

1 **EXPRESSION OF HEAT STRESS BIOMARKERS IN WILD AND CULTURED**

2 **AFRICAN CATFISH *Clarias gariepinus* (BURCHEL, 1822)**

3 **ABSTRACT**

4 The expression of heat stress biomarkers in wild and cultured African catfish *Clarias gariepinus*
5 was investigated in this study. Twenty wild and cultured fish species of average weight of
6 400±50g were obtained from Owena dam, (Latitude: 7°20'46.04"Longitude: 4°59'54.99") and a
7 reputable fish farm in Akure, Ondo State. Ten male and female fish from the two source were all
8 conditioned for 3days in concrete tanks. The fish were stocked in concrete tanks of 2m x 2m x
9 1m with the stocking density of 5 in each tank and the water quality parameters were monitored.
10 Fish were subjected to hyperthermia-induced shock at 39°C with the aid of a 2-kW heating rod
11 (Binatone, Japan). At the end of the hyperthermia-induced stress. Blood samples were collected
12 to determine the glucose level and the expression of Heat Shock Protein (HSP). The highest
13 glucose level of 50mg/l was found in the cultured male African catfish and the lowest glucose
14 level of 18mg/l was found in wild female African catfish. **There was higher diversity and**
15 **expression of HSP in cultured female fish than the** wild male. **The result of this study showed**
16 that the expression of stress biomarkers in African catfish *Clarias gariepinus* was influenced by
17 the gender and the environment where the fish was found with the male and wild fishes showing
18 more resistance to stress.

19 **Keywords:** Wild, Cultured, African Catfish, Heat Shock Proteins, hyperthermia

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Comment [M1]: THAN THE WILD FEMALE?? I
DON'T UNDERSTAND THIS COMPARATION

22 INTRODUCTION

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

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44 MATERIALS AND METHOD

45 Experimental site and procedure

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

54 Hyperthermia- induced stress

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

63 Protein gel electrophoresis

64 SDS-PAGE (Sodium dodecyl sulfate polyacrylamide gel electrophoresis) was used for the
65 protein gel electrophoresis. The protein concentration of freeze dried mucus samples were

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

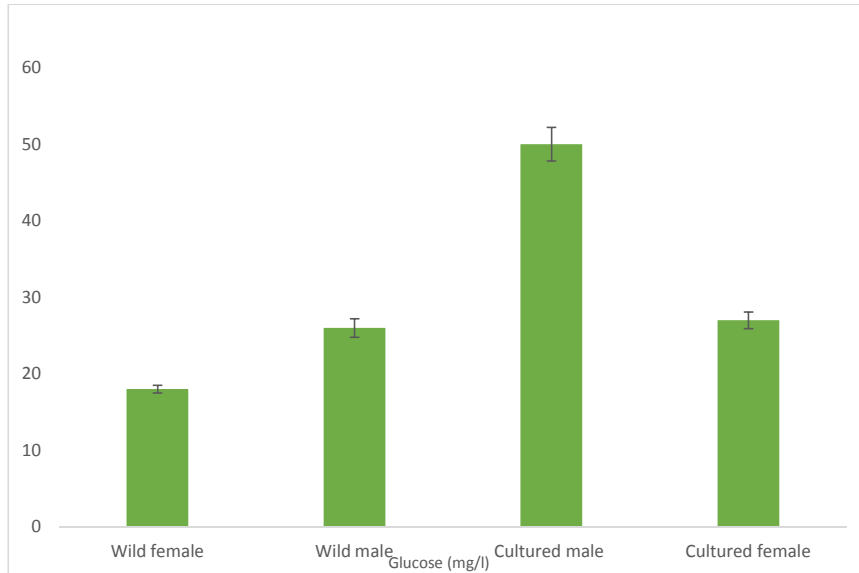
71 **Statistical analysis**

72 This experiment was designed to test for differences in the mean value of glucose and diversity
73 of in the treatments. The values were recorded as a mean \pm standard deviation. Photographed gel
74 was processed for gel diversity and migration pattern using GelAnalyzer 2010a[®], and gel
75 electrophoresis image analysis software. Minitab 18[®] statistical software was used to plot the
76 web - profile radar plot for the diversity of the SDS-PAGE gels.

