for respective sub-habitats. The abundance of all fishes at each site or station was calculated as the mean abundance from six replicates. Therefore, mean abundance of all fishes was expressed as individuals per $150m^2$. Species richness was a count of total species found in each site. Results of all census were combined to obtain the information on a) Species composition and species richness (S) of the selected area, b) Total abundance (TA), c) Percentage abundance, d) Diversity index ($H' = -\sum pi \ln pi$) of Shannon-Weiner (1949) [20] where pi is the proportion of each species in the sample (N). e) Evenness index ($J' = H'/\ln S$) of Pielou (1966) [21], f) Richness index [$d=(S-1)/\ln N$] of Margalef (1968) [22] and g) Coral Fish Diversity Index (CFDI) of Allen (1998) [23] which is counted as the total no of species of the six major key reef fish families: Chaetodontidae, Pomacanthidae, Pomacentridae, Labridae, Scaridae, and Acanthuridae. Appropriate regression formula (4.234 x CFDI–114.446) is used to obtain expected number of fishes for a particular area.

3. Results

3.1. Assemblage Structure

A total of 4130 individuals belonging to 31 families were found. There was a total of 159 species recorded in the study area. Overall mean abundance for the entire reef was recorded to be 826±118.00. Total abundance of individuals in stations were 879 (146.50±49.60, mean±SE), 472 (78.67±7.86), 715 (119.17±22.39), 869 (144.83±31.73) and 1195 (199.17±34.52) respectively for stations A, B, C, D and E (Table 1).

Major families contributing to the total abundance are depicted in Figure 2. Family Pomacentridae (Figure 3A) dominated the assemblages followed by Pempheridae (Figure 3B) Caesionidae (Figure 3C), Acanthuridae (Figure 3D), Chaetodontidae (Figure 3E) and Siganidae (Figure 3F). Families contributing less than 5% to the total abundance were clubbed together and designated as category others. This category contributed a total individual of 1001 (24%) numbers**.** Species belonging to the family Pomacentridae were highest with 28 species followed by Serranidae with 17 species, Chaetodontidae with 15 species, Acanthuridae and Labridae both with 8 species. The species showing highest abundance in the North Bay reef was *Chromis nigrura* (Pomacentridae) with a total of 448 individuals and 10.84%.

3.2. Community Parameters

Diversity index (H´) for entire reef was 1.57 (Table 2). This indicated less heterogeneity of species and individuals were unevenly distributed. The evenness index for the reef was 0.31 (Table 2). So, there

was variation on communities between the species. The CFDI, obtained for the reef was 69, and the appropriate regression formula (4.234 x 69 – 114.446) predicts an approximate total of 178 species, indicating that at least 19 more species can be expected.

4. Discussion

Local populations of marine reef fishes often show great spatial variation in abundance [24] due to combined effect of many physical and biological factors. This study dealt with the general distribution pattern and diversity of some coral reef fishes on the North Bay reef of A & N Islands which is the first of its kind from this Islands.

The prominent features of the North Bay reef fish assemblages were low species richness and absence of many reef fish taxa such as Gerreidae, Kyphosidae, and Diodontidae etc. The total species recorded was 159 species under 31 families, averaging 94.8 species per site. Vijay Anand and Pillai (2002) [19] recorded from reef slope of Kavaratti atoll during January1991 to June 1992, a total of 27 families represented by 121 species. Vijay Anand and Pillai (2005) [25] reported a total of 46 species from rubble sub-habitat of the Atoll. Reports also revealed a high total species richness of 564 (Allen and Stone, 2005) [26] from Thailand's East Andaman Sea.

This report on community structure from a single reef of these Islands was the first of its kind which could not be compared due to paucity of published records. Only Rao (2003) [27] identified 705 species belonging to 90 families encountered on the reefs of these Islands which also included other habitats adjacent to coral areas besides coral reef. Dorairaj and Soundararajan (1997) [12] reported 55 fish species from various Islands of MGMNP, Wandoor.

Species and area relationship is an important ecological factor [28, 29]. The abundance and diversity of fishes can be correlated with habitat composition and complexity [30, 31, 32, 33]. The reason for low diversity in the North Bay reef is its geographic location, only 1 km^2 in area having limited connection to nearby reefs and human disturbance. In this regards habitat availability [34] and habitat preferences [35, 36] also play a role. The variability in total abundance and species richness over 3 months was because of variation in settlement and recruitments. Settlement as well as recruitment increases towards the area far from boat anchorage area. Jennings et al. (1996) [37] demonstrated that the differences in the diversity and abundance of reef fish communities may be attributed to spatial and temporal variations in recruitment [38], habitat effects [31] and other factors. A variety of other factors, such as reef types or environmental conditions, can also interact with habitat associations to influence patterns of distribution and abundance [39].

Out of total abundance of 4130 individuals, most individuals belonged to the family Pomacentridae (40.61%). Alwany (2003) [40] also reported highest abundance of Pomacentridae (52.1%). This is in conformity to the situation on the Great Barrier Reef in New Caledonia, where Pomacentridae was the dominant fishes (pomacentrids contribute the highest percentage of species [41, 42]. Similar result was also recorded in six study stations in the vicinity of Maptaput, Thailand during 2002 [43] and Khangkao Islands [44]. In all the Islands of MGMNP, 5 species of Pomacentridae dominated the group [12]. Out of about 43 species of groupers forming 10% of the reef fishes of A & N Islands [13], only 17 species could be recorded in this study.

Pomacentridae (28 species), Serranidae (17 species) and *Chaetodon*tidae (15 species) dominated the fish fauna in terms of species richness along the North Bay reefs, and individuals of family Pomacentridae were most abundant (Table 1). The species *Chromis nigrura* (448 individuals and 10.85%) was found highest on the reef indicating the richness of ornamental species in the study area. The highest average abundance was recorded at station E (199.17±34.52 individuals/150m^2) followed by station A (146.50±49.60 individuals/150m^2) (Table 1). It was observed that the station A

was very nearer to the boat anchorage area, even though showed increased pattern of total individuals, dominated mainly by small pomacentrids (Mean 90.17±33.13 individuals/150 m^2). This can be attributed due the complexity of the reef which is very nearer to the mangrove area which may act as breeding ground for the fishes. Probably reef receives settlement from these areas. This result is in conformity to the findings by Grober-Dunsmore et al., (2006) [45] who detected fish-habitat relationships as far as 1 km from study reefs.

There was increasing trend of diversity indices towards the stations far from boat anchorage area (Station B). Overall Diversity index values in stations (Table 1) coincided the actual range of Shannon-Weiner (0-4.6). According to them values near to '0' would indicate that every species in samples are the same. A value near 4.6 would indicate that the number of individuals is evenly distributed among all the species. So, the present findings imply some sort of heterogeneity of species composition and uneven distribution of species on stations.

The overall reef status in the study site was found to be healthy with average live coral cover of 45.25 % [46] and fair with coral cover of 44.47±14.12 % [47]. Sites with very high percentage (>75%) of live coral cover are frequently composed of large mono-specific stands of corals such that their coral biodiversity is low [48, 49]. So, it indicates that when the coral diversity is high and not dominated by mono-specific strands of coral, it should support a high habitat complexity as well as high reef fish diversity. But low overall diversity indices (Table 2) indicated alteration in assemblage structure in the reef which may be attributed due to the anthropogenic activities that includes fishing pressures and tourism, sedimentation, influx of freshwater and nearness to ship passages.

The worldwide demise of coral reefs can largely be attributed to human activity, both direct and indirect intervention [50]. The reef area of North Bay was found to be impacted by various factors; notable of them were anthropogenic activities, sedimentation over years and pollution. Oil pollution, tourism, over fishing and sedimentation were the sources of stress in this reef [47]. The impacted reefs also continue to support a relatively diverse fish fauna [26]. The total species richness (159 species) of this reef area was found in agreement to average number of species of 167, 176 and 173, from three impact categories (severe, moderate, and zero or light) respectively [26].

The most significant finding was the complete absence of bigger sized food fishes, and recruits were mainly dominated by only schooling small sized fishes. These small sized fishes are of immense importance in maintaining the ecology [17] and have ornamental value because of their appalling morphology and may be considered as an item to promote scope for ornamental fish trade.

5. Conclusion

The results of this study show that the North Bay reef is a very small structurally complex reef. The reef is of fair type and supports moderate coral diversity. But corresponding to coral diversity, fish diversity was not found satisfactory, less heterogeneity of the fish species and individuals were unevenly distributed. This complex habitats supported small schooling fishes resulting protection from predation and greater availability of resources opening new avenues towards ornamental fish trade. The reef was found to be impacted with many factors; notable of them are tourism and other anthropogenic activities. Measures offering the best prospects for management of this reef are management measures, gear limitation, limited entry, closed season, shift resource use, pollution control, siltation control and establishment of organization governing for sustainable use of available resources.

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Tables

Table 1: *Total abundance (TA), Species richness, Diversity Index (H´) and Evenness Index (J´) of reef fish species of the North Bay Reef. (Mean of six replicates ± standard error/150 m^2).*

Fish Family and Fish Species	Station A	Station B	Station C	Station D	Station E	TA	%
Acanthuridae							
Acanthurus dussumieri	-	-	2.00	5.33±1.33	5	27	0.654
Acanthurus leucosternon	-	-	-	3.50±1.50	2.00	15	0.363
Acanthurus lineatus	-	1	1.00	1.75±0.48	1.67±0.67	16	0.387
Acanthurus mata	-	-	-	6.00	4.00	10	0.242
Acanthurus triostegus	1.67±0.33	1	1.50±0.50	2.00	1.50±0.50	14	0.339
Ctenochaetus striatus	5.60±2.01	7.00±2.38	3.33±0.67	5.60±1.16	7.00±2.07	129	3.123
Naso lituratus	-	5.00	-	5.50±3.51	3.50±0.50	23	0.557
Zebrasoma scopas	1.67±0.42	3.00	2.25	2.60±0.40	3.00±1.53	47	1.138
Apogonidae							
Apogon cookii	1.00	2.00	-	3.50±1.50	1.50±0.50	13	0.315
Apogon fraenatus	1.00	-	1.00	5.00	-	12	0.291
Apogon lateralis	-	2.00	1.00	-	-	3	0.073
Cheilodipterus macrodon	1.00	-	1.00	1.50±0.50	-	6	0.145
Balistidae							
Balistapus undulatus	1.40±0.40	2.50±0.50	1.25±0.25	1.33±0.33	-	26	0.630
Balistoides viridescens	1.00	-	1.00	-	1.00	3	0.073
Melichthys indicus	1.50±0.50	2.00±0.58	1.50±0.50	3.00±1.00	2.00	22	0.533
Rhinecanthus aculeatus	-	1.00	1.00	1.00	-	5	0.121
Rhinecanthus verrucosus	-	2.00	1.00	4.00	-	7	0.169
Belonidae							

Tylosurus crocodilus crocodilus	1.00	-	1.00	-	-	2	0.048
Centriscidae							
Aeoliscus strigatus	1.00	-	-	-	-	1	0.024
Caesionidae							
Caesio caerulaurea	-	-	-	15.00	15.00	30	0.726
Caesio lunaris	-	3.00	50.00	62.00	30.67±18.84	207	5.012
Caesio cuning	-	-	25.00	10.00±5.01	20.00	65	1.574
Casio teres	-	-	-	10.00	-	10	0.242
Carangidae							
Caranx ignobilis	-	-	-	4.00	4.00	8	0.194
Chaetodontidae							
Chaetodon auriga	2.00	2.00	1.50±0.50	6.00	4.67±1.20	29	0.702
Chaetodon collare	2.50±0.50	5.00±3.01	4.00	5.00±3.01	4.50±0.50	43	1.041
Chaetodon decussatus	1.50±0.50	-	2.00	-	-	5	0.121
Chaetodon lineolatus	-	-	-	2.00	2.00	4	0.097
Chaetodon meyeri	-	-	-	-	1.33±0.33	4	0.097
Chaetodon triangulum	3.00±1.00	1.00	3.00	2.00	2.50±0.50	20	0.484
Cheetodon rafflesii	-	3.00	1.00	2.00	2.50±0.50	11	0.266
Chetodon falcula	-	2.00	-	-	2.67±0.67	10	0.242
Chetodon guttatissimus	1.00	-	-	-	2.00	3	0.073
Chetodon octofasciatus	-	5.00	1.50±0.50	-	2.50±0.50	13	0.315
Chetodon plebeius	-	4.00	-	1.00	2.00	9	0.218
Chetodon trifasciatus	1.67±0.33	3.00±0.58	3.00	2.00	3.50±0.50	26	0.630
Heniochus acuminatus	1.00	2.00	2.00±1.00	1.50±0.50	3.00±1.00	18	0.436
Heniochus monoceros	-	-	-	4.00	-	4	0.097
Heniochus pleurotaenia	2.00±	4.00	4.00	4.50±0.96	6.00±1.16	50	1.211
Ephippidae							
Platax orbicularis	-	-	-	-	2.00	2	0.048
Platax pinnatus	-	-	-	2.00	3.00	5	0.121
Fistulariidae							

Fistularia commersonii	1.00	1.00	1.00	1.00	-	4	0.097
Haemulidae							
Diagramma pictum	-	-	-	2.00	-	2	0.048
Plectorhinchus chaetodonoides	-	2.00	-	-	4.00	6	0.145
Plectorhinchus diagrammus	-	-	-	-	1.00	1	0.024
Plectorhinchus orientalis	1.00	2.00	1.00	1.00	1.75±0.48	13	0.315
Plectorhinchus schotaf	-	-	2.00	-	3.00±2.01	8	0.194
Holocentridae							
Myripristis adusta	2.00	-	2.00	-	-	4	0.097
Myripristis murdjan	-	5.00±1.00	2.00	6.00	2.00	20	0.484
Sargocentron ittodai	-	3.00±1.00	2.00	4.00	-	12	0.291
Sargocentron rubrum	-	-	-	9.00	-	9	0.218
Labridae							
Coris gaimard	-	-	-	1.00	1.00	2	0.048
Gomphosus caeruleus(Dark phase)	-	-	-	2.00	2.00	6	0.145
Halichoeres hortulanus	-	-	-	2.00	2.00	4	0.097
Halichoeres ornatissimus	-	-	-	-	2.00	2	0.048
Halichoeres scapularis	-	1.00	1.00	1.50±0.50	1.00	8	0.194
Labroides dimidiatus	2.00±0.71	2.00	2.20±0.49	2.60±0.68	2.40±0.24	48	1.162
Thalassoma hardwicke	-	-	1.00	1.75±0.25	1.00	13	0.315
Thalassoma lunare	1.33±0.21	1.75±0.48	2.00	2.00±0.71	1.67±0.33	36	0.872
Lethrinidae							
Gnathodentex aureolineatus	-	-	-	5.00	20.00	25	0.605
Lethrinus ornatus	-	-	-	6.00	-	6	0.145
Lethrinus variegates	-	3.00		4.00	-	7	0.169
Lethrinus xanthochilus	1.00	1.00	2.50±1.50	-	2.00	9	0.218
Lethrinus borbonicus	1.00	1.00	2.00	-	6.00	10	0.242
Lethrinus obsoletus	-	-	-	-	4.00	4	0.097
Monotaxis grandoculis	2.00	-	1.00	1.00	2.50±0.50	11	0.266
Lutjanidae							

Lutjanus lemniscatus	-	-	-	-	2.00±0.58	6	0.145
Lutjanus bohar	1.00	2.00	1.25±0.25	4.00	2.67±0.88	24	0.581
Lutjanus decussatus	2.50±0.50	2.75±0.25	1.25±0.25	6.00	2.00±0.45	37	0.896
Lutjanus fulviflamma	1.33±0.33	1.00	2.00	2.00	2.00	13	0.315
Lutjanus kasmira	-	-	-	-	7.00	7	0.169
Lutjanus russellii	-	-	5.00	-	13.50±8.53	32	0.775
Macolour niger	-	-	-	2.00	1.00	4	0.097
Mullidae							
Mulloidichthys flavolineatus	-	-	7.00	-	-	7	0.169
Parupeneus rubescens	-	2.33±0.33	1.00	1.00	2.00	11	0.266
Parupeneus barberinus	5.00±	3.50±0.50	2.00	2.50±0.50	1.00	20	0.484
Parupeneus cyclostomus	-	2.00	4.00	7.00	12.50±6.52	38	0.920
Parupeneus indicus	3.50±0.50	2.00	7.00±5.01	-	20.50±0.50	64	1.550
Muraenidae							
Gymnothorax fimbriatus	-	-	-	-	1.00	1	0.024
Gymnothorax flavimarginatus	-	-	-	-	1.00	2	0.048
Nemipteridae							
Nemipterus bipunctatus	-	-	-	-	13.00±6.02	26	0.630
Scolopsis xenochrous	1.00	1.00	1.00	3.00	1.33±0.33	13	0.315
Scolopsis bilineata	1.50±0.50	2.00	2.00±0.41	2.80±0.86	3.50±0.50	37	0.896
Scolopsis ciliate	1.67±0.33	1.67±0.67	1.75±0.48	5.20±1.93	3.00±1.00	52	1.259
Scolopsis ghanam	-	-	1.00	1.00	2.00	6	0.145
Scolopsis lineata	1.00	2.00	1.00	-	2.00±0.58	10	0.242
Ostraciidae							
Ostracion cubicus	-	1.00	1.00	1.00	1.00	5	0.121
Ostracion meleagris	-	-	1.00	-	1.00	2	0.048
Pempheridae							
Pempheris vanicolensis	46.67±34.30	19.50±5.52	65.00±35.10	30.00	30.00	369	8.935
Pinguipedidae							
Parapercis hexophthalma	1.00	1.00	1.00	-	1.00	6	0.145
Pomacentridae							

Abudefduf bengalensis			5.00	10.00	-	15	0.363
Abudefduf sordidas	-	7.00	7.00	-	-	14	0.339
Abudefduf vaigiensis	10.20±1.43	17.75±4.23	9.00±1.92	8.50±0.87	3.67±0.88	212	5.133
Amblyglyphidodon aureus	4.00±0.58	4.33±0.88	4.00	8.25±3.17	3.00±1.00	68	1.646
Amphiprion akallopisos	-	6.00	2.00	-	-	8	0.194
Amphiprion ephippium	-	-	-	2.00	-	2	0.048
Chromis dimidiata	2.00	2.00±	5.50±1.50	-	4.50±2.18	35	0.847
Chromis nigrura	26.20±8.78	12.67±1.45	13.25±3.33	17.00±4.19	28.20±9.11	448	10.847
Chromis opercularis	-	13.00	-	-	-	13	0.315
Chromis atripectoralis	76.50±63.69	2.00	14.00±2.89	7.80±1.39	15.00	251	6.077
Chromis margaritifer	3.00±1.00	-	5.50±3.51	5.67±3.29	-	34	0.823
Chromis viridis	14.00±11.03	4.00	7.00±3.01	-	5.00	51	1.235
Chrysiptera caeruleolineata	5.00	-	3.00	-	2.00	13	0.315
Chrysiptera leucopoma (Yellow phase)	-	-	7.33±1.45	-	23.00±2.01	68	1.646
Dascyllus aruanus	-	2.00	-	3.00	2.00	7	0.169
Dascyllus carneus	5.00	-	-	21.00	7.00	33	0.799
Dascyllus trimaculatus	2.00	3.50±0.50	2.00	2.67±0.67	4.00	31	0.751
Dischistodus perspicillatus	2.00	2.00	2.00	6.00±3.06	4.67±1.77	42	1.017
Neoglyphidodon melas	4.50±0.50	-	2.00	2.00	4.50±0.50	24	0.581
Neoglyphidodon nigroris	-	-	-	8.00	-	8	0.194
Plectroglyphidodon chrysurus	-	-	-	-	11.00	11	0.266
Plectroglyphidodon dickii	-	-	4.00	6.00	6.75±3.25	37	0.896
Plectroglyphidodon lacrymatus	-	-	3.00±1.00	8.00	4.00	18	0.436
Pomacentrus ambionensis	-	-	1.00	-	7.00	8	0.194
Pomacentrus moluccensis	42.00±29.40	5.00	2.00	14.50±6.52	12.00±7.02	188	4.552
Premnas biaculeatus	-	4.00	2.00	2.00	-	8	0.194
Stegastes insularis	-	-	-	-	10.00	10	0.242
Stegastes nigricans	2.50	-	2.00	3.00	10.00	20	0.484
Pomacanthidae							

Centropyge bicolour	-	-	1.50±0.50	-	1.50±0.50	6	0.145
Pomacanthus imperator	-	-	2.00	1.00	1.00	5	0.121
Pygoplites diacanthus	1.00	2.00	1.00	3.00	1.00	10	0.242
Priacanthidae							
Priacanthus hamrur	-	-	-	-	2.00	2	0.048
Scaridae							
Chlorurus enneacanthus	-	1.00	-	-	2.00	3	0.073
Scarus atrilunula	-	1.00	1.00	-	1.33±0.33	7	0.169
Scarus ghobban	1.00	1.00	1.00	1.00	1.50±0.50	8	0.194
Scarus gibbus	-	-	1.00	-	2.00	3	0.073
Scarus globiceps	-	-	-	-	1.50±0.50	3	0.073
Scarus niger	1.00	1.00	1.00	1.00	1.00	8	0.194
Scarus psittacus	-	-	1.00	2.00	1.00	4	0.097
Scorpaenidae							
Pterois volitans	-	-	-	1.00	1.00	2	0.048
Serranidae							
Anyperodon leucogrammicus	-	-	1.00	1.00	1.00	3	0.073
Cephalopholis boenak	1.00	1.00	2.00	-	-	5	0.121
Cephalopholis cyanostigma	-	2.00±0.55	1.50±0.50	1.00	2.50±0.50	19	0.460
Cephalopholis leopardus	-	1.00	-	-	2.00	3	0.073
Cephalopholis argus	-	1.00	1.00	2.00	1.67±0.33	10	0.242
Cephalopholis miniata	2.00	1.50±0.50	1.00	2.00	2.00±1.00	13	0.315
Epinephelus bleekeri	1.00	1.67±0.33	-	-	1.00	7	0.169
Epinephelus fasciatus	-	-	-	-	2.00	2	0.048
Epinephelus itajara	-	-	-	2.00	1.00	3	0.073
Epinephelus longispinis	-	-	-	1.00	1.00	2	0.048
Epinephelus merra	-	3.00	1.00	4.00	-	8	0.194
Epinephelus ongus	-	1.00	-	-	1.00	2	0.048
Epinephelus quoyanus	1.00	-	-	1.50±0.50	-	4	0.097
Epinephelus coeruleopunctatus		-	1.00	2.00	3.00	6	0.145

Epinephelus faveatus	1.00	-	1.00	2.00±1.00	2.00	12	0.291
Epinephelus spilotoceps	1.00	-	-	1.00	1.00	3	0.073
Plectropomus areolatus	-	1.00	-	1.00	1.00	3	0.073
Siganidae							
Siganus argenteus	2.00	-	2.00	1.00	7.00	14	0.339
Siganus canaliculatus	-	-	-	-	1.00	1	0.024
Siganus corallinus	-	2.00	-	-	12.67±3.93	40	0.969
Siganus doliatus	4.00	-	6.00±2.00	6.00±1.00	9.00	47	1.138
Siganus fuscescens	-	-	2.00	-	1.50±0.50	5	0.121
Siganus guttatus	20.00	8.00	6.00	6.00	22.67±2.73	108	2.615
Siganus virgatus	-	8.00	2.00	8.00±4.01	-	26	0.630
Syngnathidae							
Corythoichthys haematopterus	1.00	-		-	-	1	0.024
Tetradontidae							
Arothron immaculatus	-	-	1.00	-	1.33±0.33	5	0.121
Arothron stellatus	-	2.00	-	-	1.00	4	0.097
Canthigaster solandri	1.00	-	-	-	1.00	2	0.048
Zanclidae							
Zanclus cornutus	-	1.00	-	3.00	3.67±1.45	15	0.363
Mean ± SE	146.50±49.60	78.67±7.86	119.17±22.39	144.83±31.73	199.17±34.52		
Total Species	66	80	99	102	127		
Species Richness index (d)	9.59	12.83	14.91	14.92	17.78		
Total abundance of individuals	**879**	**472**	**715**	**869**	**1195**	**4130**	
Diversity Index (H´)	2.83	3.63	3.65	3.99	4.06		
Evenness Index (J´)	0.68	0.83	0.79	0.86	0.84		

Table 2: *Diversity indices for the entire reef of North Bay Islands*

Samples	S	N	d	H´	J´
Total	159	4130	18.98	1.57	0.3097

Note: S (Total species), N (Total number of individuals), d (Species Richness Index), H´ (Diversity Index), J´ (Evenness Index)

Figure Legends

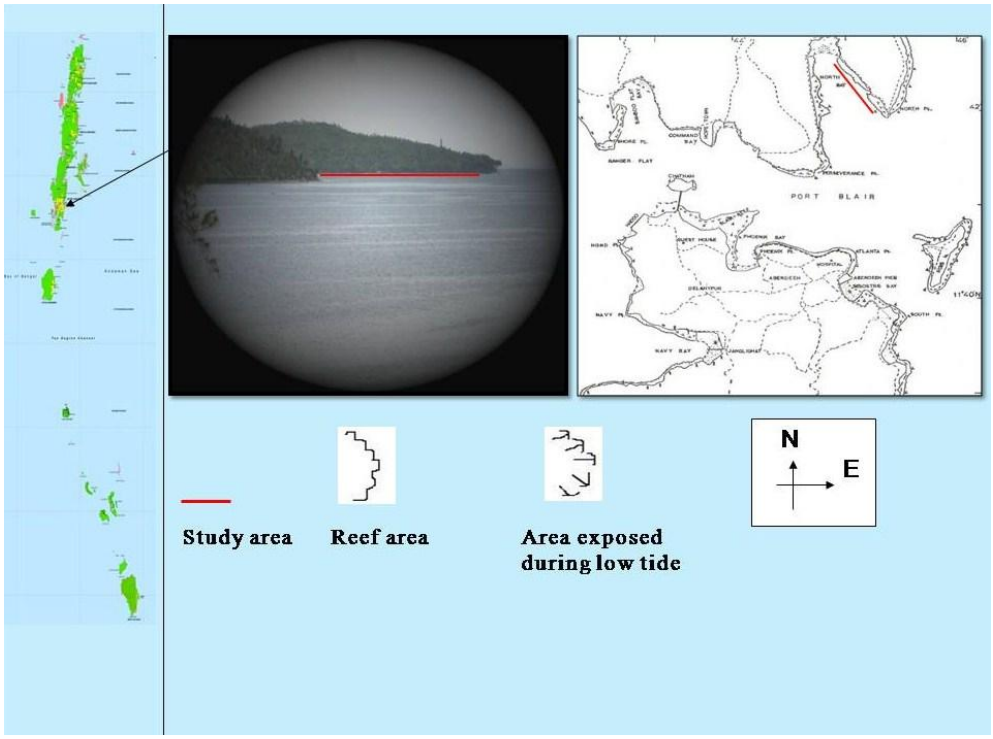

Figure 1: *Andaman and Nicobar Islands showing study area, the North Bay Islands*

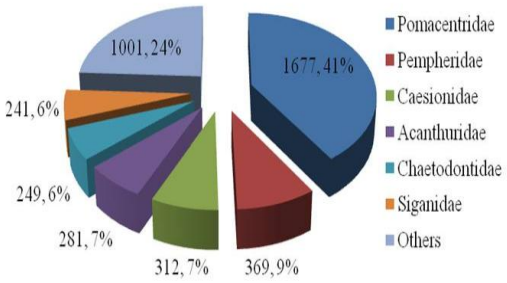

Figure 2: *Total percentage abundance of major families in the North Bay Reef of A & N*

Figure 3A

Figure 3B

Figure 3C

Figure 3D

Figure 3E

Figure 3F

Figure 3: *Species representing major families encountered in the North Bay Reef of A & N Islands:* ***3A.*** *Amblyglyphidodon aureus (Pomacentridae),* ***3B.*** *Pempheris vanicolensis (Pempheridae),* ***3C.*** *Caesio spp. (Caesionidae),* ***3D.*** *Ctenochaetus striatus (Acanthuridae),* ***3E****. Chaetodon collare (Chaetodontidae),* ***3F.*** *Siganus virgatus (Siganidae)*

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Research Article | Open Access

Sexual Differentiation in Blood Biochemistry of Rainbow Trout (*Oncorhynchus mykiss*)

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Abstract The sexual differentiation in blood biochemical values were assessed in rainbow trout (*Oncorhynchus mykiss*) cultured in Laribal Trout Culture Farm, Dachigam National Park, Srinagar, India. The blood from the healthy rainbow trout brood was collected for serum and analyzed & expressed as mean±SD in male and female fish. The present investigation revealed some significantly higher in males than female fish (P≤0.05), including total protein (6.8±2.1, 4.4±0.8 g/dl), albumin (0.52±0.1, 0.35±0.2 mg/dl), glucose (5.8±0.3, 4.14±0.5 mg/dl), phosphorous (1.5±0.1, 1.4±0.1 mg/dl), magnesium (4.2±0.3, 3.7±0.2 mg/dl). There were no significant differences with higher values in male than female of the C3 (39.2±3.7, 37.3±3.6 mg/dl), C4 (26.3±2.5, 36.1±3.4 mg/dl), alkaline phosphatase (23.2±2.5, 19.8±2.3 IU/L), amylase (161.4±22.4, 149.2±11.8 IU/L), cholesterol (54.2±2.8, 48.5±2.8 mg/dl) and creatinine phosphokinase (55.2±3.4, 49.8±2.8 IU/L). The other biochemical parameters were higher in female and the differences were not significant, in case of aspartate aminotransferase (70.3±7.2, 88.3±4.8 IU/L), alanine aminotransferase (21.9±3.6, 29.6±6.2 IU/L), IgM (80.1±8.2, 92.1±8.4 mg/dl), Iron (25.4±2.1, 29.2±0.5 µg/dl), calcium (6.9±0.2, 6.8±0.3 mg/dl). The findings would be helpful to establish a baseline to draw any conclusive remarks against the health status of rainbow trout, showing deviation from the normal parameters.
Keywords *Rainbow Trout, Biochemical Parameters, Serum, Trout Farm, Laribal*

1. Introduction

The Laribal trout culture farm is located in the picturesque surroundings of the world famous Dachigam National Park, the only abode of the highly endangered deer species, Hangul (*Cervus elaphus hanglu*). The farm is one of the largest trout breeding and rearing facilities in Asia producing about 6 to 7 tonnes of table size fish annually in addition to about 3.0 lacs of rainbow and brown trout fry. The farm is spread over an area of 40 kanals with water spread area of 5 kanals.

Trout are the most commonly cultured fish in the world, and are a food staple in many parts of Africa, Asia and South America [Talas and Gulhan, 2009]. Aquaculture of trout, as with other species of

finfish, is adversely affected by production related disorders and infectious diseases. Unfortunately, there are few diagnostic tools available to veterinarians and fish health professionals to evaluate disease in fish. Many of the clinical tools used to evaluate mammalian health are not developed for use in fishes. As the aquaculture industry expands, there is an increasing need for improved diagnostic methods. Hematology and clinical chemistry analysis, although not used regularly in fish medicine, can provide substantial diagnostic information once reference values are established. In this study, we determined reference intervals for hematologic and plasma chemistry analytes in cultured trout.

