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Comparative Estimation of Flow Rate Mediated Oxidative Stress Amongst Palaemonid shrimps

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ABSTRACT

The study explored, examined and simulated some selected abiotic factors observed at biodiversity hotspots of Osse River, located in the tropical Rainforest, Edo State, Nigeria. The study was aimed at providing information required for sustainable shrimp husbandry. The research rationale was that the stress levels in shrimps are absolutely a function of flow rates and depths. Shrimp samples were caught from Osse River at night and early morning periods. The shrimp samples were identified, sorted, and counted. Stress levels impacted by abiotic variables such as regulated flow rates and depths were investigated employing Glutathione-S-Transferase, Cytochrome P450 mono-oxygenase, Catalase, and Superoxide dismutase. *M. vollenhovenii* managed the stress the most, followed by *M. macrobrachion*. These potentials can be attributed to the relatively large and tough morphological characteristics and the animals. Proficiency of regulation of antioxidant enzymes in the animals must have contributed appreciably to the hardiness of the animals. Cultivation of *M. vollenhovenii,* followed by *M. macrobrachion* is very much achievable, with optimal results at flow rate of 0.1 m/s. The species have an appreciably wide spectrum of flow rates due to their ability to cope in slightly harsh conditions. The study provided vivid information on the flow rate of shrimp pond to support artificial rearing of the Palaemonid shrimps.

Keywords: *Palaemonid shrimps; abiotic stressors; depth; flow rate; cultivability; resilience.*

1. INTRODUCTION

Nigerian freshwater shrimps can be grouped into families Atyidae (De Haan, 1849), Alphedae (Rafinesque, 1815), Hippolytidae (Dana, 1852), and Palaemonidae (Rafinesque, 1815). However, the big and strong nature of the Palaemonidae puts them in the scientific and commercial spotlight (Isibor, 2016). Cultivability; hence profitability potentials of the palaemonids is a function of their resilience in unfavourable conditions (Isibor and Oluowo, 2016). Nigerian major Palaemonid shrimps include many groups however study has shown that the genus *Macrobrachium* are outstandingly notable for feasible cultivability and profitability to shrimp farmers. The group comprises of *Macrobrachium macrobrachion* (Herklots, 1857): brackish water prawn, *Macrobrachium vollenhovenii* (Herklots, 1857): African River prawn, *Macrobrachium felicinium* (Holthuis, 1949): Niger River prawn, and *Macrobrachium dux* (Lenz, 1910): Congo River prawn. *Nematopalaemon hastatus* (Aurivillius, 1898) and *Palaemon*

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maculatus have also are also of some appreciable cultivability tendencies (Isibor, 2016; Isibor and Oluowo, 2016).

Shrimp entrepreneurship is a sustainable recommendation for a recessed economy (CII, 2002; FDF, 2007). Growing rate of unemployment, inflation, dwindling standards of living, retrogressive access to healthcare facilities, growing mortality and epidemic rates are all symptoms of an ill economy. Proportional pressure on available natural resources which are the backbone of the economy is a noteworthy trend in developing countries such as Nigeria. Viable economic options other than total reliance on fossil fuel have been suggested by many experts (Oyedeji, 2000). Nevertheless, which way to go is a question of the most sustainable option (CII, 2002).

Empowerment schemes such as shrimp aquaculture if properly managed, has the capacity to recuperate economy of a nation from a recessed state, cushion the impacts of overfishing, while enhancing biological control of life threatening schistosomiasis (Habig *et al,* 1974; Isibor, 2016). However, shrimp husbandry is at its infancy in many developing countries. This is partly due to unavailability of technological facilities and mainly due to poor technical know-how; which is a function of unavailability of vital information on the shrimps (Pringle et al., 2000). A few researchers have made commendable efforts on investigating the abiotic factors suitable for the wellbeing of shrimps (Mérigoux, 2009). Mallet *et al.* (2014) investigated the hydraulic preferences of some marine shrimp species. They 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 are adapted to competing against water current for food, mates and prey, while escaping predators (Kogbe *et al.*, 1976). This might imply that the larger the morphometrics (particularly pleopods/ swimmerets), the better the adaptation to stressors. On this basis, depth and flow rate preference gradients can also be functions of age, size, species (Isibor *et al;* 2017) and sex.

Life on earth is mainly supported by oxygen for its existence. Paradoxically, oxygen is a highly reactive molecule that damages tissues through oxidative phosphorylation; with the aid of by-products known as Reactive Oxygen Species (ROS) i.e. reception of an electron by an oxygen molecule in the process of reduction. The resulting ROS have important roles in homeostasis and cell signalling. ROS are chemically reactive oxygen isotopes such as superoxide, hydrogen peroxides, and hydroxyl radicals. The natural ROS are classified as endogenous reactive oxygen species and they are managed through homeostasis. ROS can also be formed as result of stressors on shrimps such as unsuitable depths and flow rates which can knocking off an electron from water molecule through oxidation; a reverse of the natural trend. The resulting electron thirsty hydroxyl radical then wreaks havoc on the cell membrane, DNA and other proteins (Block *et al;* 2007); initiating cell damage through lipid peroxidation. This occurs when the abiotic stressors such as depth and flow rate cause ROS levels in shrimps to outstrip the natural homeostatic carrying capacity. Homeostasis offers organisms a complex network of antioxidant metabolites and enzymes that sequester the oxidative damage in concert (Lamoroux *et al.,* 2010) i.e. antioxidant enzymes such as superoxide dismutase, catalase and glutathione peroxidase mitigate the devastation of superoxide, hydrogen peroxide and hydroxyl radicals respectively (Crowl *et al.,* 2006; Boulton *et al.,* 2008). In a nut shell, antioxidant systems are charged with the responsibility of combating the reactive species by prevention through stabilization or ejection through detoxification. This is due to the fact that optimum level of ROS is essential for cellular redox signalling (Block *et al.,* 2007).

