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Expression of Heat Stress Biomarkers in Wild and Cultured African Catfish *Clarias gariepinus* (Burchel, 1822)

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Authors' contributions

This work was carried out in collaboration between both authors. Author KGO designed the study, performed the statistical analysis, wrote the protocol, and wrote the first draft of the manuscript. Author RON managed the analyses of the study and managed the literature searches. Both authors read and approved the final manuscript.

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ABSTRACT

The expression of heat stress biomarkers in wild and cultured African catfish *Clarias gariepinus* was investigated in this study. Twenty wild and cultured fish species of average weight of 400±50g were obtained from Owena dam, (Latitude: 7°20'46.04"Longitude: 4°59'54.99") and a reputable fish farm in Akure, Ondo State. Ten male and female fish from the two source were all conditioned for 3days in concrete tanks. The fish were stocked in concrete tanks of 2m x 2m x 1m with the stocking density of 5 in each tank and the water quality parameters were monitored. Fish were subjected to hyperthermia-induced shock at 39^{o}C with the aid of a 2-kW heating rod (Binatone, Japan). At the end of the hyperthermia-induced stress. Blood samples were collected to determine the glucose level and the expression of Heat Shock Protein (HSP). The highest glucose level of 50mg /l was found in the cultured male African catfish and the lowest glucose level of 18mg/l was found in wild female African catfish. There was higher diversity and expression of HSP in cultured

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female fish than the wild male. The result of this study showed that the expression of stress biomarkers in African catfish *Clarias gariepinus* was influenced by the gender and the environment where the fish was found with the male and wild fishes showing more resistance to stress.

Keywords: Wild; cultured; African catfish; heat shock proteins; hyperthermia.

1. INTRODUCTION

The issue of stress is central to most discussions on the welfare of wild and intensively farmed animals, including fish [1]. Stress in this context is perceived as an undesirable consequence of an unsatisfactory regime. As a result of the widely held view that there is an inverse association between stress and well-being, the detection of stress has been employed as a tool in assessing the welfare of animals. However, what must not be overlooked is that the stress response is a normal and frequently utilized element of an animal's adaptive repertoire – although activation of the stress response signals that the animal is responding to a challenge, detection of a stress response cannot be considered to be an unambiguous marker of an actual or potential decline in well-being [2]. Therefore, it is important to study the effects of stress on the expression of the stress biomarkers in wild and cultured catfish. Heat shock protein is a family of conserved ubiquitously expressed heat shock protein in response to stressful condition [3]. In spite of many studies that has been done in mammalian species systematic analysis among teleost fish species like the African catfish has been lacking. In this present study, expression of stress biomarker in wild and cultured catfish. African catfishes are a diverse group of ray-finned fish. They are named for their prominent barbels, which resemble a cat's whiskers, Catfish are of considerable commercial importance; many of the larger species are farmed or fished for food [4]. They are the most commercially important cultivated fish in Nigeria [5]. Therefore, the objective of this study is to assess and compare the expression of stress biomarkers in wild and cultured African catfish.

2. MATERIALS AND METHODS

2.1 Experimental Site and Procedure

The study was carried out at the hatchery room of the Teaching and Research Farm of the department of Fisheries and Aquaculture Technology, Federal University of Technology Akure, Ondo State. Ten wild fish (5 males and 5 females) samples were sourced from the Owena Dam, (Latitude: 7°20'46.04"Longitude: 4°59'54.99") Ondo State, Nigeria and the 10 cultured samples from a reputable fish farm in Akure Ondo-state. With average weight of 400g±0.75, they were stock based on sexes and acclimatized for 14 days in a concrete tank in the Department of Fisheries and Aquaculture Teaching and Research Farm. They were not fed during the acclimatization period. Each sample of the used samples was weighed.

2.2 Determination of Glucose

Two fish specimen from each tank were removed for blood glucose analyses. Blood were collected by puncture of the caudal blood vessels .This was done with the aid of 2ml disposable syringe. Glucose concentration was measured according to [2] using Bio-La-Test oxochrome GLUCOSA (Glu 250E). Based on the oxidation of glucose catalyzed by glucose oxidase to hydrogen peroxide and gluconate. The peroxide produced was determined by oxidation coupling with substituted phenol and 4-amino antipyrin. The coupling was catalysed by peroxidase.

