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Application of Antioxidant Enzymes as Biomarkers in Cultivability Assessment of Palaemonid Shrimps

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Authors' contributions

This work was carried out in collaboration between all authors. Author IPO designed, conducted, and analyzed the research. He also interpreted the data and prepared the manuscript. Authors IJO, AOS, TOS and IOJ conducted and interpreted the laboratory analysis and searched for relevant literatures. All authors read and approved the final manuscript.

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ABSTRACT

The scope of the study was to explore and simulate some selected abiotic factors from the natural home of the shrimps (a tropical rainforest river) with a view to providing information required for sustainable shrimp husbandry.

Hypothesis: Abundance of shrimps is solely a function of the physico-chemical (abiotic) characteristics of the river.

Methodology: Water, sediment and shrimp samples were collected on monthly basis from the Osse River, in Edo State, Nigeria. The samples were collected between April and December, 2015; at night and early morning periods. The physico-chemical properties of the water and sediment samples were analyzed in the laboratory. The shrimp samples were identified, sorted, and counted.

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Analysis of variance was employed in analyzing the descriptive statistics of the physico-chemical properties. Sex distribution patterns amongst the shrimp species and the ratio of male to female distribution for each species was analyzed mathematically. Stress levels impacted by abiotic variables were investigated employing Cytochrome P450 mono-oxygenase (CYP450), Glutathione-S-Transferase (GST), Catalase (CAT), Superoxide dismutase (SOD) and Lipid Peroxide (LPO).

Results: Availability of the shrimps in the study area was: *Macrobrachium vollenhovenii* > *Macrobrachium macrobrachion* > *Macrobrachium lux* > *Macrobrachium fellicinium* > *Nematopalaemon hastatus* > *Palaemon maculatus*. The female shrimps outnumbered the males in among all the species captured from the natural environment. Order of abundance of shrimps in the river was Station 2 (41%) > Station 1 (32%) > Station 3 (16%) > Station 4 (11%). Abundance of shrimps at Station 2 can be attributed to the predominant abiotic factors such as flow rate (0.1 ± 0.01 m/s), depth (72.5 ± 3.1 cm), water pH (6.7- 7.5), water temperature (27.6 ± 1.07 °C), and primary productivity nutrients (oligotrophic). Laboratory results conform to field observations on the basis of depth having greater impacts on the organisms than flow rate.

Conclusion: Palaemonid shrimps are littoral organisms which have considerable cultivability. Depth of 72 cm and flow rate of 0.1 m/s are recommended for aquaculture of the shrimps. Notwithstanding, sufficient information about their feeding habits and breeding conditions are imperative.

Keywords: Palaemonid shrimps; abiotic ecology; cultivability; oxidative stress; antioxidant enzymes.

1. INTRODUCTION

Shrimp entrepreneurship is a sustainable strategy of poverty eradication in developing countries such as Nigeria. Shrimp husbandry has the capacity to cushion the impacts of overfishing, while enhancing biological control of life threatening schistosomiasis [1]. However, attempts to boost Nigeria's shrimp production and export by giant investors have proven abortive despite tremendous research efforts.

There has been rising need for shrimp husbandry over the years due to shrimp's nutritional and biological values in diet and evolving biological control of schistosomiasis respectively [2]. Shrimps are safe animal proteins due to the fact that their circulatory system is made up of haemocyanin, hence they are free from health implications associated with consumption of animals with haemoglobin.

Total revenue from shrimps export in 2008 was \$384 Million; creating 83, 950 jobs in Nigeria. The demand for shrimps is on a steady rise with increasing population. Currently, the demand far outstrips the supply [3]. Estimates have put the average demand for fishery products in Nigeria at about 1.5 million tons annually. Unfortunately, local fish supply has only amounted to about a trifling 0.5 million tons per year [4]; consequently necessitating fish importation to fill the gap. This factor has made Nigeria the largest importer of frozen fish in Africa and its annual import bill was about \$208 million in 2001 [5]. Aquaculture is the most viable sustainable option which may bridge

the gap between supply and demand, preserve the shrimp resource base from overfishing, and create jobs opportunities.

Nigerian freshwater shrimps can generally be grouped into families Atyidae (De Haan, 1849), Alpheidae (Rafinesque, 1815), Hippolytidae (Dana, 1852), and Palaemonidae (Rafinesque, 1815). However, of all the families Palaemonidae have attracted the most scientific and commercial attention due to its unique morphometrics and physiology i.e. they have relatively large body size and they are stronger than other groups hence have higher cultivability potentials than other families [6]. Nigerian major Palaemonid shrimps include many groups, however the genus *Macrobrachium* are most reputable for relatively high commercial values. The group comprises of *Macrobrachium macrobrachion* (Herklots, 1857): brackish water prawn, *Macrobrachium vollenhovenii* (Herklots, 1857): African River prawn, *Macrobrachium fellicinium* (Holthuis, 1949): Niger River prawn, and *Macrobrachium dux* (Lenz, 1910): Congo River prawn. Species such as *Nematopalaemon hastatus* (Aurivillius, 1898) and *Palaemon maculatus* have also exhibited some cultivability tendencies [1]. These species were well represented in Osse River, Edo State, Nigeria. The largest of all is *M. vollenhovenii* which could attain 182 mm in standard length at adult stage; followed by *M. macrobrachion* which could grow up to 138 mm at adult stage [1].

Palaemonid shrimps are detritivorous animals which feed on epibenthic organisms such as

polychaetes, molluscs and other crustaceans [7]. Current researches aimed at establishing male shrimp population with a view to ameliorating the endemicity of Bilharziasis (schistosomiasis) are still at infancy [6]. Palaemonid shrimps are very abundant in Nigerian rivers and besides being promising agents of biological control of Mollusca of medical importance, they also serve as source of relatively cheap animal protein requirements; hence income generation for shrimpers [6] and exotic export commodity [8].

Vulnerability of these unique species to anthropogenic perturbations is however noteworthy [2]. There is high variability in perturbation tolerances among shellfish species [9]. Palaemonid shrimps fall in the category of shellfishes with relatively low tolerance to unregulated anthropogenic activities. Hence their abundance and life cycle depends greatly on the abiotic factors of their environment. Anthropogenic activities noticed around Osse River during the period of this study include overfishing, dredging, oil production, agricultural practices and lumbering.

The perpetual impediments facing establishment of shrimp aquaculture is a function of unavailability of data on their hydraulic preferences. Although Mallet et al. [10] made a laudable attempt on investigating the hydraulic preferences of some marine shrimp species. There is dearth information on the hydraulic preferences of tropical freshwater species [11,12,13]. Research into these species has mainly focused on the altitudinal distribution of species, impacts of habitat alteration [6,7], the importance of maintaining upstream/downstream connectivity [14], trophic links and the role of macro-consumers [15,16].

Mallet et al. [10] attributed the preferences of *Atya scabra* and *Macrobrachium heterochirus* for high flow rates and *Xiphocaris elongate* for low flow rates to their capacity to resist currents i.e. larger and more tenacious species prefer the challenges of competing against the current for food and prey, while escaping predators. This might imply that the larger the morphometrics (particularly pleopods/ swimmerets), the better the adaptation to higher flow rates. On this basis, depth and flow rate preference gradients can also be functions of age, size and species; amidst other factors.

Generally, Palaemonid shrimps prefer shallow waters which support the growth of floating

macrophytes such as *Eichhornia crassipes*, *Paspalum vaginatum*, and *Pistia stratiotes* [17]. These plants provide shelter to the animals particularly during breeding activities. The floating macrophytes also render services such as temperature and pH regulation; as well as support proliferation of plankton which constitute the diet of the shrimps.