Information on the ecological study of palamonid shrimps abound but there is restricted transferability of habitat-specific data between rivers due to myriads of heterogeneous biotic and abiotic factors (Lamouroux *et al*., 1999; Jowett, 2003; Lamouroux *et al*., 2010). Furthermore, shrimp aquaculture requires regulated conditions, hence the need for stringently controlled experimental set-up for data verification.

Earlier investigation on the oxidative stress induced in palaemonid shrimps at varied depths and flow rates showed that depth of 72 cm and flow rate of 0.1 m/s were most suitable for palaemonid shrimp cultivation (Isibor *et al*., 2017). There is need to further compare the cultivability of the various shrimps with a view to ascertaining the most viable species for aquaculture. This study was therefore built on the platform of previous findings; to investigate and compare the performances of selected shrimp species at depth 72 cm and a range of flow rates. The study was aimed at employing the oxidative stress enzymes such as Super Oxide Dismutase (SOD), Catalase (CAT), Glutathione-S-Transferase (GST), and Cytochrome P450 Mono-oxygenase (CYP450) as biomarkers (Pandey *et al.,* 2003) in assessing the impacts of the flow rates on the wellbeing of the selected shrimp species. The research seeks to provide a cultivability profile among the different species.

2. MATERIALS AND METHODS

2.1. Collection and identification of shrimps

Shrimp samples were collected from Osse River, Edo State, Nigeria during night periods and early mornings using local fishing gears such as woven cylindrical non-return valve traps, baskets and scoop nets. Baits used include coconut, cassava and earthworm. Excess shrimp catch was intentional to make up for shortages that might ensue from unprecedented mortality. Morphomerics such as pleura arrangement and number; shape of rostrum, and number of spines on the rostrum of each species were used for identification to species level (Bello-Olusoji et al., 2004) using identification manuals such as FAO (1981) and Powel (1982). Catch assessment was evaluated on weight measurement to the nearest 0.01 g unit using sensitive weighing balance (model pl440 w).

All experimental procedures were conducted in compliance with the guidelines provided by National Animal Ethics Advisory Committee (2010). Procedures also conformed strictly to the stringent guidelines provided by the Institute for Laboratory Animal Research (2010).

2.2. Acclimatization

Shrimps were sorted, identified and screened before recruitment into research. *Macrobrachium macrobrachion* (8.14±0.3g), *Macrobrachium vollenhovenii* (16.2±2.1g), *Macrobrachium felicinium* (12.2±2.2g), *Macrobrachium dux* (4.5±0.12g), *Nematopalaemon hastatus* (6.5±0.1g) and *Palaemon maculatus* (3.2±0.2g) were inspected for general fitness before being subjected to acclimatization. Unfit individuals were discarded while viable individuals were acclimatized for 14 days under natural day and night photoperiods (12/12-hrs) before the commencement of the experiment in a glass aquarium (breadth = 55 cm, length = 72cm and height= 72cm). The shrimps were fed with slices of coconut, cassava and agro-feed. The physico-chemical parameters of the water such as temperature was maintained at 24- 28 $^{\circ}$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).

Previously suggested depth of 72 cm was conclusive but flow rate of 0.1 m/s was somewhat speculative; as test for preferences towards higher flows rate was suggested by Isibor et al., 2017. This therefore necessitates imperative investigation on an ideal range across the flow rate. Thus, sixty (60) shrimps; ten (10) representatives of each species were periodically released into the aquarium at flow rates of 0.05, 0.1, 0.5 and 1.0 m/s at depth of 72 cm. Each flow rate regime lasted for 96 hours. Afterwards, the shrimps were retrieved, new acclimatized shrimps (60) were released from the prepared stock for next flow rate regime and the experiment was repeated for the categories of the selected flow rates.

2.3. Analysis of antioxidant enzymes

2.3.1. Preparation of post-mitochondrial supernatant (PMS)

After the shrimps were retrieved 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 $-10\ ^{\circ}C$ prior to further biochemical analysis. Whole tissue was homogenized in chilled TRIS buffer (100 mM, pH 7.8; 1:10 w/v); with the aid of an Ultra-Turrax tissue homogenizer. Afterwards, the homogenates were centrifuged at $10,500 \times g$ for 20 min at $4\ ^{\circ}C$ to obtain the post-mitochondrial supernatant (Graham, 2002).

2.3.2. Antioxidant enzymes.

Catalase (CAT) induction rate was assessed using the procedures demonstrated by Beers and Sizer (1952). Reduction in H_2O_2 concentration in samples was measured using Spectrophotometer at 240 nm, and 1.0 mL quartz cuvettes with a light path of 1.0 cm. CAT concentrations were reported in nmol H_2O_2 consumed/min/mg protein. Cytochrome P450 (CP450) was measured using spectrophotometer at 400–490 nm; by measuring the difference between absorbance readings at 450 nm. Superoxide dismutase (SOD) concentration was measured using the xanthine oxidase–cytochrome method as demonstrated by McCord and Frodovich (1969). The assessment followed the reaction of Xanthine with 2-[4-iodophenyl]-3-[4-nitrophenyl]-5-phenyltetrazolium chloride to form superoxide radicals which further reacted to forming a reddish formazan; which was used to determine the SOD activity in the assayed tissues. SOD bound with superoxide radicals to reduce the availability of superoxide radicals, consequently inhibiting formation of formazan. SOD activity was assessed with the aid of spectrophotometer at 505 nm, which was calculated as inhibition percent of formazan formation. Concentration of Glutathione-S-Transferase (GST) in the tissues samples was analyzed by extracting the tissues separately using a phosphate buffer (pH 7.2), which was homogenized and centrifuged at 10,500g for about 20 min at a stable temperature of $4\ ^{\circ}C$. GST level was then investigated in supernatants spectrophotometer (Habig *et al.*, 1974).

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2.4. Statistical analysis

Change in concentrations of antioxidant enzymes represented accumulated stress (stress factor), which was calculated thus:

$$\Delta S = \frac{C2}{C1}$$

Where ΔS = stress factor, C2= final concentration antioxidant enzyme, and C2= initial concentration of antioxidant enzyme.