We also evaluated clinical chemistry results from a small group of trout raised under different culture conditions. To our knowledge, this is the first study to determine complete hematologic and clinical chemistry results for trout from Kashmir province of Jammu and Kashmir State, India and to report the values as reference intervals suitable for diagnostic use. Rainbow trout *(Onchorhynchus mykiss)* are an important aquaculture species yet there are few diagnostic tools available to assess their health.

Hrubec (1970) envisaged the need for new diagnostic tools corresponding to increase in aquaculture industry. To understand the species-specific response to various factors including nutritional and farm management practices, the knowledge of haematology is of utmost importance [3]. The concept of 'metabolic profile' in this regard was given by Payne *et al*. (1970). By this method, the productive and reproductive performances of the haemothermic animals could be protected by ensuring the deficiencies which may occur from time to time [3]. The practical utility of this diagnostic technique is this clear, as it permits the verification of possible errors in the farming practice so that they can be dealt with before they show up clinically [4]. So great attention has been recently paid to biochemical characterization of fish blood as an index of the state of internal milieu [5].

2. Materials and Methods

Healthy rainbow trout brooders (Length 24±2.0 cm and Weight 1.95±50 g) were collected from brood ponds of Laribal fish farm, Srinagar. Water temperature, oxygen and pH ranged from 9-10°C, 8-9 mg/l and 6.8-7.5 respectively. Fish were not fed up to sampling to reduce any dietary influences on metabolic status. Clinical and pathological testing was performed on apparently looking healthy brood fishes only.

Individual rainbow trout was rapidly netted and carefully placed in a circular tank and was anesthetized with MS 222. Ten male and ten female with an average weight of 1.950±50 g were bled using 2 ml syringes from the caudal vein. Serum was separated by centrifugation at 3000 g for 15 min at 4°C. After separation all sera were maintained at 20°C until processed in the laboratory. Sex and maturity stage of the samples were determined by necroscopy. The females had distinct eggs and males had lobulated testes.

2.1. Analysis Methods

The blood of the test fishes was collected by cardiac puncture. The blood as frozen before further use. The serum of the blood was collected for the estimation of total protein (TP), albumin (Alb), glucose (Glu), creatinine (CREA), aspartate aminitransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), creatine phosphokinase (CPK), cholestrol (CHOL), complement C3, complement C4, blood urea nitrogen (BUN), immunoglobulin (IgM), amylase, calcium (Ca), iron (Fe), phosphorous (P) and magnesium (Mg).

2.2. Statistical Analysis

One way ANOVA was used to evaluate the biochemical values of rainbow trout. The results were presented as mean±SE.

3. Results

During the present experimental work, male and female rainbow trout were used to analyze the changes in blood values depending upon the sex (Table 1). Significantly higher values (P≤0.05) in males were observed for total protein, albumin, globulin, phosphorous and magnesium. The results were higher but not significant in males in case of C3 (38.3±8.5, 37.2±9.5 mg/dl), C4 (24.5±10.2, 35.7±17.7 mg/dl), alkaline phosphatase (22.3±6.9, 19.5±5.8 IU/L), amylase (157.3±44.3, 140.4±17.5 IU/L), cholesterol (55.3±13.0, 49.4±9.5 mg/dl) and creatinine phosphokinase (54.3±12.6, 50.0±9.4 IU/L). In contrast to higher values in males, some of the biochemical values showed higher values, though non-significant, in females which include aspartate aminotransferase (67.6±325.6, 89.9±23.6 IU/L), alanine aminotransferase (22.8±9.8, 30.17±15.98 IU/L), IgM (78.8±22.5, 91.2±6.28 mg/dl), Iron (26.5±6.4, 30.6±8.5 µg/dl), calcium (6.8±0.6, 6.7±0.9 mg/dl), and blood urea nitrogen (2.7±0.6, 2.8±0.9 mg/dl). The results of the present investigation set up a baseline data for future use in fisheries science to assess the stressors by analyzing any changes in normal haematological/biochemical values in male and female rainbow trout.

Table 1: *The Blood Biochemistry of Brood Male and Female Rainbow Trout (Oncorhynchus mykiss)*

Parameters	Male	Female
TP (g/dl)	6.8±2.1 a	4.4±0.8 b
Albumin (mg/dl)	0.52±0.1 a	0.35±0.2 b
Glucose (mg/dl)	5.8±0.3 a	4.14±0.5 b
Creatinine (mg/dl)	0.46±0.9	0.51±0.3
ALT (IU/L)	21.9±3.6	29.6±6.2
AST (IU/L)	70.3±7.2	88.3±4.8
C3 (mg/dl)	39.2±3.7	37.3±3.6
C4 (mg/dl)	26.3±2.5	36.1±3.4
IgM (mg/dl)	80.1±8.2	92.1±8.4
ALP (IU/ml)	23.2±2.5	19.8±2.3
CHOL (mg/dl)	54.2±2.8	48.5±2.8
CPK (IU/L)	55.2±3.4	49.8±2.8
BUN (mg/dl)	2.6±0.2	2.8±0.4
Amylase (IU/L)	161.4±22.4	149.2±11.8
Ca (mg/dl)	6.9±0.2	6.8±0.3
Fe (µg/dl)	25.4±2.1	29.2±0.5
P (mg/dl)	1.5±0.1a	1.4±0.1 b
Mg (mg/dl)	4.2±0.3a	3.7±0.2 b

The difference alphabet, indicate the significance (P<0.05) in two sample.

4. Discussion

Blood biochemistry is the most economical and authentic tool to assess the health status of fishes. The blood profile of any fish can change with the fish species, age, the cycle of sexual maturity and health conditions [6]. This is through biochemical constituents of the fish blood that the metabolic disturbances of fishes could easily be assessed [8]. Sex of a fish is a great differentiation in various components of the blood of fish, depending upon the quantum of metabolic activities taking place in the organism [5].

The present study revealed significant differences (P≤0.05) in serum protein levels in male and female trout. Plasma protein is the protein component of the blood and is vulnerable to increase with starvation or any other stress [10]. In the present case, plasma protein concentration in rainbow trout ranged from 2 to 8 g/l, as determined through refractometry [9]. Plasma protein gives an index of the health status of the brood fish [11] and as indicator of nutritional status [12]. In this study total protein for rainbow trout were consistent with those of previous study in Lake trout (*Salvelinus nemaycush*) [13]. Lupi *et al*. [3] found lower total protein in immature rainbow trout in comparison with this study. This difference is mainly due to an increase in the globulin fraction and to some extent the albumiun fraction [2, 6, 14]. Zorriehzahra *et al*. [40] observed in Persian sturgeon (*Acipensor percius*) protein levels increased with age as it is in agreement with rainbow trout and hybrid stripped bass [2, 14]. Sano [14] compared serum total protein in two sized of rainbow trout and found serum total protein in fingerling fish is lower than bigger one and increase of serum total protein coincides with age.

Albumin in fish blood performs the transportation of lipids [16] and helps in the general metabolism of fish. The rise in albumin concentration in animals due to loss through urine or faeces or through break down may result in impaired synthesis [15]. Swain *et al*. [11] reported higher albumin concentration in *Lebeo rohita* at the time of reproduction. In our study, total albumin levels of male and female rainbow trout were 0.54±0.1, 0.34±0.9 (mg/dl) respectively (P≤0.5). Similar to our finding Asadi *et al*. [17] found significantly higher albumin in male Beluga (*Huso huso*) serum. Studies on Persian sturgeon [40] revealed higher albumin values in males than female fish [6]. Manera and Britti (2006) reported a value of 1.38±0.05 (g/dl) of albumin in rainbow trout with an average weight of 240 g [18]. In contrast, Valisek and Svobodova [19] reported a value of 0.4 g/dl, which supports our finding.

Percin and Konyalioglu (2008) reported that glucose in blood serum is the best indicator of stress in fish [20]. Generally, glucose is continuously required as an energy source by all body cells and must be maintained at adequate levels in the plasma [20]. Our reference interval for glucose concentration was much lower than that for some freshwater fish for example Lake trout [13], Rainbow trout and Tilapia [2], but was consistent with the mean value of other fish like Adriatic sturgeon [21], Tench [5], Persian sturgeon and starry sturgeon [22]. Glucose concentration also varies because of size, age and nutritional and reproductive status [9]. In our study glucose levels of male and female rainbow trout were 5.6±0.7, 4.0±0.8 (mg/dl) respectively. Total protein and glucose in male were higher than female fish. The higher concentration of glucose and total protein in males than female sturgeons is attributed to higher growth rate and higher food conversion efficiency [23, 24, 25].

The liver plays a major role in cholesterol homoestatis [26] by regulating plasma lipoprotein metabolism and lipid output in bile [27, 28]. The cholesterol concentration in rainbow trout in this study was inconsistent with data reported by Manera and Britti [18] and Rehulka *et al* [29]. Cholesterol concentration varies both among and within fish species because of variations in diet activity and sexual development [9]. Alkaline phosphatase is important for active transport of nutrients, and is a cell membrane associated glycoprotein [30]. Sknoberg (1991) documented various factors including age as a factor influencing the ALP concentration [31]. Our results showed non-significant but higher values in males than females. More or less similar results were obtained for Tench [5] and bluefin tuna [20].

Creatinine levels in fish blood may show a rise after severe kidney damage [31]. The reference interval for creatinine concentration in rainbow trout in this study was 0.46±0.9 (mg/dl) for male rainbow trout (P>0.05). They were consistent with data reported by other studies foe examples; Rehulka *et al*. [29] reported creatinine concentration 0.31 (mg/dl) for rainbow trout and also it is reported 0.41 and 0.46 (mg/dl) for brook trout and brown trout respectively. Also Manera and Britti [18] reported serum creatinine concentration for rainbow trout 0.29 (mg/dl).

Similarly, the P levels of male rainbow trout were higher than those of female fish rainbow trout ($P<0.05$). Our results are supported by the work of [6, 17] who worked on Beluga and Persian sturgeon. In our study, magnesium concentration of male and female rainbow trout were (4.1±0.6, 3.6±0.7 mg/dl) respectively ($P\leq0.05$). Only data obtained from female fish is consistent with Manera and Britti [18] and male data is much higher. Also Hrubec and Smith [35, 39] found magnesium concentration for rainbow trout 3.1 mg/dl. Zorriehzahra *et al*. [17, 34, 40] found male Persian sturgeon had significantly ($P<0.05$) higher magnesium concentration, in comparison with female fish that is consistent with this study.

In our study calcium levels of male and female rainbow trout were 6.8±0.6, 6.7±0.9 mg/dl respectively ($P>0.5$), while calcium measured in other study were higher for rainbow trout like 9.92 mg/dl [35] and 12.52 mg/dl [18]. Neither stress nor circardian fluctuations have negligible effects on calcium levels [9]. Because about one half of total plasma calcium is ionized and one half is bound to plasma proteins [35-37], a decline in plasma proteins in fasting fishes should also lower plasma calcium concentrations and also increased values can be seen with acute stress [2]. Concentrations of total magnesium are lower than for total calcium in freshwater species and are tightly regulated [9]. This is a consistent with our study.

Fishes are the best indicators of environment and best experimental animals to assess the impact of various stressors which may arise in any ecosystem. A baseline data of the hematology and biochemistry of the fish are mandatory to get an idea of the impact of various stressors [38], hence evaluation of environment besides other factors seems necessary. In conclusion, the current findings can provide a helpful reference for evaluating the health, nutritional status, physiological status of individuals and routine metabolic levels of rainbow trout in aquaculture condition.

5. Conclusion

The present research revealed significant differences in blood profile of the rainbow trout, owing to the sexuality of the fish. The findings of the present research make a baseline for further research in fish haematology and may act as a baseline for the normal hematological indices of this commercially important fish.

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Maturation and Spawning Biology of a Hill Stream Major Carp *Labeo dyocheilus* (McClelland 1839) from Central Himalaya, India

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Abstract Fishes adapt to reproduce under most variable environmental conditions. Teleost gonadal structure, function and reproductive processes are controlled by biological rhythms as well as various environmental factors. Study area was W. Ramganga, Chaukhutia (Latitude: 29°53'55"N and Longitude: 79°21'22"E), for a period of three years. GSI, an indicator of sexual maturity, reported in our experiment varied from 0.008 in October to 3.933 in July for male fish and from 0.099 in October to 10.508 in July for female. Thus, onset of rainfall (Aug-Sep) was considered as the breeding season. We also found out that the maturity of ovaries and testis exist in seven stages, namely Immature or Virgin, Maturing Virgin, Developing, Maturing, Ripening, Spawning or Running, and Spent. The spawning sites were found to be in the small patches of slow running water. Specific ranges of Physico-chemical parameter were found to be responsible for spawning in this study. The experimental fish was observed to show sexual dimorphism during breeding season. During this period, the male fish became slightly brighter in color while the female fish became darken in color. This study would helpful for productive aquaculture, scientific based fishery management, and commercial utilization.

Keywords *Breeding Season; Gonado-somatic Index; Labeo Dyocheilus; Maturity Stages; Sexual Dimorphism; Spawning Sites*

1. Introduction

It is well established that the reproductive processes in fishes are controlled by endogenous biological rhythms as well as by environmental factors [1]. Teleost have inherent mechanisms for correlating gonadal structure and functions with the environmental factors. This strategy allows for spawning when conditions are optimal for survival and growth of the progeny [2]. Fishes are adapted to reproduce under most variable conditions and with these reproductive peculiarities, the structure and mode of life of their embryos, fries, fingerlings and adults are highly related to the environment [3].

Labeo dyocheilus has been categorized as vulnerable species [4, 5, 6]. For commercial utilization of any fish species, it is highly essential to have a prior knowledge of its spawning behavior, which includes the month, frequency and ecology of spawning. The knowledge on the reproductive biology of any fish is also important for productive aquaculture and scientific-based fishery management of any water body. It includes the maturation biology based on the study of developing eggs in ovary, season and frequency of spawning, reproductive capacity and ecology of the spawning grounds. The knowledge of its spawning time, frequency and viability is essential for the fish culture at the commercial level.

2. Material and Methods

The sampling was carried out at Chaukhutia (Latitude: 29°53'55"N and Longitude: 79°21'22"E) in Uttarakhand state of India, annually between September 2009 to August 2012 for the period of three years. About 12 adult individuals (25-30 cm in length) in each month were collected.

2.1. Gonado-Somatic Index (GSI)

Gonado-somatic index (GSI) is an indicative of gonadal maturation and spawning period. This includes the physical observation of fish and gonads, so as their length-weight was measured. The GSI value was calculated with the help of following formula.

$$GSI = \frac{Weight\ of\ Gonads}{Weight\ of\ Fish} \times 100$$

2.2. Determination of Maturity Stages

The maturity stages were estimated on the basis of growth rate of ova throughout the year. The different maturity stages were determined according to modified ICES scale [7].

2.3. Spawning Season

Spawning season was determined by analyzing the GSI value followed by calculating the monthly variation of different maturity stages of fish samples. The months were considered as spawning season, in which the spent fishes were available.

2.4. Determination of Spawning Grounds

Spawning grounds were examined by visual observation. The physico-chemical characteristics of the water of spawning grounds were determined according to Welch's method [8] and studied in order to have an idea of the optimum conditions required for successful spawning.

2.5. Sexual Dimorphism

The Sexual dimorphism of *Labeo dyocheilus* were examined by visual observation throughout the period of investigation.

3. Results

3.1. Gonado-Somatic Index

Minimum value of GSI for male was calculated as 0.008 ± 0.002 in October, whereas the maximum value 3.933 ± 0.665 in July. It can also be seen from the GSI data that it increases regularly from the

month of October to till July and then further decreases. GSI for female was minimum 0.099 ± 0.016 in month of October, where onwards from which it increased till a maximum value of 10.508 ± 1.881 in month of July. Thus, above result showed the Monsoon (GSI peak value in July) was the spawning season for both male and female fishes. Moreover, the GSI value of female fish was found to be always higher than that of male fish throughout the study. (Table 1)

Table 1: *Average Monthly Variation on Gonado-Somatic Index (GSI)*

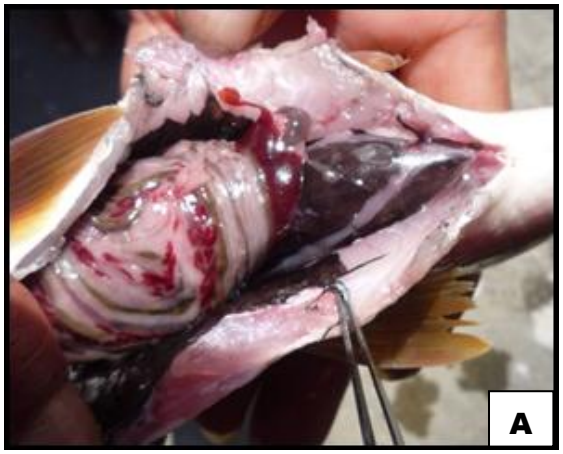
Month	GSI of Male	Average ± S.D.	GSI of Female	Average ± SD
Sep	0.035-0.056	0.042±0.006	0.178-0.254	0.213±0.034
Oct	0.005-0.013	0.008±0.002	0.075-0.124	0.099±0.016
Nov	0.084-0.123	0.101±0.011	0.271-0.365	0.315±0.036
Dec	0.248-0.345	0.292±0.034	0.625-0.876	0.745±0.112
Jan	0.498-0.591	0.546±0.037	0.784-1.241	0.982±0.089
Feb	0.897-1.542	1.245±0.285	1.013-1.924	1.574±0.281
Mar	1.808-2.428	2.023±0.254	2.865-4.752	3.927±0.817
Apr	2.572-3.412	2.847±0.154	3.942-6.545	5.175±1.023
May	2.902-3.537	3.261±0.112	5.546-9.548	7.857±2.021
Jun	3.216-3.882	3.568±0.321	8.513-11.966	9.995±1.058
Jul	3.241-4.521	3.933±0.665	8.581-12.768	10.508±1.881
Aug	0.712-1.211	0.920±0.153	2.482-3.687	3.102±0.410

3.2. Determination of Maturity Stages

Seven maturity stages namely immature 1st (Immature or Virgin), immature 2nd (Maturing Virgin), maturing 1st (Developing), maturing 2nd (Maturing), mature 1st (Ripening), mature 2nd (Spawning or Running) and spent (Spent) were identified for both male and female fishes. (Table 2 and Figure 1)

Table 2: *Different Stages of Maturity of Labeo Dyocheilus*

Stages	Maturity Stages		Ova Diameter (OMD) (1 OMD = 0.016 mm)	Peak Value
	ICES Scale (Wood, 1930)	Stages Name in Present Study		
Stage - I	Immature 1st	Immature or Virgin	3 OMD – 25 OMD	20 OMD
Stage - II	Immature 2nd	Maturing Virgin	5 OMD – 55 OMD	42 OMD
Stage - III	Maturing 1st	Developing	15 OMD – 80 OMD	58 OMD
Stage - IV	Maturing 2nd	Maturing	25 OMD – 100 OMD	75 OMD
Stage - V	Mature 1st	Ripening	35 OMD – 140 OMD	110 OMD
Stage - VI	Mature 2nd	Spawning or Running	60 OMD – 170 OMD	155 OMD
Stage - VII	Spent	Spent	15 OMD – 60 OMD	48 OMD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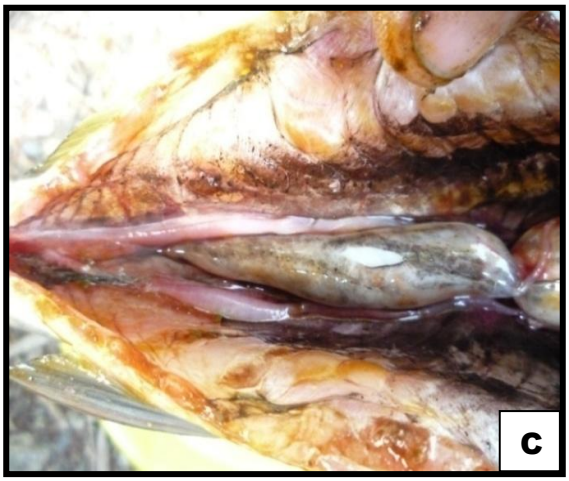
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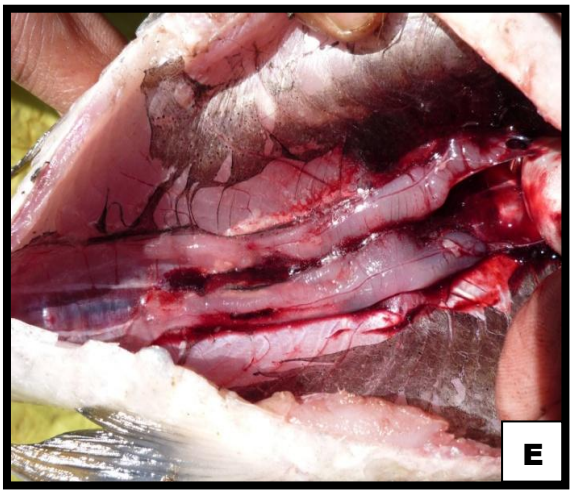
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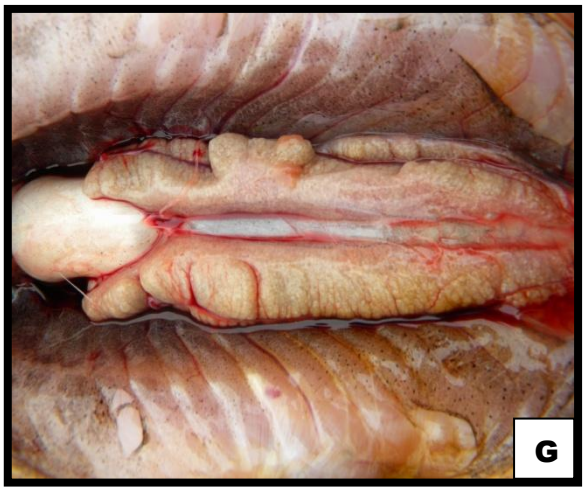
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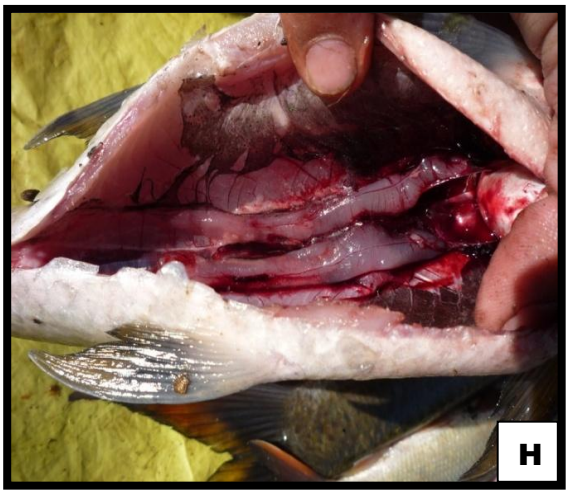
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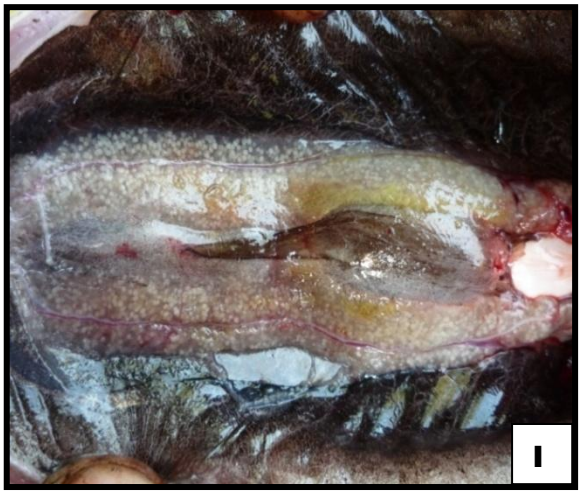
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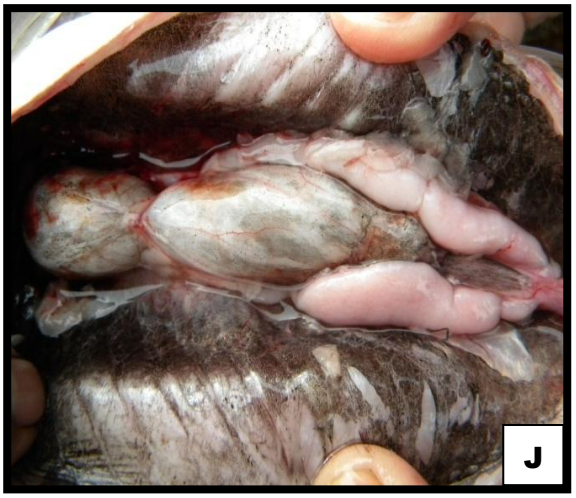
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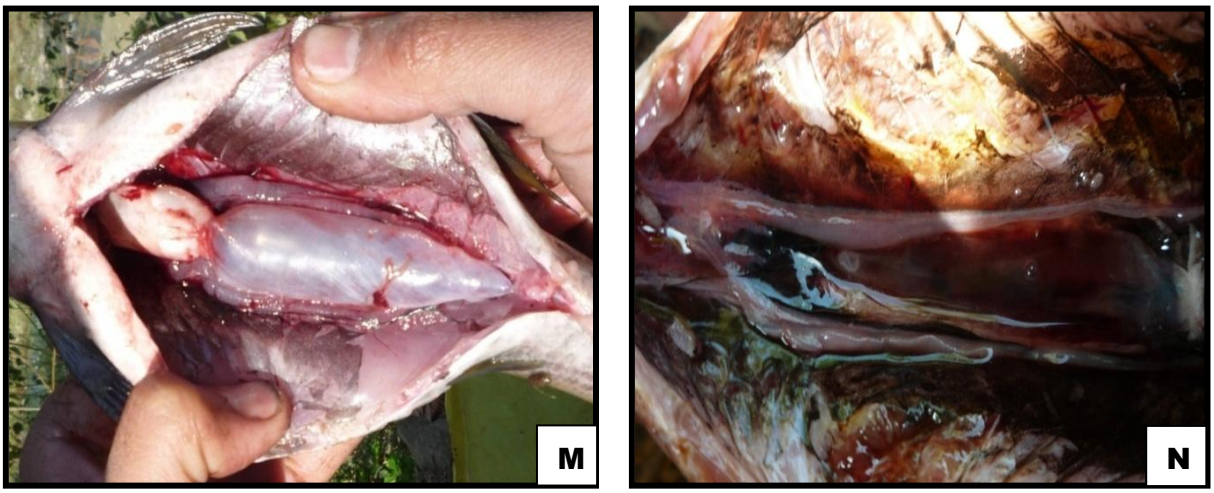

Figure 1: *Stage I - Immature or Virgin (A= Ovary, B= Testis), Stage II - Maturing Virgin (C= Ovary, D= Testis), Stage III - Developing (E= Ovary, F= Testis), Stage IV – Maturing (G= Ovary, H= Testis), Stage V - Ripening (I= Ovary, J= Testis), Stage VI - Spawning or Running (K= Ovary, L= Testis), Stage VII - Spent (M= Ovary, N= Test*

Stage I: Ova diameters were vary from 3 to 25 OMD (ocular microscope diameter), not visible by naked eyes. We obtained a peak at 20 OMD, i.e. maximum number of ova of this diameter. The testes fall into this category were thread-like in structure, cylindrical and extending about two third of the body cavity, not differentiated with naked eye.

Stage II: The first batch of ova separated from the general egg stock with a diameter range of 5-55 OMD, peak at 42 OMD was obtained. Testes were slightly thicker, slightly whitish and increased in volume.

Stage III: Ovaries were slight dark yellow color and the ova collected were ranges from 15-80 OMD, peak at 58 OMD. The testes observed in this stage were thicker, whitish and increased in volume and weight.

Stage IV: The ova diameters varied from 25 to 100 OMD with a peak value of 75 OMD. Testes became much thicker, lobulated and were irregular in shape, dark white in color.

Stage V: The ovary got slightly dark yellow color and enlarge, diameter varied from 35-140 OMD with peak at 110 OMD. The testes were deep white in color, having the maximum weight and occupied entire length of the body cavity.

Stage VI: Large sized ova with full yolk were found in each ovary and come out by applying a slight pressure on the abdomen, ova diameter vary from 60-170 OMD and peak at 155 OMD. Testis also released milt by applying high pressure on abdomen. Milt was thick, white and adhesive.

Stage VII: Ovary got shrunken; flaccid, dirty light yellow in color. Un-spawned ova diameter was varied from 15 to 60 OMD and peak at 48 OMD. Testes appeared flaccid and thin due to extruded milt.

3.3. Spawning Season

It is clear that in the month of October all fishes were immature, with the minimum GSI value in this month. From November to June, collected samples were in increasing percentage from immature through maturing to matured stage. It also observed that there was maximum number of matured fishes in the month of July, in which the GSI value of fish samples was highest. In the month of August and September, the matured fishes started spawning. Thus, these two months were collectively called as breading season of *Labeo dyocheilus* in which the GSI started decreasing from its maximum value. So *Labeo dyocheilus* pattern of spawning was single spawning or short durational spawning or protracted spawning. (Table 3)

Table 3: *Percentage Occurrence of Different Maturity Stages of Labeo Dyocheilus*

S. No.	Month	Percentage of Fish						
		Stage I	Stage II	Stage III	Stage IV	Stage V	Stage VI	Stage VII
1	Sep	-	-	-	-	-	5	95
2	Oct	100	-	-	-	-	-	-
3	Nov	90	10	-	-	-	-	-
4	Dec	82.5	17.5	-	-	-	-	-
5	Jan	75	25	-	-	-	-	-
6	Feb	-	65.5	34.5	-	-	-	-
7	Mar	-	28	52	20	-	-	-
8	Apr	-	-	61	39	-	-	-
9	May	-	-	-	74.4	25.6	-	-
10	Jun	-	-	-	32.75	67.25	-	-
11	Jul	-	-	-	-	24	65	10
12	Aug	-	-	-	-	-	22.25	77.75

3.4. Spawning Grounds and Ecology of Spawning Sites

The spawning sites were found in the slow running water and the eggs of the fish samples were observed on small and medium sized stones nearby the confluence of small tributaries in side of riverbank at a depth of 24 to 30 cm. During the spawning season, the physico-chemical parameters of water of spawning ground were recorded. The water temperature responsible for breeding was varied from 21.58°C to 19.92°C, velocity of water current ranged between 0.899 to 0.741 m/s, turbidity ranged from 94.4 to 58.4 NTU, pH varied from 7.9 to 7.86, dissolved oxygen content from 8.08 to 8.92 mg/l, total alkalinity from 59.34 to 67.24 mg/l and total hardness from 80.3 to 83.03 mg/l. Thus, a specific range of these physico-chemical parameters is responsible for spawning. (Figure 2)

Figure 2: *Spawning Ground*

3.5. Sexual Dimorphism

Labeo dyocheilus was observed to show sexual dimorphism only during the breeding season. During this period, the male fish became slightly brighter in color while the female fish became darken in color, but no sexual dimorphism was seen in other months of the year. Hence, the differentiation between male and female fishes was difficult round the year except during breeding season. (Figure 3).