77 **RESULTS**

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

Comment [M2]: IT IS NECESSARY TO MAKE A STATISTICS ANALYSIS



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Figure 1: Glucose levels in wild and cultured hyperthermia –stressed African catfish

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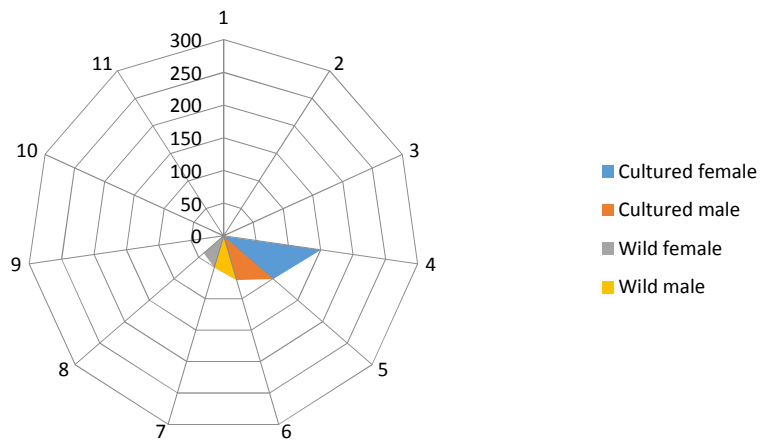
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Heat Shock Protein expression in wild and cultured African catfish is presented in Figure 2. Web radar analysis showed the distributions of the HSP markers with intensity and molecular mass in Kilodactylum (kDa). The web radar revealed that cultured 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 Figure 3. The lowest band diversity is recorded in wild male catfish.

Comment [M3]: ???

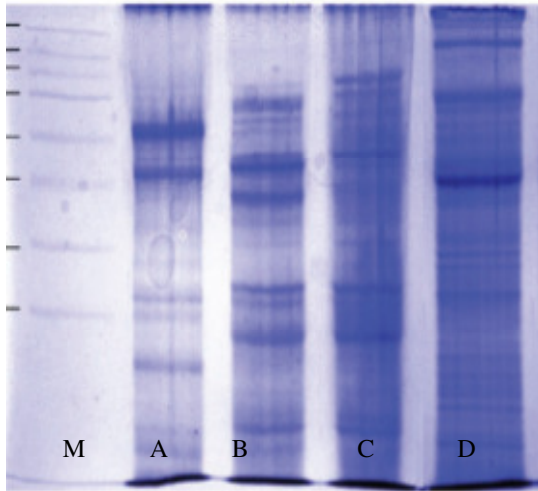


90

91 **Figure 2:** Web radar analysis showing the distributions of the HSP markers with intensity and
 92 molecular mass in *Kilodactylum* (kDa).

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

Comment [M4]: WHERE IS THE BAND?? THE MOLECULAR WEIGHT MUST BE POINTING

99 DISCUSSION

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

108 Higher glucose levels were recorded in wild and cultured male fish compared with the female
109 fish. The aggressiveness and glucose level in male catfish increase with activity like chasing of
110 female for mating, competition for foods, escape from predation, territorialism and defense [7-
111 9]. It was observed in present study that the HSP levels, diversity and the expression of stress
112 biomarkers in the cultured fish are more than those recorded in the wild fish, showing that the
113 wild catfish are more stress resistant and hardy compared to the cultured fish. Under stressful
114 conditions such as heat shock, pH shift or hypoxia, increased expression of HSPs protect the
115 cell by stabilizing unfolded proteins, giving the cell time to repair or re-synthesize damaged
116 proteins [10-12]. This may be due to fact that stress can function as a metabolic rate
117 suppression which modify the behavior, physiology and cellular biochemistry of fish in order
118 to reduce the whole organism's energy expenditure and maintain homeostasis resulting in a
119 more resilient wild stock [3, 9]. Furthermore, environmental stress creates an alarm responses,
120 an important component which enhances survival in fish when induced [9, 12].

121 CONCLUSION

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

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Comment [M5]: HOW DID YOU MAKE THIS COMPARATION? WHY DID YOU MAKE A COMPARATION MALE TO MALE AND FEMALE TO FEMALE IN BOTH WILD AND CULTURE ENVIRONMENT

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Comment [M6]: ONLY TWO REFERENCES NEW

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