Results obtained were presented as the mean ± S.E. The differences in concentrations of antioxidant enzymes among the different species of shrimps at different flow rates 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 (p< 0.05).

3. RESULTS AND DISCUSSION

The rationale behind the research is that the stress levels induced in the shrimps will vary according to the flow rates they are subjected to. This was used as a basis for assessing the comparative resilience of the species and their respective suitability for aquaculture. The stress levels exerted by the various flow rates also served as guide in the determnation of the most suitable flow rate for the tested species. Combination of both information promises to give a reinforced reliable information on shrimp aquaculture.

They keys used in the report are: *Macrobrachium macrobrachion*= MM, *Macrobrachium vollenhovenii*= MV, *Macrobrachium felicinium*= MF, *Macrobrachium dux*= ML and *Nematopalaemon hastatus*= NH.

3.1. Stress level assessments at flow rate of 0.05 m/s

Palaemon maculatus exhibited an oustandingly high (P<0.05) induction of Cytochrome P450 CYP450 at flow rate of 0.05 m/s (Figure 1). *M. macrobrachion* and *M. vollenhovenii* exhibited the lowest profile of oxidative stress after subjected to stressor. While *M. felicinium*, *M. dux*, and *N. hastatus* were averagely impacted.

The levels of catalase Catalase (CAT) were outstandinly high in only in *M. dux* and *P. maculatus*. While low concentrations were observed in other shrimp species (Figure 2). *M. macrobrachion*, *M. vollenhovenii*, particlularly *M. dux* maintained the stress levels sgnificantly in terms of GST concentration (Figure 3). Results also show that *M. macrobrachion*, *M. vollenhovenii, M. felicinium* and *N. hastatus* maintained a fairly stable resilience in terms of SOD concentration (Figure 4).
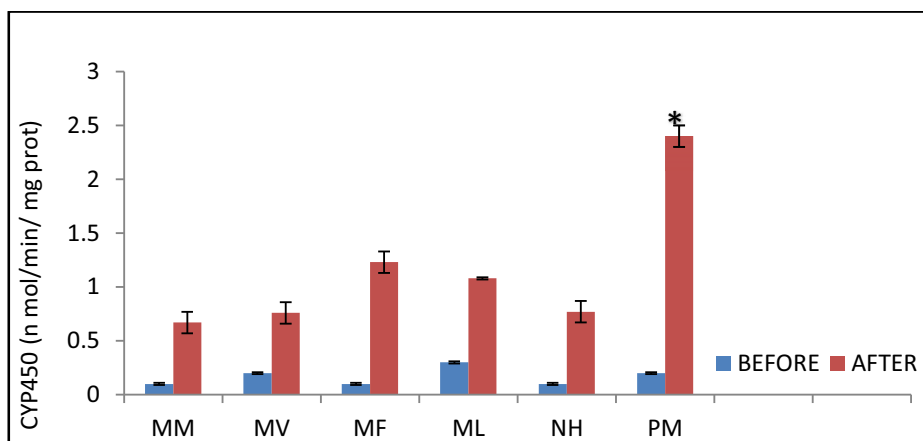
Figure 1: Concentrations of CYP450 in shrimps at flow rate of 0.05 m/s
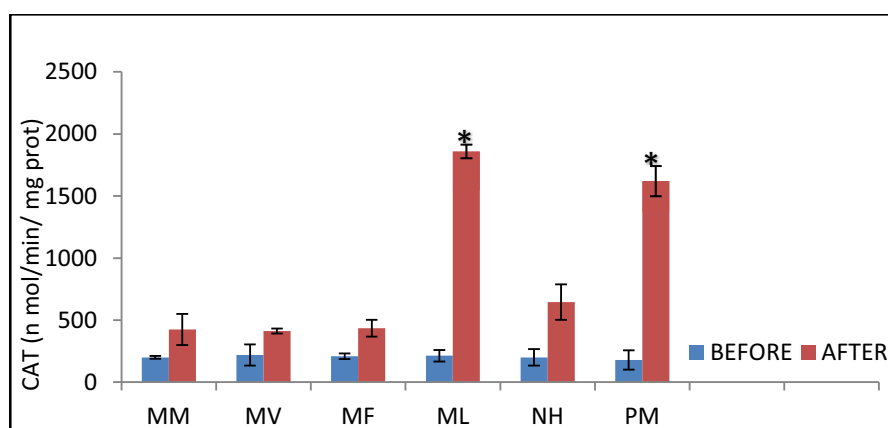* = *Significant difference at P < 0.05. N= 10*

Figure 2: Concentrations of CAT in shrimps at flow rate of 0.05 m/s
* = *Significant difference at P < 0.05. N= 10*