Figure 3: *Sexual Diamorphism in Male and Female*

4. Discussion

The maximum GSI values for both the sexes of *L. dyocheilus* were observed when the fish attained full maturity in the month of July. In the present work the spawning fall in GSI values was noticed during August indicating the complete spawning of fish. GSI value of female fish was found to be always higher than that of male fish because of larger size of ovary than testis. Our findings were supported by literature [9, 10, 11].

For the determination of maturity stages, most of the ichthyologist follows the trend set in ICES scale [7]. In the present work, seven maturity stages were determined for Labeo *dyocheilus.* Seven maturity stages are frequently observed in the literature for a number of fishe viz. *Crossocheilus latius latius, S. curvifrons, P. sulcatus* from Jhelum River and Alaknanda River etc., [10, 11, 12].

It was observed in the present study that *Labeo dyocheilus* spawns during August-September in the Western Ramganga River. We arrive at this conclusion because the first spent fish was collected in the month of August and the percentage of spent fish in the collection was regularly increased up to September. Thus during the spawning season (August-September), the fish changes its stage from stage VI to stage VII. A study on *Tor khudree* shows that it breeds during August to September which also supports our findings [13]. *Tor putitora* and *Tor mosal* also breed during same period August to September [14, 15]. We can also summarize the breeding season (August-September) of *Labeo dyocheilus* was the season of onset of rainfall. In the literature [16, 17, 18, 19] synchronization of sexual maturation and reproduction with onset of rainfall has also been documented in genus *Labeo.*

We observed in our study that from October onwards there were no spent fish and maximum fish were either in resting phase (stage I and II) or started developing new batches of eggs (stage III and IV), which is obvious from Table 3. We can also conclude that during the winter season (November to January); the growth of ova was slow due to the low temperature of water. There was a rapid growth of ova during the month of May to July due to favorable environmental conditions.

Thus, the present study concludes that *Labeo dyocheilus* is a single spawner i.e. has a single frequency of spawning, and spawns from August to September. This type of spawning is referred as single spawning or short durational spawning or protracted spawning. Majority of teleost is seasonal breeders but in the Indian subcontinent a vast majority of the freshwater fishes breed during the monsoon season [20]. Similar pattern of spawning has also been reported in *Sardinella longiceps*, which was observed as single spawner [21]. A study on *Glyptothrox pectinopterus* proved information that it was also a single spawner species [22].

In the present work it was noticed that *Labeo dyocheilus* spawns in the slow running water with the velocity of 0.741 to 0.899 m/s under stones and pebbles and the required water temperature is ranged from 19.92°C to 21.58°C, turbidity ranged from 58.4 to 94.4 NTU, pH was from 7.86 to 7.9, dissolved oxygen content from 8.08 to 8.92 mg/l, total alkalinity from 59.34 to 67.24 mg/l and total hardness from 80.3 to 83.03 mg/l. Thus it was concluded that the spawning of *Labeo dyocheilus* depends upon specific range of physico-chemical parameters. These values were noticed from habitat side during spawning time. Many possible environmental factors viz. photoperiod, seasonal rainfall and temperature play crucial role in regulating the reproductive cycle in teleost fishes [3]. It has been noticed for *Tor chilinoides,* spawns in the shallow of River Nayer along with rising level of water, turbidity and lowering down of pH are prime factors responsible for spawning [23]. *Crossocheilus latius latius* spawns in monsoon with flooded river and low pH initiate spawning [24].

5. Conclusion

In summary, the monthly variation in the GSI of male and female fishes were computed and found that the GSI value of female fish is always higher than that of male fish. We also concluded that the maturity of ovaries and testes exist in seven stages. It is also summarized that in the month of July the maximum number of fishes were in maturing stage, having highest GSI value and spawning season of fish was found to be from August to September. The study also investigated the breeding season of *Labeo dyocheilus* as the season of onset of rainfall. Moreover, the spawning of this species also found to depend upon several physico-chemical parameters such as seasonal rainfall, water velocity, temperature, pH, hardness, and alkalinity. Study fish was observed to show sexual dimorphism during the breeding season only.

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Research Article | Open Access

Low Cost Innovative Technology for Seed Production of *Cirrhinus Reba* (Hamilton, 1822) at Backyard of Murshidabad District, West Bengal, by using Ovaprim

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Abstract Induced spawning of fresh water Raikhor Bata, *Cirrhinus Reba* was carried out in captivity using Ova prim (sGnRH+ Domperidone). The optimum dose of Ovaprim (0.3 ml/kg of body weight for male and 0.5 ml/kg for female) were standardized based on three experiments, viz., fecundity (relative fecundity), and response time (hrs) and fertilization rate at different doses. Maximum fecundity (9120) obtained at 0.5 ml/kg of body weight. Hatching conducted in a specially designed inverted at 2 liter thumps up bottle following the mechanism of glass jar hatchery and the spawns are reared at nursery pond by placing the hatchlings in the hapa. This innovative and cost effective technology will certainly add a new era in the seed production of *Cirrhinus Reba* including some other indigenous fishes of fresh water sector.
Keywords *Raikhor Bata; Induced Breeding; Relative Fecundity; Hatching*

1. Introduction

Cirrhinus reba (Hamilton, 1822), commonly known as Kharkebata or Raikhor bata, is a widely distributed endemic freshwater minor carp India, Bangladesh, Pakistan & Nepal. In India, it is a very common species of rivers and ponds in the Gangetic belt of the northern region and also in the river Cauvery at the south. In West Bengal, especially in the northern part, it fetches considerable market demand for its consumer preference due to good taste as well as soft and less bony flesh (Chondar, 1999). The initial growth rate is quicker than Catla and due to its compatibility the fish can be considered as a major candidate species for polyculture with Indian Major Carps (Job, 1954). In addition, *C. reba* is adorned with attractive appearance along with beautiful hexagonal scales and

bluish longitudinal bands above lateral line which offers the fish an ornamental value. All these qualities allure the farmers to consider the fish as a potential species for the diversified farming. Non-availability of quality seed supply, due to want of any standard procedure for breeding in captivity, the population of such an important item for culture is decreasing by days.

C. Reba is a perennial breeder while the single spawning period restricted to south-west monsoon during June to August in W. Bengal. The fish does not spawn in captivity even though attain full maturity. (Chondar, 1999) reported responsive result following inducing breeding in captivity using piscine pituitary gland extract even at a small dose. In this study, an attempt was made to standardize the breeding of the fish, using synthetic inducing agent Ovaprim followed by stripping method. Subsequently hatching of the fertilized eggs conducted in a low cost glass jar hatchery system at the backyard to assure ready to hand seed supply of *C. Reba*.

1.1. Systematic Position

Phylum: Chordata
Class: Actinopterygii (Ray-finned fishes)
Order: Cypriniformes (Carps)
Family: Cyprinidae (Carps and minnows)
Genus: *Cirrhina*
Species: *C. Reba*

1.2. Habitat

Cirrhinus Reba is found in large streams, rivers, tanks, lakes and reservoirs (Menon, 1999; Talwar and Jhingran, 1991). It attains a total length of 30 cm (Menon, 1999) and breeds once in a year in the flooded shallows regions of river during June to early September (Gupta, 1975). The fish feeds on plankton and detritus (Talwar and Jhingran, 1991). No information is available on the definite habitat trends of the fish and is available in all the rivers and clear streams of Bangladesh (Bhuiyan, 1964) including tanks, canals, ponds, beels and inundated fields (Talwar and Jhingran, 1991; Rahman, 1989).

1.3. Food and Feeding

Feeds mainly on plankton and detritus (Talwar and Jhingran, 1991) but also on mud, vegetables, crustaceans and insect larvae (Bhuiyan, 1964). Feed ingredients mainly composed of algae (10%), higher plants (70%), protozoa (5%), crustaceans (10%) and mud and sands (5%) (Mookerjee *et al*., 1946)

2. Materials and Methods

2.1. Collection of Brood Fishes and their Acclimatization

Disease free, healthy and gravid male (average length 15 cm and weight 135 gm) and female (average length 17 cm and weight 150 gm) were collected from local fish ponds in the month of June and were acclimatized for 48 hours, prior to breeding operation, in the glass aquarium (120 cm x 45 cm x 45 cm) filled with unchlorinated tap water (pH 7.2 + 0.15, DO 5.72 + 0.80 mg/L, CO_2 10.16 + 2.3 mg/L, total alkalinity 146 + 6.13 mg/L as $CaCO_3$ and hardness 107 + 8.2 mg/L as $CaCO_3$) having aeration facilities. During acclimatization male and female brooders were kept in separate aquaria side by side to enhance the desire for mating and no feed was applied. Before release into aquaria the fishes were treated with 1% $KMnO_4$ solution for 5 minutes to make pathogen free.

(***Figure** 1a)*

***(Figure** 1b)*

***Figure 1a and b**: Brood Fish of Raikhor Bata, C. Reba*

2.2. Selection of Brooders

Gravid males were identified by the roughness on the dorsal side of pectoral fins at the base and stout abdomen with elongated, introvert and whitish vent. On slight pressure on the abdomen prior to vent milt oozes out (Figure 1a, Figure 2). The scales on the flanks, nape and anterior-dorsal side are rough and sandy in texture. Fully gravid female brooders, on the other hand, are characterized by tender bulging abdomen with extrovert, fleshy, round and pinkish vent. On slight pressure eggs comes out one by one. Pectoral scales are smooth to touch (Figure 1b, Figure 3).

***Figure 2:** Mature Male Raikhor bata, C. Reba*

***Figure 3:** Mature Female Raikhor bata, C. Reba*

2.3. Breeding Operation

Generally breeds at the beginning of summer and extends from June - September when the temperature is high and rainfall is excessive (Bhuiyan, 1964). The fish breeds along the shallow inundated region along the river (Talwar and Jhingran, 1991). Artificial breeding in captivity was conducted between 10.00 pm and 10.30 pm due to availability of cool and congenial environment for breeding. Ovaprim, a synthetic inducing agent [combination of salmon gonadotropin releasing hormone analogue (D-Arg6 Trp7 Leu8 Pro9-Net) and domperidone dissolved in calibrated quantities of non-toxic organic solvent] (Nandeesha et al., 1990), a product of Syndel Laboratories, Canada, was administered to the brooders intramuscularly in the caudal peduncle above the lateral line at 45^0 angle. The required doses were 0.3 ml and 0.5 ml per kg body weight to male and female fishes respectively. Both male and female fishes were given single dose and the induced fishes (male & female) were kept in separate aquaria. A sex ratio of 2♂:1♀ was maintained.

On the very next day, between 5 to 6 o'clock in the morning, eggs were stripped out into a dry and clean stainless steel plate and subsequently milt were oozes out over the eggs (Figure 4). The milt and eggs were mixed thoroughly with the help of avian feather (Figure 5). A little freshwater was added to facilitate fertilization process. The fish indicate their readiness for stripping by rubbing body against the wall (cloth) of hapa.

Figure 4: *Mature Female Brood Fish before Stripping*

Figure 5: *Fertilized Egg after Stripping*

2.4. Hatching Operation

Fertilized eggs were released into plastic thumps up bottles (2 L capacity) @ 500 ± 50 per unit for hatching. Bottles were arranged upside down in a row over a wooden platform having holes depending upon the number of bottles while the bottom end were being attached linearly to a iron rod. The mouth of each bottle remains inserted into the hole on wooden plank while a slice is removed from the bottom end. The mouth at the lower end remained connected to a water flow by rubber tube connected to water line having screw valve to control flow and to maintain constant circular upside down motion within the bottle. This can be termed as mini glass jar hatchery, easy to handle at the backyard with less requirement of water and ensures higher hatching rate (Figure 6). This is a very low cost glass jar hatchery for poor farmers. On campus training at KVK, Murshidabad farmers are well trained and practically shone its operation (Figure 7, 8).

During incubation period water quality parameters were tested following standard method (APHA, 1995). The whole cycle of the hatching experiment was repeated thrice in order to overcome methodological errors and the results obtained from all three sets of experiment were statistically analyzed.

Figure 6: *A Single Thumps Up Bottle with Fertilized Egg*

Figure 7: *Demonstration of Low Cost Hatching Unit to Farmers*

Figure 8: *Sets of Inverted Thumps up Bottle Used as Glass Jar Hatchery*

In the present study, both male and female *C. reba,* injected with Ovaprim, responded well to stripping with complete release of eggs. Rate of fertilization was 86.4%. Fertilized eggs were demarsal, non-adhesive, non-floating and transparent. Water hardened eggs were spherical in shape and 2 mm in diameter. Eggs were gradually transformed into elongated tubular in structure. Hatching took place after 8-10 hours of fertilization at 27^{o}C with a success rate of 91.6%. Newly hatched spawns were transparent and without any pigmentation over the body, which started after 5-6 hours. At hatching body length measured 2.75 mm.

Physico-chemical parameters of water during incubation and hatching recorded a more or less congenial temperature of 27^{0} C, dissolved oxygen 5.5 mg/L, and pH 7.6.

(i) Physico-Chemical Parameter

Temperature: 26.5-27.8C
pH: 7.2-7.4
D.O.: 5.2-6.4 mg/L

3. Results

3.1. Relative Fecundity (No. of Ova)

In this experiment, it was observed that relative fecundity vary in different observations at 0.3 ml/kg of body weight dose (Table 1). Fecundity was highest at 10th observation is 9,120 (No. of ova) and

lowest fecundity was observed at 6th observation is 3,254 (No. of ova) (Figure 9).

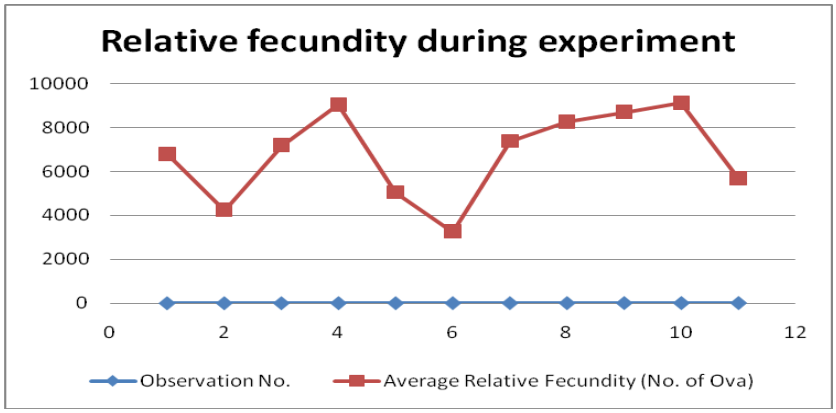

Figure 9: *Relation between Relative Fecundity and Different Observation*

Table 1: *Relative Fecundity of Female Raikhor Bata at the Dose of 0.3 ml/kg of Body Weight*

Observation No.	Average Relative Fecundity (No. of Ova)
1	6780
2	4250
3	7181
4	9023
5	5042
6	3254
7	7374
8	8256
9	8691
10	9120
11	5680

3.2. Eggs Diameter (mm)

Fully developed eggs (10-15) were collected in 5 ml plastic test tube containing Simpson solution (2 ml) as preservative. The swollen egg diameter was measured by ocular micrometry. It was observed that, the maximum (3.0 mm) and minimum (2.5 mm) fertilized egg diameter were found (Table 2). The fertilized egg diameter (mm) and relative fecundity was negatively correlated. In Figure 10 showed that there is an inverse relation between relative fecundity (No. of ova) and egg diameter (mm).

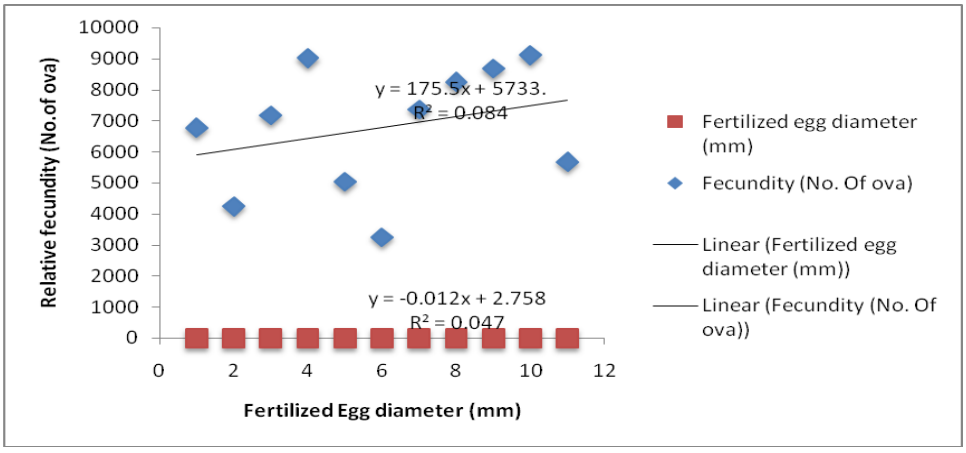

Figure 10: *Correlation between Relative Fecundity (No. of Ova) and Egg Diameter (mm)*

Table 2: *Fecundity in Relation to Egg Diameter*

Fecundity (No. of ova)	Fertilized Egg Diameter (mm)
6780	2.6
4250	3.0
7181	2.6
9023	2.5
5042	2.8
3254	3.0
7374	2.7
8256	2.5
8691	2.5
9120	2.5
5680	2.8

3.3. Spawn

In this experiment, it was observed that spawn length (mm) was varied with relative fecundity (Table 3). The spawn length (mm) and relative fecundity was negatively correlated. It showed that there is an inverse relation between relative fecundity (No. of ova) and spawn length (mm).

Table 3: *Length of Spawn as Observed During Experiment*

Fecundity (No. of ova)	Spawn Length (mm)
6780	3.2
4250	3.4
7181	3.3
9023	3.1
5042	3.0
3254	3.2
7374	3.2
8256	3.1
8691	3.2
9120	3.0
5680	3.2

4. Description

In this present study, induced breeding was done using Ovaprim a synthetic hormone at 0.3 mi/kg of body weight and 0.5 ml/kg of body weight for male and female respectively. The highest relative fecundity (No. of ova) was found (9120) and lowest fecundity was observed 4250. Here a negative correlation was found between relative fecundity (No. of ova) and fertilized egg diameter (mm). It showed that there is an inverse relation between relative fecundity (No. of ova) and spawn length (mm).

5. Conclusion

This is very useful and eco-friendly technology for the fish farmers. To use this technology farmer can produce huge numbers of quality fish seed for their own farm.

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Diversity of Macrozoobenthos in Morand River- A Tributary of Ganjal River in Narmada Basin

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Abstract The aim of present study was to assess the diversity of macrozoobenthos in Morand River which is the major tributary of Ganjal River and comes under Narmada basin. For the present investigation, eight sampling stations were selected at different locations and results revealed that 31 taxa of macrozoobenthos were recorded from these sampling stations. During the rapid study, it was observed that phylum arthropoda was in dominant position than phylum mollusca and annelida. Macrozoobenthos diversity was assessed by using Shannon diversity index and Margalef diversity index.

Keywords *Diversity; Macrozoobenthos; Morand River*

1. Introduction

River ecosystem encircles a wide spectrum of habitats spanning a continuum from small mountain springs to large lowland rivers [1]. Rivers are the most important fresh water resources for living being and provide a home to many plants and animals including macrophyte, plankton, insects and molluscs etc. Benthic communities are very important in aquatic ecosystem and common inhabitants of lakes and streams. These organisms usually inhibiting the bottom substrate for at least part of their life cycle [2]. Macrozoobenthos are generally visible with the naked eyes, and have limited mobility which plays a significant role in the food chain because of their ability to convert low quality and low energy detritus into better quality food for higher organisms in the food web. The abundance and distribution of macrozoobenthos have been used as biomonitoring tool for fresh water pollution.

In the present study, diversity of macrozoobenthos was carried out on the Morand River with the objective to collect first hand and baseline information about diversity of macrozoobenthos as there is no previous data on this river.

2. Material and Methods

2.1. Study Area

Morand river is a perennial river which originates from Satpura mountain range near Chicholi village in Betul district of Madhya Pradesh at 78^0 16'E longitude and 22^0 00'N latitude. Morand is a rain fed river having length of 136.29 km, watershed area of 1143.52 sq km which makes this river the only major tributary of Ganjal River, which is a tributary of River Narmada in the central region of Narmada basin. After covering different types of landscapes Morand River meets the Ganjal River near Timarni Bridge in Hoshangabad district. Local people called Morand River *"The Baghin of Satpura"* because of its sudden increase in water level and flow during monsoon season. The location of the study area is shown in Figure 1 (Map 1).

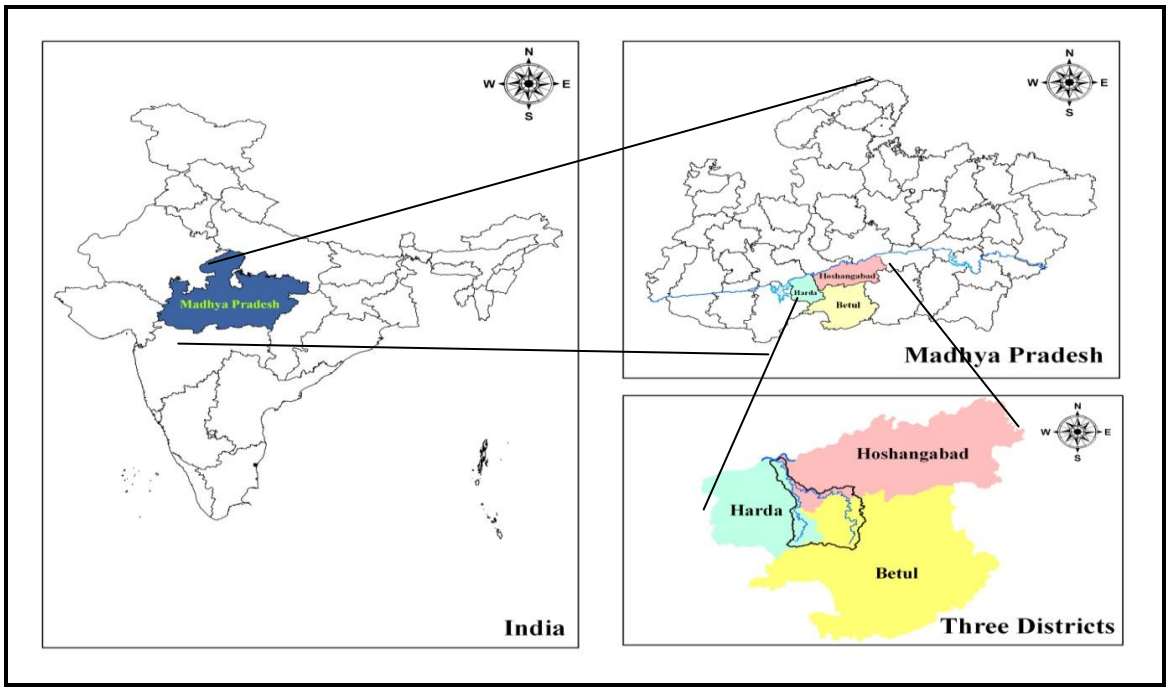

Figure 1: *(Map 1) Location of the Study Area*

2.2. Sampling Stations

Eight sampling stations were selected for the present study which is shown in Map 2. Geographical co-ordinates of all sampling stations are shown in Table 1, whereas these stations are systematically arranged from origin of the river up to the confluence with Ganjal River.

Table 1: *Geographic Position of the Sampling Stations*

S. No.	Sampling Station	Longitude	Latitude
1.	Kondhar	77^0 39' 53.6" E	22^0 1' 39.52" N
2.	Jhapal	77^0 39' 39.07" E	22^0 7' 10.08" N
3.	Sangwani	77^0 38' 1.85" E	22^0 12' 11.47" N
4.	Bainth	77^0 36' 12.13" E	22^0 21' 30.49" N
5.	Lokhartalai	77^0 26' 12.13" E	22^0 21' 40.21" N
6.	Ranipur	77^0 24' 0.8" E	22^0 21' 25.6" N
7.	Amlara Khurd	77^0 20' 46.44' E	22^0 22' 38.55" N
8.	Confluence with Ganjal	77^0 18' 59.49" E	22^0 24' 6.05" N

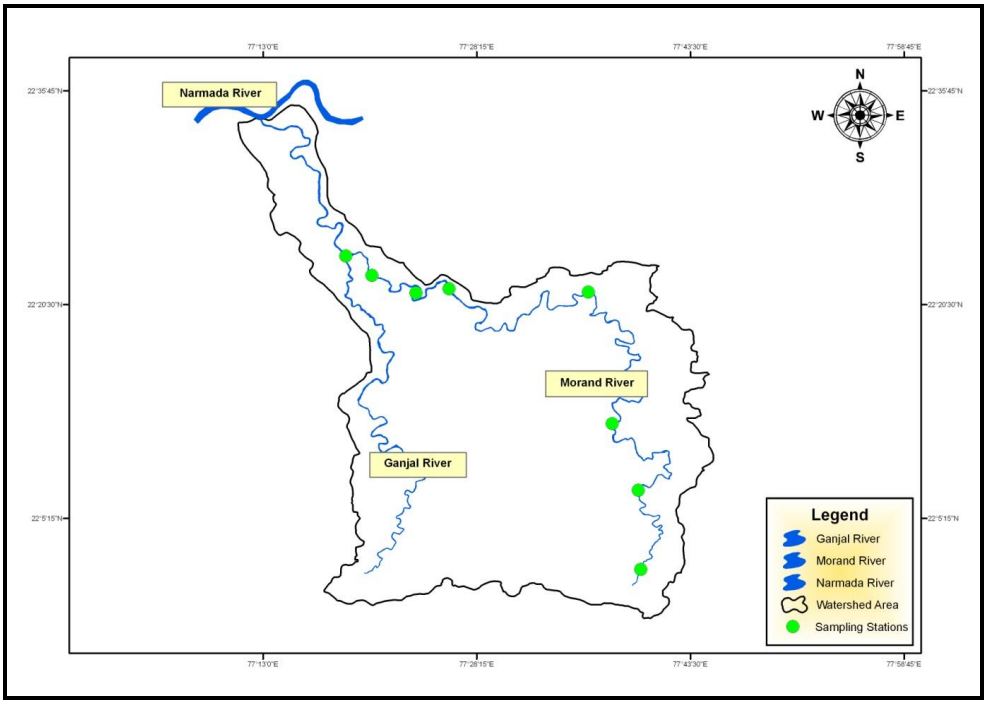

***Figure 2**: (Map 2) Selected Sampling Stations on Morand River*

2.3. Collection, Sieving, Sorting, Preservation and Identification of Macrozoobenthos

First of all, habitats of macrozoobenthos were identified in the river to collect samples. Different gears were used to collect macrozoobenthos from different types of habitats. Where the depth was less than 1 meter; Surber sampler was used, from macrophytes where macrozoobenthos fauna was in attached form D- Frame net was used, in some areas where large stones, pebbles were found, here Kick net was used to collect the macrozoobenthos fauna [3].

Collected samples were sieved from brass sieve having mesh size of 0.5 to 0.6 micron. Animals were washed properly and sorting was made on the field using forceps and brushes. Separate screw cap wide mouth reagent grade plastic bottles were used for storage of animals followed by 5% formalin as preservative.

After completion of field procedure samples were transferred to the laboratory with utmost care. Macrozoobenthos fauna were identified to the lowest possible taxonomic levels as per requirement. Stereo microscope and hand lens with 6x zoom capacity were used to observe the finest details about the animals. In this process, organisms were identified up to the genus or species level using different monographs or key which are subject of availability in the laboratory [4, 5, 6, 7, 8].

2.4. Data Analysis

Analysis of raw data is a logistic support towards making proof of any work. After generation the raw data were compiled properly. In the process of statistical analysis two diversity indices were analyzed i.e. Shannon and Margalef. Shannon index is an index applied to biological systems by a mathematical formula used in communication area by Shannon in 1948 [9]. This is the most preferred and common index among the other diversity indices and its values are between 0.0 and 5.0. Generally, results come between 1.5 and 3.5, and it exceeds very rarely up to 4.5 [10]. Above 3.0 value indicates about the structure of stable and balanced habitat whereas, under 1.0 value indicates about the pollution and habitat degradation in habitat structure.

$$H' = \sum [(n_i / N) * (\ln n_i / N)]$$

H'= Shannon Diversity Index
n_i = number of individuals belonging to i species
N = Total number of individuals

Margalef diversity index has no limit value and it shows a variation depending upon the number of species and used to calculate species richness. It can be used for comparison of sites [10].

$$d = (S - 1) / \ln N$$

d = Margalef Diversity Index
S = Total number of species
N = Total number of individuals

3. Results and Discussion

During the investigation, 31 taxa of macrozoobenthos were recorded from eight sampling stations with total of 295 individuals belonging to three phyla *viz.,* mollusca, annelida and arthropoda. Among them 10 species are of molluscan community which are represented by two classes *viz.,* gastropoda and bivalvia. Class gastropoda was represented by only one order mesogastropoda with two families, four genera and five species. Class bivalvia was represented by two orders *viz.,* trigoinoida and veneroida with three families, four genera and five species. Phylum annelida was represented by only one class, order, family and genera. As we know that phylum arthropoda is the largest phylum in animal kingdom and here it has been represented through three classes *viz.,* insecta, crustacea and arachnida. Class insecta is represented by five orders with thirteen families and seventeen genera, while class crustacea is represented by only one order, family and genera whereas, class arachnida is represented by two orders, two families and two genera. During the study, phylum arthropoda was in dominant condition than mollusca and annelida and its percent composition is shown in Figure 3. Similar findings were observed in the study of macroinvertebrate fauna in Ken River of central India [11]. Class level distribution with numbers of taxa is shown in Figure 4. Diversity of various macrozoobenthos species at different sampling stations is shown in Table 2.