Thus, this study sought to compare results of abiotic factors of Palaemonid shrimps obtained from Osse River, Edo State, Nigeria with results obtained from laboratory studies; with a view to ascertaining the hydraulic preferences.

2. MATERIALS AND METHODS

The research was designated into two phases; phase 1 was the field study which entailed collection of surface water, bottom sediment and shrimp samples at the selected stations of Osse River in order to have a cognizance of the abiotic ecology. While phase 2 entailed ascertaining of the results obtained from field studies under controlled laboratory conditions.

2.1 Phase 1 (Field Study)

2.1.1 Study area

The field study was carried out on Osse River; which is known for high productivity of shrimps. The river is a link between Benin River and Ughoton stream. It traverses from Ijaw fishing camp, through Ekehuan and Gelegele; and terminates at Izedema community. It is a fresh oligotrophic water [18], with a thick tropical vegetation cover along its bank. The area encompassed is located in Ovia North-East local Government Area of Edo State; within the tropical rainforest belt of Southern Nigeria. The river is located between 5°16'40" E and 5°23'20" E; 6°2'0" N and 6°14'0" N.

The geology of the study area is made up of the Benin formation which is of Miocene- recent age. The thickness is about 200 m [19]. The climate of the study area is a humid tropical climate; characterized by two different seasons, which are the wet and dry seasons. The wet season occurs between April and October; with a break in August and an average rainfall of 1704 mm; with a range of 1562 – 1867 mm. The dry season on the other hand lasts from November to March with a cold harmattan spell which occurs between December and January. The average temperature is 25°C (77 F) in the rainy season

and about 28°C (82 F) in the dry season; with a mean daily temperature ranging from 23°C minimum in the rainy season to 30°C maximum in the dry season.

Four (4) stations were chosen were labelled Stations 1, 2, 3 and 4. Station 1 was located at Ijaw fishing camp (06° 13.432'N, 05°20.826'E). A few surface macrophytes were observed at this station. Station 2 was located at the Ekehuan section (06° 11.398'N, 05° 21.781'E). This station is characterized by shallow depth and abundance of surface water macrophytes. Activities such as laundering, fishing, and boating were the predominant activities observed at this station. Station 3 was located at the Gelegele section (06° 09.323'N, 05°20.584'E). This station constantly received effluents from an Oil Exploration Company named Dubri Oil Company is situated near it. Station 4 was located at the Izedema section (06° 08.936'N, 05°19.939'E). This area was perturbed with logging activities.

2.1.2 Collection and analysis of samples

Samples were collected from all stations on monthly basis to cover some part of rainy and dry seasons i.e. from April to December, 2015. Shrimp samples were collected from 4 stations during night periods and early mornings using local fishing gears such as woven cylindrical non-return valve traps, baskets and scoop nets; in conjunction with coconut, cassava and earthworm baits.

The samples were preserved in four different coolers with ice; appropriately labeled to indicate the source stations and were transported immediately to the laboratory for immediate sorting, identification and counting. Palaemonid shrimps in the sites were identified by taxa to species levels, using taxonomic keys provided by FAO [20] and Powel [21]. Morphometrics such as pleura arrangement and numbers, shape of rostrum, and number of spines on the rostrum of each species were used for identification to species level [22]. Catch assessment was evaluated on weight measurement to the nearest 0.01 g unit using sensitive weighing balance (model pl440w). Sex distribution pattern amongst the shrimp species and the ratio of male to female distribution for each species was analyzed using Chi Square method. The sex variations across the stations were further analyzed using the Students' T-test. Bartlett's F-test was used to test for homogeneity of variance [1]. Species composition, spatial distribution and relative abundance were used as tools to

analyze the impacts of abiotic ecological variation among the stations.

Temperatures (°C) of water and sediment at each station were measured by immersing the tip of a mercury-in-glass thermometer into the water and left for about 2 minutes; for a stable reading. The depth (cm) was measured with the aid of a rope with a weight of lead attached to its lower end and lowered into the water till the lead just touched the bottom. The distance between the water mark on the rope and the lead was recorded as the depth. For quality assurance purpose, the Geographical Positioning System (GPS) equipment was used to cross check the possible errors in the depth assessment methodology. The flow rate was determined by placing a floating plastic on the surface of the water at a marked point of a meter rule (calibrated in cm) and the reading on a stop watch was recorded after the object covered a readable distance. The flow rate was then calculated thus:

$$\text{Flow rate m/s} = \frac{\text{distance covered(m)}}{\text{Time (s)}}$$

The analysis of water quality parameters such as pH, total dissolved solids (mg/L), nitrate (mg/L), phosphate (mg/L), and sulfate (mg/L) were determined in situ by using Hydro-lab water quality meter (Electronic Probe, Hanna HI98106 model). Water samples were collected in stopper bottles enclosed in black polythene bags containing ice and were immediately transported to the limnology laboratory for chemical analysis of Carbonate (mg/L), dissolved oxygen (mg/L), and biological oxygen demand (mg/L); using procedures recommended by APHA [23].

2.2 Phase 2 (Laboratory Study)

There is restricted transferability of habitat-specific data between rivers [24,25,26] due to myriads of biotic and abiotic factors [27]. This necessitates further observations under regulated conditions.

Therefore, the laboratory phase of the study entailed analysis of depths and flow rates induced oxidative stress on the selected Palaemonid shrimps, while other factors were held constant. Identification of the shrimps was carried out using identification manuals such as FAO [20] and Powel [21]. More specimens than required for the experiment were recruited into the acclimatization phase to make up for shortages that might ensue from unprecedented deaths.

2.2.1 Ecological simulation

Identified *M. macrobrachion* (8.14±0.3g), *M. vollenhovenii* (16.2±2.1g), *M. felicinum* (12.2±2.2g), *M. dux* (4.5±0.12g), *N. hastatus* (6.5±0.1g) and *P. maculatus* (3.2±0.2g) were inspected for disease and general fitness prior to acclimatization period. Viable individuals were acclimatized for 14 days under natural day and night photoperiods (12/12-hrs) prior to the commencement of the bioassay in a glass aquarium of four (4) interconnected chambers (Fig. 1). Chambers 2 and 3 were simulated after Stations 1 and 2 respectively of the study area while Chambers 1 and 4 were upper and lower limits extensions respectively, to accommodate possible ecological inadequacies due to anthropogenic perturbations. The shrimps were fed with slices of coconut, cassava and tiny earthworms throughout the experiment. The physico-chemical parameters of the water such as temperature was maintained at 24- 28°C, dissolved oxygen was 5- 8.5 mg/L and the hydrogen ion concentration (pH = 6.8 - 8). The temperature and the dissolved oxygen of the water were measured on daily basis with a Model JPSJ-605 DO-Analyzer, while the pH was measured using the Electric Probe Hydro-lab water quality meter (HANNA HI 9813 GRO). Same procedure as the field phase was used in determining the flow rates for the laboratory experiment.

The entire experiment conformed strictly to the stringent guidelines provided by the Institute for Laboratory Animal Research [28].

2.2.1.1 Inducing stress with varied depths

Thirty (30) individuals which comprised of five (5) representatives of each of the six (6) species were released into each chamber of the aquarium containing non-chlorinated freshwater. In order to eliminate the chances of stress variability which could be induced by varied stocking density, all chambers were constructed with equal volume (length X breadth X height = 389, 620 cm³). For quality assurance purpose

the variability of the heights (depths) was compensated by the lengths to maintain constant volume of 389, 620 cm³ across all chambers (Table 1).