Generally, the concentrations of SOD in all the shrimp species were higher than other antioxidant enzymes. However, significantly higher concentration was observed in *N. hastatus* (Figure 4). Analysis of SOD showed that *M. macrobrachion, M. vollenhovenii* and *M. dux* adapted best.
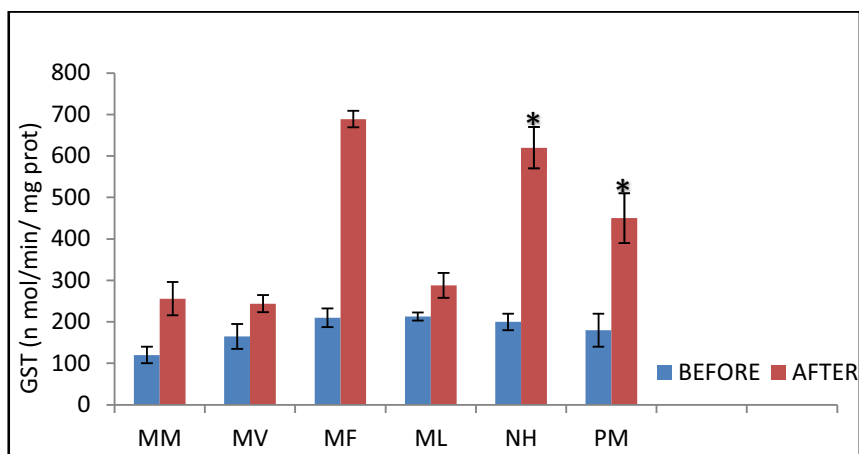
Figure 3: Concentrations of GST in shrimps at flow rate of 0.05 m/s
* = Significant difference at P < 0.05. N= 10
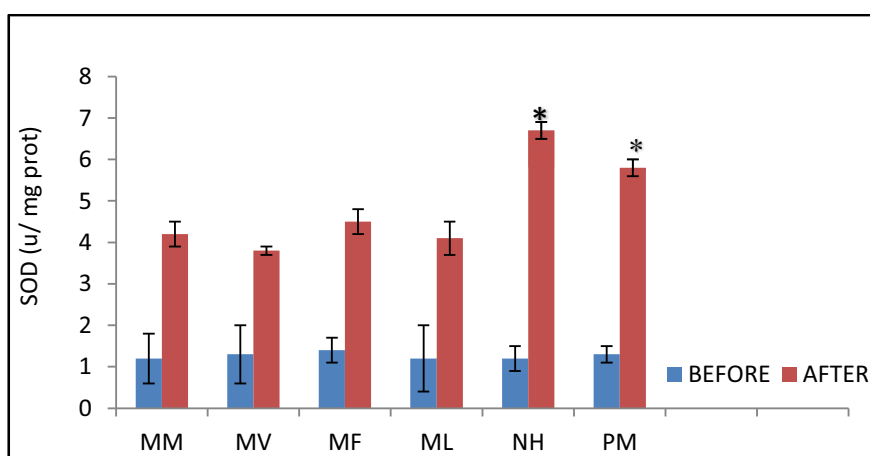
Figure 4: Concentrations of SOD in shrimps at flow rate of 0.05 m/s
* = Significant difference at P < 0.05. N= 10

3.2. Stress level assessments at flow rate of 0.1 m/s

After exposure to flow rate condition of 0.1 m/s, *M. macrobrachion* exhibited the lowest concentartion of CYP450, followed by SOD. Outstandingly higher concentrations were recorded in other species, particularly in *N. hastatus* (Figurre 5); *M. dux and M. felicinium* (Figure 6). This showed a consistent result with observations at 0.05 m/s, which indicates that *M. macrobrachion* and *M. vollenhovenii* were consistently resilient.
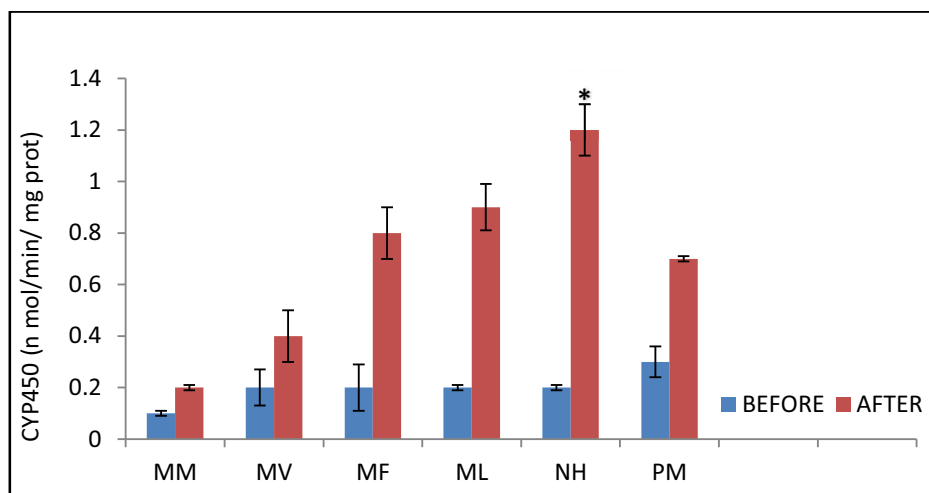
Figure 5: Concentrations of CYP450 in shrimps at flow rate of 0.1 m/s
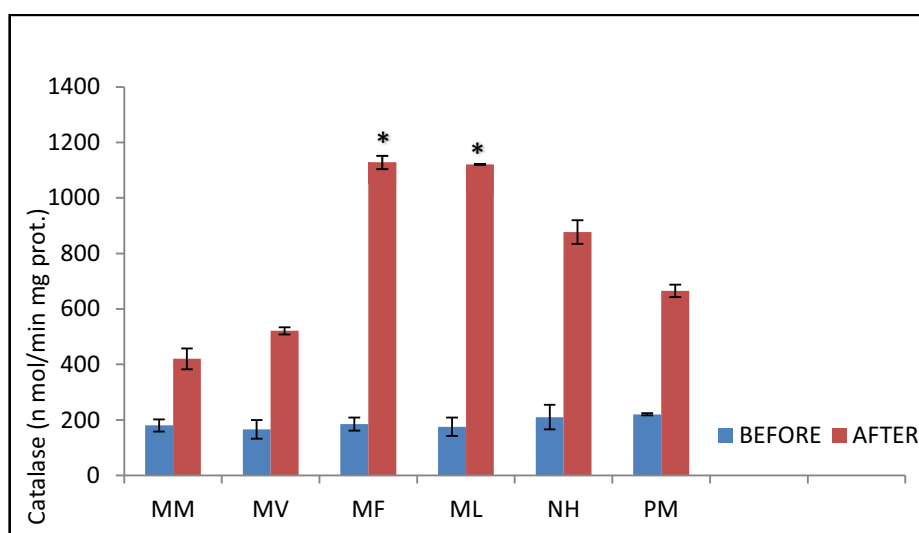** = Significant difference at P < 0.05. N= 10*

Figure 6: Concentrations of CAT in shrimps at flow rate of 0.1 m/s
* = Significant difference at P < 0.05. N= 10