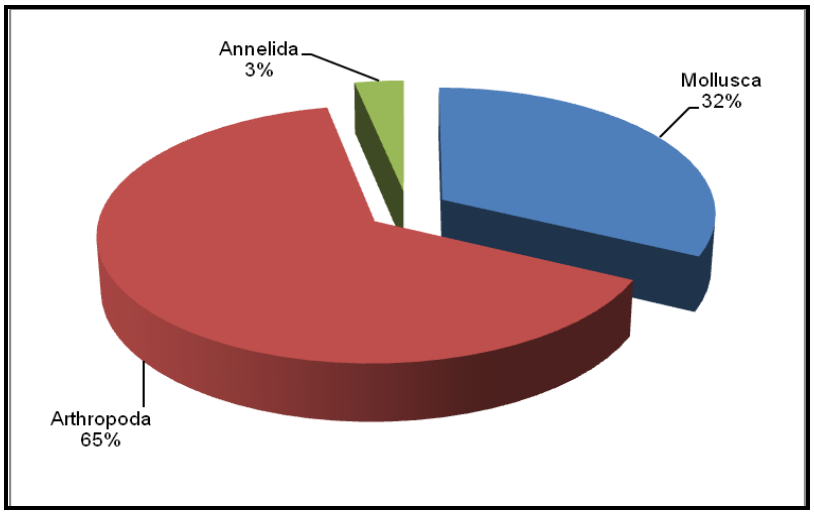

Figure 3: *Percent Composition of Higher Taxonomic Groups*

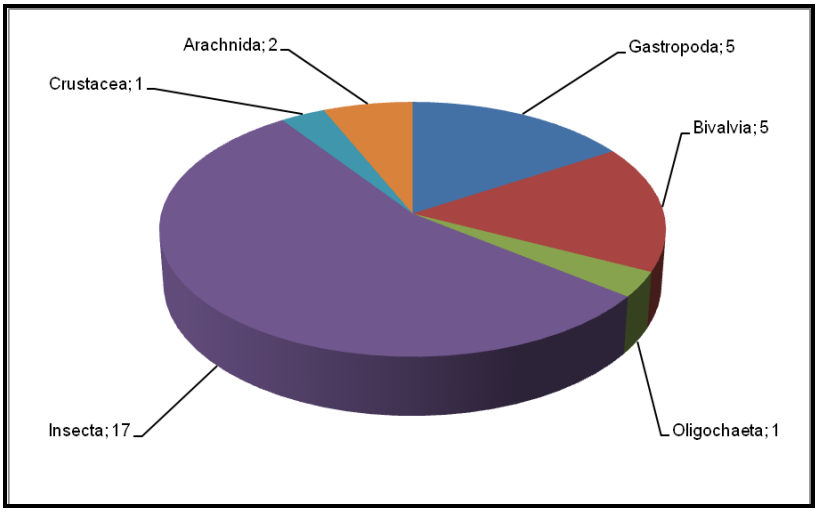

Figure 4: *Class Level Distribution of Macrozoobenthos*

Table 2: *Diversity of Macrozoobenthos Fauna*

S. N.	Taxa	Sampling Stations							
		Station-1	Station-2	Station-3	Station-4	Station-5	Station-6	Station-7	Station-8
Phylum	**Mollusca**								
Class	**Gastropoda**								
Order	**Mesogastropoda**								
1	Bellamya *bengalensis*	+	-	+	+	-	-	-	+
2	Thiara *scabra* (Muller)	+	-	-	-	-	-	-	+
3	Thiara (Melanoides) *tuberculata* (Muller)	+	+	-	-	-	-	-	+
4	Tarebia *lineata* (Gray)	+	+	-	+	-	+	-	+
5	Tarebia *granifera* (Lamarck)	+	-	-	+	-	+	-	+
Class	**Bivalvia**								
Order	**Trigoinoida**								
6	Parreysia (Radiatula) *occata* (Lea)	-	-	-	-	+	-	-	-
7	Parreysia *corrugata*	+	-	-	-	+	+	-	+
8	Parreysia (Radiatula) *shurtleffiana* (Lea)	-	-	-	-	+	+	-	-
9	Lamellidens *corrianus* (Lea)	-	-	-	-	+	+	-	-
Order	**Veneroida**								
10	Corbicula *striatella* (Deshayes)	+	-	-	-	-	+	-	+
Phylum	**Annelida**								
Class	**Oligochaeta**								
Order	**Haplotaxida**								
11	Tubifex *sps.*	-	-	+	-	-	-	-	-
Phylum	**Arthropoda**								
Class	**Insecta**								
Order	**Odonata**								
12	Gomphus *sps.*	-	+	-	-	-	+	+	-
13	Cordulegaster *sps.*	-	+	-	-	-	+	+	-
14	Anax *sps.*	+	-	-	-	-	+	+	+
15	Enallagma *sps.*	-	+	+	-	-	-	-	-

16	Lestes *sps.*	-	+	-	-	-	-	-	-
Order	**Hemiptera**								
17	Notonecta *sps.*	-	-	-	+	-	-	+	-
18	Ranatra *sps.*	-	-	+	-	+	-	-	-
19	Nepa *sps.*	-	-	+	-	-	-	-	-
20	Sigara *sps.*	-	-	-	+	-	+	+	-
21	Belostoma *sps.*	-	+	-	-	-	+	-	-
Order	**Diptera**								
22	Culex *sps.*	-	-	-	+	-	-	-	-
23	Tabanus *sps.*	+	+	+	-	-	-	+	+
Order	**Ephemeroptera**								
24	Ephemerella *sps.*	+	+	+	+	-	-	+	+
25	Caenis *sps.*	-	-	-	-	-	-	+	+
Order	**Coleoptera**								
26	Dytiscus *sps.*	+	+	-	-	-	-	-	+
27	Berosus *sps.*	-	+	+	+	-	+	-	+
28	Stenelmis *sps.*	-	-	-	-	-	+	-	-
Class	**Crustacea**								
Order	**Decapoda**								
29	Palaemonetes *sps.*	-	+	+	+	-	-	-	-
Class	**Arachnida**								
Order	**Araneae**								
30	Dolomedes *sps.*	-	+	-	-	+	-	-	-
31	Tetragnatha *sps.*	-	+	-	-	+	-	-	-
	Total	**11**	**14**	**9**	**9**	**7**	**13**	**8**	**13**

Observations revealed that phylum arthropoda is in dominant position and out of eight sampling stations genus Ephemerella (16 individuals) recorded from 6 sampling stations, Tabanus (8 individuals) recorded from 5 sampling stations, Berosus (11 individuals) recorded from 5 sampling stations, Anax (54 individuals) recorded from 4 sampling stations and Palaeomonetes (31 individuals) recorded from 3 sampling stations. High dominance of phylum arthropoda was observed in Ken River [11], in River Narmada [12], in Tons river [13] and in streams of a national park in Turkey [14].

Phylum mollusca is in second position after arthropoda and observations depicted that out of eight sampling stations in class gastropoda Tarebia *lineata* (Gray) with 26 individuals present on 5 sampling stations, Tarebia *granifera* (Lamarck) with 12 individuals present on 4 sampling stations and Bellamya *bengalensis* with 8 individuals recorded from 4 stations respectively. On the other hand in class bivalvia Parreysia *corrugata* with 10 individuals recorded from 4 stations and Corbicula *striatella* (Deshayes) with 6 individuals recorded from 3 stations. In River Narmada dominance of Tarebia *lineata* (Gray), Tarebia *granifera* (Lamarck), Bellamya *bengalensis* and Corbicula *striatella* (Deshayes) was also reported [15]. Similar observations were reported in River Barak and its tributary in Assam [16] and in River Nile [17].

At station 2, 6 and 8 taxonomic richness was higher than other stations due to presence of heterogeneous substrate type (Boulder>Cobble>Pebble>Gravel>Sand) and dense macrophytic growth with negligible human disturbance. It is well known that land use and land cover of catchment area and habitat structure largely affect the diversity of macrozoobenthic fauna. Similar results have been obtained in Ken river [11], Tons river [13] and in Barna stream network comes under Narmada basin [18].

3.1. Statistical Findings

Shannon Diversity Index

In this study, the value of Shannon diversity index was between 1.40 and 2.26 (Figure 5, Table 3). The highest value of index was found at station 2 which shows diversified species composition of macrozoobenthos whereas, lowest value of index was observed at station 5 which shows minimum diversity of organisms rather than others. Observations revealed during the study of River Narmada the value of Shannon diversity index was between 1.14 and 2.75 [12] and in Mouri river of Khulna, Bangladesh with the range of 1.20 to 1.49 [19].

Margalef Diversity Index

The value of Margalef diversity index was between 2.41 and 3.18 (Figure 5, Table 3). This index depends on the number of species or species richness recorded at different sampling stations at different sampling occasions. During the investigation, highest value of index was recorded at station 2 while minimum value of this index was recorded at station 5 in comparison with all sampling stations. In the Semenyih River of peninsular Malaysia the species richness as reflected by the value of Margalef index ranged between 0.08 and 1.90 at seven sampling stations [20].

Table 3: *Numeric Data of the Study*

Sampling Stations	S	N	H'	d
Station -1	11	48	1.97	2.58
Station -2	14	60	2.26 **	3.18**
Station -3	9	26	1.93	2.46
Station -4	9	20	2.04	2.67
Station -5	7	12	1.40 *	2.41*
Station -6	13	54	2.00	3.01
Station -7	8	18	1.95	2.42
Station -8	13	57	2.22	2.97

Where,

S: Number of species
N: Number of individuals
H': Shannon diversity index
d: Margalef diversity index
* The lowest biodiversity diversity index value
** The highest biodiversity index value

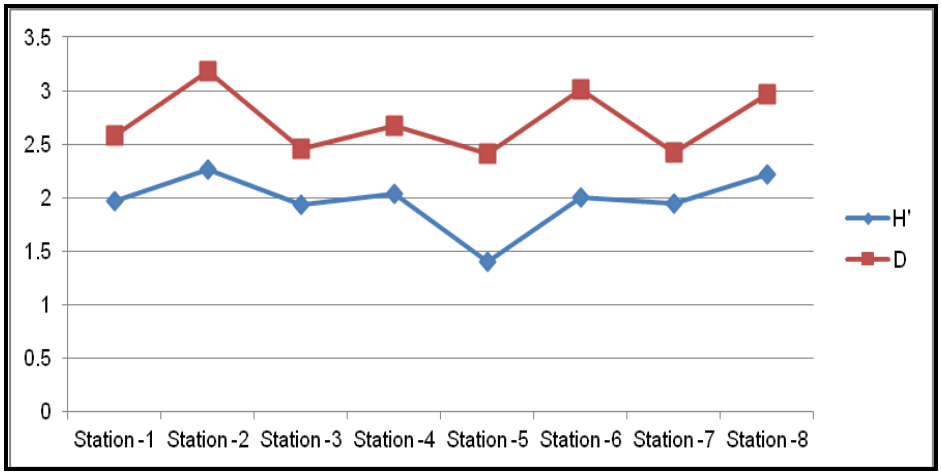

Figure 5: *Graphical Representation of Statistical Findings*

4. Conclusion

The findings of this rapid study will form baseline information as the catchment of river is experiencing gradual changes in land use pattern. The agriculture activities and road connectivity will increase human disturbance in this area. Moreover, dams are also proposed on tributaries of River Narmada and this will change habitat structure of the pristine stream condition.

Acknowledgement

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Effect of Parathion on Physio-Biological Aspects of *Notopterus notopterus* (Pallas, 1769)

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Abstract During the present experiment, *Notopterus notopterus* were exposed to lethal concentration (0.1 ppm) of parathion for a period of 60 min in triplicates. A marked reduction in the opercular beat frequency (90.0 to 30.0) and tail beat frequency (7.3 to 2.0) was observed at the end of 60 min. exposure time. The results on the hematological aspect of the experiment (5 replicas) revealed that significant ($P < 0.05$) increase in WBC (6.06 to 7.8), and decrease in Hb, RBC, PCV, MCV, MCH, MCHC and other non-specific defence cells. The increase in the WBC count is due to the non-specific immune response of the fish.

Keywords *Parathion; Toxicity; Lethal Dose; Hematology*

1. Introduction

The pesticide or pesticide residues are added ceaselessly to the aquatic environment, thereby polluting fish and water bodies. Fish and water bodies around the world offer not only food to people but also act as a means of generating employment and money for thousands. Unfortunately fish and water bodies are in grave danger because of bioaccumulation of pesticides. Pesticides suspend in water and bind with soil particles, thereby making the aquatic environment unfit for the aquatic inhabitants, which get badly affected.

Water is an important medium to affect the fish metabolism, as the epidermal cells have direct contact with the obnoxious materials carried by it, which in turn affect the fish. Fish being closely associated with the water, the bosom contact assuages the manoeuvre of chemicals into and through the mucous, skin and other external layers and becomes a pitfall to the aquatic inhabitants when nefarious chemicals, pollutants and contaminates enter the aquatic environment. The pollutants in turn disturb the physiological pathways of the fish, resulting in alteration of some important defence mechanisms including specific and non specific defence routes, hence making fish vulnerable to different stresses.

Blood scrutiny is indispensable in many fields of ichthyologic research, including toxicology, environmental monitoring, and fish farming, as blood is the only rightful indicator of the changes occurring in fish physiology (Adedeji *et al*., 2000). Researchers who have appraised the consequence of various pesticides on the behaviors and hematological responses of different species of fish include (Anees, 1978; Benarji and Rajendranath, 1990; and Svoboda *et al*., 2001). The authors found diverge riposte after exposing the fish to diverge sub lethal concentrations using the 96 h acute toxicity tests. The present paper on the effect of parathion on the blood profile of fish *Notopterus notopterus* Pallas" will be an engender in the field of fish toxicology and a value addition to the haematobiochemical profiles of fish exposed naturally and artificially to sub lethal/lethal concentrations of different pesticides.

2. Materials and Methods

Healthy *Notopterus notopterus* (Pallas) fish were purchased from local hatchery and accustomed for two weeks prior to the start of experiment. Balanced pelleted diet was given to fishes @ 2% body weight per day, containing 35% crude protein.

Parathion ($C_{10}H_{14}NO_5S$) is manufactured by Shivalic Agro Chemical Industries. The parathion is broad spectrum organophosphate pesticides used to control many insects pests. Parathion is used as non-systematic and as fumigant. For the present experimentation, 0.1 ppm of parathion was prepared. 30 L of the diluents water was used as control. 30 fishes were used for each treatment, kept in aquariums in triplicates. 0.1 ppm of the stock solution was introduced separately in each experimental tank. Mortality of the fish post-exposure was observed from 1-5 hours for any mortality.

In order to appraise the influence of pesticides on the physiological requirement of oxygen, opercular beat frequency (OBF) was computed by observing the opercular beats before and after the exposure. The OBF was enumerated using the stop watch, scrutinized for one minute after every 20 min post exposure. Tail beat frequency (TBF) is an inventory of enumerating the incidence of tail movements of the fish before and after the exposure to pesticides. Like OBF, TBF was calculated using stock watch and observing the change in movement of the fish after the exposure.

Blood samples from the confronted fishes were collected after every 10, 20 and 30 min. in fishes exposed to mixed solution of the pesticides. Blood samples were collected from the caudal fin with 21 gauge needles and 3 cc syringes before ventilatory riposte was noticeably disconsolate. PCV (%) was determined by centrifuging the blood for three minutes (3000 rpm). The hemoglobin content (Hb) of erythrocytes was determined by the haemoglobinimeter in g/100 ml. RBC value was determined by counting all the cells lying to the left and below the demarcation line of counting chamber. MCV, MCH and MCHC were calculated by the standard formulas (Blaxhall and Daisley, 2006).

The Neubauer counting chamber was used to count leucocyte, demarcated by triple lines (1 mm^2). For differentiating small and large lymphocytes, Unna-Ziehl staining was used. Standard method of Romeis (1968) was used for differentiating neutrophils by using € Granulation staining. *∂*-granulation staining was employed for differentiating monocytes and thrombocytes. The total serum protein was estimated by Gornall's biueret method (Ryan and Chopra, 1976).

3. Results and Discussion

Unpropitious effect of parathion on the physio-biological activities of *N. notopterus*, are depicted in the Tables 1 and 2. The results of the opercular beat frequency (OBF) for parathion are presented as mean±SE in Table 1. In case of 0.1 ppm parathion exposure to *N. notopterus*, the OBF decreased from 90.0±0.6 (0 min) to 58.3±2.9 (20 min post parathion exposure). The OBF showed further increase to 102.7±3.8 (40 minutes post parathion exposure) and again decreased to 30.0±0.5 (60 min

post parathion exposure). Same was the case with tail beat frequency (TBF). In case of 0.1 ppm parathion exposure to *N. notopterus*, the TBF reduced from 7.3±0.6 (0 min) to 4.7±0.6 (20 min post parathion exposure). The TBF showed further increase to 5.0±2.0 (40 min post parathion exposure) and again decreased to 2.0±0.1 (60 min post parathion exposure).

Table 1: *Summary of OBF Values of N. notopterus Exposed to 0.01 ppm of Three Pesticides*

Pesticide	Exposure Duration			
	00 min	20 min	40 min	60 min
OBF	90±0.5	58.3±2.9	102.7±3.8	30.0±0.5
	00 min	20 min	40 min	60 min
TBF	7.3±0.6	4.7±0.6	5.0±2.0	2.0±0.1

Table 2: *Mean Hematological Parameters of Notopterus notopterus Exposed to Five Trials of 0.1 ppm Parathion*

Parameter	Control	20 min			40 min			60 min		
		Min.	Max	Mean±SE	Min.	Max	Mean±SE	Min.	Max	Mean±SE
PCV (%)	25.0±0.83	21.5	24.5	23.0±0.25 ^{a}	18.8	23.2	21.0±2.11 ^{b}	16.8	19.2	18.0±0.98 ^{a}
Hemoglobin (g/dL)	8.3±0.23	6.8	7.6	7.20±0.12 ^{a}	6.65	6.95	6.80±1.25 ^{ab}	5.8	6.8	6.3±0.25 ^{a}
RBC (X 10^{6}/µL)	2.61±0.06	2.15	2.45	2.30±0.03 ^{a}	1.75	2.25	2.00±0.92 ^{a}	1.5	1.7	1.6±0.69 ^{a}
MCV (fL)	95.8±1.25	93.3	94.5	93.9±0.75 ^{ab}	76.4	85.4	80.9±0.45 ^{a}	86.3	90.5	88.4±0.45 ^{a}
MCH (pg)	31.8±0.92	28.3	29.3	28.8±1.20 ^{ab}	19.6	25.6	22.6±1.11 ^{a}	21.8	25.6	23.7±1.10 ^{ab}
MCHC (g/dL)	33.2±1.37	27.2	32	29.6±1.10 ^{ab}	26.5	29.3	27.9±0.19 ^{a}	25.3	28.3	26.8±1.21 ^{b}
WBC (X 10^{3}/µL)	6.06±0.24	6.13	6.85	6.49±0.12 ^{b}	6.99	7.45	7.22±0.05 ^{b}	7.3	8.3	7.8±0.10 ^{b}
Small lymphocytes (X 10^{3}/µL)	25.3±0.02	28.6	30.2	29.4±0.08 ^{ab}	29.7	32.1	30.9±0.02 ^{b}	34.7	37.3	36.0±0.08 ^{b}
Large lymphocytes (X 10^{3}/µL)	1.5±0.020	1.38	1.82	1.6±0.018 ^{b}	1.65	1.95	1.8±0.010 ^{ab}	2.1	2.5	2.3±0.018 ^{b}
Neutrophils (X 10^{3}/µL)	1.9±0.014	2.05	2.35	2.2±0.010 ^{a}	2.47	2.73	2.6±0.010 ^{b}	2.8	3.6	3.2±0.010 ^{ab}
Monocytes (X 10^{3}/µL)	1.65±0.02	1.8	2.2	2.0±0.020 ^{a}	2.67	2.93	2.8±0.20 ^{b}	3.2	3.8	3.5±0.010 ^{a}
Eosinophils (X 10^{3}/µL)	0.5±0.020	0.7	0.9	0.8±0.01 ^{a}	0.88	1.02	0.95±0.02 ^{b}	0.8	1.2	1.0±0.001 ^{ab}
Thrombocyte like cells (X 10^{3}/µL)	1.8±0.021	1.24	1.56	1.4±0.014 ^{a}	1.79	2.21	2.0±0.020 ^{ab}	3	3.4	3.2±0.014 ^{a}
Thrombocytes (X 10^{3}/µL)	34.9±0.02	28	32	30.0±0.04 ^{ab}	37.9	42.1	40.0±0.15 ^{a}	45.1	46.9	46.0±0.01 ^{a}
Plasma protein (g/dL)	3.8±0.024	2.95	3.45	3.2±0.010 ^{b}	2.05	2.35	2.2±0.010 ^{a}	1.58	1.92	1.75±0.020 ^{ab}

Note: Results expressed as mean±SD of five replications (d.f. 5, 30).
*The values of the MCV, MCH and MCHC are calculated by the formulae, corresponding to the appropriate values of Hb, PCV and RBC.

Fifteen blood parameters were studied for appraisal into the effect of parathion on the hematological indices. The mean±SD value of normal PCV (%) was 25.0±0.83, which reduced after 60 min. of exposure, ranging from 16.8 - 19.2 with a mean±SD of 18.0±0.98, showing 'variance', 'regression equation' and 'correlation coefficient of 308.9, Y = –0.115X + 25.2 and 0.99 respectively. The normal haemoglobin (Hb) expressed in g/dL was 8.3±0.23, which decreased after 60 min. of exposure, ranging from 5.8 - 6.8 with a mean±SD of 6.3±0.25, with 'variance', 'regression equation' and 'correlation coefficient of 435.2, Y = –0.032X + 8.11 and 0.97 respectively.

The RBC count (x10^{6}/µL) was 2.61±0.06, which decreased after 60 min. of exposure, ranging from 1.5 - 1.7 with a mean±SD of 1.6±0.69 showing 'variance', 'regression equation' and 'correlation coefficient of 507.7, Y = –0.016X + 2.627 and 0.99 respectively. Likewise MCV (fL) was 95.8±1.25, which showed a decrease after 60 min of exposure, ranging from 86.3 - 90.5 with a mean±SD of 88.4±0.45 showing 'variance', 'regression equation' and 'correlation coefficient' of 1324.4, Y = –0.176X + 95.03 and 0.68 respectively. The normal MCH (pg) was 31.8±0.92, which reduced after 60 min of exposure, ranging from 21.8 - 25.6 with a mean±SD of 23.7±1.10 with 'variance', 'regression

equation' and 'correlation coefficient' of 296.8, Y = –0.152X + 31.3 and 0.90 respectively. The MCHC (g/dL) was 33.2±1.37, which showed a decrease after 60 min of exposure, ranging from 25.3 - 28.3 with a mean±SD of 26.8±1.21 showing 'variance', 'regression equation' and 'correlation coefficient' of 289.18, Y = –0.104X + 32.51 and 0.96 respectively.

The normal WBC ($\times 10^3/\mu L$) was 6.06±0.24 which showed an increase after 60 min. of exposure, ranging from 7.3 - 8.3 with a mean±SD of 7.8±0.10 with 'variance', 'regression equation' and 'correlation coefficient' of 437.79, Y = 0.030X + 6.023 and 0.98 respectively. The small lymphocytes count ($\times 10^3/\mu L$) was 25.3±0.02, which showed an increase after 60 min. of exposure, ranging from 34.7 - 37.3 with a mean±SD of 36.0±0.08 with 'variance', 'regression equation' and 'correlation coefficient' of 294.13, Y = 0.168X + 25.36 and 0.98 respectively. The large lymphocyte count ($\times 10^3/\mu L$) was 1.5±0.02, which later showed an increase after 60 min of exposure, ranging from 2.1 - 2.5 with a mean±SD of 2.3±0.018, with 'variance', 'regression equation' and 'correlation coefficient' of 512.9, Y = 0.013X + 1.41 and 0.94 respectively. The normal neutrophil count ($\times 10^3/\mu L$) was 1.9±0.014 which showed an increase after 60 min. of exposure, ranging from 2.8 - 3.6 with a mean±SD of 3.2±0.010 with 'variance', 'regression equation' and 'correlation coefficient' of 502.3, Y = 0.021X + 1.83 and 0.98 respectively.

The monocytes count ($\times 10^3/\mu L$) was 1.65±0.002 which later showed an increase after 60 min. of exposure, ranging from 3.2 - 3.8 with a mean±SD of 3.5±0.010 with 'variance', 'regression equation' and 'correlation coefficient' of 502.2, Y = 0.031X + 1.535 and 0.98 respectively. The eosinophils count (0.5±0.02) showed an increase after 60 min. of exposure, ranging from 0.8 - 1.2 with a mean±SD of 1.0±0.001 with 'variance', 'regression equation' and 'correlation coefficient' of 529.13, Y = 0.008X + 0.565 and 0.94 respectively. The thrombolytic like cells (1.8±0.021) showed an increase after 60 min. of exposure, ranging from 3.0 - 3.4 with a mean±SD of 3.2±0.014 with 'variance', 'regression equation' and 'correlation coefficient' of 508.3, Y = 0.024X + 1.38 and 0.80 respectively. The thrombocytes (34.9±0.02) showed an increase after 60 min. of parathion exposure, ranging from 45.1 - 46.9 with a mean±SD of 46.0±0.01 with 'variance', 'regression equation' and 'correlation coefficient' of 322.9, Y = 0.216X + 31.23 and 0.81 respectively. The normal plasma protein content (g/dL) was 3.8±0.024, which showed a decrease after 60 min. of parathion exposure, ranging from 1.58 - 1.92 with a mean±SD of 1.75±0.020 with 'variance', 'regression equation' and 'correlation coefficient' of 498.4, Y = –0.035X + 3.81 and 0.98 respectively.

4. Discussion

The increase in OBF and TBF upon exposure to different pesticides either individually or in groups has earlier been reported by (Omoregie, 1995). The initial increases in OBF and TBF may be analogous with the response to shock. The change in behavioural response to different pesticides with prevalent change in the rate of OBF and TBF from control imputes an adjustment in physical fitness as a result of the stress condition (Leight and Van Dolah, 1999). (Grillitsch *et al*., 1999) suggested that organisms unveil behavioural responses to chemical stress both at acute and sub lethal toxicity. This elicits the potency and sensitivity of the fish, *N. notopterus* to the test chemical, witnessed by the change in OBF and TBF.

During the present experiment the hematological parameters of *N. notopterus* were greatly disturbed on exposure to 0.1 ppm of parathion. Haemoglobin (g/dL) showed a decrease from 8.3 to 6.3; RBC ($\times 10^6/\mu L$) from 2.61 to 1.6; PCV (%) from 25.0 to 18.0; MCV (fL) from 95.8 to 88.4; MCH (pg) from 31.8 to 23.7; MCHC (g/dL) from 33.2 to 26.8; and plasma proteins (g/dL) from 3.8 to 1.75. The work of (Murty *et al*., 1984) on the toxicity of methyl parathion and fensulfothion to the fish *Mystus cavasius* reveals oxygen stressor in fishes subjected to the pesticides because of the decrease in number of RBC's and reduction in haemoglobin titer. (Prasada Rao and Ramana Rao, 1984) reported the

inhibitory mechanism of acetylcholinesterase activity of parathion in the tissues of the teleost (*Tilapia mossambica*).

Calumpang *et al*., 1997 reported significant (P < 0.5) decrease in the values of Hb and RBC after the exposure of fish and frogs to chlorpyrifos, fenubucarb, monocrotophos, and methyl parathion. The concept regarding the hematological changes and related metabolic dysfunctioning was assessed by (De La Vega Salazer *et al*., 1997) who studied the bioaccumulation of methyl parathion and its toxicology in several species of the freshwater community in Ignacio Ramirez dam in Mexico. The study of (De La Vega *et al.* Salazer *et al*., 1997) was further strengthened by the recommendation of (ATSDR, 2001) who investigated the complete toxicological profile for methyl parathion. Later on (Castillo *et al*., 2002) studied the behavioural effects of exposure to endosulfan and methyl parathion in adult rats.

Extensive study on the effect of parathion on hematological parameters in the serum of male Bagrid fish (*Psudobagrus fulvidraco*) has been carried out by (Kyu-Seok Cho *et al*., 2004). A significant (P < 0.1) decrease in RBC, Hb, PCV, MCV and MCH in the fish in their study. (Edwards & Tchounwou, 2005) further strengthened the study, who worked on environmental toxicology and health effects associated with methyl parathion exposure. The work of (Monteiro *et al*., 2006) lend complete support to our findings, who worked on oxidative stress as biomarkers in the freshwater characid fish, *Brycon cephalus*, exposed to organophosphorus insecticide Folisuper 600 (methyl parathion). (Janice *et al*., 2007) investigated parathion and methyl parathion toxicity to insecticide resistant and susceptible mosquitofish (*Gambusia affinis*) and observed that the resistant population demonstrates a 1.3 fold greater tolerance of methyl parathion than the susceptible population. This statement justifies the alteration in hematological parameters of the fish exposed to different pesticides.

A significant decrease was observed by (Bhat *et al*., 2012) in values of hematological parameters like Hb, Hct, RBC and plasma protein throughout the exposure of methyl parathion. The authors reported an increase in leukocyte initially, which later recovered, showing a significant decrease at the end of the experiment. Same trend was observed in case of MCV and MCH, whereas MCHC value was more or less similar to control group up to the 21st day, and then a significant decrease was observed in the remaining study period. Plasma glucose values increased up to the 28th day (13.37 %) and then declined. The observations of the above also stand true for our results. The results are further strengthened by the work of (Xiang & He-Qing, 2012) who observed the alteration of the kidney membrane proteome of *Mizuhopecten yessoensis* induced by low-level methyl parathion.

5. Conclusion

The present investigation revealed a marked reduction in the opercular beat frequency (90.0 to 30.0) and tail beat frequency (7.3 to 2.0) in *N. Notopterus* exposed to parathion at the end of 60 min. exposure time. The results on the hematological aspect of the experiment (5 replicas) revealed that significant (P < 0.05) increase in WBC (6.06 to 7.8), and decrease in Hb, RBC, PCV, MCV, MCH, MCHC and other non-specific defence cells.

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Diagnosis, Growth and Exploitation Rate of the Sapater (*Chloroscombrus chrysurus,* Linnaeus 1766) Fishing by Purse Seine in the Nearshore Waters of Benin

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Abstract *Chloroscombrus chrysurus* (Linnaeus 1766) is one of the most abundant species of Carangidae family occurring in the nearshore waters of Benin. To ascertain the exploitation level of this fishery resource, some demographic parameters of the species were studied for management and conservation measures. 605 specimens were collected randomly from commercial catches from August to November 2014. Morphometric, meristic and weight data were analyzed using SPSS software and the ELEFAN I routine from FiSAT. The population of *C. chrysurus* was not heterogeneous with a sex-ratio of 1.4:1 ($p < 0.05$; $\chi^2 = 312.485$) and masculinity and femininity rates were 58.31% and 41.69% respectively. Length frequencies showed low percentage of small sized individuals and independent distribution of sex ($r = 0.16$, $p < 0.05$). The monthly variations of the gonad-somatic index (GSI) and the condition factor (Kc) showed a decreasing trend over the review period and corresponding probably to the period of gametes emission. von Bertalanffy growth parameters estimated were $L_\infty = 28.35$ cm, $K = 0.49$ year^{-1}, $t_0 = -0.33$ year and $\varphi' = 2.59$. Total mortality, natural mortality and fishing mortality are 1.39, 1.17 and 0.22 respectively. The exploitation rate ($E = 0.16$) showed that the resource was underexploited implying that the purse seine is not a danger for this species.
Keywords *Chloroscombrus chrysurus; growth parameters; mortalities; underexploited*

1. Introduction

Fisheries and aquaculture make a decisive contribution to the welfare and prosperity of the inhabitants of this world and fish is now an important source of nutritious foods and animal protein for a large part of the world population (FAO, 2012). This also explains the continual increase of fishery resources sampling rates which endanger the balance of the oceans. Because of the services so rendered whose importance and diversity made them a global appropriation stake, marine and coastal biodiversity is under pressure affecting the integrity of its resources (Etoga, 2009).