Connected to the outer end of Chamber 1 is a small tap which served as the in-flow of clean water and flow rate regulator. The link (flow regulator) between Chambers 1 and 2, 2 and 3; and 3 and 4 were at heights 92, 82, and 72 cm respectively, while the ultimate out-flow from Chamber 4 was at height 62 cm. All conjunctions of water flow including the ultimate inlet at Chamber 1 and outlet at Chamber 4 were fortified with sieves of mesh size 10 mm to retain the shrimps in their respective chambers. A homogenous flow rate of 0.1 m/s (adopted from Station 2 of the study area) was applied across all chambers with the aid a flow regulators with 10 mm mesh size. After 96 hours, the shrimps were retrieved from the aquarium for further analysis.

2.2.1.2 Inducing stress with varied flow rates

A homogenous depth of 72 cm (adopted from Station 2 of the study area) was simulated across all chambers of the aquarium. The outlet of Chamber 4 was locked so that the water level would be made up to the 72 cm water mark. Four (4) mini hoses with specialized adjustable pressure valves were regulated to generate 0.05, 0.1, 0.5 and 1 m/s flow rates at Chambers 1, 2, 3 and 4 respectively. Having adjusted the depths of all chambers to 72 cm, the volume of water in each which was initially 389, 620 cm³ was altered i.e. Volume of Chamber 4 > Chamber 3 > Chamber 2 > Chamber 1. For quality assurance purpose, the stocking density was maintained across the chambers as a check; to ascertain a constant condition (Table 2).

Member of the earlier acclimatized groups were recruited into the chambers of the aquarium as specified in Table 2. At least 5 representatives of each of the 6 species were present in each chamber. After 96 hours, the shrimps were retrieved from the aquarium for further analysis.

Table 1. Architectural statistics of the aquarium showing varied lengths with heights (depths)

Chambers	Length (cm)	Breadth (cm)	Depth (cm)	Volume (cm ³)
1	77	55	92	389, 620
2	86.34	55	82	389, 620
3	98.39	55	72	389, 620
4	114.26	55	62	389, 620

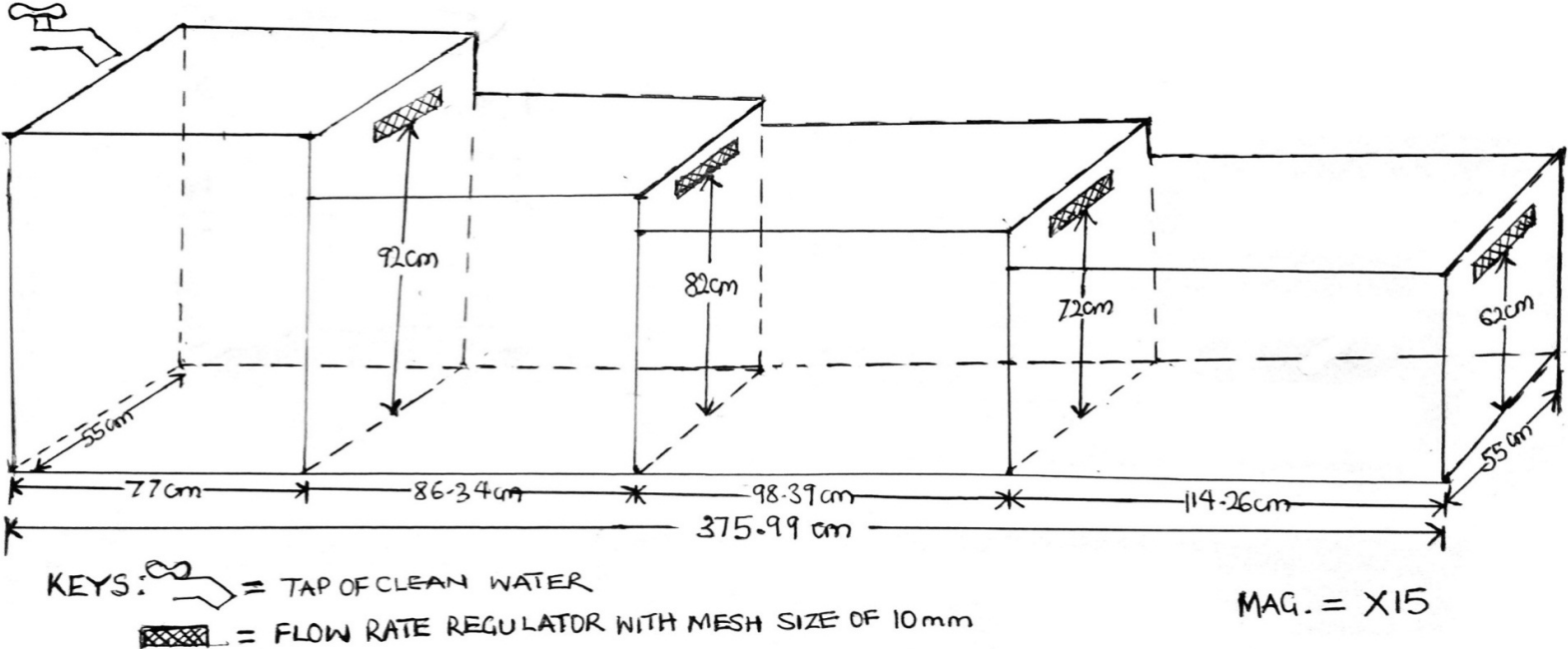


Fig. 1. 3- dimensional architecture of experimental aquarium
NOTE: 92 cm height= Chamber 1, 82cm = Chamber 2, 72cm = Chamber 3, and 62 cm = Chamber 4

Table 2. Stocking density of aquarium chambers with respect to volume of water

Chambers	Length (cm)	Breadth (cm)	Depth (cm)	Vol. (cm ³)	Shrimps	Stocking density
1	77	55	72	304, 920	31	0.103
2	86.34	55	72	341, 906	35	0.103
3	98.39	55	72	389, 620	40	0.103
4	114.26	55	72	452, 469	46	0.103

$$\text{Stocking density (Shrimps/ cm}^3\text{)} = \frac{\text{Num. of shrimps}}{\text{Volume of water}}$$

2.2.2 Biochemical analysis

2.2.2.1 Preparation of post-mitochondrial supernatant (PMS)

After retrieval from the aquarium, the shrimp samples were sedated with 40 % methanol, stripped of their exoskeleton, telson, mouth parts and other hard cuticles. They were rinsed with distilled water, weighed and thawed in freezer at -10°C prior to further biochemical analysis. The whole tissue was homogenized in chilled TRIS buffer (100 mM, pH 7.8; 1:10 w/v) using an Ultra-Turrax tissue homogenizer. The homogenates were centrifuged at $10\ 500\times g$ for 20 min at 4°C to obtain the post-mitochondrial supernatant (PMS) for various biochemical analyses.

2.2.2.2 Antioxidant enzymes

Catalase (CAT) activity was assayed by the procedures demonstrated by Beers and Sizer (1952). Spectrophotometer was used to read the reduction of hydrogen peroxide (H_2O_2) at 240 nm; using 1.0 mL quartz cuvettes with a light path of 1.0 cm. CAT levels were expressed in nmol H_2O_2 consumed/min/mg protein. Superoxide dismutase (SOD) activity was measured using the xanthine oxidase-cytochrome method as described by McCord and Frodovich [29]. Xanthine reacted with 2-[4-iodophenyl]-3-[4-nitrophenyl]-5-phenyltetrazolium chloride to form superoxide radicals which reacted to form a red coloured formazan. The red formazan was used to determine the SOD activity in the tissues i.e. presence of SOD readily bound with superoxide radicals, thereby reducing the availability of superoxide radicals, ultimately inhibiting formation of formazan. SOD activity was measured spectrophotometrically at 505 nm and calculated as inhibition percent of formazan formation. The concentration of Cytochrome P450 (CP450) was quantified spectrophotometrically at 400–490 nm, on the

basis of the difference between absorbance readings at 450 nm. Glutathione-S-Transferase (GST) activities in the tissues samples were analyzed by extracting the tissues separately with a phosphate buffer (pH 7.2), homogenized and centrifuged at $10,500\ \text{g}$ for 20 min at 4°C . The activity of GST was investigated in supernatants spectrophotometrically according to the recommendation of Habig et al. [30].