Downward regulation in concentrations of GST and SOD at flow rate of 0.1 m/s shows a slight deviation from usual trend (Figures 7 and 8). *M. vollenhovenii* had lower concentrations of GST and SOD than *M. macrobrachion.*
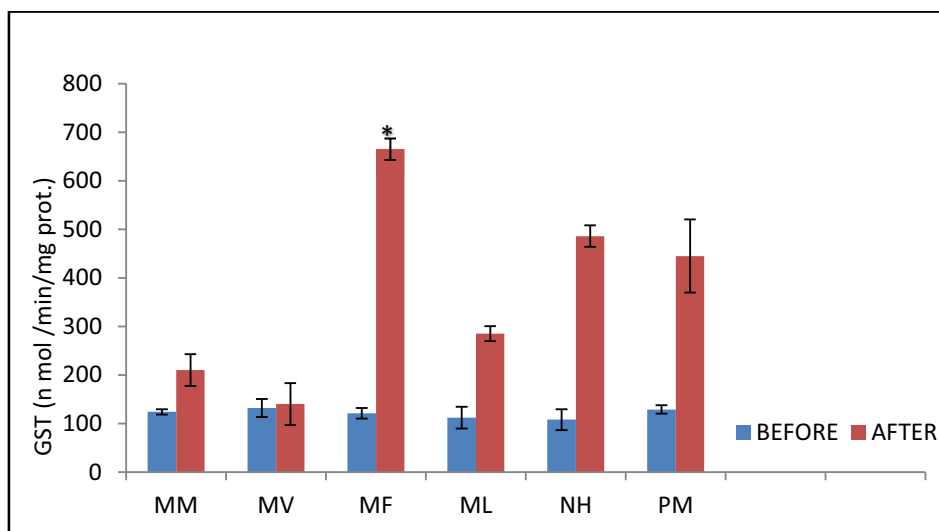
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Figure 7: Concentrations of GST in shrimps at flow rate of 0.1 m/s
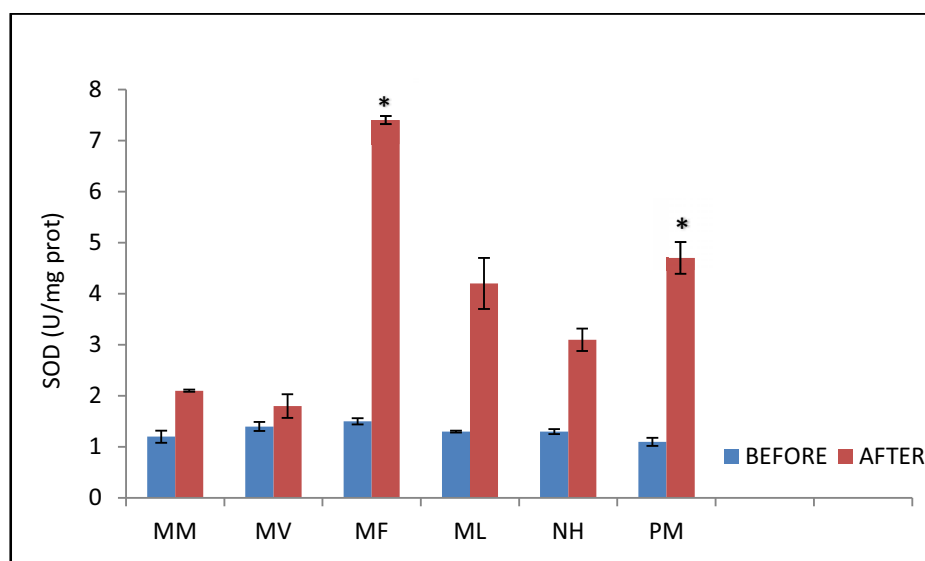** = Significant difference at P < 0.05. N= 10*

Figure 8: Concentrations of SOD in shrimps at flow rate of 0.1 m/s
* = Significant difference at P < 0.05. N= 10

3.3. Stress level assessments at flow rate of 0.5 m/s

Stress levels exerted at flowrate of 0.5 m/s showed a general rise in the level of CYP450 above 1 nmol/min/mg/prot. (Figure 9), as against concentrations lower than 1 nmol/min/mg/prot. recorded at 0.05 and 0.1 m/s. This might be an indication that the stress exerted by the flow rate is the highest of all three.
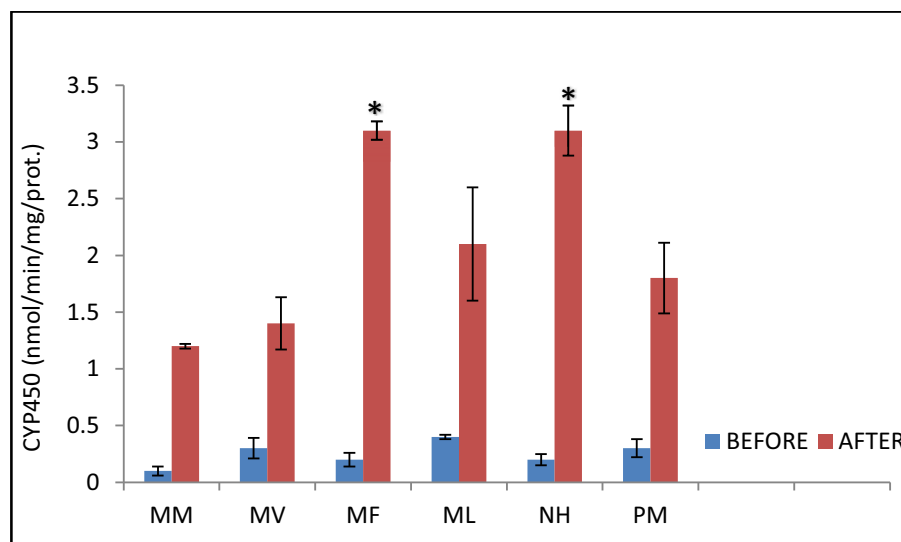
Figure 9: Concentrations of CYP450 in shrimps at flow rate of 0.5 m/s
* = *Significant difference at P < 0.05. N= 10*