Marine and coastal biodiversity is experiencing strong erosion (FAO, 2006). Many stocks are in a poor state at varying degrees. This can result in either reduced reproductive biomass, or by an unsustainable rate of exploitation in the medium term, or a combination of both effects. Two essential causes that led to this situation are recognized: on one hand, fishing capacities implemented far outweigh the stocks renewal potential; on the other hand the captures of young population are important. These two causes have led to a scarcity of many resources, landings significantly lower than what they should be in a better balanced exploitation and a weakening of the populations boosting their abundant natural fluctuations.

Samples taken by the fisheries reduce stock biomass. These react by increasing their rate of natural increment in order to regain the equilibrium position they had when there was no fishing. A new apparent equilibrium is then established at a lower level of biomass and captures match that faculty of resources to recover their level of abundance in a virgin state (Graham, 1935).

In addition to the increase in fishing effort, the decrease in landings could be explained by the degradation of fish habitat and the use of non-selective or unfavorable machine for the preservation of the resource.

In Benin as in all the countries of the Gulf of Guinea, two types of fishing are being practiced: industrial fishing and artisanal fishing. The latter is exerted on the continental shelf, usually in coastal waters below five miles (Okpeitcha, 2010). Artisanal fishing in Benin nearshore waters takes place throughout the year through various fishing machines most of which are unfavorable for the preservation of fishery resources.

The purse seine often used in the nearshore waters of Benin has proved an unrivaled productivity in artisanal fishing, sometimes even exceeding that of small industrial units (sardine fishers) (Freoj and Weber, 1981). They can capture on a haul several tons of fish and Carangidae are one of the main species caught by that machine according to the statistics from the Fisheries Department.

The biological material of this study, *Chloroscombrus chrysurus*, is the subject of a major fisheries activity. On Benin nearshore waters, it is subjected to a relatively intense artisanal activity. *Chloroscombrus chrysurus* species is highly consumed in Benin due to its abundance and relatively cheaper cost.

Rational management of renewable fishery resources is required to provide animal protein and an economic and social well-being to the population (Ekouala, 2013). The estimate of the age and growth of aquatic animals is an indispensable operation for the study of demography and dynamics of natural populations (Lamotte, 1975). Knowledge on the terms of their growth, longevity and all the events marking their lives (age at recruitment, age at sexual maturity, mortality, etc.) is useful for assessing the biomass likely to be fished but also to determine the optimal captures size. Therefore it is necessary to study the selectivity of fishing gear used in relation to the exploitation of fishery resources.

The aim of this study is to assess some demographic parameters of *C. chrysurus* fished by the purse fishing gear and propose conservation and management measures.

2. Materials and Methods

2.1. Study area, Fish sampling and Data collection

The Artisanal Fishing Port of Cotonou (AFPC) in the southern Benin (Figure 1), which is the core area of this study, is a public utility infrastructure located in the dock on the East side of industrial fishing port. It is a space developed in 1972 to facilitate canoes landing.

The basic biological material of this study comprises *Chloroscombrus chrysurus* (Carangidae) specimens caught with purse seining haul in the nearshore waters of Benin and landed at AFPC.

The data collection equipment is made of fish length measuring board, a stop ruler and a tape measure to perform the various measurements on fish namely: the fork length (Lf); the eye diameter (O) and head length (HL). A scale of Sartorius type (Model 6200S LP) 0.1 g precision and scope 500g allowed taking the total weight (Wt), eviscerated weight (We) and organ weight (gonads and stomach). No secondary sexual external characteristic could be detected so far for sex identification with *Chloroscombrus chrysurus*, the examination of gonads is necessary for this purpose. To this effect, the fish were dissected and gonads were subjected to macroscopic observation.

The monthly sampling of 150 fish specimens was made randomly from purse seining haul, from August to November 2014 (four months). In total of 605 fish specimens were sampled throughout the period. Each fish specimen was identified according to the determining keys of Paugy et al. (2003). Individuals were immediately transferred under ice to the Zoology Laboratory for different measurements, counting and dissection.

a) Benin in Africa b) Republic of Benin c) Artisal Fishing Port of Cotonou

Figure 1: *Location of the sampling site*

2.2. Population structure and sex ratio

The size frequency was analyzed at 2 cm interval total length (TL, cm) class using a histogram to determine the type of distribution which characterizes the fish population. Whit numerical abundance by sex, the following ratio was computed:

$$Sex\ ratio = \frac{\text{number of males}}{\text{Number of females}}$$ (Chakroun et al., 2003)

2.3. Length-weight relationship

The commonly used relationship $W = aL^b$ (Ricker, 1975) was applied to establish the length-weight relationship by sex, where W is the ungutted weight (Wt, g), L is the total length (TL, cm) and “a” and “b” are intercept end slope of the regression curve of the length and the weight of the fish, respectively. Tests for differences between sexes were performed to consider pooled population together. The correlation (r^2), which is the degree of association between the length and weight, was computed from the linear analysis.

In fact this relationship is closely related to the biological and physiological condition of the fish (degree of the stomach repletion, degree of development of genital glands and stage of maturation).

Among the applications of length-weight relationships in fisheries biology, knowledge of this relationship is useful for estimating fish weight from its length, this value is an indicator of the fish

living conditions or status of the fish stock (Petrakis and Stergiou, 1995; Froese and Pauly, 2006), also allows assessing the fish biomass.

2.4. Fish condition factor (Kc)

To check the stoutness, the condition factor (Kc) was calculated using the following formula:

$$Kc = 100 * Wt / TL^{b}$$ (Chakroun et al., 2003)

Where Wt = total fish weight (g); **Lt** = total length (cm).

To assess the wellbeing of the fish, "b" is compared to 3 and the evolution of Kc is analyzed in the sample size range. If "b" is not significantly different from 3, the species has a good adaptation with regard to the dominant ecological habitat condition. In contrast, if "b" is significantly different from 3, there is less adaptation (Bijot et al., 1994).

2.5. Growth and mortality parameters

The estimate of the age and growth of aquatic animals is an indispensable operation for the study of demography and dynamics of natural populations (Lamotte, 1975). Knowledge on the terms of their growth, their longevity and on all the events marking their lives (age at recruitment, age at sexual maturity, mortality, etc.) is useful for assessing the biomass likely to be fished but also to determine the optimal captures size.

Fish growth is expected to follow the growth function of von Bertalanffy (VBF), whose equation is:

$$L_t = L_\infty \{1-\exp [-K (t-t_0)]\}$$ (King, 1995)

With L_t = length of the fish at time t (mn); $L\infty$ = theoretical asymptotic length (mm) **K** = coefficient of growth or growth rate; t_0 = hypothetical age at which the total length is zero or fictitious age corresponding to the size zero.

This model is based on an assumption that the instantaneous speed of growth is the result of two opposing physiological processes: anabolism proportional to organisms' surface and catabolism proportional to the volume of their body (weight).

Pauly empirical equation below permits to determine the hypothetical age when fish size is zero.

$$\text{Log}_{10} (-t_0) = - 0.392 – 0.275 \text{ Log}_{10} L\infty - 1.038 \text{ Log}_{10} K$$ (Pauly, 1979)

The estimated **K** and **L∞** are used to determine the growth performance index (**φ'**) of the species from the equation developed by Pauly and Munro (1984) is as follows:

$$\varphi' = \text{Log}_{10} K + 2\text{Log}_{10} L_\infty$$

The growth performance index is an indication of the wellbeing of the species with regard to its external environment.

2.6. Mortality Estimation parameters (Z, M and F)

Total mortality **(Z)** was determined using the software FiSAT (Gayanilo et al., 1995). The instantaneous natural mortality **M** was calculated by the empirical equation of Pauly (1980) using an average surface temperature in the following manner:

$$\mathbf{Log_{10}\ M = - 0.0066 – 0.279\ Log_{10}\ L\infty + 0.6543\ Log_{10}\ K + 0.463\ Log_{10}\ T}$$

The instantaneous mortality rate by fishing, **F**, was estimated from the relationship:

F=Z-M (Pauly, 1980)

2.7. Longevity (t_{max})

To get an independent estimation of the instantaneous natural mortality rate (**M**), Pauly (1983) established that M is in correlation with the fish longevity defined as follows

$$\mathbf{t_{max} = 3 / K}$$ (Anato, 1999)

The assessment of stock conditions and mortality rate was done by the calculation of the exploitation rate (**E**) from:

E = F/ Z (Pauly, 1983)

With **F** = mortality annual rate by fishing; **Z** = total mortality annual rate.

Value of E is approximately equal to 0.5 supposing that the performance is optimized when F is approximatively equal to M (Gulland, 1971).

2.8. Capture probability and size at first capture (Lc or $L_{50\%}$)

The capture probability gives a clear idea on the estimate of the actual size of the fish in the fishing area and that are caught by specific devices. At the same time, it is an important tool for fisheries managers who in regulating the minimum mesh size of a fishing fleet can roughly conclude what should be the minimum size of the target species fishing. The probability of capture was estimated by extrapolation back to the descending portion of the linearized curve length converted in capture.

Selectivity curve generated using linear regression fitted to the data points in ascending capture probability and the length is used to estimate the final value of L25, L50 and L75 (that is to say, lengths at which 25%, 50% and 75% of those fish are proving vulnerable to the fishing machine used. Estimates of the length at first capture (L50) are derived from the probabilities of capture generated by analyzing the capture curve produced by FiSAT.

2.9. Statistical analysis

SPSS statistical software was used for χ^2 test (Scherrer, 1984) and averages correlation and comparison. The assumption of heterogeneity and the sex ratio 1:1 were tested by the χ^2 test (Scherrer, 1984).

Assuming an equal distribution of males and females within the sampling and by reference to properties normal distribution, we will say that there is a 5% chance that a difference between the observed and theoretical values is purely coincidental. We will also say that the difference in the probability level of 5% is significant if it is superior to $\chi^2 = 3.842$.

Allometric coefficients "b" of males and females were compared with the theoretical value "3" by the T test for single sample and between the sexes by T test for independent sample.

Monthly distributions of length frequency were analyzed to have indirect growth. The amplitude of the classes was arbitrarily defined taking into account the different sizes of fish in the population.

Von Bertalanffy growth parameters namely asymptotic length (L∞) and the growth coefficient (K), are obtained using the routine **ELEFAN1** of **FiSAT** (Pauly and David, 1981) recommended by FAO, the software FiSAT is based on Von Bertalanffy equation (1938), it permits to provide initially the assessments of different parameters and uses iterative algorithms.

3. Results

3.1. Diagnosis

***Table 1**: Diagnosis of Chloroscombrus chrysurus (Linnaeus, 1766) of the nearshores waters off Benin*

	Parameters used	Minimum	Maximum	Average + Gap-type
Meristics Data	Rays (1st dorsal fin)	6	7	6.9±0.28
	Rays (2nd dorsal fin)	24	29	27.0±0.77
	Rays (anal fin)	24	29	27.0±0.77
	Rays (pectoral fin)	15	21	17.6±0.80
	Rays (pelvic fin)	4	6	4.9±0.25
	Rays (caudal fin)	12	14	12.0±0.19
	Branchiospines (inferior arc)	21	39	31.9±1.94
	Branchiospines (superior arc)	0	12	8.8±1.27
	Branchiospines (whole racher)	27	45	40.7±2.39
Morphometric Data	Total Length (TL)	13.7	27.7	21.0±2.28
	Fork Length (FL)	11.0	23.5	17.5±1.96
	Standard Length (SL)	10.3	21.1	16.2±1.80
	Pre-dorsal Distance (DP)	3.5	6.8	5.3±0.56
	Pre-anal Distance (PA)	5.4	10.9	8.4±0.90
	Pre-Pectoral Distance (PP)	2,5	4.8	3.7±0.38
	Pre-ventral Distance (PV)	3.6	6.9	5.3±0.54
	Head Length (HL)	2.4	4.6	3.7±0.37
	Pre-orbital Distance (Pr-O)	0.6	1.40	1.0±0.12
	Eye Diameter (O)	0.7	1.7	1.0±0.11
	Post-orbital Distance (Pt-O)	0.7	1.4	1.1±0.18
	Inter-orbital Distance (I-O)	1.0	2.0	1.5±0.13
	Great Height (H)	4.7	8.4	6.7±0.64
	Small height (h)	0.4	1.0	0.7±0.09
	Circumference (Circ)	10.2	18.8	14.5±1.38
	Density (Thick)	0.9	2.2	1.6±0.23

To confirm the characteristics of *Chloroscombrus chrysurus* species (Linnaeus, 1766) which constitutes the biological material on which we conducted our study, a number of observations, measurements and counting were performed on specimens that make up the sample. Table 1 summarizes the various distinctive characteristics of the species.

Generally, *Chloroscombrus chrysurus* of the nearshore waters off Benin has a sufficiently compressed oval body, the ventral profile more convex than the dorsal profile with an oblique mouth and a small eye. The top of the head and trunk are colored black, while the bottom is a shiny gray. Specimens have a black spot on the upper caudal peduncle. They have two dorsal fins; the first ray is 6.9±0.28 and the second 27±0.77. The upper lobe of the caudal fin is longer than the lower lobe. The pectoral fins have 17.6±0.80 rays and the pelvic fins have 4.9±0.25 rays. There is an anal fin with 27.0±0.77 rays. Counting of gill rakers permitted to observe on the upper blade of the 1st branchial arch 8.8±1.27 branchiospines. It could be noted that cases where the upper plate does not exist are rare. The lower blade has 31.9± 1.94 branchiospines. Scutes could not be counted because of their very small size.

3.2. Population structure and sex ratio

The assumption of heterogeneity and that of sex ratio 1: 1 were tested by the X^2 test (Scherrer, 1984). A total of 605 specimens of *Chloroscombrus chrysurus* comprising 347 males, 248 females and 10 unsexed were sampled. The difference between the number of males and females, was statistically significant ($p < 0.05$, $\chi^2 = 312.485$). The population is not sexually heterogeneous and sex ratio (number of males/number of females) is 1.4: 1.

The proportions of males (masculinity rate) and females (femininity rate) are expressed in percentages as follows: Males = masculinity rate (M / M + F) * 100 = 58.31%; Females = femininity rate = (F / M + F) * 100 = 41.68%.

Size distribution of population of males, females and total population are reported in Figs 2-4 of the study sample.

The size distribution of the population of males and females and pooled sex are unimodal. The most represented classes range from 18 to 24 totaling 82.4% of the total number of the population. The sizes of females vary from 13.7 to 25.9 cm, while those of the males range from 15.3 to 27.7 cm with the same modal class 20, 22.

Sex seems to play any role in the determination of any difference in size distribution ($r = 0.16$; $n = 595$, $p < 0.05$).

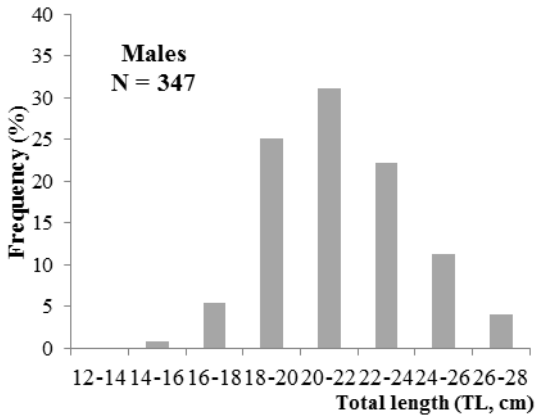

Figure 2: *Size structure of males*

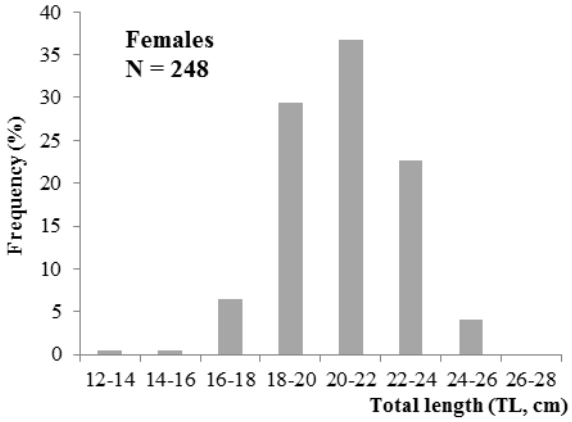

Figure 3: *Size structure of females*

Figure 4: *Size structure of pooled sexes (males and females)*

3.3. Length-weight relationship

The length-weight relationship is $Wt = 0.007TL^{3.027}$ ($r = 0.96$; $n = 347$) for males, $Wt = 0.005TL^{3.141}$ ($r = 0.945$; $n = 248$) for females and $Wt = 0.006TL^{3.038}$ ($r = 0.949$; $n = 605$) for both sexes. The value of the slope "b" is significantly different between the sexes (t-test: $t = 14.97$, $p < 0.05$) and is significantly higher than the theoretical value of 3 for males (t-test: $t = 21.401$; $p < 0.05$) and females (t-test: $t = 50.544$; $p < 0.05$) for all the curves (Figures 5, 6 and 7), the experimental points are strongly ordered around the theoretical curve (regression lines), this is explained by the fact that the lowest value of the coefficient of determination r^2 is very close to 1 ($r^2 = 0.945$)

The calculated equations reflect a positive or majored allometric growth for males, females and for the whole population (male + female). The fish, all sex put together, grows faster in weight than in length.

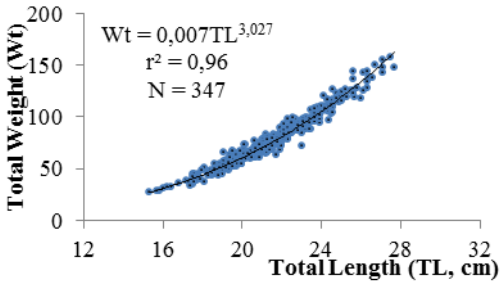

Figure 5: *Length-weight relationship for males*

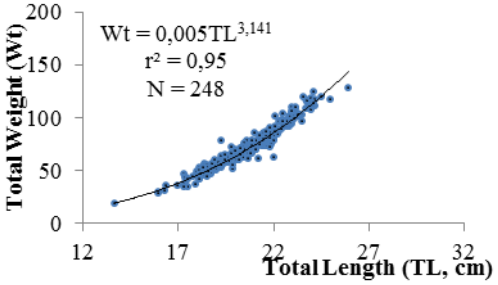

Figure 6: *Length-weight relationship for females*

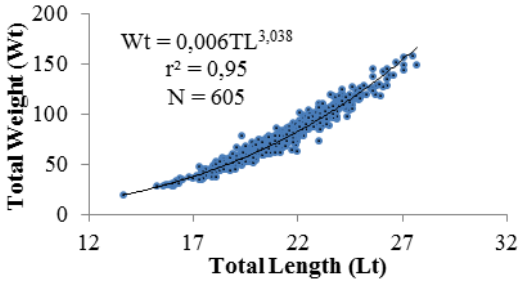

Figure 7: *Length-weight relationship for pooled sexes (males+ females)*

3.4. Fish condition factor (Kc)

The condition factor (Kc) in males, females and both sex (Figure 8), even though they were not significantly different from one month to the other ($p > 0.05$) decreased from August to November.

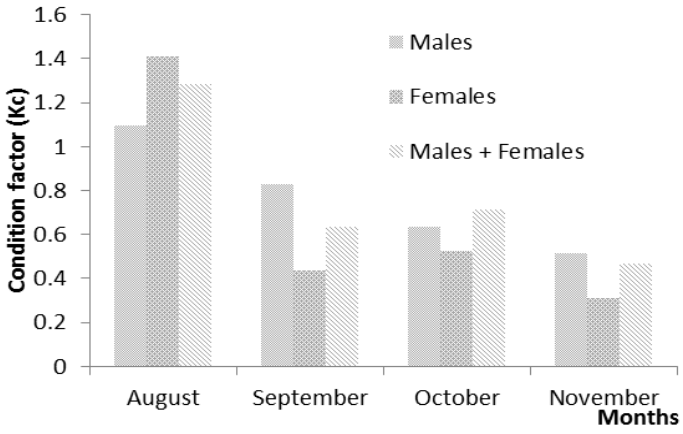

Figure 8: *Monthly variations of the condition factor (Kc)*

3.5. Gonad-somatic index relationship (GSI)

The monthly evolution of the gonad-somatic values (GSI) of *Chloroscombrus chrysurus* from Benin nearshore waters is reported in Figure 9.

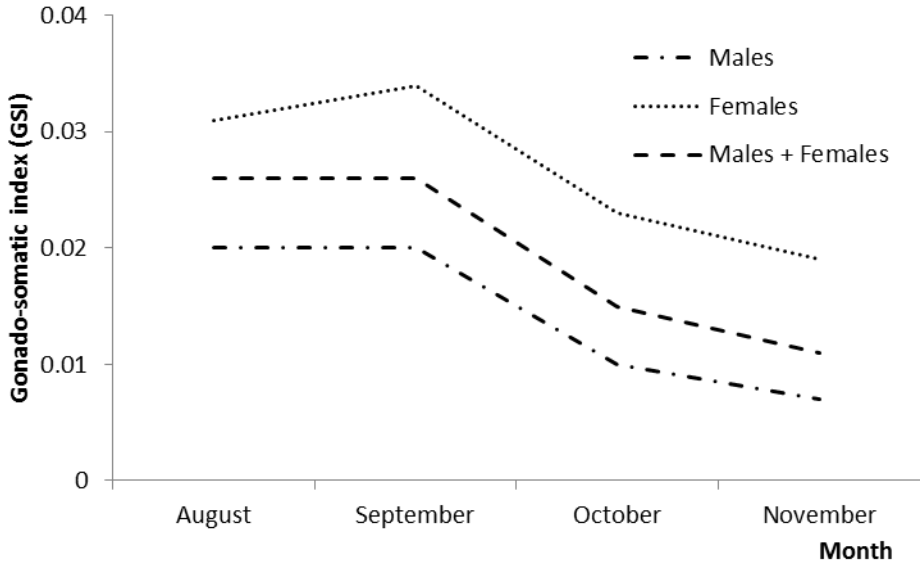

Figure 9: *Monthly variations of gonad-somatic index (RGS)*

The values of gonad-somatic index of males (0.020 ± 0.008) and females (0.031 ± 0.009) remained almost static from August to September with a slight increase with female to reach 0.034 ± 0.011. From September to November they decreased (Figure 12) to reach 0.007 ± 0.004 and 0.019 ± 0.008, respectively, for males and females corresponding probably to the period of gametes emission. Generally, the values of gonad-somatic index (RGS) of C. chrysurus females are higher than those of males.

3.6. Estimate of growth and mortality parameters

Figure 13 shows the curves from which the parameters of The von Bertalanffy growth function for *Chloroscombrus chrysurus* (Figure 10) show five (5) curves representing five cohorts started in January with no fish size group represented. The first group of modal size 12 cm (Total length) appears from September and goes to August of the following year with a modal size of 18 cm (Total length).

$L\infty$ and K output from FiSAT were 28.35 cm and 0.490 $year^{-1}$ respectively. The parameters of von Bertalanffy equation are reported in Table 6. The hypothetical age (t_0) and the growth performance index (φ') were -0.33 year and 2.595 respectively.

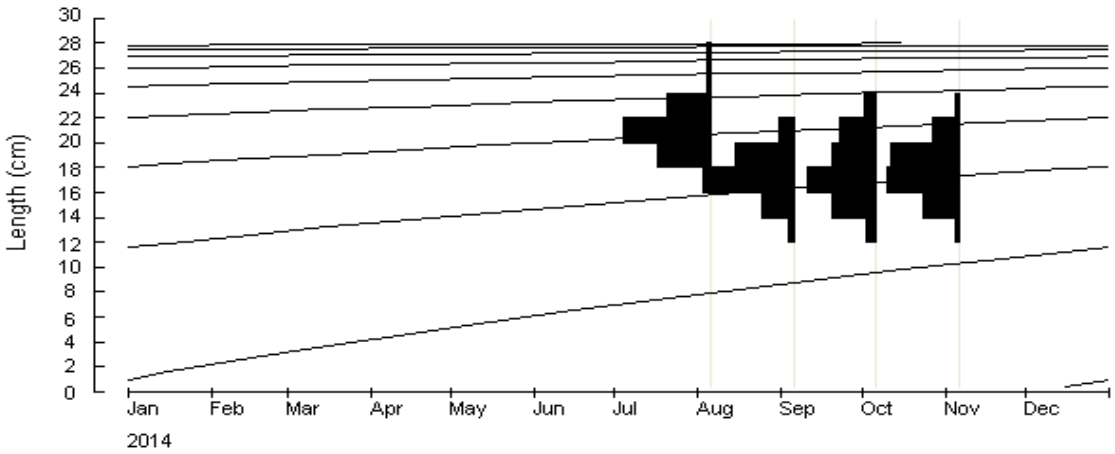

Figure 10: *Monthly increase of modal class according to routine ELEFAN 1 from FiSAT II of Chloroscombrus chrysurus*

Table 4: *Von Bertalanffy equation parameters*

Mortality Parameters	Asymptotic Length (L_∞)	Growth Coefficient (K)	Hypothetical Age (t_0)	Growth performance Index (φ')
Values	28.35 cm	0.49 $year^{-1}$	-0.33 year	2.59

The von Bertalanffy growth function equation is: **Lt = 28.35{1– exp [-0.490(t+0.328)]}**

3.7. Estimate of mortality parameters (Z, M and F) and exploitation rate (E)

The total mortality (Z), natural mortality (M) mortality by fishing (F) and exploitation rate (E) of *C. chrysurus* from the nearshore waters off Benin are estimated by FiSAT and generated (Figure 11) and reported in Table 6.

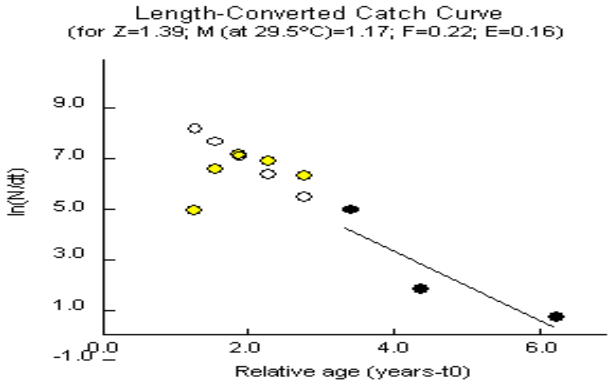

Figure 11: *Mortality parameters of Chloroscombrus chrysurus of Benin nearshore waters*

Longevity (t_{max})

The maximal lifespan (t_{max}) of *Chloroscombrus chrysurus* calculated according to Anato, (1999) (t_{max} = 3/k) is 6.12 years.

Probability of capture and size on first capture

The probability of capture estimated by FiSAT (Figure 12) show that 50% of *C. chrysurus* individuals are vulnerable to seining haul used in Benin nearshore waters at length of first capture L_{50} = 15.06 cm

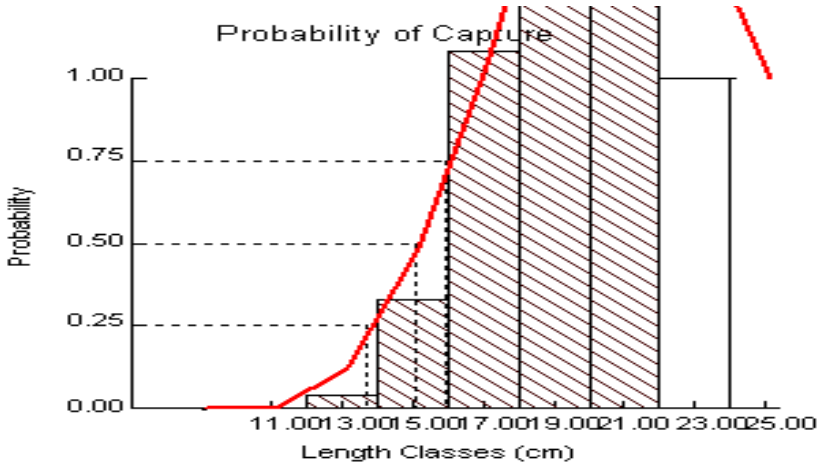

Figure 12: *Probability of capture of C. chrysurus of Benin nearshore waters*

4. Discussion

4.1. Diagnosis

After measurements it was noted that the morphometric and meristic distinctive characteristics found coincide with those given by Acosta et al. (2008) and by Smith-Vanish (2002). The species of *C. chrysurus* from the nearshore waters off Benin is not different from what described on other coasts such as those of South-East Brazil.

4.2. Population structure and Sex ratio

C. chrysurus size structure in Benin nearshore waters has unimodal distribution with modal class 13.7 - 27.7 cm (Total length). The size range between 18 and 24 cm represents over 82% of the capture (Table 3). There were very few small sized individuals in the samples (7%). Cunha et al. (2000) observed in contrast to current study, ranges including enough small sized fish (3-17 cm) in the bay of North-East of Brazil.

Moreover, the range of fish sizes observed in Benin nearshore waters seems smaller than those reported in other geographical environments. da Costa et al., (2005) observed for example a greater range (2-30 cm) in the Bay of Sepetiba in Northern Brazil. This restriction of the size range could be related either to the selectivity of the fishing gear used for fishing (purse seining haul) or the scarcity of small individuals in areas where fisheries were conducted. In addition, the maximum size found in Benin nearshore waters (27.7 cm) is lower than that reported in Brazil (30 cm) which may be due to better living conditions on the Brazilian coast. The lack of juveniles in the captures is due to the area where fisheries were conducted (full sea area) which are not areas of abundance of juvenile. According to Johnson (1978), the distribution of adults is done from coastal waters to marine waters up to a depth of 180 m. Juveniles of *Chloroscombrus chrysurus* are abundant in coastal areas close to estuaries, lagoons and bays (Flores-Coto and Sanchez-Ramirez, 1989). Sex ratio 1.4: 1 observed in *C. chrysurus* shows that there is dominance of males with respect to females. According to Aka et al. (2004), the variation of sex ratio is dependent upon to the physiological condition of the fish. In general, with the Teleost, males predominate during reproduction period while during sexual rest period; females are predominant (Paugy, 1980; Santos et al., 2007). Several factors such as moves to find food, differential growth and sex mortality rate also influence fish sex ratio (Mellinger, 2002).

4.3. Length-weight relationship

Values of "b" calculated being greater than the theoretical value 3, the calculated equations reflect a positive or increasing allometric growth for males, females and for the whole population (male + female). *Chloroscombrus chrysurus* in the nearshore waters of Benin for both sex, grows faster in weight than in length. This value is an indicator of the fish living condition or the condition of the fish stock (Petrakis and Stergiou, 1995; Froese and Pauly, 2006) may vary over time and space. It is also possible that the sampling mode influences the length-weight relationship.

4.4. Fish condition factor (Kc) and gonad-somatic Index (RGS)

Monthly variations of gonad-somatic index and Fulton condition factor indicate that the months of August, September, October and November probably correspond to the period of reproduction of the species and specifically the period of gametes emission. Although these results are consistent with those found by Conand and Franqueville (1973) that determined the period from July to October as the reproduction period and *Chloroscombrus chrysurus* larva abundance, this study needs to be conducted on a more extended period (at least one year) for confirmation of these results. Moreover, apart from the insufficient period of the study, it must also be complemented by a thorough study of reproduction by determining the monthly variation in the percentage of sexual maturity stages, the absolute fertility and oocyte diameters and distribution frequency.