2.2.2.3 Lipid peroxide

The malondialdehyde (MDA) concentration; which indicates the level of lipid peroxidation, was measured following the method described by Esterbauer and Cheeseman [32] on homogenized tissues. 0.5 mL of homogenized shrimp tissue previously treated with 25 μL of butylhydroxytoluene 1% v/v in glacial acetic, was mixed with 0.2 mL of sodium lauryl sulfate (8%), 1 mL of acetic acid (20% v/v) and 1 mL of 0.8% thiobarbituric acid. The mixture was heated at 95°C for 30 minutes. The resulting chromogen was extracted with 3 mL of *n*-butyl. It was then centrifugation at $1500\times g$ for 10 minutes. The absorbance of the organic phase was determined at 532 nm. 1,1,3,3-Tetramethoxypropan was used as a standard. Values were presented as $\mu\ \text{mol}$ MDA formed/g tissue.

2.3 Statistical Analysis

Results were presented as the mean \pm S.E. The differences between in shrimp statistics, physico-chemical parameters were analyzed by one-way Analysis of Variance (ANOVA) and Duncan Multiple Range (DMR) test was used to ascertain the actual locations of the significant differences.

3. RESULTS AND DISCUSSION

3.1 Field Observations

The mean catch composition by weight (g) of individuals was 18% *M. macrobrachium*, 22% *M.*

vollenhovenii, 14% *M. dux*, 12% *M. felicinium*, 18% *P. maculatus*, and 16% *N. hastatus* per catch. The sex ratio patterns observed was 1 male: 4 females among *M. macrobrachion*; 1 male: 5 females among *M. vollenhovenii* and *M. lux*; 1 male: 2 females among *M. felicinium*, *Nematopalaemon hastatus*, and *Palaemon maculatus*. Although it is ecologically healthy for female population to out-number males, as this would minimize competition for mates among males and increase successful mating. However, extreme cases of sex ratio might be counter-productive. A fair margin in the population ratio has the potentials for a stable population growth i.e. 1 male: 2 females. Results showed that sex ratios among *M. felicinium*, *Nematopalaemon hastatus*, and *Palaemon maculatus* of Osse River have potentials for a sustainable population growth. This sex ratio has earlier been recommended for trial induced shrimp aquaculture by [33].

The abrupt rise in the population of shrimps between the periods of August and October was distinct among *M. macrobrachion* and *M. vollenhoveii* (Figs. 2 and 3). This rise was accompanied by a stabilized temperature; between 27 – 27.6°C; mainly at Stations 1 and 2 (Fig. 5). Stations 1 and 2 had more stable temperatures throughout the study period; therefore supported the shrimps. Regardless of the relatively high temperatures recorded at Stations 3 and 4, *M. macrobrachion* and *M. vollenhovenii* were present, though in scanty numbers. This conforms to the established facts that these species are the most resilient among others [33]. Extreme temperatures recorded at Stations 3 and 4 (Fig. 3) might be responsible for the absence of others. The stable temperature observed at Stations 1 and 2 can be attributed to the presence of floating aquatic macrophytes; which take up the carbon dioxide (CO₂) produced by the entire aquatic biota and converts it to oxygen through photosynthesis thereby stabilizing the temperature and oxygen levels for the survival of the shrimps. Table 4 shows that the mean temperatures at Stations 1 (27.2°C) and 2 (27.6°C) were consistent with the recommendation of Eniade and Bello-Olusoji [33], and it is about the room temperature in the tropics; hence achievable in shrimp farms. There was a general rise in shrimp population during periods of high rainfall (September and October), followed by an abrupt drop in November (Fig. 2). *M. macrobrachion* and *M. vollenhovenii* dominated the study area throughout the sampling period. This trend was more apparent

on the basis of distribution of the shrimp according to stations (Fig. 3). A steady decline from in the shrimp population, from Station 1 towards Station 4 was also apparent.

The dissolved oxygen at Station 1 was significantly higher than the concentration at Station 2, which was higher than Station 4, which was in turn higher than Station 3 ($P < 0.05$). Relatively high concentrations of oxygen at Stations 1 and 2 over the study period can be attributed to the presence of the floating macrophytes.

The depth of Station 4 was significantly higher than other stations ($P < 0.05$) throughout the period of the study (Table 3). This trend was concurrent with low abundance of shrimps at the station throughout the field survey. On the other hand, Station 2 which had the shallowest depth ($P < 0.05$) harboured the highest number of shrimps. The mean depths at Stations 1 (82 ± 1.4 cm) and Station 2 (72.5 ± 3.1 cm) were closer than the depths of other stations to the recommended depth of 67 ± 0.25 cm observed at Ilahe Estuary in Ondo by Eniade and Bello-Olusoji [33]. Station 2 only fairly outnumbered Station 1 in terms of the spatial distribution of the shrimps. Station 2 was the closest to all previously recommended depths [17]. Higher population of shrimps at Station 2 than other stations can be attributed to its relatively shallow depth, which supports floating macrophytes, which in turn flourished the shrimps. There was a decline in the depths of all stations in dry season; due to reduced rains, which was accompanied by a general rise in the shrimp population [1].

The flow rate at Station 2 was significantly lower than the flow rate of Station 1, which was lower than Station 4, Station 3 had the highest flow rate. The highest abundance of shrimps observed at Station 2 can be attributed to the minimal flow rate at the station (Table 3). Furthermore, the flow rate had negative significant correlation with the abundance of all the shrimp species except *M. vollenhovenii* (Table 4). Given these facts, coupled with the spatial distribution of the shrimps (Fig. 3) which favoured Station 2 the most, the flow rate at Station 2 (0.01m/s) and the depth (72.5 ± 3.1 cm) were noted for adoption in the laboratory phase of the experiment. The depths had higher correlations with distribution of the shrimps than the flow rates (Table 4). This implies that the depth might be a more influencing factor than the