Outstandingly higher concentrations of CAT were observed in *N. hastatus* and *P. maculatus* at flow rate of 0.5 m/s. As usual, the lowest concentrations were observed in *M. macrobrachion* and *M. vollenhovenii* i.e. they exhibited the highest resilience even in the less favourable condition (Figure 10).
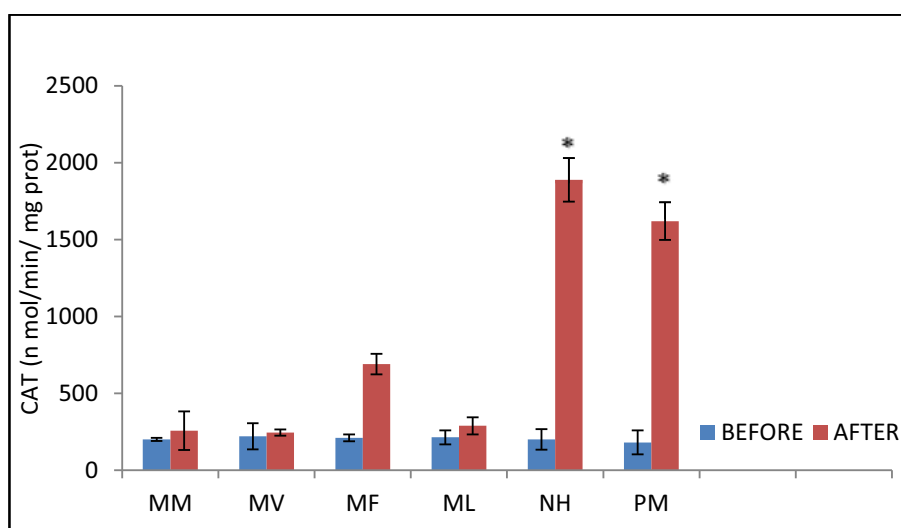

Figure 10: Concentrations of CAT in shrimps at flow rate of 0.5 m/s
* = *Significant difference at P < 0.05. N= 10*

Higher concentrations of GST were observed in all the shrimps at 0.5 m/s relative to 0.05 and 0.1 m/s (Figure 11). This implies that the flow rate might have exerted the highest levels of stress on the shrimps, thereby resulting in release of the antioxidant enzymes to mitigate the stress. Very high concentration levels were also recorded in SOD (Figure 12). Although results show that the conditions appeared unfavourable for the shrimps, *M. macrobrachion* and *M. vollenhovenii* coped best with the seemingly unfavourable condition.
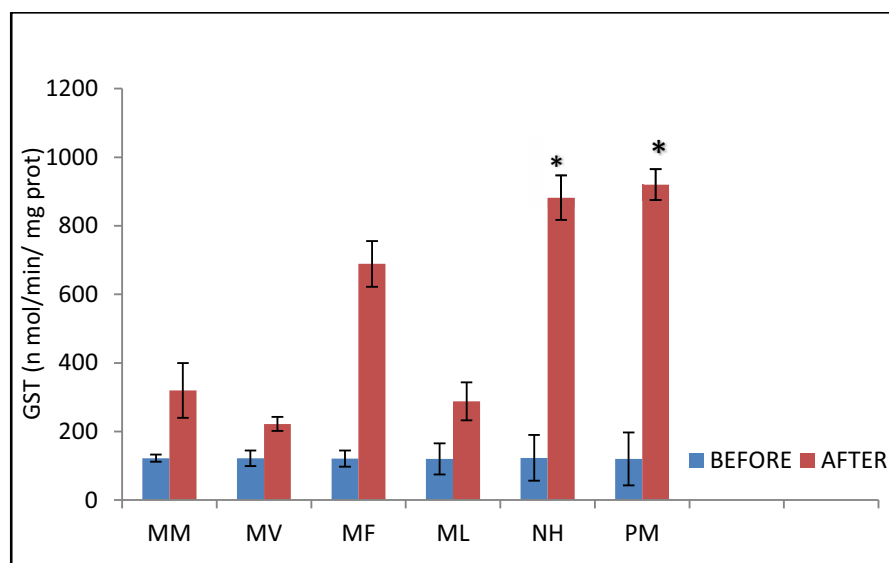
Figure 11: Concentrations of GST in shrimps at flow rate of 0.5 m/s
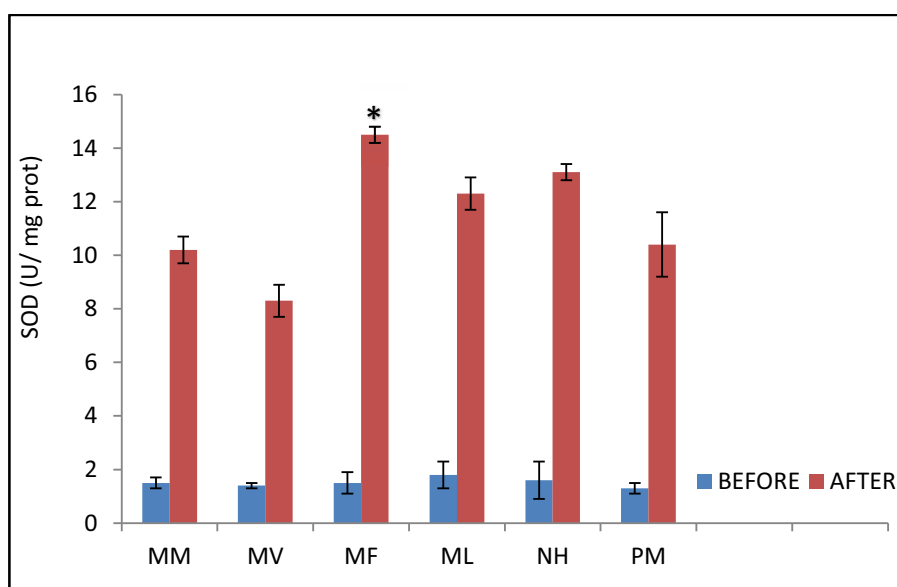** = Significant difference at P < 0.05. N= 10*