2.3 Hyperthermia- induced Stress

At the end of the feeding trial, fish from each treatment were kept in plastic tanks for hyperthermia- induced stress according to a modified method [1] using a 2-kW heating rod (Binatone, Japan). The rate of heating ramp was about 3^{o} C/h. Water temperature was maintained at 39 ± 0.05°C throughout the hyperthermia – induced stress period. No fish died during the hyperthermia treatment. Fish were taken randomly at 2h after exposure from the tanks. Two fish per tank were euthanized by overdose (200 mg / liter of water for 10 min) of tricaine methane sulphonate (MS222; Pharmaq, Fordingbridge, UK). Mucus samples were removed and weighed immediately after hyperthermia- induced stress from fish for SDS-PAGE analysis.

2.4 Protein Gel Electrophoresis

SDS-PAGE (Sodium dodecyl sulfate polyacrylamide gel electrophoresis) was used for the protein gel electrophoresis. The protein concentration of freeze dried mucus samples were determined using bovine serum albumin as the standard. The protein profile of epidermal mucus was examined using sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE). The gel was run in a Bio-Rad electrophoresis apparatus for 4 h at 150 V. The gel was then stained with Coomassie Brilliant Blue and the protein banding profile were compared with standard markers (Spectra multicolor High Range Protein Ladder, Fermentas).

2.5 Statistical Analysis

This experiment was designed to test for differences in the mean value of glucose and diversity of in the treatments. The values were recorded as a mean ± standard deviation. Differences between mean were considered significant at $P<0.05$ using one way analysis of variance (ANOVA). Follow– up procedure was performed where significant differences occurred in the means using Tukey by SPSS software version 13 (SPSS Inc.). Photographed gel was processed for gel diversity and migration pattern using GelAnalyzer 2010a®, and gel electrophoresis image analysis software. Minitab 18® statistical software was used to plot the web – profile radar plot for the diversity of the SDS-PAGE gels.

3. RESULTS

The result of the study shows that the cultured fish has the highest glucose level than the wild fish sample after the hyperthermia-induced stress. The male cultured species has the highest glucose level with 50mg/l while the wild female has the least with 18mg/l as shown in Fig. 1.

Heat Shock Protein expression in wild and cultured African catfish is presented in Fig. 2**.** Web radar analysis showed the distributions of the HSP markers with intensity and molecular mass in Kilodactylum (kDa). The web radar revealed that cultured fish has higher expression of HSP than the wild fish. This result also revealed that female fish has higher expression of HSP than male catfish. The cultured female has highest band diversity than any other samples as shown in Fig. 3. The lowest band diversity is recorded in wild male catfish.

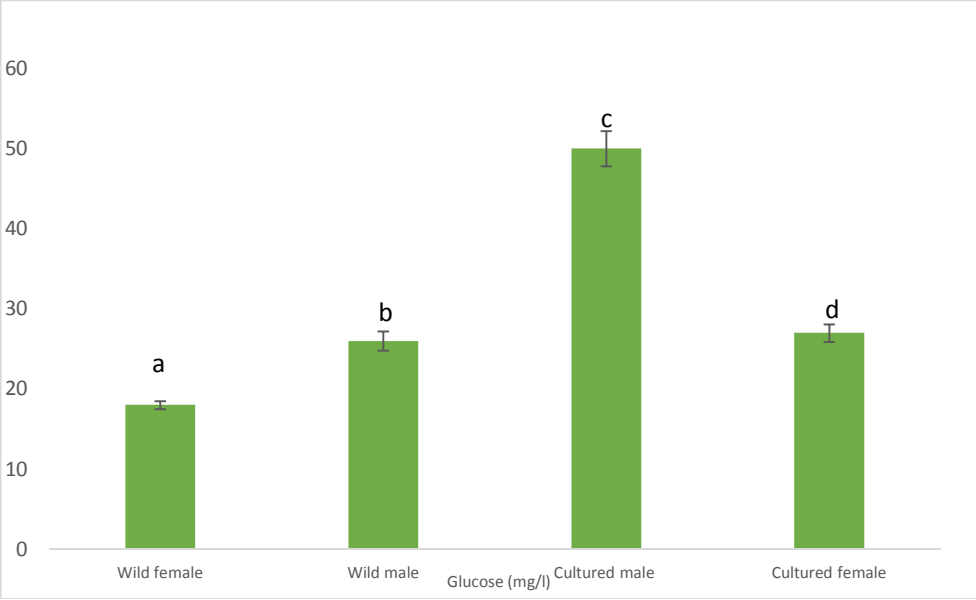

Fig. 1. Glucose levels in wild and cultured hyperthermia –stressed African catfish

a,b,c values on each bar with the different superscript are significantly different (P<0.05) using ANOVA Post Hoc (Tukey test).