Table 3. Summary of physico-chemical properties of study area

Parameters	Station 1 Mean±S.E(Range)	Station 2 Mean±S.E(Range)	Station 3 Mean±S.E(Range)	Station 4 Mean±S.E(Range)	P- Value	FEPA [31]
Air Temp. (°C)	30 ± 0.4 ^C (29.5- 31.7)	30.8 ± 2.01 ^C (28.5- 32.7)	36 ± 1.3 ^A (35.2- 37)	32.4 ± 0.65 ^B (30.7- 34.2)	P < 0.05	-
Water Temp. (°C)	27.2 ± 0.26 ^C (25- 31)	27.6 ± 1.07 ^C (27.5- 28.2)	32.8± 0.73 ^A (24- 35.3)	29.8± 0.86 ^B (23- 37.1)	P < 0.05	< 40
Depth (cm)	82± 1.4 ^C (22- 175)	72.5±3.1 ^D (24.5-121)	114±2.7 ^B (100- 445)	399±3.1 ^A (167.6- 738.4)	P< 0.001	-
Transparency (%)	68±6.02 ^A (47– 78)	75.5±8.91 ^A (56.9-95.5)	4.23±4.04 ^C (2.31–7.5)	12.3±2.9 ^B (8.5 – 17.5)	P<0.01	-
Flow Rate (m/s)	0.52±0.01 ^C (ND-0.79)	0.1±0.01 ^D (ND-0.003)	2.58±0.03 ^A (0.14-4.32)	1.89±0.02 ^B (0.35-2.88)	P< 0.05	-
DO (mg/L)	3.2 ± 0.2 ^B (0.8 – 3.3)	6.3±0.6 ^A (3.3 – 11.1)	7.8±0.4 ^D (4.3 – 11.2)	2.3±0.5 ^C (2.1 – 5.1)	P<0.05	7.5
BOD (mg/L)	3.25±0.31 ^B (1.5 – 5.2)	2.19±0.9 ^B (1.67 – 6.32)	4.39±0.7 ^A (1.7 – 8.5)	4.62±0.7 ^A (1.7 – 7.9)	P< 0.05	30
TSS (mg/L)	34.8±1.4 ^B (23.5 – 45.5)	38.2±10.1 ^B (13.5 – 68.3)	139±16.7 ^A (82.6 – 204.6)	143.5±20.12 ^A (72 – 210)	P< 0.01	2000
EC (µS/cm)	46.21 ^B ±4.8(22 – 77)	22.64 ^C ±20.2(29 – 300)	113.8 ^A ±33(49 – 670)	108.5 ^A ±3.7(20 – 770)	P< 0.001	400
pH	6.5±0.1 ^B (6 – 7.2)	7±0.2 ^A (6.7 – 7.5)	6.1±0.2 ^B (4.89 – 7.5)	6.4±0.1 ^B (5.3 – 7.5)	P< 0.05	6- 8
Sulfate (mg/L)	3.62±0.17 ^B (0.2 – 5.8)	5.47±0.38 ^A (2.1 – 8.5)	2.14±0.37 ^C (0.7 – 4.2)	2.22±0.57 ^C (0.8-4.8)	P< 0.01	500
Phosphate (mg/L)	3.2±11.02 ^B (0.7– 5.8)	4.5±4.91 ^A (2.9-6.5)	1.22±4.04 ^C (0.8–2.5)	1.56±0.9 ^C (1.1 – 2.5)	P < 0.001	< 5
Nitrate (mg/L)	3.12±0.4 ^B (1.5 – 5.6)	4.47±0.6 ^A (2.1 – 8.9)	1.21±0.07 ^D (0.1 – 2.2)	2.32±0.11 ^C (1.6 – 3.6)	P < 0.01	20

Note: Values with same superscript have no significant difference, while different superscripts have significant difference.

P > 0.05 means there is no significant difference, P < 0.05 means there is significant difference, P < 0.01 means there is much significant difference, and P < 0.001 means there is very much significant difference.

Sample replicates N = 9.

Table 4. Correlation between variables and shrimp population

	Air Temp.	W.Temp	W.pH	W.Depth	W.Trans.W.Flow.	W.Cond.	W.DO	W.BOD	W.TSS	W.NO ₃	W.PO ₄	W.SO ₄	S.Temp	S.pH	S.Cond.	S.NO ₃	S.SO ₄	S.PO ₄	MM	MV	MF	ML	NH	PM	
Air Temp.	1																								
W. Temp	0.58	1																							
W. pH	0.05	0.03	1																						
W. Depth	-0.43	-0.54	0.04	1																					
W. Transp.	0.32	0.48	0.07	-0.89	1																				
W. Flowrate	0.04	0.54	0.04	-0.72	-0.32	1																			
W. Cond.	0.24	0.08	0.56	-0.34	-0.22	0.02	1																		
W.DO	-0.55	-0.75	0.01	-0.76	0.54	0.52	0.06	1																	
W.BOD	0.36	0.36	0.03	0.66	-0.45	0.01	0.01	0.01	1																
W.TSS	0.22	0.03	0.69	0.45	-0.75	0.06	0.43	0.43	-0.12	1															
W.NO ₃	0.02	0.04	0.12	0.34	-0.62	0.02	-0.41	-0.41	0.64	0.06	1														
W.PO ₄	0.08	0.04	0.22	0.76	-0.42	0.21	-0.22	-0.22	0.73	0.03	0.21	1													
W.SO ₄	0.02	0.32	0.35	0.27	-0.78	0.12	-0.45	-0.45	-0.04	0.04	0.39	0.06	1												
S. Temp.	0.65	0.72	0.02	-0.54	0.02	0.42	0.22	-0.01	0.02	0.04	0.03	0.03	0.45	1											
S. pH	0.24	0.03	0.53	-0.43	0.01	0.04	0.02	0.02	0.22	0.03	0.29	0.22	0.08	0.04	1										
S. Cond.	0.21	0.34	0.65	-0.62	-0.02	0.05	0.39	0.07	0.08	0.01	0.32	0.08	0.21	0.01	0.04	1									
S.NO ₃	0.19	0.72	0.14	0.24	0.07	0.07	0.08	0.03	0.13	0.56	0.12	0.11	0.14	0.02	0.45	0.22	1								
S.SO ₄	0.09	0.42	0.13	0.07	0.04	0.02	0.05	0.07	0.23	0.07	0.19	0.07	0.03	0.24	0.35	0.17	0.56	1							
S.PO ₄	0.03	0.08	0.33	0.03	-0.03	0.11	-0.01	0.01	0.07	0.02	0.11	0.12	0.12	0.04	0.22	0.34	0.62	0.55	1						
MM	0.03	0.04	0.13	-0.54	0.45	-0.24	0.12	0.21	0.06	0.04	0.03	0.03	0.45	0.06	0.15	0.07	0.75	0.76	0.54	1					
MV	0.08	0.02	0.66	-0.67	0.34	-0.42	0.22	0.04	0.03	0.01	0.69	0.04	0.34	-0.35	0.23	0.56	0.88	0.69	0.77	0.06	1				
MF	-0.54	-0.81	0.31	-0.77	0.78	-0.67	0.35	0.23	0.04	0.06	0.12	0.01	0.76	-0.22	0.57	0.03	0.71	0.78	0.04	0.03	0.03	1			
ML	-0.45	-0.76	0.87	-0.86	0.67	-0.78	0.13	0.51	0.12	0.03	0.22	0.82	0.06	-0.57	0.78	0.01	0.93	0.99	0.57	0.04	0.02	0.45	1		
NH	-0.76	-0.74	0.76	-0.92	0.98	-0.82	0.04	0.78	0.22	0.04	0.56	0.03	0.03	-0.87	0.35	0.22	0.59	0.71	0.62	0.01	0.06	0.34	0.67	1	
PM	-0.32	-0.84	0.04	-0.85	0.69	-0.95	0.01	0.88	0.35	0.04	0.73	0.77	0.04	-0.98	0.84	0.44	0.66	0.54	0.51	0.44	0.07	0.76	0.78	0.65	1

Note: P value <0.5 represents insignificant correlation, P > or = 0.5 represents significant correlation. Emboldened numbers show significance. MM= *M. macrobrachioin*, MV= *M. vollenhovenii*, MF= *M. felicinium*, ML= *M. lux*, NH= *N. hastatus*, PM= *P. maculatus*, W= water, and S= sediment