Figure 12: Concentrations of SOD in shrimps at flow rate of 0.5 m/s
* = Significant difference at P < 0.05. N= 10

3.4. Stress level assessments at flow rate of 1.0 m/s

A futher increase was recorded in the general concentrations of the antioxidant enzymes at flow rate of 1.0 m/s. This implies a further drift away from the somewhat suitable condition. Exceedingly higher concentrations of CYP450 (Figure 13), CAT (Figure 14), GST (Figure 15) and SOD (Figure 16) indicate a further drift away from the prefered flow rate. Despite the

increasing unfavourability, *M. macrobrachion* and *M. vollenhovenii* maintained the best poisitions of relatively higher resilience.
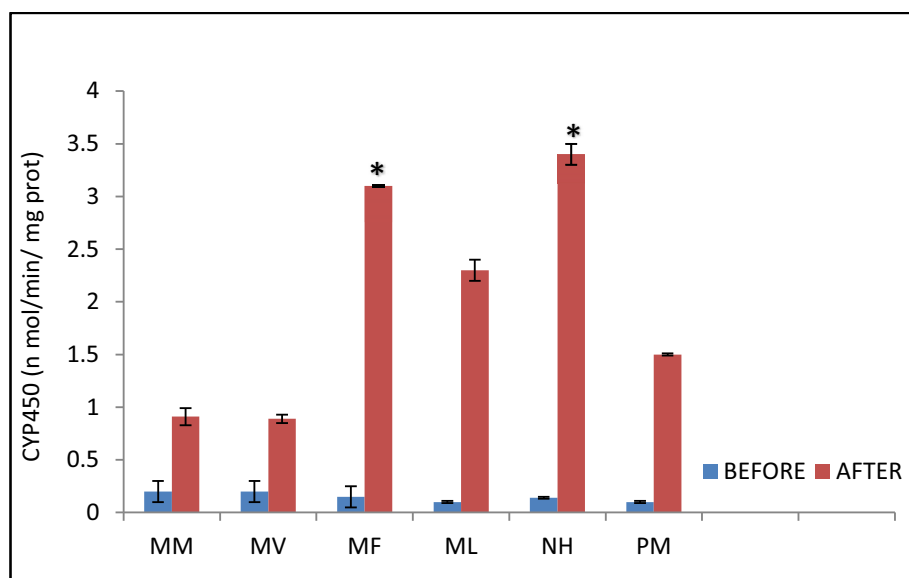

Figure 13: Concentrations of CYP450 in shrimps at flow rate of 1 m/s
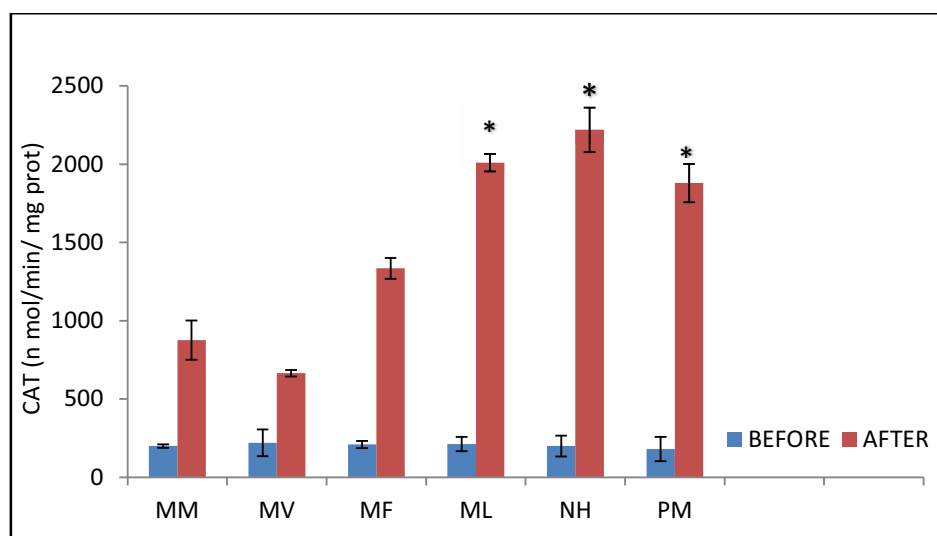** = Significant difference at P < 0.05. N= 10*

Figure 14: Concentrations of CAT in shrimps at flow rate of 1 m/s
* = Significant difference at P < 0.05. N= 10

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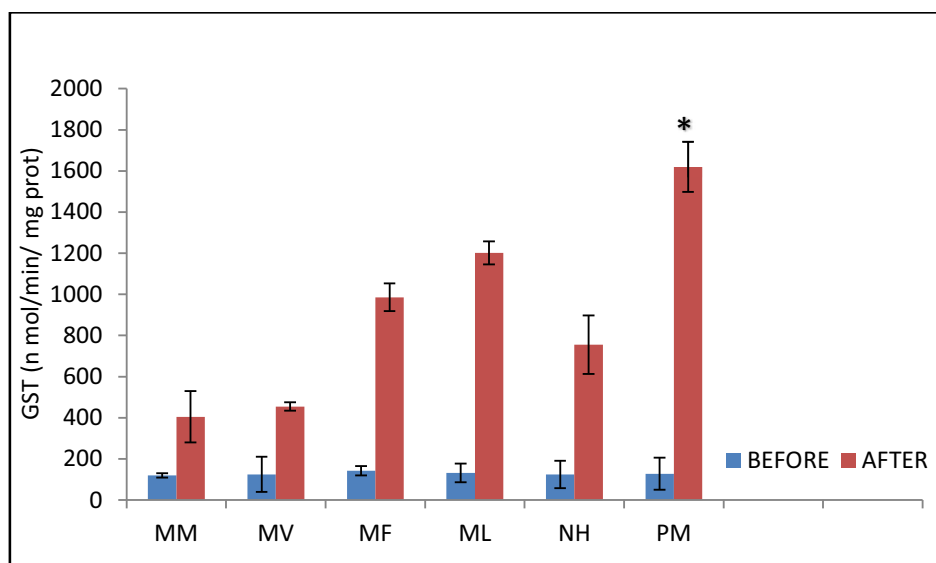doi:10.1088/1755-1315/210/1/012011