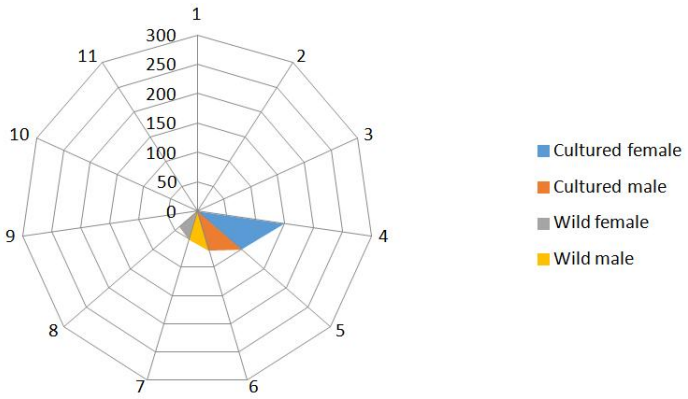

Fig. 2. Web radar analysis showing the distributions of the HSP markers with intensity and molecular mass in Kilodactylum (kDa)

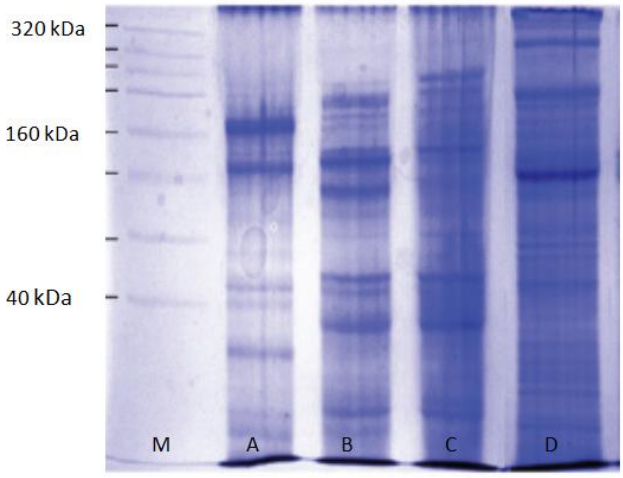

Fig. 3. SDS-PAGE showing the HSP profile of wild and cultured African catfish species; M = marker molecular mass [kDa], A =Cultured female *Clarias gariepinus,* B= Cultured male *Clarias gariepinus,* C = Wild female *Clarias gariepinus* D= Wild male *Clarias gariepinus*

4. DISCUSSION

For effective stress management, it is important to study the stress response in fish, its functional role, how the response can be measured, and whether detection of a stress response provides information relevant to the assessment of the stress resistance and health of fish [6]. The current study revealed that the cultured male fish had higher glucose level than their counterpart from the wild habitat, these may be due to the size of the enclosure of the rearing facilities compare to the large expanse of space in the wild habitat. The cultured sample are fed at regular interval but the wild fish have no regular food, they tend to scavenge and do not always feed on nutritionally complete diet.

Higher glucose levels were recorded in wild and cultured male fish compared with the female fish. The aggressiveness and glucose level in male catfish increase with activity like chasing of female for mating, competition for foods, escape from predation, territorialism and defense [7- 9]. It was observed in present study that the HSP levels, diversity and the expression of stress biomarkers in the cultured fish are more than those recorded in the wild fish, showing that the wild catfish are more stress resistant and hardy compared to the cultured fish. Under stressful conditions such as heat shock, pH shift or hypoxia, increased expression of HSPs protect the cell by stabilizing unfolded proteins, giving the cell time to repair or re-synthesize damaged proteins [10-12]. This may be due to fact that

stress can function as a metabolic rate suppression which modify the behavior, physiology and cellular biochemistry of fish in order to reduce the whole organism's energy expenditure and maintain homeostasis resulting in a more resilient wild stock [3,9]. Furthermore, environmental stress creates an alarm responses, an important component which enhances survival in fish when induced [9,12].

5. CONCLUSION

Environmental factors, gender and the habitat are important factors which affect the expression of stress biomarkers in African catfish. The result of this study showed that the expression of stress biomarkers in African catfish is influenced by the gender and the environment where the fish is found.

ETHICAL APPROVAL

As per international standard or university standard written ethical approval has been collected and preserved by the author(s).

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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