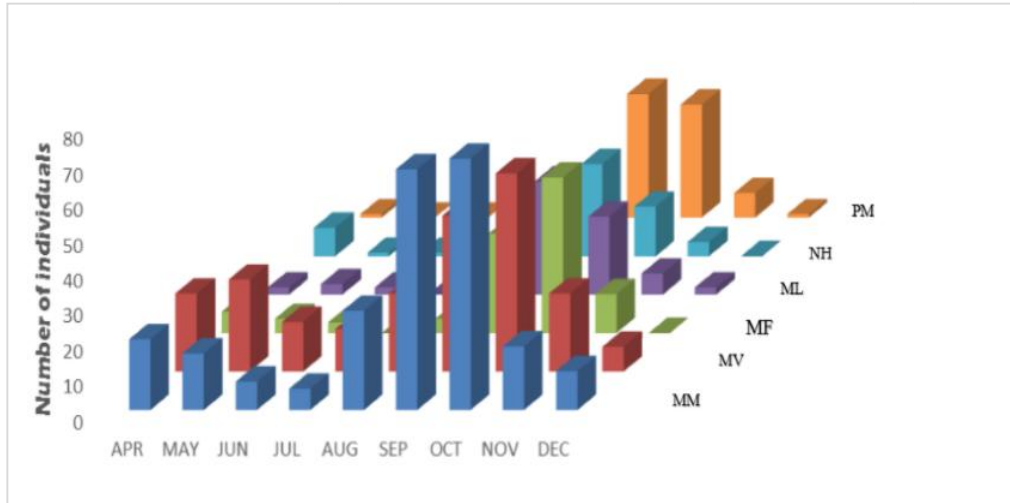


Fig. 2. Temporal variation in shrimp population

NOTE: MM= *Macrobrachium macrobrachion*, MV= *Macrobrachium vollenhovenii*, MF= *Macrobrachium felicinum*, ML= *Macrobrachium lux*, NH= *Nematopalaemon hastatus*, PM=*Palaemon maculatus*

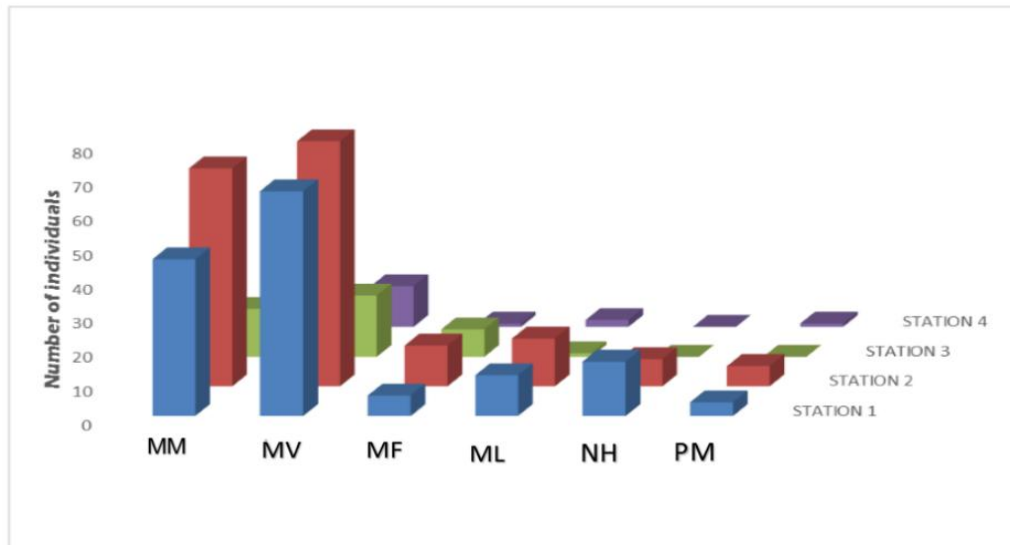


Fig. 3. Spatial distribution of shrimp population

NOTE: MM= *Macrobrachium macrobrachion*, MV= *Macrobrachium vollenhovenii*, MF= *Macrobrachium felicinum*, ML= *Macrobrachium lux*, NH= *Nematopalaemon hastatus*, PM=*Palaemon maculatus*

flow rate. Station 2, followed by Station 1 also had the highest primary productivity nutrients (Table 3), which determine the availability of food [34] for the shrimps. The abiotic variables observed at Station 2 are most consistent with the recommended conditions for shrimp cultivation [33]. This justifies the highest population of shrimps observed at Station 2 i.e. Station 2 (41%) >

Station 1 (32%) > Station 3 (16%) > Station 4 (11%).

Water temperature had a significant negative correlation (-0.75) with dissolved oxygen and positive correlation (0.86) with biological oxygen demand (Table 4). This showed that the available oxygen for the shrimps reduced with increasing temperature; thereby increasing the

biological oxygen demand. Consequently, this must have been a contributing factor to the significant negative correlations of temperature with the population of *M. felicinum* (-0.81), *M. lux* (-0.76), *N. hastatus* (-0.74), and *P. maculatus* (-0.84). Only *M. macrobrachion* and *M. vollehovenii* showed no significant correlation with water temperature. This is an indication of higher tolerance to temperature than the others that showed significant impacts. This result conforms to earlier findings of Adebola and Olaniyan [17]. The primary productivity nutrients (nitrate, phosphate and sulfate) positively correlated with the number of shrimp individuals at all stations. There was a significant positive correlation between nitrate and numbers of *M. vollehovenii* (0.69), *N. hastatus* (0.56), and *P. maculatus* (0.73). Field results showed that the palaemonid shrimps are littoral animals which are cultivable in captivity. There was also a significant positive correlation of phosphate with *M. lux* (0.82) and *P. maculatus* (0.77). Sulfate only showed a positive significant correlation with *M. felicinum* (0.76). Station 2 hosted the highest number of shrimps throughout the period of study. The order of abundance of shrimps in the aquatic ecosystem was: Station 2 (41%) > Station 1(32%) > Station 3 (16%) > Station 4 (11%).

3.2 Phase 2 (Laboratory Observations)

3.2.1 Depth-induced oxidative stress

Cytochrome P450 (CYP450) was significantly induced in all the treatments except depth 72 cm (Fig. 4). The activities of the antioxidant enzymes were also significantly higher in the shrimps exposed to depths 92, 82 cm than that the levels observed in shrimps exposed to 62 cm depth, which was significantly higher than those exposed to 72 cm depth. This implies that depth 72 cm was the most conducive depth for the shrimps. Catalase (CAT) activity levels were also minimal in the shrimps at depth 72 cm. Significant rises in CAT levels at other depths indicate substantial levels of stress inflicted on the shrimps (Fig. 5). Glutathion-S-Transferase (GST) induction was insignificant only in shrimps at depth 62 and 72 cm, significant induction of GST was observed in shrimps at other depths (Fig. 6).

The levels of Sodium Oxide Dismutase (Fig. 7) and Lipid Peroxide (Fig. 8) in the shrimps at depth 72 cm were also significantly lower than

that observed in shrimps at other depths. This implies that depth 72 cm was most suitable for the shrimps. These observations are consistent with the findings earlier observed in the field study. Current observation also correlates with the most suitable depth (67 cm \pm 0.25 cm) observed at Oluwa Creek, Igbokoda by Adebola and Olaniyan [17]. This is however much higher than 49 cm \pm 0.26 observed at Ilaje Esatuary in Ondo by Eniade and Bello-Olusoji [33]. This indicates that some better results might be obtainable between 49 and 72 cm depth.

3.2.2 Flow rate-induced oxidative stress

Significant CYP450 activity levels were induced at flow rates of 0.05 and 1 m/s, while the levels in shrimps subjected to flow rates of 0.1 and 0.5 m/s exhibited no significant difference (Fig. 9). In the cases of other antioxidant enzymes such as CAT (Fig. 10), GST (Fig. 11), SOD (Fig. 12), and LPO (Fig. 13), significant stress levels were observed in the shrimps exposed other flow rates except 0.1 m/s, which exerted no significant stress on the shrimps. These results indicate that flow rates of 0.1 m/s is the most suitable for the shrimps. This quite corresponds with the observations from the field studies which showed that Station 2, which had the minimal flow rate (0.1 m/s) was also the most conducive location for the shrimps (Table 2).