Figure 15: Concentrations of GST in shrimps at flow rate of 1 m/s
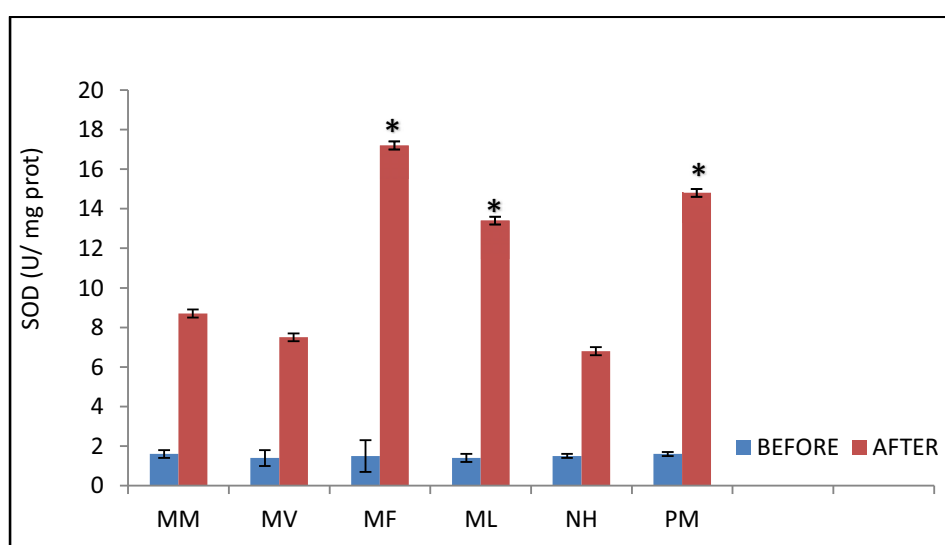** = Significant difference at P < 0.05. N= 10*

Figure 16: Concentrations of SOD in shrimps at flow rate of 1 m/s
* = Significant difference at P < 0.05. N= 10

Results of the induced antioxidant enzymes at the various flow rates have consistently showed that *M. macrobrachion* and *M. vollenhovenii* significantly strived to main balance in the various levels of stress. Figure 17 depicts at a glance, comparative analysis of change in antioxidant concentrations in the shrimp species at different assessed flow rates. Result shows that flow rate of 0.1 m/s is the most suitable for the survival of the shrimp species. On the basis of comparison of the resilience of the shrimps in handling the stress exerted at different flow rates. *M. vollenhovenii* managed the stress the most, followed by *M. macrobrachion*. These potentials can be attributed to the relatively large and tough morphological characteristics and the animals.

Proficiency of regulation of antioxidant enzymes in the animals must have contributed appreciably to the hardiness of the animals. Cultivation of *M. vollenhovenii,* followed by *M. macrobrachion* is very much achievable, with optimal results at flow rate of 0.1 m/s.
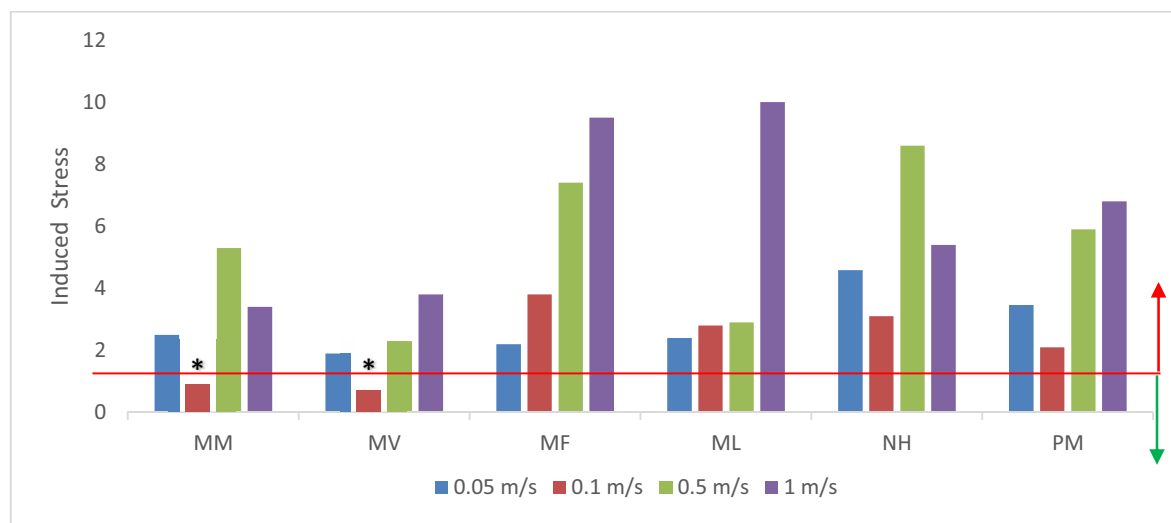

Figure 17: Comparative induced stress levels among shrimp species at various flow rates
Note: Stress level of 1 and above is significant, while below 1 is insignificant. Red arrow indicates significant margin while green arrow indicates insignificant margin. Asterisked bars indicate insignificant stress levels.

4. CONCLUSION

M. vollenhovenii, followed by *M. macrobrachion* has considerable cultivability at flow rate of 0.1 m/s which can be simulated in shrimp aquaculture. The species have an appreciably wide spectrum of flow rates due to their ability to cope in slightly harsh conditions. The study provided vivid information on the flow rate of shrimp pond to support artificial rearing of the palaemonid shrimps.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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