4.5. von Bertalanffy growth parameters ($L\infty$, K and t_0) and growth performance index (φ')

von Bertalanffy growth parameters ($L\infty$, K and t_0) and growth performance index (φ') show a better adjustment due to the non-existence *Chloroscombrus chrysurus* of the alternation effect marked by cold season and dry season.

The estimated value of the asymptotic length from Benin coasts ($L\infty$ = 28.35 cm) is less than what found by da Costa et al. (2005) in the Bay of Sepetiba in the South of Brazil ($L\infty$ = 31.6 cm). In other words older fish in the Brazilian bay are of greater height than those studied of Benin coasts. This difference may be due either to age classes used in curve fitting or to the difference in environmental conditions in the two areas.

Growth coefficient (K) calculated in this study (K = 0.490 year $^{-1}$) is greater than the one reported by da Costa et al. (2005) in the Bay of Sepetiba (K = 0.380 year $^{-1}$), reflecting a faster growth of the species from the nearshore waters of Benin and indirectly a faster achievement of the asymptotic length. The growth performance index (φ') is the same as what was reported by da Costa et al. (2005) and this could be attributed to environmental and dietary conditions comparable in these two environments.

4.6. Mortality parameters (Z, M and F)

Total mortality (Z), natural mortality (M) and fishing mortality (F) (Table 7) determined by FiSAT software (Gayanilo et al., 1995) for *Chloroscombrus chrysurus* from Benin coasts were 1.39 year^{-1}, 1.164 year^{-1} and 0.226 year^{-1} respectively. Factors that affect the determination of the total mortality are among others, variations in the lengths of fish in the same cohort (Gabche and Hockey, 1995). As for natural mortality, it depends on both physiological factors such as diseases and old age and environmental factors namely temperature and water flow and finally, factors due to hazard such as encounters with predators. But fishing mortality results from fishing effort. It is obvious that the fishing effort on this species is relatively low, as confirmed by a low rate of exploitation (E = 0.16) compared to the optimal exploitation rate (E = 0.5). *Chloroscombrus chrysurus* is underexploited on Benin coasts and seining haul is not a threat to this fish resource.

On the other hand, (Sossoukpè E. et al., 2013; 2016) found higher exploitation rate for *Pseudotholithus senegalensis* (E1 = 0.91; E2 = 0.82) and low exploitation rate for *Sardinella maderensis* (E = 0.33 per year) respectively in the nearshore waters of Benin. *C. chrysurus* is less exploited than those above-mentioned species because of its relatively low economic value but nevertheless it has a high fishing potential and a significant ecological value (Yañez-Arancibia and Sánchez-Gil, 1986; Flores-Coto and Sánchez-Ramírez, 1989; García-Tapia, 1991).

The longevity of *Chloroscombrus chrysurus* from Benin coasts (t_{max} = 6.12 years) is less than what reported da Costa et al. (2005) on Brazilian coasts (t_{max} = 7.89 years). There is a strong correlation between natural mortality rate and longevity. In the case of fish, natural mortality is inversely proportional to the longevity; therefore, it is related to K. It goes without saying that this mortality is inversely related to the size of the fish, as those of greater size have fewer predators. This natural mortality is also correlated with the environmental temperature (Pauly, 1980).

4.7. Probability of capture and length at first capture

Probability of capture and different corresponding sizes generated by the FiSAT software were used to determine the length at first capture (L_{50}), length at which 50% of individuals of this species are vulnerable to purse seining haul used in the nearshore waters of Benin, L_{50} = 15.06 cm. This value must be compared to the length at first sexual maturation. Knowing the size of first sexual maturation is important in the management of fishery resources (Dadebo et al., 2003). It was adopted as the minimum sized specimen not to be captured. This comparison would show whether the individuals of this species are massively captured before reaching their first sexual maturation. In case of need would be symptomatic of poor exploitation of the stock. Failing to have this data in this study, we will compare it to the first sexual maturity size found by Magro et al. (2000) in the South of Brazil (11.5 cm) and Cunha et al. (2000) in the North-East of Brazil (9.5 cm). It appears that these values are below the size at first capture on Benin coasts (L_{50} = 15.06 cm). On the basis of these comparisons, we can say with a minimum reservation that the resource is not badly exploited on Benin coasts with the use of seining haul. In other words fish have chance to reproduce at least once to ensure the renewal of the stock before being captured. This result, which is against all expectation, could be due to the relatively small size of *C. chrysurus* juveniles and therefore are not massively captured by seining haul although being recognized as a devastating machine. However it would be interesting to extend the study on different fishing machines used on Benin coasts.

The length-weight relationship reflects a positive allometric growth which suggests that the species is in a reproduction period. This assumption is partially confirmed by the monthly changes in Kc and GSI values, corresponding to a decreasing trend over the period from August to November.

The exploitation rate obtained in this study is low and seining haul seems harmless for the stock of that resource. Indeed, the size classes mostly captured by this machine are between 18 and 24 cm, highly above the size of first sexual maturity.

As adjustment measures, it is suitable to develop and implement an effective management plan for C. chrysurus in particular and species not fully exploited in general before considering to intensify the exploitation of these stocks, if we want to avoid overfishing similar to the one currently among the numbers of stocks overfished such as *P. typus and P. senegalensis.*

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Research Article

Butrylacetycholinesterase Activity in Liver and Plasma, Liver Glycogen and Plasma Glucose Content, Haematology and Behaviour of Clariid Catfish Clarias Gariepinus to Dichlorvos

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Abstract Semi-static bioassay experiment was conducted to ascertain Butrylacetycholinesterase enzyme activity in liver and plasma, liver glycogen and plasma glucose content, haematology as well as behaviour of Clarias gariepinus (mean weight 24.52±1.64 g) to varying concentrations (0, 0.4, 0.8, 1.6 mg L^{-1}) of Dichlorvos for 96 hours. Butrylacetycholinesterase activity in liver was significantly ($P < 0.05$) inhibited all through the exposure duration to 0.4, 0.8 and 1.6 mg L^{-1} of DDVP compared with control group of fish. Similarly, plasma BuChE activity was inhibited all through the exposure duration to 1.6 mg L^{-1} DDVP compared to control. There was a significant ($P < 0.05$) inhibition in HB, RBC and MCV of exposed fish after 24,48, 72 and 96h to 1.6 mg L^{-1} DDVP compared to the control but PCV and WBC were significantly elevated after 72 and 96h to 1.6 mg L^{-1} DDVP compared to the control. Plasma cortisol and liver glycogen were significantly inhibited after 72 and 96 h to 0.8 and 1.6 mg L^{-1} compared to the control but plasma glucose was elevated after 24, 48, 72 and 96 h exposure to 0.4, 0.8 and 1.6 mg L^{-1} compared to the control group of fish. The 96 h LC_{50} of DDVP to the exposed fish was found to be 0.66 mg L^{-1} with safety value estimated to be 0.01 mg L^{-1} while lower and upper confidence limits gave 0.29 and 1.49 mg L^{-1}, respectively. The 96 h LT_{50} values for 0.8 and 1.6 mg L^{-1} was shown to be 79.23 and 60.26 hours with safety values of 3.98 and 3.01 hours respectively. WBC value was inversely related to HB ($r=-0.997$, $p < 0.05$, RBC ($r=-0.999$, $p < 0.05$) and PCV ($r=-0.953$, $p < 0.05$). RBC related positively to HB ($r=0.998$ and PCV ($r=0.959$), and inversely to MCH ($r=-0.996$) and MCHC ($r=0.995$). Plasma BuChE activity related positively to PCV ($r=0.979$) and inversely to MCHC ($r=-0.995$ and TBF ($r=984$). Liver BuChE related positively with plasma BuChE ($r=0.978$), plasma cortisol ($r= 0.970$) and liver glycogen ($r= 0.975$) and inversely to TBF ($r=-0.998$). Changes in BuChE activity may serve as surrogate information for projecting potential hazards in the health status of C. gariepinus.

Keywords *Butrylacetycholinesterase; Clarias gariepinus; Dichlorvos; Haematology; Liver glycogen; Plasma glucose*

1. Introduction

Majority of insecticides used today in many developing countries are organophosphorus insecticides (Ops) including dichlorvos which were developed as a substitute for nicotine (Costa, 1987) because of

their relatively nonpersistent characteristics in the environment. Although these compounds offer the advantage of rapid degradation, they generally lack target specificity and have high acute toxicity toward many species. Thus, many terrestrial and aquatic organisms may be at risk for intoxication caused by exposure to these compounds in the environment. Dichlorvos (dimethyl-2,2-dichlorovinyl phosphate) is an organophosphate (Ops) insecticide used against insect pests to stored products of plants and animals as well as outdoor and greenhouse fruits and vegetable crops. The aquatic environment is under a constant threat as a result of indiscriminate use of synthetic pesticides including dichlorvos (Cerejeira et al., 2003; Pandey et al., 2011), which enter through run off and in most cases, predisposes non-target organisms to potential toxicity. In the water, its molecules may accumulate in sediments or be absorbed by the aquatic organisms with attendant patho-physiological changes (Jordan et al., 2013). Its toxicity to freshwater and estuarine fish is moderate to high (Roth, 2000; (Das, 2013)), but does not bioaccumulate in fish (Lakshmanan et al., 2013). Most authors while describing toxicity of commercial formulations of dichlorvos, reported altered behavioural responses in various fish species (Das, 2013). Among various biomarkers of pesticide exposure, the family of cholinesterase's have been widely used to evaluate the noxious effects of pesticides especially organophosphates. Butrylcholineterase enzyme (BuChE, EC 3.1.8) is synthesized in the liver and present in the plasma and other tissues. Although, its physiological function is not very clear, it is present as one of the most effective detoxifying enzymes that scavenges a broad range of xenobiotics compounds (Inacio Lunkas et al., 2006). Plasma cortisol is widely used as a general indicator of stressful conditions in fish (Pickering et al., 1998). Under stress condition, body of fish produces primary and secondary responses (Martinez et al., 2009). The primary response is the perception of an altered state by the central nervous system to release of stress hormone, cortisol into the blood stream (Martinez et al., 2009). Secondary response occurs as a consequence of the released stress hormone causing changes in the blood and tissue chemistry (Babujanarthanam et al., 2010) such as increase in plasma glucose (Begg and Pankhurst, 2004). The exposure of fish to several types of chemical agents may induce changes in several haematological and physiological parameters, which are frequently used to evaluate fish health (Banaee et al., 2011; Ezike, 2017). The present study was therefore designed to determine butrylacetycholinesterase enzyme activity in liver and plasma, liver glycogen and plasma glucose content, haematology and behaviour of C. gariepinus to Dichlorvos.

2. Materials and Methods

2.1. Experimental Fish and Pesticide

A total of one hundred and twenty (120) juveniles of African catfish (mean weight 24.52 ± 1.64 g; mean length 18.48 ± 1.01 cm) were obtained from a local outskirt in Enugu Nigeria and transported to Fisheries Wet Laboratory of the Department of Animal/Fisheries Science and Management, Enugu State University of Science and Technology ESUT, Enugu Nigeria. They were held in four fibers reinforced plastic (FRP) tanks, containing 500 L of de-chlorinated tap water. Aeration was provided to all tanks round the clock in order to maintain dissolved oxygen contents. Before the commencement of the study, the fish were acclimatized for two weeks and were fed with commercial fish diet composed of 40% crude protein. The faecal matter and other waste materials were siphoned off daily to reduce ammonia content in water. Dichlorvor (2,2-dichlorovinyldimethyl phosphate) obtained from Boehringer-Mannheim (Germany) was dissolved in distilled water to make a stock solution that was used in the study. Ethical clearance from the Enugu State University of Science and Technology Committee on Experimental Animal Care was obtained and followed.

2.2. Acute Toxicity Test

Toxicity of Dichlorvor to C. gariepinus was carried out according to the OECD guideline for testing of chemicals No. 203 in a semi-static renewal system by using 200L capacity glass aquaria. Three different concentrations (0.4, 0.8 and 1.6mgL^{-1}) and control 0.00mgL^{-1} were selected and prepared in triplicates for definitive exposures after range-finding test and ten (10) fish were exposed to each

replicate. One group was exposed to clean freshwater which served as control. Feed was not offered to the fish for 96 h of test period. Dead fish were immediately removed to prevent deterioration of water quality. The exposure solution was renewed each day and was also analyzed using LC–MS/MS to ensure the agreement between nominal and actual concentrations of the pesticide in the aquaria (Li et al., 2011). The experiment was conducted under the natural photoperiod of 12:12 light-dark cycle. The physico-chemical parameters of the test water were analyzed daily, using standard methods APHA (2005) and were recorded (dissolved oxygen 7.50 ± 0.45 mg L^{-1}, temperature 27.75 ± 0.5 ^{0}C, pH 7.8 ± 0.13 and free carbon dioxide 4.28 ± 0.6 mg L^{-1}). The test fish were sampled on hours 24, 48, 72 and 96 in each replicate to determine the toxic effects of DDVP on the fish. The behavioural response in exposed and control fish were observed and recorded daily. The LC_{50}/LT_{50} was determined by Probit analysis (Finney, 1971). The 95% confidence of 96h- LC_{50} was determined according to Sokal and Rohlf (1994). The safe level was estimated by applying the safety application factor (AF) suggested by CCREM (1991).

2.3. BChE Activities

The activity of butrylcholineaterase (BChE) (EC. 3.1.1.8) in liver and plasma respectively was measured at 540 nm according to Hestrin (1949) as modified by Augutinsson (1957). The activity is expressed as µmole of butrylcholine hydrolyzed mg protein^{-1} min^{-1}.

2.4. Plasma Cortisol, Glucose and Liver Glycogen

The determination of cortisol was performed in plasma using a diagnostic ELISA direct immunoenzymatic kit (Diametra, Italy). Plasma glucose and liver glycogen was measured using a diagnostic kit (Granutest, E. Merck-Darmstadt, Germany).

2.5. Haematological Analysis

Blood was collected from fish through the caudal vein by means of heparinized plastic syringe after the administration of clove oil in order to reduce stress. It was then stored in ethylenediaminetetracetic acid (EDTA) tubes. The blood samples were analyzed, using automated blood analyzer (Pentra XL 80, Pentra 60C+, BIORAD D-10HPLC, Automated Coagulometer, Japan). The following parameters were measured: red blood cell count (RBC), haemoglobin (Hb), packed cell volume (PCV), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC) and total white blood cell count (WBC).

2.6. Statistical Analysis

Data are expressed as mean ± standard deviation and were analyzed using the statistical package SPSS 20.0 computer program (SPSS Inc. Chicago, Illinois, USA). Differences in the test concentrations and control were subjected to one-way analysis of variance (ANOVA), followed by Turkey's multiple range tests to determine significant mean differences. The Pearson correlations between the test biomarkers and blood parameters as well as the principal component analysis to assess the variability associated with each biomarker exposed to different concentration of DDVP were determined on day 4 (after the study) using XLSTAT® 2017. The statistical significance was determined at 95% level of probability.

3. Results

3.1. Behavioural Changes and LC_{50}

Exposed fish to acute concentrations of dichlorvos for 96 h indicated varying degree of behavioural disorder prior to death such as mucus secretion, uncoordinated swimming, air gulping, restlessness

and loss of balance (Table 1), hyperventilation and increased tail fin frequency (Figure 1). Behavioural irregularities of test fish were elevated with increasing concentration of DDVP (Figure 1), thus exhibiting a positive correlation with the concentration. However, butrylcholinestterse and plasma cortisol activities indicated inversely correlated tendency with TBF (r= -0.984 and -0.968 respectively, p <0.05, Table 4). No visible abnormal behavioural disorder was observed in the control group of fish during the study.

At 0.8 mg L^{-1} and 1.6 mg L^{-1} of DDVP, 60% and 90% mortalities respectively were observed in exposed fish (Table 2, Figure 2) while no mortality was recorded in the control group. The 96 h LC_{50} of DDVP to the exposed fish was found to be 0.66 mg L^{-1} with safety value estimated to be 0.01 mg L^{-1} while lower and upper confidence limits gave 0.29 and 1.49 mg L^{-1}, respectively. The 96 h LT_{50} values for 0.8 and 1.6 mg L^{-1} (Table 3, Figure 3) was shown to be 79.23 and 60.26 hours with safety values of 3.98 and 3.01 hours respectively.

Table 1: *Behavioral changes of C. garipinus exposed to different concentrations of dichlorvos for 96 hours*

Behavioural changes	DDVP (mgL^{-1})			
	0.0	0.4	0.8	1.6
Mucus secretion	-	-	xxx	xxxx
Uncoordinated movement	-	-	xx	xxx
Air gulping	-	-	xx	xxx
Coughing	-	-	xx	xxx
Restlessness	-	-	xx	xxxx
Swimming with back	-	-	-	xx
Loss of balance	-	-	xx	xxx

- none, x - mild, xx - moderate, xxx - strong, xxxx - very strong

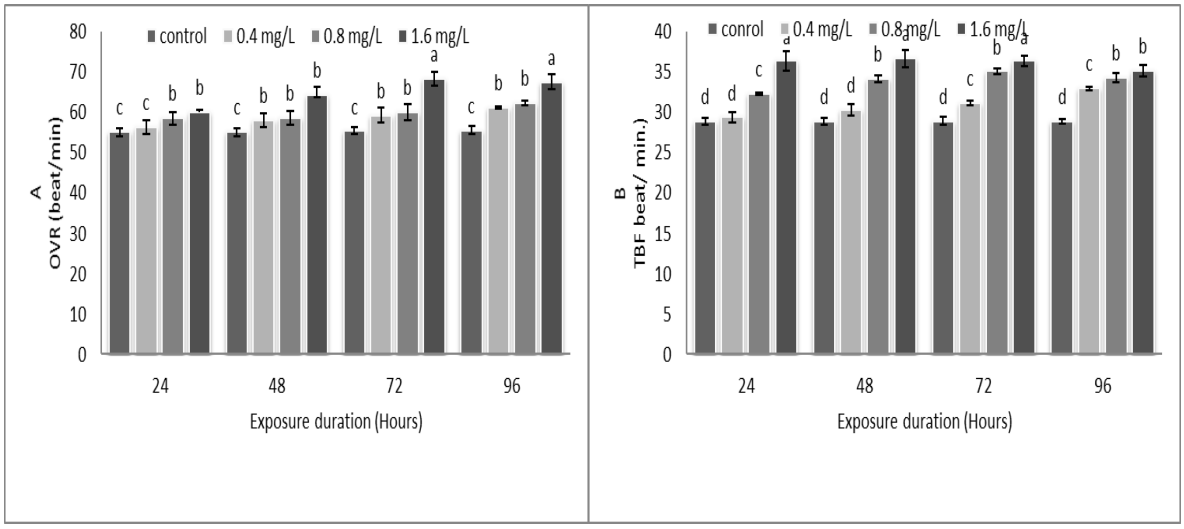

Figure 1: *(A) OVR and (B) TBF of exposed fish to dichlorvor for 96 h*

Table 2: *Mortality of Clarias gariepinus exposed to different concentration of Dichlorvos*

Concentration mgL^{-1}	Log concentration	%mortality	Probit
0.00	0	0	0
0.4	-0.398	30	4.48
0.8	-0.097	60	5.26
1.6	0.204	90	6.28

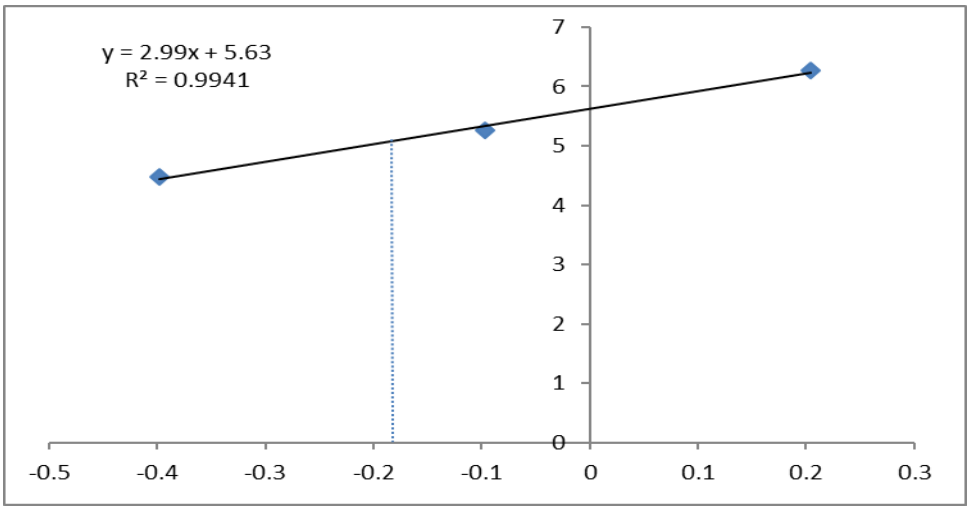

Figure 2: *Logarithmic probit line for determination of 96-h LC50 DDVP to C. gariepinus*

Table 3: *Cumulative mortality and time for 0.8 and 1.6mgL^{-1} DDVP exposed to C. gariepinus for 96 hours*

Time (hrs)	Log time	Cumulative mortality (%) for 0.8mgL^{-1} DDVP	Probit	Cumulative mortality (%) for 1.6mgL^{-1} DDVP
24	1.39	10	3.12	10
48	1.68	20	4.16	30
72	1.86	30	4.48	60
96	1.96	60	5.25	90

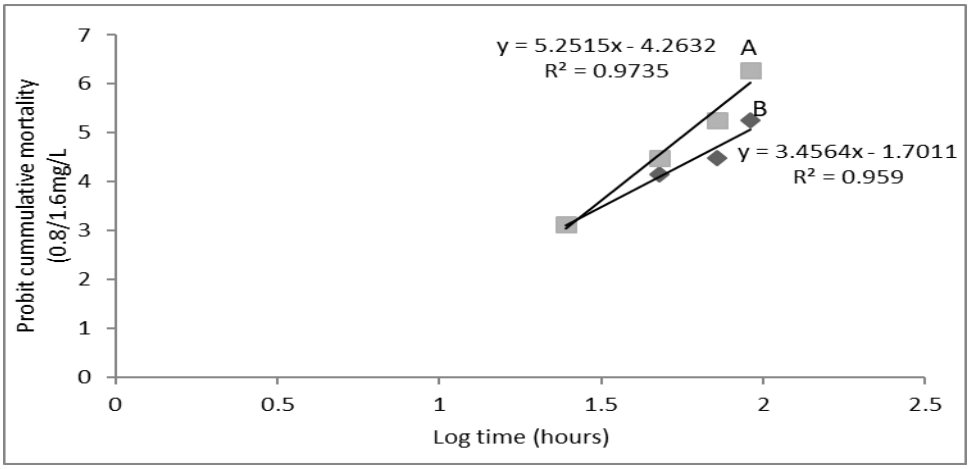

Figure 3: *Logarithmic probit line for determination of 96-h LT50 at (A) 1.6 and (B) 0.8mgL-1 DDVP to C. gariepinus*

PG plasma glucose, OVR opercular ventilation, TBF tail beat frequency, p BuChE plasma butrylcholineaterase, CS cortisol, l BuChE liver butrylcholineaterase, l GLY liver glycogen.

3.2. Plasma Cortisol, plasma Glucose and Liver Glycogen Responses

Plasma cortisol reduced significantly ($p < 0.05$) in exposed fish to 0.8 and 1.6 mg L^{-1} of dichlorvor when compared to control from 72 to 96 h exposure duration. The percentage reduction of 14.7 and 29.3% for 0.8 mg L^{-1} and 26.7 and 44.0% for 1.6 mg L^{-1} was recorded (Figure 4A).

Plasma glucose was elevated throughout the exposure duration and in all test concentration when compared to the control group of fish. The percentage elevation for 0.4 mg L^{-1} was shown to be 25.0, 50, 25 and 6.5% at 24, 48, 72 and 96 exposure periods while those exposed to 0.8 and 1.6 mg L^{-1} rose by 50, 62.51, 62.56, 37.51% and 75.0, 77.5, 75.15, 52.57% respectively from 24,48 72 and 96 h periods (Figure 4B). Liver glycogen was inhibited by 33.8, 35.6, 39.4 and 40.7% at 24, 48, 72 and 96h respectively to 0.4 mg L^{-1} DDVP and by 35.6, 36.4. 41.2, 42.4% for 0.8mg L^{-1} and 53.4, 54.2, 58, 58.5% for 1.6 mg L^{-1} at 24, 48, 72 and 96 h respectively.

Cortisol correlated inversely with OVR r =-0.991 and TBF r= -0.968 and positively to liver BuChE r=0.970 and liver glycogen r=0.964. Liver glycogen correlated inversely to OVR (r=-0.963) and TBF(r=-0.985) and positively to plasma BuChE (r=0.969), CS (r=0.964) and liver BuChE(r=0.975).

Table 4: *Pearson correlation between activities of BuChE, liver glycogen, plasma cortisol, plasma glucose and behavioural response*

Variables	PG	OVR	TBF	p BuChE	CS	l BuChE	l GLY
PG							
OVR	0.895						
TBF	0.853	0.945					
p BuChE	-0.757	-0.883	-0.984				
CS	-0.930	-0.991	-0.968	0.909			
l BuChE	-0.875	-0.942	-0.998	0.978	0.970		
l GLY	-0.805	-0.963	-0.985	0.969	0.964	0.975	

Values in bold are different from 0 with a significance level alpha=0.05

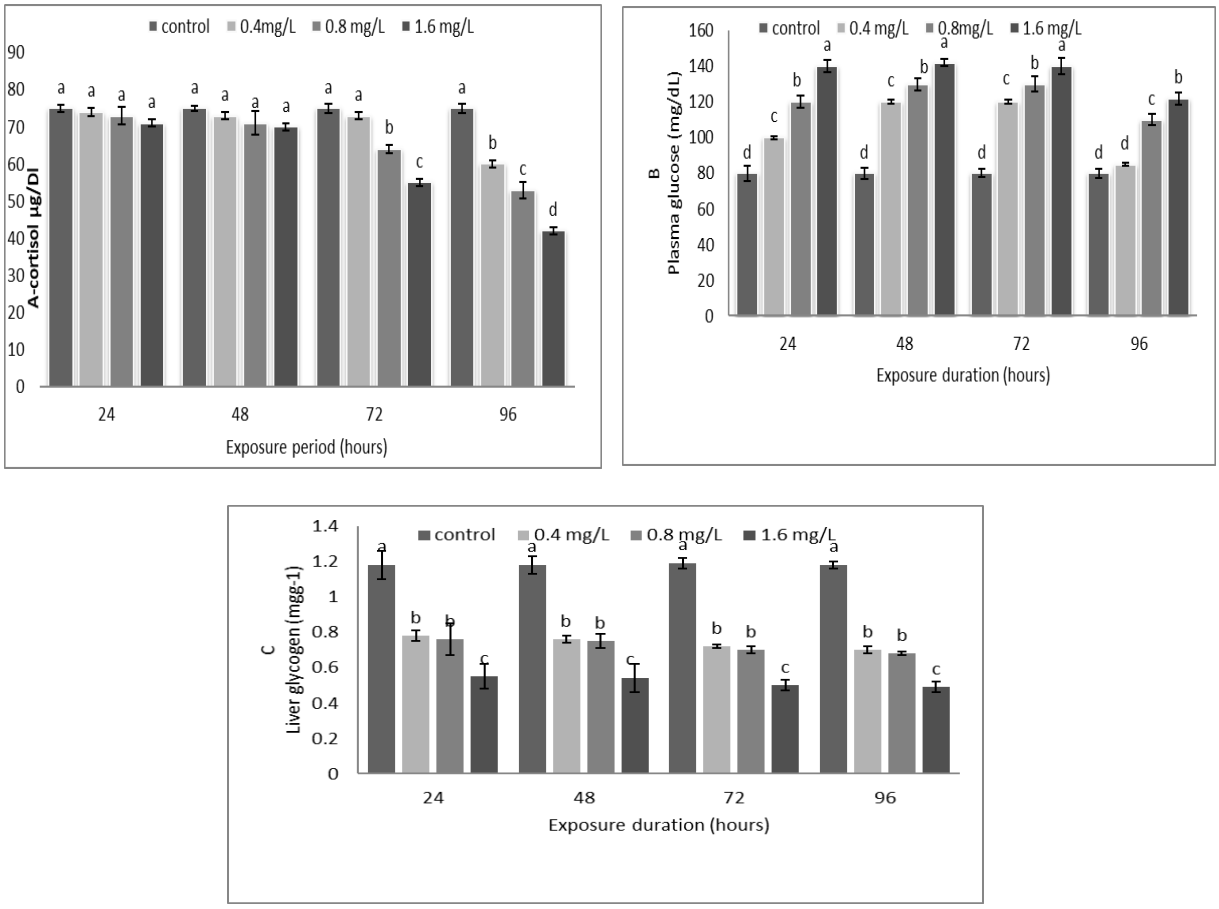

Figure 4: *(A) plasma cortisol (B) plasma glucose and (C) liver glycogen of exposed fish to DDVP for 96 hours*

3.3. BuChE Activities in Liver and Plasma

Butrylcholineaterase activity was significantly lower in fish liver exposed to various concentrations of DDVP throughout the exposure duration when compared to control group of fish. Inhibition by 20, 30.9, 49.3, 54.5% for 0.4 mg L^{-1}, 41.8, 53.7, 63.6 and 78.2% for 0.8 mg L^{-1} and 54.5, 67.3, 78.2, 89.1% for 1.6 mg L^{-1} at 24, 48, 72 and 96 h exposure durations respectively (Figure 5A). Butrylcholineaterase activity was significantly lower in fish plasma exposed to 1.6 mg L^{-1} throughout the exposure duration when compared to control group of fish. Its values decreased by 51.0%, 55.0%, 56.0% and 73.0% respectively at 24, 48, 72 and 96 h of exposure. Decrease recorded in fish exposed to 0.4 and 0.8 mg L^{-1} did not vary significantly when compared with control group of fish (Figure 5B). Plasma BuChE correlated inversely to TBF r=-0.984.and positively to liver BuChE r=0.978 and liver glycogen r=0.970. Liver BuChE correlated inversely to TBF r=-0.998 and positively to CS r=0.970, liver glycogen r=0.975and plasma BuChE r=0.978 (Table 4).