The concentrations of CYP450 induced by the depths: 0.2– 0.86 n mol/min/mg prot. (Fig. 4) was higher than that induced by flow rates: 0.22- 0.55 n mol/min/mg prot (Fig. 9). Depth also induced higher CAT range: 210- 878 n mol/min/mg prot. (Fig. 5) than flow rates: 136- 428 n mol/min/mg prot (Fig. 10). Depth also induced higher GST: 213 – 656 n mol/min/mg prot. (Fig. 6), SOD: 2.18- 8.26 U/mg prot. (Fig. 7), and LOP: 0.32- 4.51 μ mol/MDA/ g tissue (Fig. 8) than the stress induced by the flow rates which were 152- 538 n mol/min/mg prot. (Fig. 11), 2.2- 5.3 U/ mg prot. (Fig. 12), and 0.65- 2.34 μ mol/MDA/ g tissue (Fig. 13) respectively. These results imply that depth is a more superior factor to flow rate in Palaemonid shrimp aquaculture. It is noteworthy that these laboratory observations are quite consistent with field observations. This fact is evident in the higher correlation values of depths with shrimp distribution than flow rates (Table 4). This does not however undermine the fact that flow rate of 0.1 m/s might be best suitable for Palaemonid shrimp cultivation.

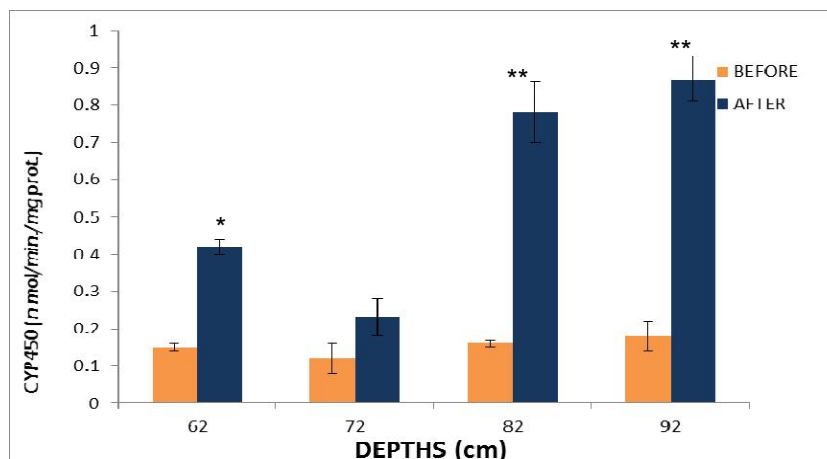


Fig. 4. Activity level of CYP450 at varied depths
 * = Significant difference at $P < 0.05$. $N = 30$.

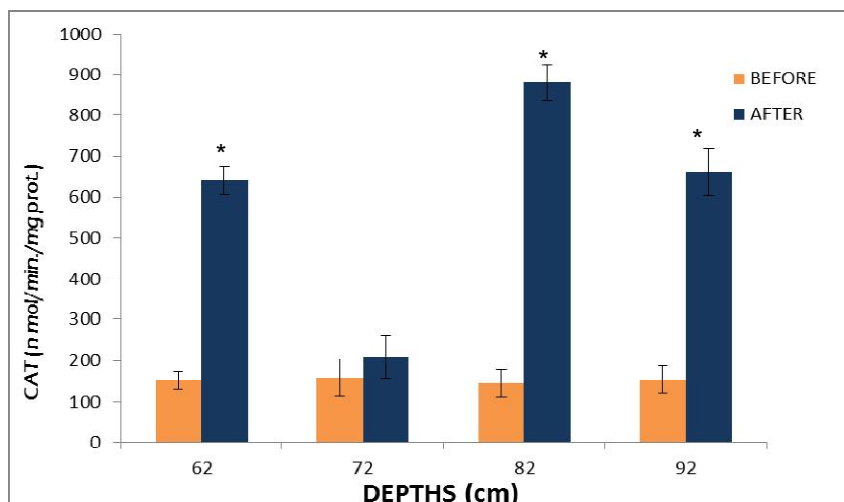


Fig. 5. Activity level of CAT at varied depths
 * = Significant difference at $P < 0.05$. $N = 30$.

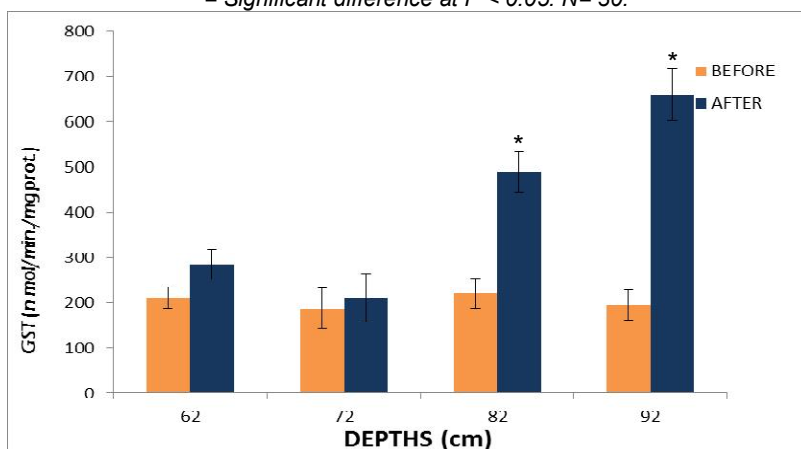


Fig. 6. Activity level of GST at varied depths
 * = Significant difference at $P < 0.05$. $N = 30$.

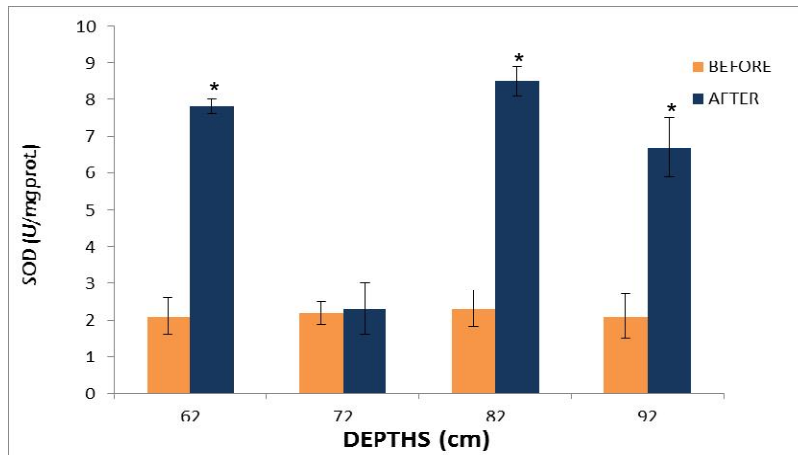


Fig. 7. Activity level of SOD at varied depths
 * = Significant difference at $P < 0.05$. $N = 30$.

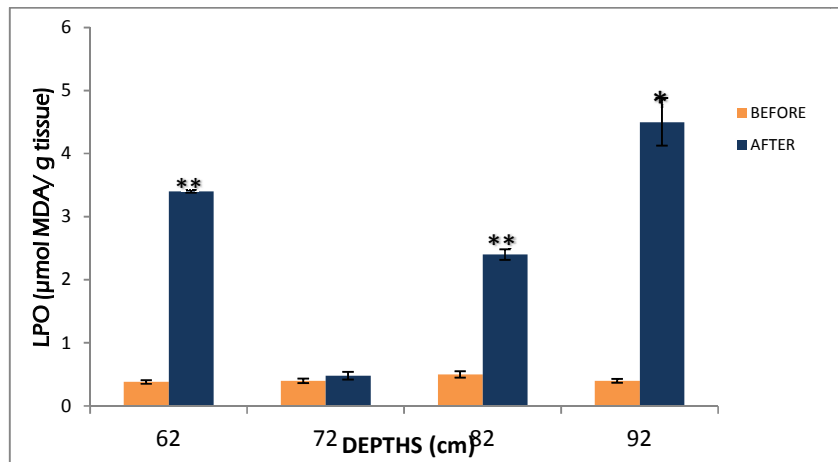


Fig. 8. Activity level of LPO at varied depths
 * = Significant difference at $P < 0.05$. $N = 30$.

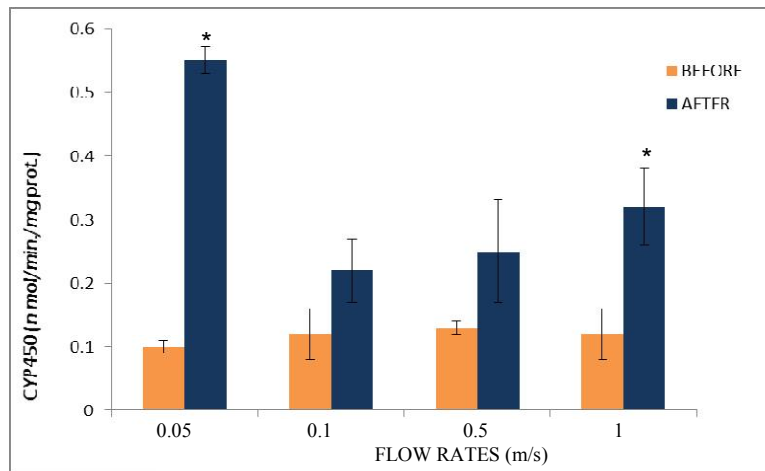


Fig. 9. Activity level of CYP450 at varied flow rates
 * = Significant difference at $P < 0.05$. $N = 30$.

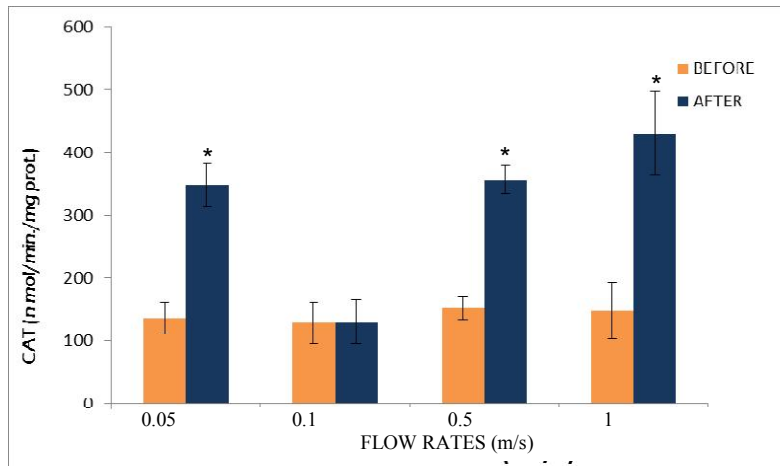


Fig. 10. Activity level of CAT at varied flow rates
* = Significant difference at $P < 0.05$. $N = 30$.

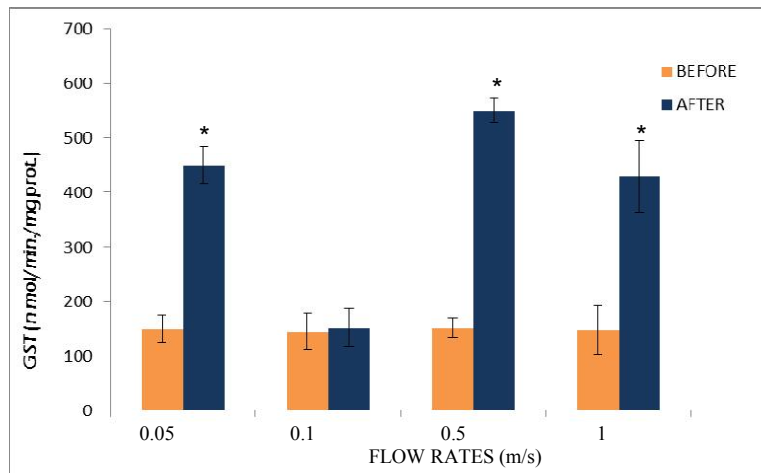


Fig. 11. Activity level of GST at varied flow rates
* = Significant difference at $P < 0.05$. $N = 30$.

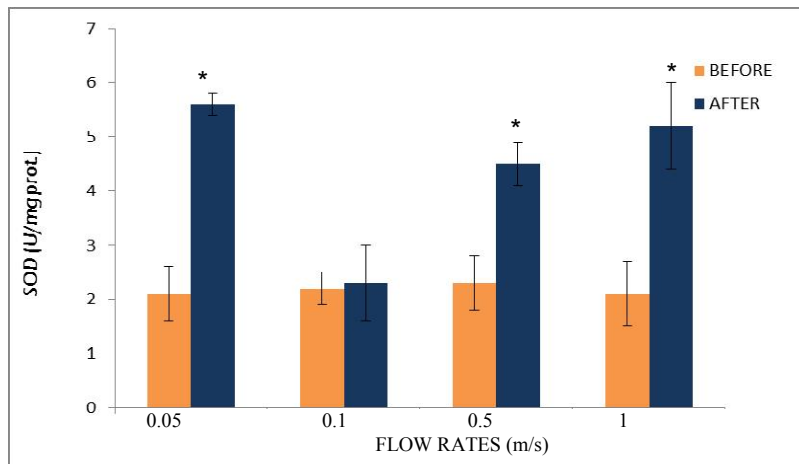


Fig. 12. Activity level of SOD at varied flow rates
* = Significant difference at $P < 0.05$. $N = 30$.

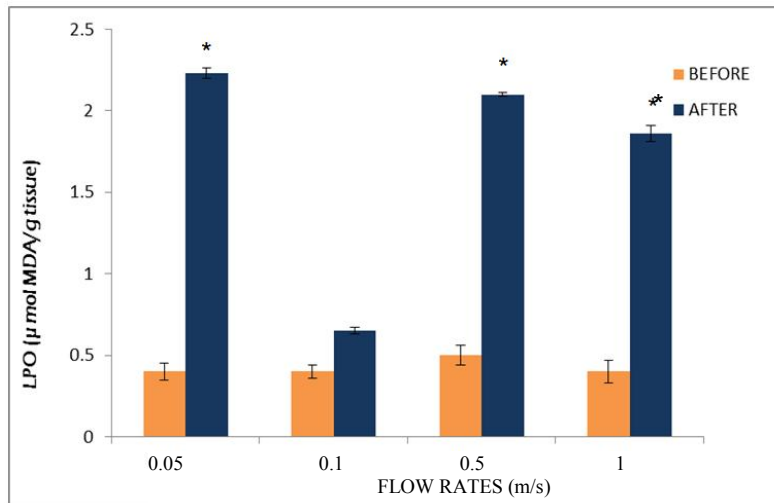


Fig. 13. Activity level of LPO at flow Rates

* = Significant difference at $P < 0.05$. $N = 30$.

4. CONCLUSION

Results showed that Palaemonid shrimps are littoral organisms which have considerable cultivability due to the fact that depth of 72 cm and flow rate of 0.1 m/s can be simulated in shrimp aquaculture. However further research on inter-specific stress comparisons is recommended for optimal results.

The study has provided vivid information to support artificial breeding of the Palaemonid shrimps for biological control of schistosomiasis disease and shrimp entrepreneurship in a recessed economy.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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