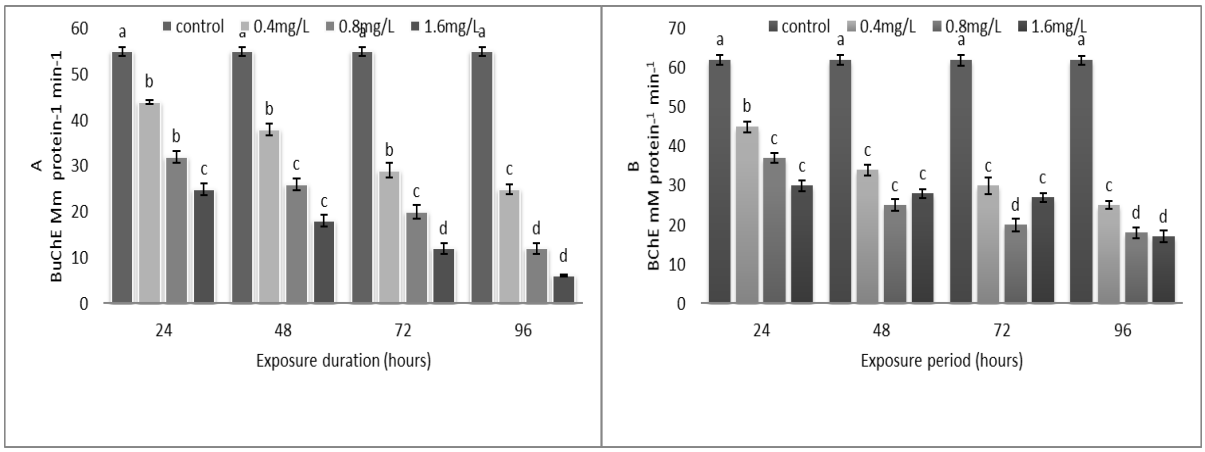

Figure 5: *BuChE (liver) and BuChE (Plasma) activities of exposed fish to DDVP for 96 hours*

3.4. Haematological Responses

There was significant inhibition in the values of HB, RBC and MCV of exposed group of fish compared to the control group. inhibition of 10.2% HB was observed in test fish to 0.8 mg L^{-1} at the 96h period while 10.8% and 13.7% reduction of HB was recorded at 72 and 96 h exposure periods for 1.6 mg L^{-1} exposed fish (figure 6A). Reduction of RBC and PCV was significant in group of fish exposed to 1.6 mgL^{-1} of DDVP, RBC reduced by 19.1%, 40.0%, 50.0% while MCV reduced by 16.6%, 25.2%, and 36.3% from 48, 72 and 96 exposure duration (Figure 6B and 6C). Significant elevation of PCV and WBC was observed in fish exposed to 1.6 mg L^{-1} of DDVP from 72 and 96 h of exposure when compared to the control group of fish. MCV was elevated by 24.6% and 28.1% while WBC increased by 36.3 and 58.1% respectively from 72 and 96 h duration periods. WBC value was inversely related to HB (r=-0.997, p <0.05, Table 5), RBC (r=-0.999, p <0.05) and PCV (r=-0.953, p <0.05). RBC related positively to HB (r=0.998 and PCV (r=0.959), and inversely to MCH (r=-0.996) and MCHC (r=0.995). BuChE activity related positively to PCV (r=0.979) and inversely to MCHC (r=-0.995, p <0.05, Table 5).

Table 5: *Pearson correlation between activities of plasma BuChE and haematological responses*

Variables	Hb	RBC	PCV	WBC	MCV	MCH	MCHC	p BuChE
Hb								
RBC	0.998							
PCV	0.939	0.959						
WBC	-0.997	-0.999	-0.953					
MCV	-0.957	-0.936	-0.815	0.935				

MCH	-0.999	-0.996	-0.938	0.994	0.963		
MCHC	-0.941	-0.962	-0.994	0.960	0.806	0.935	
p BuChE	0.914	0.938	0.979	-0.939	-0.756	-0.904	-0.995

Values in bold are different from 0 with a significance level alpha=0.05

Hb - haemoglobin, RBC - red blood cell, PCV - packed cell volume, MCV - mean cell volume, WBC - white blood cell, MCH - mean cell haemoglobin, MCHC - mean cell haemoglobin concentration, pBuChE - plasma butrylcholineaterase

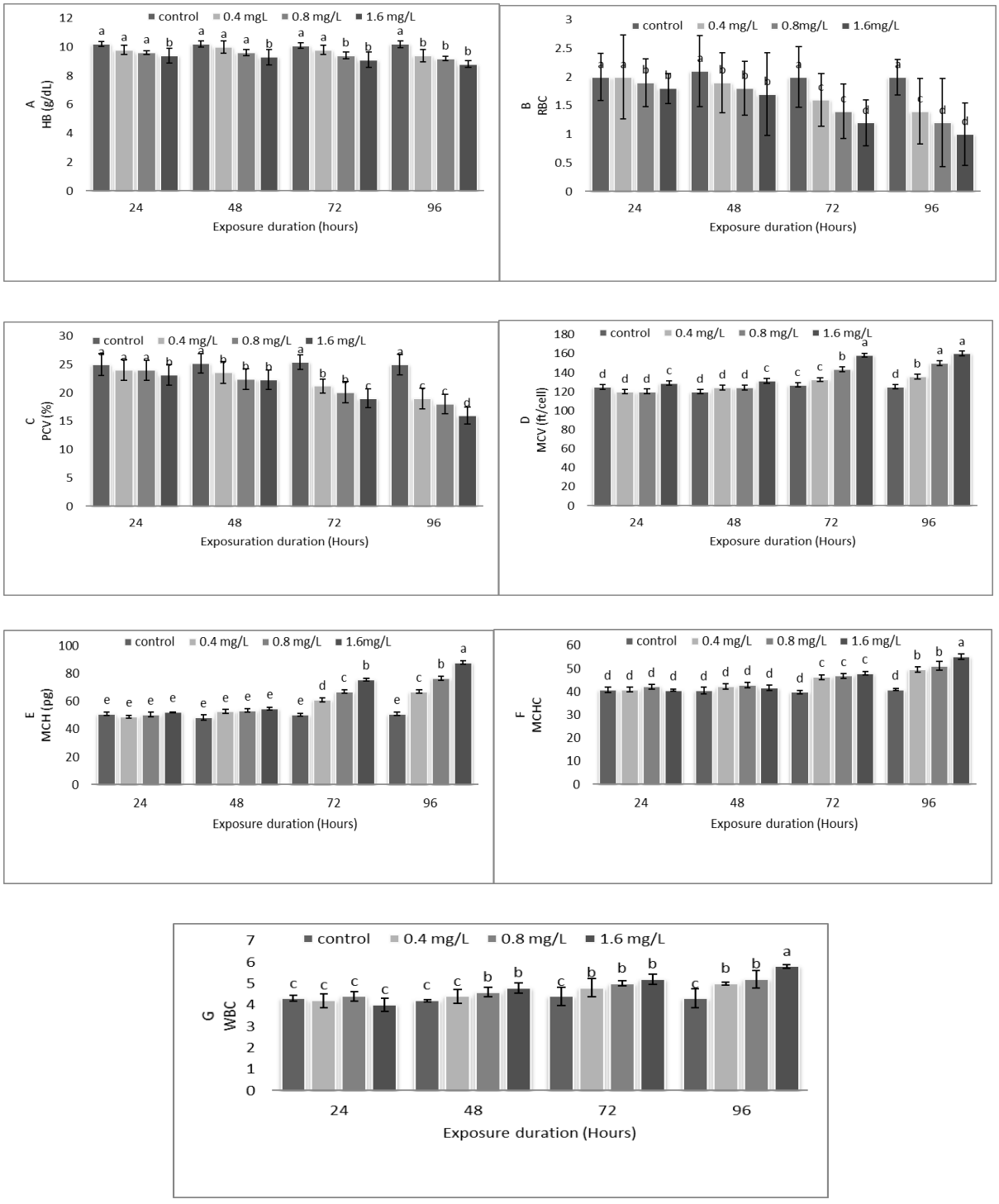

Figure 6: *Haematological responses (A) HB, (B) RBC, (C) PCV, (D) MCV, (E) MCH, (F) MCHC and (G) WBC of fish exposed to DDVP for 96 h*

3.5. Principal Component Analysis in the Blood of Fish

Figure 7 shows the biplot of principal component analysis (PCA) which represents 99% of the total variance of blood parameter and BuChE in the fish after 96 h exposure to different concentrations of DDVP. The first component (PC1) depicts approximately 94% of the total variance which indicate the separation between 0.8 and those exposed to 0.68 mg^{-1} of DDVP through the distribution of the negatively related MCHC and positively related PCV, RBC and BuChE values of fish. The second component (PC2) explains 5% of the total variance, showing the separation of the 1.6 with positively correlated MCV, MCH and WBC and 0.4 mg L^{-1} with negatively related HB value of the fish (Table 5).

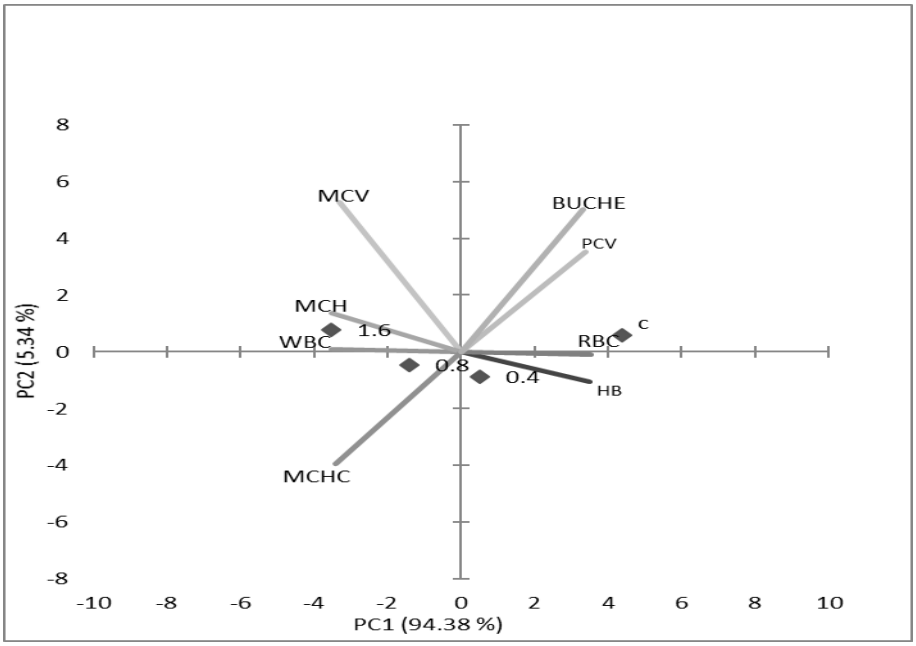

Figure 7: *PCA of blood parameters and BuChE activity of fish exposed to DDVP for 96h*

4. Discussion

4.1. Behavioural Responses and LC_{50}

The present study has demonstrated that organophosphate insecticides available in aquatic ecosystem can elicit various damages to freshwater fish in aquatic environment. Behavioural disorders are vital tools used for assessing the functional status of fish in contact with toxic materials Robinson, 2009). Behavioural irregularities such as uncoordinated swimming and restlessness observed in this context could be a preventive strategy formed by the exposed fish in response to ops and other toxic compounds Aziz et al., 2014). Elevated opercular beats, tail fin beats frequency and respiratory distress observed in the study may be attributed to the effects of DDVP on the fish as it might have influenced impulse stimulating enzymes and available oxygen to treated fish. The exposed fish could have increased opercular and tail beat rates in order to argument for the deficient in oxygen concentration in the gill (Akpa et al., 2010). Omoregie (2009) noted that fish in unsuitable environment usually increase their opercular ventilation. In this study, 0.66 mg L^{-1} was estimated as the 96 h LC_{50} of DDVP to C. gariepinus. This data is lower than 2.35 and 3.17 mgL^{-1} reported for Anabas testudineus (Patar et al., 2015) and Aphanius iberus (Varó et al., 2008) but higher than 0.48 and 0.55mgL^{-1} in bluegill and spots respectively (Kenaga, 1979). Toxicity of compounds on aquatic biota has been documented to have effects on water quality, chemical formulations, age and size of the exposed species (Wekler, 2000; Pandey et al., 2011). The toxic effects of DDVP on C. gariepinus may have caused the mortality observed in the investigation. Firat et al. (2011) found that DDVP had

profound effects on the fish even at lower concentrations and need to be prevented from becoming a potential environmental pollutant.

4.2. BuChE Activity

Our study has indicated that DDVP inhibited BuChE activity in plasma and liver of treated fish which may have triggered accumulation of butrylcholine and sister esters capable of protecting the exposed fish consequently causing altered changes in carbohydrate metabolism (Lucic et al., 2002); haematology (Thiermann et al., 2007) and nervous system functions (Glusczak et al., 2006). BuChE is an enzyme without known biological substrate in animals (Lucic et al., 2002) but it hydrolyses a variety of esters including butyryl thiocholine, butyrylcholine, acetylcholine propionyl thiocholine, propionyl choline, and pharmacologically important succinylcholine (Lucic et al., 2002). It has been suggested that BuChE is the precursor of AChE in the nervous system, with an important role in the regulation of slow impulse conduction in the nervous system (Kutty et al., 1994). Several studies have shown the inhibitory effect of chemicals on plasma BuChE activity (Katalinic et al., 2014). BuChE is found in higher concentrations in the liver and plasma than in other tissues (Inacio Lunkas et al., 2006; Santarpia et al., 2013) because it is synthesized in the liver and released into the bloodstream in free form. Changes in BuChE activity may serve as surrogate information for projecting potential hazards in the health status of C. gariepinus.

4.3. Haematological Responses

The assessment of haematological parameters in fish is important means of understanding the normal, pathological processes and toxicological consequences due to toxic substances (Svobodava, 2001; Sudova et al., 2009). Alterations in blood biochemical parameters serve as an important diagnostic tool that can also be used for the detection of abnormalities in liver and other tissues (Banaee et al., 2011). Our results revealed that the effects of DDVP on the haematology of the fish resulted in a decrease of HB, RBC count and PCV level. Decreases in the above parameters of the blood are indicators of anemia as observed by (Lakshmanan et al., 2013) after exposure of Clarias gariepinus to dichlorvos. The reduction may also be attributed to the limit in erythrocyte synthesis, as well as impaired osmoregulation across the gill epithelium, due to accumulation of the toxicant in the gill region (Pereira et al., 2013). Also, the exposure led to increase in MCHC and MCV. Increase in MCV may be attributed to the increase in immature RBC (Caravalho and Fernandes, 2006). Changes in MCV values are frequently used to estimate possible causes of anemia (Aslan et al., 2002). Increase in WBCs in DDVP-treated fish indicates an immune response to the toxic effects of the insecticide. Our results are in agreement with Mallum et al. (2016) who reported decrease in HB, RBC, and PCV in O. niloticus to DDVP for 96h exposure duration, and also with reported increase in WBCs in O. niloticus exposed to channel blocker pharmaceutical drug, verapamil (Ajima et al., 2016). Contrary to our report, Lakshmanan et al. (2013) found reduction in leukocyte counts in O. niloticus exposed to DDVP which they attributed to immune suppression of the WBCs by the toxicant. The BuChE may be considered as a potential biomarker of adverse health effects of red blood since it related to red blood cells (Tanasorn et al., 2013).

4.4. Plasma Cortisol Response

In most fishes, cortisol levels increase an hour after stress, and return to normal six hours later (Iwana et al. 2006). This response in fish is due to stimulation of the hypothalamus, as a result of combined neuro -endocrine system activation and accompanied by metabolic changes (Lowe and Davison, 2005) which assist behavioural adaptation of fishes to environmental variation and maintenance of homeostasis (Pickering 1998). The primary stress response is the perception of an altered state by the central nervous system and the release of stress hormone cortisol ((Iwana et al., 2006). Our results however indicated inhibition of cortisol in exposed fish to DDVP probably due to impairment of the adrenal area responsible for cortisol secretion. Hontela et al. (1992) stated that only fish

chronically exposed to pollutants exhibited impaired cortisol function. Long-term stress may suggest an inhibition in the protein system responsible for cortisol transport, due to energy utilization and exhaustion in the protein synthesis pathway and difference in functionality in terms of affinity and binding potential (Lynn et al., 2003; Aaron et al., 2004). Aluru et al. 2004 noted that cortisol response-impairment was due to exhaustion of the cortisol-producing system and pituitary corticotrope atrophy, possibly as a result of its prolonged hyperactivity). Our results however do not agree with the previous statement because cortisol responses are complex, and may depend on fish species and tested pesticide rather than on short-term vs. long-term exposure (Teles et al., 2003). Several studies have corroborated the impairment in the cortisol synthesis and secretion due the action of chemicals. Gravel and Vijayan, (2006) studied the impacts of three pharmaceuticals (acetaminophen, ibuprofen, and salicylic acid) in rainbow trout and supported the hypothesis that these pharmaceuticals disrupt steroidogenesis in fish internal tissue. These findings were also tested in vitro and observed that salicylic acid produced a depression of ACTH stimulation in cortisol secretion and a lower gene expression of steroidogenic acute regulatory (StAR) protein, which is involved in steroidogenesis of cortisol (Hontela, 2006). StAR protein may be a sensitive target of many environmental pollutants, ranging from pesticides to pharmaceuticals (Hontela, 2006). Considering the present experimental design, it is not possible to clarify the mechanism involved in this endocrine disruption; therefore complementary parameters should be evaluated.

4.5. Plasma Glucose and Liver Glycogen Responses

Hyperglycemia is a typical response caused by the exposure of fish to several pesticides (Sriwastwa and Singh, 1981; Sharma, 1999). The occurrence of hyperglycemia is an important phenomenon in animals subjected to pesticide stress. Our result shows that an increase in the rate of glycogenolysis in liver may have caused an increase in the blood sugar level and it is in agreement with the results obtained in Barbus conchonius exposed to endosulfan (Lakshamann et al., 2013) in Clarias batrachus, Saccobranchus fossilis and Mystus vittatus exposed to sub lethal concentrations of thiotox and dichlorvos. It is evident from the present investigation that Dichlorvos has a specific impact on BuChE activity causing increased glycogenolysis or decreased glycogenesis on tissue glycogen to enhance blood glucose level in test fish. It is suggested in the present study that carbohydrate metabolism plays an important role in energy yielding process to overcome the severe energy crisis at the cellular level due to pesticide stress (Glusczak et al., 2006). A stressful situation in an animal elicits neuroendocrine responses, which in turn induces disturbances in carbohydrate metabolism (Lakshamann et al., 2013). An overall lowering in glycogen level in tissues might be due to the prevalence of hypo toxic or anoxic conditions which in turn increase the carbohydrate utilization (Martinez et al., 2009). It is believed in the present study that depletion in glycogen content in liver may be due to an increased demand for glucose to meet the energy requirements or may be due to disturbance in the mechanism of glycogenesis. Such findings have also been observed by Medda et al. (1993) who established rapid utilization of liver and other tissues glycogen in fishes exposed to Ops.

Although previous studies have shown a positive correlation between BuChE activity and glucose levels (Cwiertnia et al., 2010; Jabeen et al., 2014) this study showed significant correlation ($P < 0.05$) with liver glycogen. Although the mechanism is unknown, evidence of relationship between the activity of BuChE and carbohydrate metabolism in liver and plasma of C. gariepinus could serve as early warning of health disorder (Thiermann et al., 2007; Cwiertnia et al., 2010; Benyamin et al., 2011; Santarpia et al., 2013).

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Research Article

Haematology, Oxidative Stress and Micronuclei Frequency of Clarias Gariepinus Exposed to Glyphosate based Herbicide Glycot® GBHG

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Abstract Glyphosate (N-phosphonomethyl glycine) is a broad-spectrum systemic herbicide used extensively in weed control in Nigeria. The present study was designed to evaluate the toxicity effects of glyphosate-Glycot® on post juveniles (50±1.96g; 22±1.2cm n=300) of *Clarias gariepinus* exposed to triplicate acute doses: 31, 33, 35, 37 and 39 mg glycot L^{-1} of clean water and to 8 day sub-acute doses: 3.39, 6, 78 and 16.95mgL^{-1} of the same, corresponding to $^{1}/_{10}$ LC_{50}=3.39 mgl^{-1}, $^{1}/_{5}$ LC_{50}=6.78 mgL^{-1}, ½ LC_{50}=16.95 mgL^{-1}, A set of fish were also maintained simultaneously in water as the control (0.0 mg glycot /L of clean water), during the exposure periods. The 96 hours LC_{50} of the herbicide to the fish was determined at 33.39 mgL^{-1} which corresponds to 96h safety dose of $^{1}/_{100}$ LC_{50} = 0.35 mg/L. Blood samples were taken at intervals of day 1, 4 and 8 for assessment of micronucleus frequency, hematology and antioxidant enzymes. The hematological parameters were significantly reduced in the treated values of PCV and ranged from 20.00±0.19 – 23.33±0.55 %) below the value of 28.33±0.61% recorded in the control. Similarly, the red blood cells RBC, and haemoglobin HB recorded inhibited ranges of 8.58±0.21 - 9.10±0.03 x 10^{6} mm^{3} below their elevated respective controls of 10.06±0.03 x 10^{6} mm^{3} and 8.73±0.05 g/dL. However, the white blood cells WBC and platelets PL recorded elevated ranges of 12934.00±544.68 - 13700.00±485.72 x 10^{3} mm^{3} and 15933.33±322.79 above their respective control values of 9466.66 0.09±96.86 x 10^{3} mm^{3} and 15633.33±181.89. Catalase CAT was significantly ($p<0.05$) inhibited in treatments compared to control, and it ranged from the highest value of 0.74 umol mm^{-1} mg $protein^{-1}$ in control to the lowest value of 0.21 umol mm^{-1} mg $protein^{-1}$ in exposed group to 16.95 mg/L on day 4 but returned to control value on day 8 in all the treatments. Superoxide dismutase SOD was significantly ($p<0.05$) inhibited among exposed fish compared to control on days 1-4. A range of 11.60 U mg $protein^{-1}$ in control on day 1 to lowest value of 6.50 U mg $protein^{-1}$ returned to control value on day 8. Similarly, GPX was significantly inhibited to a lowest value of 4.25 in fish exposed to 3.39 mg/L compared to the highest value of 9.36 in control fish on day 4 but returned to control value with a high value of 9.25 $umol^{-1}$ min $protein^{-1}$. Similarly, Lipid peroxidation LPO ranged from the highest value of 6.8±0.00058 mMole/TBARS/ mg protein in 16.95mg/L to a lowest value of 6.41 mMole/TBARS/ mg protein in control on day 4, returned to the control value on day 8. The mean values of micronuclei frequency in the treatments were significantly ($p<0.05$) elevated above the control value of 8.50±0.93 to the highest value of 406.66±15.03. This finding indicated that GBHG impaired haematology, antioxidative stress enzymes and cytogenic

potential of *C. gariepinus* and could serve as an early warning signs toward the avoidance of its ecotoxicological hazards in aquatic ecosystems in Nigeria.
Keywords *Ecotoxicological hazards; Micronucleus frequency; Haematology; Anti-oxidative stress enzymes; Catfish*

1. Introduction

Glyphosate based herbicide Glycot® is a commercial formulation of glyphosate (N-phosphonomethyl glycine) based herbicide, whose main surfactant is 15% polyethoxylated tallow amine POEA without the listing of other ingredients (Cox and Surgan, 2006). It was launched in India but has wide application in Nigeria as a broad spectrum in the control of broad-leaved weeds, grasses and sedges in the cultivation of cassava, sugar cane, yam, and potatoes. Its main route into the aquatic environment is indirect from runoff of agricultural fields along waterways occasioned by extensive and unregulated usage, although Tsui and Chu (2008) reported direct use of a related glyphosate herbicide, Roundup in the control of aquatic weeds in fish ponds, lakes, canals and slow running water. Surfactants play the key useful roles and support the adhesion, wetting, spreading and uptake through the leaf in plants but many unspecified members have been reported on related herbicides to be very toxic compared to the original glyphosate and other glyphosate based herbicides GBH (Williams et al., 2000; Santos et al., 2005; Modesto and Martinez, 2010). The toxicity of GBHG is however very scarce.

The study of the alteration in fish blood by herbicides and pesticides has been widely used by several workers to evaluate fish health status (Banaee et al., 2011; Ezike et al., 2017). The use of such haematological parameters such as packed cell volume PCV, numbers of red blood cells RBC and white blood cells WBC, haemoglobin HB and platelets PL has been noted to be indicators of toxicity with wide application in environmental monitoring and aquatic animal toxicological studies (Bacellos, 2003).

Pollutants and several xenobiotics have been implicated to induce reactive oxygen species ROS including hydrogen peroxide H_2O_2, Superoxide anion and hydroxyl radical which often due to their high reactivity result to lipid, protein and carbohydrate damages. Many enzyme activities that act as antioxidants can be used as a biomarker for assessment of pesticide contamination in water (Chandrasekara and Pathirantne, 2005). Biochemical markers, like lipid peroxidation (LPO) and antioxidant enzymes such as catalase CAT, Superoxide dismutase SOD and glutathione peroxidase GPX are widely used to assess the toxic stress, integrity of the immune system and tissue damage in different organisms (Ansari et al., 2011; Dabas et al., 2012). They have the advantage of being sensitive, highly conserved between species and often easier to measure as stress indices (Agrahari et al., 2007). The antioxidant enzymes have been shown to work in synergistic manner to protect against oxidative stress and tissue specific damage. Oxidative stress develops when there is imbalance between pro-oxidants and anti-oxidants ratio, leading to the generation ROS and consequently to membrane LPO (Scandalios, 2005; Modesto and Martinez, 2010; Jaqueline and Biller, 2017) and impaired chromosomal damage (Fenech, 2011).

Micronucleus are essentially lagging whole chromosomes or its fragments which failed to correctly attach to spindle during segregation of chromosomes in anaphase in a mitotic cell division of animals, often without a true nucleus but are enclosed with a nuclear membrane, structurally similar to but smaller in size compared to conventional nucleus. Fenech et al. (2011) reported that micronucleus assay systems are very economical, require much less skill in scoring than conventional metaphase tests, and are much faster than these conventional tests. Since micronucleus assays reflect chromosomal aberrations reliably and rapidly, they are extremely useful for a quick assessment of chromosomal damage. Marked increase in the number of cells with micronuclei can be concluded that the chemical induces structural and/or numerical chromosomal damage. Since micronucleus tests

must be performed on actively dividing RBC produced from bone marrow of animals are ideal candidates for the test.

Sancho et al. (2000) noted that teleost fish have proved to be good models to evaluate the toxicity and effects of contaminants on animals since their biochemical responses are similar to those of mammals. The African catfish *C. gariepinus* is an important economic fish found in most African rivers and is considered as a potential bioindicator species (Shagbanmu et al., 2018).

2. Materials and Methods

2.1. Experimental Fish, Herbicide and Range Finding Test

The test herbicide GBHG is a commercial formulation of glyphosate 41% SL with a trade name Glycot, was produced by Sabero Organics Ltd., Gujrat, India and supplied and distributed by Afcott Nigeria PLC. The herbicide was purchased from Ogbete main market, Enugu North Enugu, Nigeria. Three hundred **(**300) post Juveniles of African catfish, *Clarias gariepinus* (Burchell, 1822) of about 8–12 weeks old with an average weight of 50g±1.96g and 22cm±1.2cm of length were sourced from Nature Blend Remedio Farms, a commercial farm in Onuagu Amon, Onyeama hills Ngwo, along Enugu-Onitsha expressway, Enugu. The fish were transported in FAO aerated fish transit tanks to Heldin Fisheries Laboratory Emene, Enugu where they were acclimatized under laboratory conditions for 2 weeks (14 days) in four plastic aquaria tanks of 300L capacity, containing dechlorinated and aerated tap water. They were fed twice daily at 3% body weight with Copens feed (3.2 mm) at 08.00hrs and 17.00 hours and water changed daily during the acclimatization period. Feeding was however terminated 24 hours before the commencement of the experimental study to empty their stomach and avoid pollution of the water with their feces. Permission from the Committee for the protection and care of animals Enugu State University of Science and Technology Enugu was observed prior to and throughout the research. A 24h range finding test was first carried out prior to the toxicity test to ascertain the concentrations of the test solution for definitive test.

2.2. Acute Toxicity

Acute Toxicity of GBHG was conducted following OECD (1992), guideline for testing of chemicals No. 203 in a semi-static renewal system by using 200 L capacity glass aquaria. Eighteen (18) fish per treatment were randomly exposed to 5 experimental treatments (31, 33, 35, 37 and 39) and a control (0.0) mgL^{-1} of GBHG. Mortality and Survival rate were monitored and recorded at 24, 48, 72 and 96h intervals respectively. The 96h median lethal concentration (LC_{50}) value was determined from the bioassay results using the Probit analysis method described by Finney (1971). The LC_{16}, LC_{50}, and LC_{84} values were read off from the Probit versus log concentration line graph. Feeding was suspended 24h prior to and during acute test. The slope function (S) and F constant were determined by the formula:

$S = \frac{1}{2} (LC_{84}) + (LC_{50})$, F = antilog (2.77log S)

LC_{50} LC_{16} $\sqrt{N}$

S = Slope; F = factor Constant and N = Number of fishes tested at concentrations whose effects are between 16% and 84% mortality (Lichfield and Wilcoxon, 1949).

2.3. Subacute Toxicity

Sub-Acute Toxicity Fish to GBHG were exposed to four (3) sub lethal treatments and a control (0.00mg/L) for 192 hours (8 days). The resultant concentrations of the herbicide were ($^{1}/_{10}$

LC_{50}=3.39mgL^{-1}, $^1/_5$ LC_{50}=6.78mgL^{-1}, ½ LC_{50}=16.95 mgL^{-1} and control 0.0 mgL^{-}1 of GBHG. The test solution was changed and re-treated every 48h to counter-balance the decreasing pesticide concentration due to its hydrolysis in water. Samples for the haematology, oxidative stress and micronuclei frequency were taken in triplicates at 24h (day 1), 96h (day 4) and 192h (day 8) to investigate the hematology and micronuclei effect of glyphosate in the peripheral blood cells and antioxidant enzyme activity and lipid peroxidization in liver during the sub-lethal test. Feeding was served at 5% of body weight once every day using a commercial diet.

2.4. Haematology Assay

Blood samples were collected by incising the caudal vein of the caudal peduncle with a heparinized syringe & EDTA vials and was used to evaluate the effects of glyphosate in the fish blood by estimating according to Ochei and Kolhatkar (2000), these hematological parameters; Packed Cell Volume (PCV), Red Blood Cell (RBC) count, Hemoglobin (Hb/dl), Platelets and total white blood cell count (WBC).

Red Blood Cells (RBC) Count

The red blood cells count was determined by the method of Ochei and Kolhatkar (2000) using a Microscope, Haemocytometer, RBC pipette and RBC diluting fluid (Sodium citrate). The blood specimen was diluted at 1:200 with RBC diluting fluid and cells were counted under (40×) magnification by using a Neuber counting chamber. The number of cells were calculated and reported as the number of red cells/cu.mm of whole blood.

Total RBC (mm^3) = N x 1 x 1 x 200

0.2 0.1

N = Numbers of cells counted; 0.1 = depth of the chamber; 0.2 = area counted; 200 = dilution factor.

The white blood cell count was determined following the method described by Ochei and Kolhatkar (2008). The glacial acetic acid lyses the red cells while gentian violet slightly stains the nuclei of the leucocytes. The blood specimen was diluted1:20 in a WBC pipette with the diluting fluid and the cells were counted under low power microscope by using a counting chamber. The number of cells in undiluted blood was reported as the number of white cell/cu.mm of the whole blood.

Total WBC (mm^3) = N × 20

0.1 × A

N = Number of cells counted; 0.1 = depth of the chamber; A = area counted; 20= dilution factor.

Packed cell volume (PCV) was estimated as described by Ochei and Kolhatkar (2000). Blood sample was taken with a heparinized capillary tube, cleaned and sealed with plasticine. The filled tubes were placed in the microhematocrit centrifuge and spun at 10,000 rpm for 5 minutes. Spunned tubes were placed into a specially designed scale and PCV was read as a percentage.

PCV% = Packed RBC column height × 100

Total Blood Column Height

Hemoglobin (Hb/dl) concentration was determined using cyanomethaglobin technique as outlined by Ochei and Kolhatkar (2000). 4ml of Drabkin's solution which contains potassium ferricyanide,

potassium cyanide and potassium dihydrogen phosphate was well mixed with the haemoglobin in a test tube and allowed to stand for 10mins at room temperature. The ferricyanide formed methemoglobin which was converted to a colored cyanmethemoglobin by the cyanide. The absorbance was measured colorimetrically at 540nm with Drabkin's solution as a blank.

2.5. Oxidative Stress

Antioxidative Stress Enzyme Assay

Fish livers were weighed and homogenized in 0.1M potassium phosphate at 15000g, 4^0C for 20minutes. The supernatant was collected for biochemical parameter of the antioxidant enzymes and lipid peroxidation. The catalase (CAT) in the blood was determined according to the method of Takahara et al. (1960) which involved H_2O_2 breakdown, and was measured spectrophotometrically at 240 nm. Enzyme activity was expressed as nanomoles of H_2O_2 decomposed min/L mg/L protein. Superoxide dismutase (SOD) activity were determined using the method of Misra and Fridovich (1972), based on the oxidation of epinephrine-adrenochrome transition by the enzymes. Superoxide dismutase activity was assed spectrophotometrically at 420 nm and expressed as the amount of enzyme mg/L of protein required to give 50% inhibition of epinephrine auto-oxidation. Glutathione peroxidase (GPX) activities was assayed according to Paglia and Valentine (1967) which was based on the oxidation of glutathione in the presence of NaN_3.

Lipid Peroxidation

Lipid peroxidase LPOX in the liver tissue was determined by estimation of thiobarbituric acid reactive substances (TBARS), according to Sharma and Krishna-Murti (1968). TBARS concentration was measured spectrophotometrically at 535 nm at molar extinction coefficient of 156 Nm cm/L in mMole/TBARS/ mg protein.

2.6. Micro Nucleus Frequency

The genotoxicity assessment/potential of the pesticide was assessed by micronuclei assay. Peripheral blood samples were collected from the caudal vein and smeared on clean, grease-free, frosted glass slides. The slides were fixed in methanol for 10mins and left to air dry at room temperature and finally stained with 6% Geimsa in Sorenson buffer (pH 6.9) for 20mins. After dehydration through graded alcohol and clearing in Xylene, slides were mounted in a mixture of Distyrene (Polystyrene), Plasticizer (tricresyl phosphate) and Xylene. From each slide, 1000 erythrocyte cells were scored under light microscope under 100 magnifications. Non refractive circular or ovoid chromatin bodies smaller than one third of the main nucleus and displaying same staining and focusing patterns as the main nucleus were scored as the micronucleus (Carrasco et al., 1990; Al-Sabti and Metcalfe, 1995; Nwani et al., 2013). The micronuclei frequency was calculated as:

MN (%) = Number of cells containing micronuclei × 100 / Total number of cells counted 1

2.7. Water Quality Parameters

The physico-chemical parameters of the test water were analyzed daily, using standard methods (APHA, 2005, AOAC, 2005) and were recorded (dissolved oxygen 7.50 ± 0.45 mg L^{-1}, temperature 27.75 ± 0.5^0C, pH 7.8 ± 0.13 and free carbon dioxide 4.28 ± 0.6 mg L^{-1}).

2.8. Statistical Analysis

The data obtained were analyzed using statistical package SPSS (Version 22). These data were subjected to one-way analysis of variance (ANOVA) to determine the significant differences among

treatments at 5%. The obtained results and mean values (±SEM) of the toxicity experiment was analyzed using the statistical package SPSS (Version 22). Significantly different results were compared by Duncan's multiple range test with accepted level of significance at $p<0.05$ (Duncan, 1955).

3. Results

Table 1: *The cumulative percentage mortality rate and Probit mortality of Clarias gariepinus exposed to different concentrations of GBHG for 96 hours*

Conc. (mg/L)	Log Conc. (mg/L)	Fish exposed	Cumulative mortality				% Survival	% Mortality	Probit mortality
			24	48	72	96			
Control 0.0	0	10	-	-	-	-	100	0	-
31	1.50	10	-	1	1	1	70	30	4.48
33	1.52	10	-	2	1	1	60	40	4.75
35	1.54	10	1	1	1	2	50	50	5.00
37	1.56	10	1	1	2	3	30	70	5.52
39	1.59	10	1	2	2	3	0	90	6.28

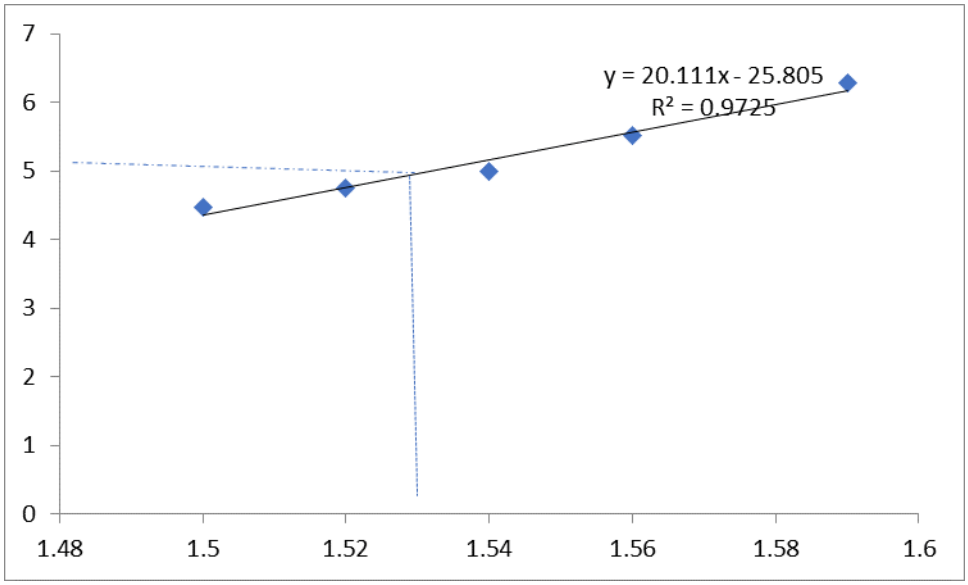


Figure 1: *Logarithmic Probit line to determine 96 h LC_{50}*

3.1. Acute Toxicity

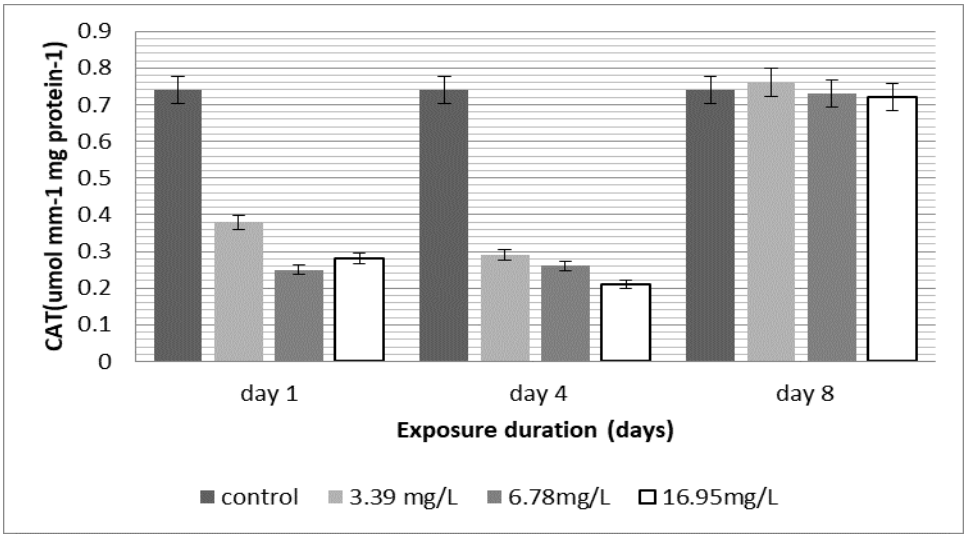
Fish were exposed to acute doses: 31, 33, 35, 37, 39 and control (0.00) mg glycot L^{-1} of clean water and obtained 96h LC_{50} value of 33.9 mgL^{-1} and a logarithmic Probit line y = 20.11x - 25.80, R^2 = 0.972. Sub -acute doses: 3.39, 6.78, and 16.95mgL^{-1} of the same, corresponding to $^1/_{10}$ LC_{50}=3.39 mgL^{-1}, $^1/_5$ LC_{50}=6.78 mgL^{-1}, ½ LC_{50}=16.95 mgL^{-1}, were generated. The 96 h LC_{50} of the herbicide to the fish corresponds to 96h safety dose of $^1/_{100}$ LC_{50} = 0.339 mgL^{-1}.

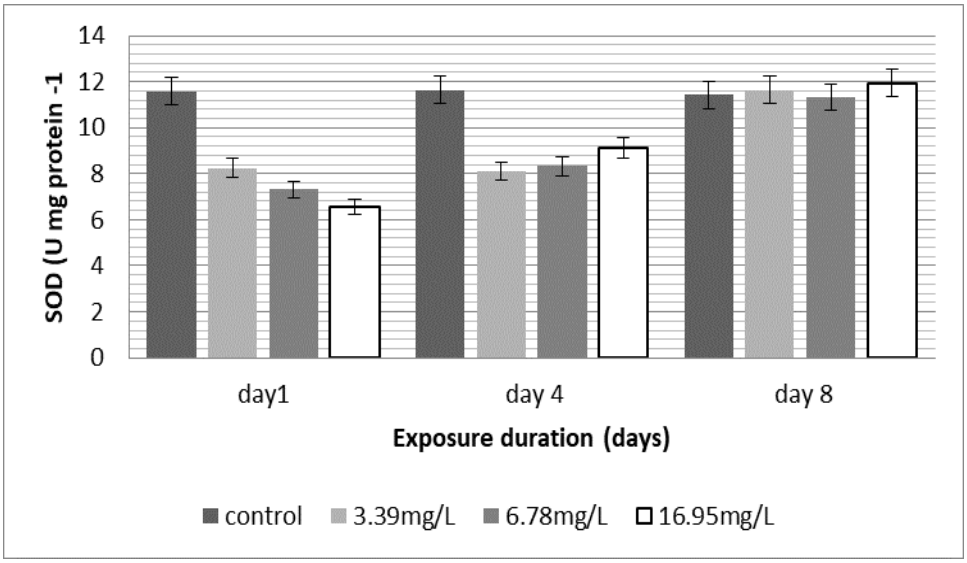
Table 2: *Hematological parameter of Clarias gariepinus exposed to different concentrations of GBHG for 8 days*

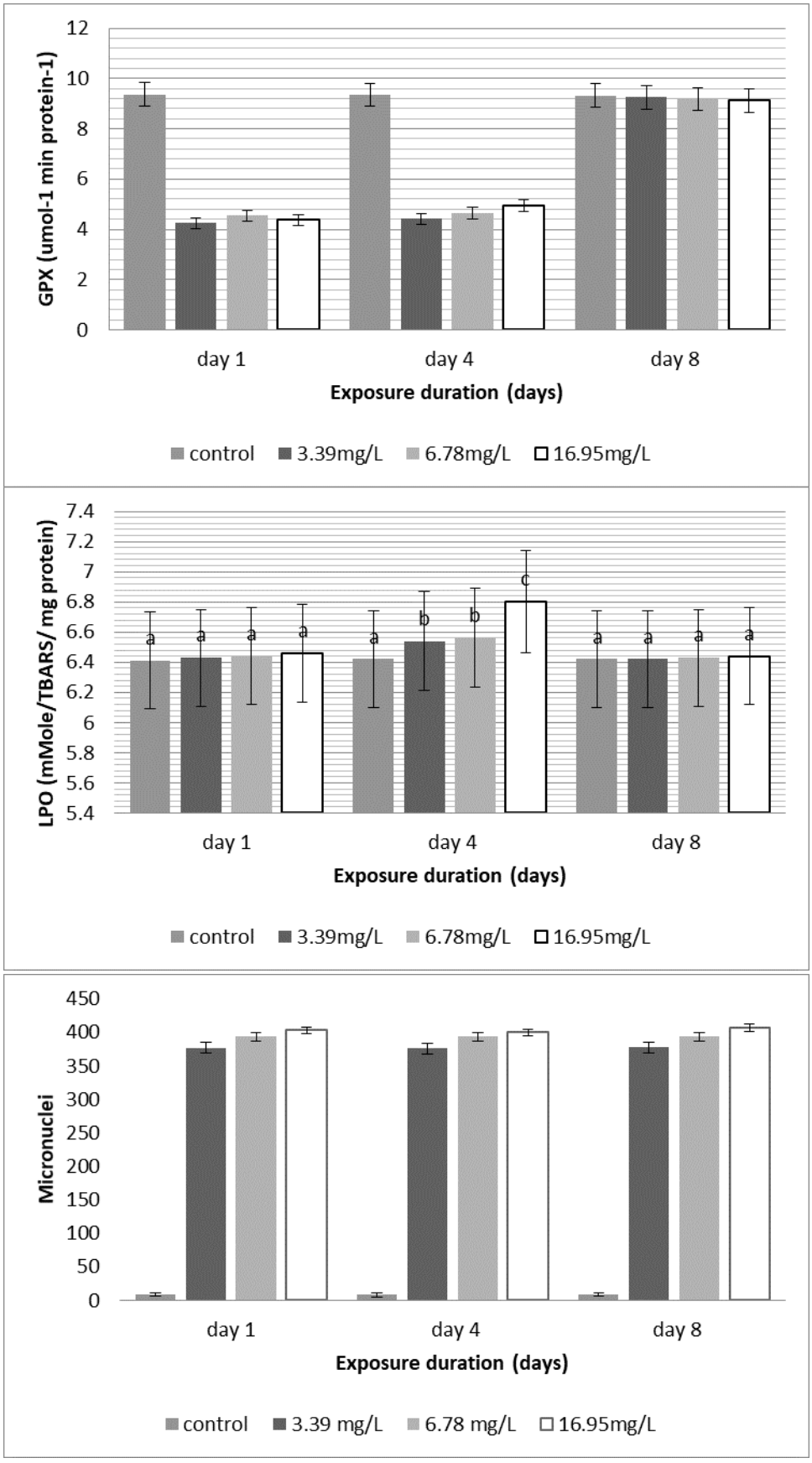
Parameters	Concentrations (mg/L)	Exposure days		
		1	4	8
PCV	Control	28.00±0.38 [a]	27.33±0.40 [a]	28.33±0.61 [a]
	3.39	23.00±0.33 [b]	23.00±0.19 [b]	23.33±0.55 [b]

	6.78	21.33±0.29 ^{b}	20.33±0,29 ^{b}	21.33±0.11 ^{b}
	16.95	21.33±0.58 ^{b}	21.00±0.66 ^{b}	20.00±0.19 ^{b}
RBC	Control	10.05±0.03 ^{a1}	10.06±0.03 ^{a1}	10.05±0.03 ^{a1}
	3.39	8.60±0.06 ^{b1}	8.61±0.06 ^{b1}	8.61±0.06 ^{b1}
	6.78	9.09±0.12 ^{a2}	9.10±0.12 ^{a2}	9.10±0.12 ^{a2}
	16.95	8.58±0.21 ^{b1}	8.58±0.21 ^{b1}	8.59±0.21 ^{b1}
WBC	Control	9433.33±125.21 ^{a1}	9400.00±153.96 ^{a1}	9466.66±96.86 ^{a1}
	3.39	13033.33±544.44 ^{a2}	12934.00±544.66 ^{a2}	13133.66±544.72 ^{a2}
	6.78	13333.33±357.66 ^{a2}	13233.33±363.28 ^{a2}	13400.00±360.55 ^{a2}
	16.95	13633.33±481.25 ^{b1}	13566.66±477.00 ^{a2}	13700.00±485.72 ^{b1}
Hb/dl	Control	8.73±0.05 ^{a1}	8.63±0.07 ^{a1}	8.73±0.05 ^{a1}
	3.39	7.43±0.09 ^{b1}	7.56±0.09 ^{b1}	7.50±0.12 ^{b1}
	6.78	7.70±0.12 ^{a2}	7.73±0.13 ^{a2}	7.70±0.11 ^{a2}
	16.95	7.86±0.11 ^{a2}	7.73±0.12 ^{a2}	0.96±0.10 ^{a2}
Platelets	Control	15600.00±203.67 ^{a}	15600.00±220.94 ^{a}	15633.33±181.89 ^{a}
	3.39	13900.00±472.58 ^{a}	13866.66±449.41 ^{a}	13933.33±496.40 ^{a}
	6.78	15866.66±327.91 ^{a}	15533.33±298.96 ^{a}	15933.33±322.79 ^{a}
	16.95	15633.33±401.07 ^{a}	15600.00±397.67 ^{a}	15600.00±384.41 ^{a}

Mean Values with different alphabetic superscripts (a1, a2, b1 and b2) differ significantly ($p<0.05$) between concentrations in each parameter.







Different alphabetic superscripts differ significantly ($p<0.05$) between concentrations in each parameter.

Figure 2: *Mean of CAT, SOD, GPX and LPO C. gariepinus to GBHG for 8 days*

3.2. Haematology

The hematological parameters were significantly reduced in the treated values of PCV and ranged from 20.00±0.19 – 23.33±0.55 %) below the value of 28.33±0.61% recorded in the control (table 2). Similarly, the red blood cells RBC, and haemoglobin HB recorded inhibited ranges of 8.58±0.21 - 9.10±0.03 x 10^6 mm^3 below their elevated respective controls of 10.06±0.03 x 10^6 mm^3 and 8.73±0.05. However, the white blood cells WBC and platelets PL recorded elevated ranges of 12934.00±544.68 - 13700.00±485.72 x 10^3 mm^3 and 15933.33±322.79 above their respective control values of 9466.66 0.09±96.86 x 10^3 mm^3 and 15633.33±181.89.

3.3. Antioxidative Enzymes and Lipid Peroxidation

Catalase CAT was significantly ($p<0.05$) inhibited in treatments compared to control, and it ranged from the highest value of 0.74 umol mm^{-1} mg $protein^{-1}$ in control to the lowest value of 0.21 umol mm^{-1} mg $protein^{-1}$ in exposed group to 16.95 mg/L on day 4 but returned to control value on day 8 in all the treatments (figure 2). Superoxide dismutase SOD was significantly ($p<0.05$) inhibited among exposed fish compared to control on days 1-4. A range of 11.60 U mg protein $^{-1}$ in control on day 1 to lowest value of 6.50 U mg protein $^{-1}$ returned to control value on day 8 (Figure 2). Similarly, GPX (Figure 2) was significantly inhibited to a lowest value of 4.25 in fish exposed to 3.5mg/L compared to the highest value of 9.36 in control fish on day 4 but returned to control value with a high value of 9.25 $umol^{-1}$ min $protein^{-1}$. Similarly, Lipid peroxidation LPO (Figure 2) ranged from the highest value of 6.8 mMole/TBARS/ mg protein in 16.95mg/L to a lowest value of 6.41 mMole/TBARS/ mg protein in control on day 4, returned to the control value on day 8.

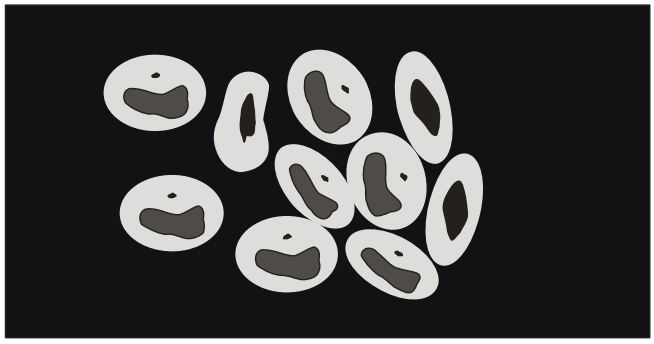


Plate 1: `*Photomicrograph of micronucleated erythrocytes in C. gariepinus exposed to GBHG*

Table 3: *Micronuclei frequencies in erythrocytes of Clarias gariepinus exposed to sub-lethal concentrations of GBHG for 8 days*

Parameters	Concentrations (mg/L)	Exposure days		
		1	4	8
Micronucleus	**Control**	8.50±0.93[a]	8.50±1.00[a]	8.50±0.88[a]
	3.39	376.66±8.01[b]	375.66±8.11[b]	377.66±7.92[b]
	6.78	393.33±6.75[b]	393.33±7.06[b]	393.33±10.45[b]
	16.95	403.33±4.84[b]	400.00±4.91[b]	406.66±15.03[b]

Mean values with different alphabetic superscripts differ significantly ($p<0.05$) between concentrations in each parameter.

3.4. Micronuclei Frequency

The mean values of micronuclei frequency in the treatments were significantly ($p<0.05$) elevated above the control value 8.50±0.93 to the highest value of 406.66±15.03 was about 48 times higher than the control (Table 3, Plate 1).

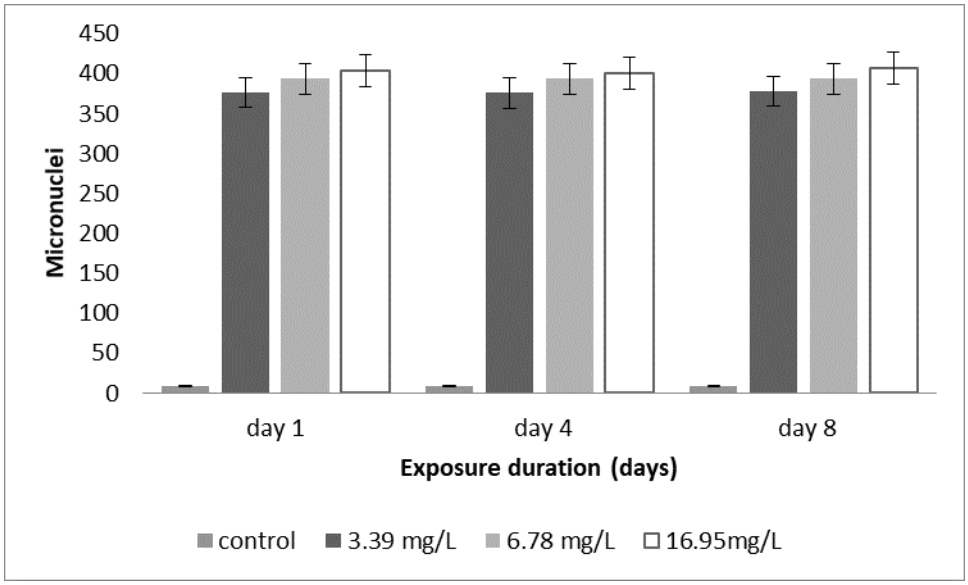


Figure 3: *Micronuclei aberration of C. gariepinus to GBHG for 8 days*

3.5. Water Quality Parameters

The physiochemical parameter of the test water in various treatment levels ranged from pH reading of 8.40 ± 0.1 – 9.8 ± 0.60; temperature reading vales of 25 ± 0.5 – 25.81 ± 0.05 and dissolved oxygen values of 5.0 ± 0.00 – 5.5 ± 0.01 respectively. These ranges of water quality were within the same standard for aquaculture.

4. Discussion

Acute of Glyphosate Toxicity based Herbicide Glycot to Exposed Fish

Substantial reports on the first and second generation groups of the original glyphosate Roundup and several other group of glyphosate based herbicides have been reported (Monsanto, 1995; Glusczak et al., 2009; Modesto and Matinez, 2010; Modesto and Martinez, 2010), but the toxicity effects of GBHG is almost unavailable in literature except maybe a lowered value of 24.6 mgL^{-1} 96h LC_{50} reported by Ani et al. (2017) on the same species of fish juveniles compared to ours of 33.39 mgL^{-1}. The present value also proved to be higher than respective values of 1.05mgL^{-1} and 13.6 mgL^{-1} by Ayoola (2008) and Langiano and Martinez (2008) when they exposed *Oreochromis niloticus and Prochilodus lineatus* to glyphosate and glyphosate-based herbicides respectively. However, the reported value of 108mgL^{-1} of glyphosate to tadpole's juveniles by Clements et al. (1997) is somewhat higher than the present, as well as respective higher values of 620 mgL^{-1} and 975 mgL^{-1} 96h LC_{50} reported by Shiogiri et al. (2012) when *Cyprinus carpio* and *Palloceroscaudi maculatus* were exposed to glyphosate and glyphosate commercial formulation (Rodeo) herbicides probably due to species and formulation variations. The present study indicated that GBHG formulation was less toxic compared with other reported cases which could be an indication of toxicity improvement on the mixture of POEA surfactants and other unspecified inert adjuvants and preservative substances. It has been shown that many manufacturers regard as a trade secret and seldom disclose (Cox and Surgan, 2006; Mesnage et al., 2013), and have been implicated to be responsible for the high level of toxicity reported on glyphosate based commercial formulations and roundup in general. Mesnage et al. (2019) noted that POEA talloamine present in GBH was more toxic compared to the original glyphosate in Roundup.

Haematological effects of glyphosate-based herbicides and indeed other pollutants have been used as a health status biomarker indicator of stressed animals (Modesto and Martinez, 2010). The present indication of inhibited PCV, RBC and HB by subacute doses of GBHG and elevated WBC and PL compared to the control corroborated with report of Glusczak et al. (2006) on the same active principle glyphosate but of different species and formulations on Leporinus obtusidens, suggest to the fact that there was a lowering in the production level from the haemopoietic areas in the exposed group because of hemodilution (Modesto and Martinez 2010) or it may have been hampered by some of unspecified inert substances in the formulation and rendered inefficient to produce sufficient parameters below the control. In order to respond to the forgoing, the fish elevated its WBC and PL to counter the effect of the herbicide and restore normalcy of the blood demand of the fish. However, it disagreed with the reported elevations on PCV, RBC and HB and lowered WBC and PL in fish exposed to a glyphosate based herbicide at short duration (Svodova, 1994). On the contrary, increased total number of leukocytes and platelets could be a defense response in the presence of surfactants and other inert portion of herbicide into the blood stream or as the organism's adaptive response ploy for towards effective immune defense (Barreto-Medeiros et al., 2005; cazenave et al., 2005; Dong et al., 2017; Li et al., 2017).

Anti-oxidative Stress Enzyme Activity Effect of GBHG

Antioxidants are substances that significantly delay or prevent the oxidation of oxidizable substrate (Franco and Martenez-Pinilla, 2017). The antioxidants produced by the body of animals act enzymatically to decrease the excess of free radicals through enzymatic components such as SOD, CAT and GPX (Halliwel et al., 1995). In our finding, inhibition of all three antioxidants CAT, SOD and GPX below their respective controls occurred on day 1 but there was further inhibition of CAT on day 4 followed by an elevation to control level on day 8 compared with elevation in both SOD and GPX on day 4, followed by further elevation to control level on day 8. Voet and Voet (1990) noted that antioxidants work in tandem to dismutase oxygen radicals in which SOD converts superoxide anion to hydrogen peroxide which is broken down to oxygen and water by catalase. The SOD– CAT system has been noted to be the first line of defense against oxygen toxicity, due to the inhibitory effects on the formation of oxygen radicals (Pandey et al., 2003), and these enzymes were frequently used as biomarkers, that indicated the production of reactive oxygen species (ROS) (Monteiro et al., 2006). The reduction in SOD activity after day 1 of exposure to the herbicide may be related to the production of oxidants. An excess of hydrogen peroxide may have reduced SOD activity, while the superoxide anion may be responsible for further decrease in CAT activity on day 4 (Bagnyukova et al., 2006; Scandalios, 2005). Thus, it may be reasonable to assume that hydrogen peroxide was responsible for the reduction observed in SOD activity while the reduction of CAT activity was due probably to accumulated superoxide anions not sufficiently neutralized by SOD. The activities of enzymes involved in animal's antioxidant system have been known to be a complex pathway of interactions among enzymes, because the activity and substrate product of one enzyme may influence the other. In the present work, the inhibition of CAT and SOD limited the antioxidant defenses of the fish during the first 4 days of exposure to the herbicide. But was restored on day 8 when the activities of the antioxidants approached the control level. Although GPX has been noted to function principally in the removal of organic peroxides, Maran et al. (2009) reported its involvement in the metabolism of hydrogen peroxide. The significant increased activity of GPX in the fish after day 4 to the herbicide indicated that the antioxidant pathway was stimulated, probably due to the increased production of peroxides. Thus, the activation of GPX may be an indication of adaptive response to compensate the inhibition of CAT at the period of exposure.

Reactive oxygen species left un-neutralized reacted with membrane lipids which produced lipid peroxidation, considered as one of the main consequences of oxidative stress (Ahmad et al., 2000; Ansari et al., 2011; Nwani et al., 2013). In this work, the occurrence of lipid peroxidation was indicated by a transient increase in LPO in fish to 17.5mgL^{-1} to the herbicide on day 4. However, LPO levels returned to control levels after day 4. Thus, it can be inferred that the antioxidant defense before day

8 of exposure was insufficient due to significant decreases in SOD, CAT and GPX activities which led to increased lipid peroxidation as a function of the presence of GBHG. However, these defenses returned to basal levels on day 8 and then were enough to combat the ROS, which prevented incidence of oxidative damage. Lushchak et al. (2009) using a similar method to quantify lipid peroxidation found that the herbicide Roundup original also did not affect lipid peroxidation in the liver of the goldfish after 96 h of exposure.

The significant increase in the number of micronuclei of exposed fish to GBHG, which was initiated on the first day of exposure, progressed to the 4th and 8th day of exposure (Cavas and Ergene-Gozukara, 2005). They may have been elicited by increased LPO that caused oxidative damage at the onset up to day 4. The restored LPO to control level on day 8 by increased activities of antioxidant enzymes of SOD and GPX could not however revert the micronucleus to basal level after day 8 (Cavas and Konen, 2007).

5. Conclusion

The results obtained in the present study may allow us to conclude that the commercial formulation of GBHG promoted alterations in hematologic and biochemical parameters of the experimental fish which were more evident in fish to the higher concentration of the herbicide. Hematologic changes occurred in decreased RBC, PVC and HB and increased WBC and PL, which probably represented the adaptive responses that assisted the organism to counteract the herbicide effects. Exposed fish showed reduction in SOD, CAT and GPX on day 1-4 which elicited LPO, however it returned to control levels after 8 days exposure to GBHG, when fish showed an increased activity of SOD and GPX apparently enough that combated ROS and prevented oxidative damage but could not restore elevated micronucleus frequency elicited at the unset of LPO, to control level.

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