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Full Length Research Paper

Organosomatic Indices, Haematological And Histological Assessment as Biomarkers of Health Status in Feral and Cultured *Clarias gariepinus*

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ABSTRACT

The project was designed to study the physiological differences and histological changes in cultured and feral *Clarias gariepinus*. The quality of the culture water was also compared. Water samples and live specimens of Clarias gariepinus of both sexes were purchased weekly for five weeks from fishermen at Eleyele River (feral) and a commercial fish pond (cultured) in Ibadan, Nigeria. The samples were subjected to haematological and histological assessments. Water and milt qualities were also determined. There were no significant differences in the organosomatic indices and haematological parameters. The spermatozoa count was significantly higher in cultured fish. However, the dissolved oxygen content of the cultured pond was significantly lower ($p< 0.05$) than feral. Histological assessment revealed macrophage hyperplasia and severe fatty degeneration with sinusoid dilation of the liver of cultured fish; which respectively, can be attributed to reaction to the presence of an antigen and malnutrition from the supplementary diet fed cultured fishes.

Keywords; *Clarias gariepinus, haematological parameter, Biomarkers, histological changes, milt quality*

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INTRODUCTION

Fish serve as an important biomarker for pollution. They represent the largest and most diverse group of vertebrates, and fishes have the ability to adapt to a wide variety of environments. Fish are relatively sensitive to changes in their surrounding environment including an increase in pollution. Fish health may thus reflect, and give a good indication of the health status of a specific aquatic ecosystem (Adams et al., 2000). Biomarkers are measures of cellular, biochemical, molecular, or physiological change in an organism that indicate exposure to or the effects of environmental contaminants. Despite the fact that a large number of biomarkers have been developed and used to monitor environmental health since the mid 1980s, there is still no consensus on the best suite of markers or on their sensitivity and reliability (Lam and Gray 2003).

Environmental stressors have an important influence on condition. A condition index is a simplified measure designed to capture most of the variation in physiological well-being. If measured appropriately, condition indices can have strong implications for individual fitness and population dynamics. (Saltz et. al., 1995). Ratios of the mass of particular organs or tissues relative to total body mass can also be used as indices of change in nutritional and energy status (Bollard et al., 1994). Blood parameters have also been commonly used to observe and follow fish health. Since variations in blood tissue of fish has been known to be caused by environmental stress (Shah, 2006).

Histopathological changes have been widely used as biomarkers in the evaluation of the health of fish exposed to contaminants, both in the laboratory (Wester and Canton, 1991, Thophon et al., 2003;) and field studies (Teh et al., 1997). One of the great advantages of using histopathological biomarkers in environmental monitoring is that this category of biomarkers allows examining specific target organs, including gills, kidney and liver, that are responsible for vital functions, such as respiration, excretion and the accumulation and biotransformation of xenobiotics in the fish (Gernhofer et

al., 2001). Furthermore, the alterations found in these organs are normally easier to identify than functional ones (Fanta et al., 2003), and serve as warning signs of damage to animal health (Hinton and Laurén, 1990). Motility of the spermatozoans is the most commonly used indicator of sperm quality, since high motility is a prerequisite for fertilization and correlates strongly with fertilization.

There is a need for a continued development, validation, and improvement in the application of biomarkers (Adams et al., 2001). It is against this background that this study was designed to comparatively assess the impact of the different environmental conditions of feral and cultured *Clarias gariepinus* on the health status of both the female and male fish using organosomatic indices, haematological parameters, sperm motility and gonadal histopathology as biomarkers.

MATERIALS AND METHODS

Sampling Of Fish and Water from the Wild and Cultured Pond

Live specimens of *Clarias gariepinus* of both sexes were purchased weekly for five weeks from fishermen at Eleyele River and from a private farmer at Oluyole area of Ibadan, Southwest, Nigeria. Also, water samples were collected from Eleyele River and the cultured pond.

Blood Collection and Haematological Analyses

2mls of blood was collected into ethylenediamine tetraacetic acid (EDTA) bottle by venous puncture from the caudal vein. Sterile needle was inserted into the musculature perpendicular to the ventral surface of the fish until the spine was reached and blood entered the syringe. Red Blood Cell (RBC), total White Blood Cell (WBC) and platelet counts were done using the Neubauer haemocytometer. The Packed Cell Volume (PCV) and Haemoglobin (Hb) values were determined by the microhaematocrit capillary tube and cyanomethaemoglobin methods respectively (Hesser, 1960). The Mean Corpuscular Volume Haemoglobin (MCV), Mean Corpuscular Haemoglobin (MCH), and Mean Corpuscular Haemoglobin Concentration (MCHC) were derived using standard formulae:

$$\text{MCV (fl)} = \frac{\text{PCV} \times 10}{\text{RBC } (\times 1012/\text{L})}$$

$$\text{MCH (pg)} = \frac{\text{Hb (g/L)}}{\text{RBC } (\times 1012/\text{L})}$$

$$\text{MCHC (g/L)} = \frac{\text{Hb (g/L)} \times 100}{\text{PCV (\%)}}$$

Blood smears stained with giemsa, were used to determine the white blood cell count (WBC), thrombocyte count, and differential WBC counts. This method of manually determining total WBC and differential count has been recommended for fish (Stoskopf, 1983).

Sperm Motility Assessment

By simple visual inspection through a microscope, it is possible to conclude whether sperm samples are motile or quiescent. After dilution of sperm in normal saline, sperm head movements were assessed with a light microscope and scored.

Histopathology

Fish were stunned and organs (liver, spleen, heart and gonads) were harvested and tissues were fixed in Bouin's fixative for 24hrs after which, they were fixed in 10% phosphate-buffered formalin (pH 7.0-7.4). Tissues were dehydrated, embedded in wax, sectioned on a microtome into 5 µm sections, mounted on clean glass slides and stained with haematoxylin and eosin. Sections were viewed with a light-microscope (Kiernan, 1990). Significant findings were documented using a digital camera.

Water Quality analysis

This was determined using Hach® Fish Farmers Water quality test kit according to manufacturer's guidelines

Statistical Analyses of Data

Data were subjected to descriptive statistics and results presented as mean ± standard deviation. They were also subjected to tests of significance (Students't-test and ANOVA).

RESULTS

The result of the spermatozoa quality assessment shows that only the sperm count was significantly higher in cultured fish (Table 1). The dissolved oxygen content of the water from feral source was significantly higher than cultured ponds, while there were no significant differences in the other water quality parameters (Table 2).

Table 1:
Spermatozoa Parameters of Cultured and Feral Fish

Spermatozoa parameters	Cultured Fish	Feral Fish
Weight (g)	517.0 ± 92.2	861.0 ± 236.5
Motility (%)	92.0 ± 2.7	96.8 ± 1.6
Live-dead	96.8 ± 1.6	96.8 ± 1.6
Count	236.4 ± 15.5	199.8 ± 12.5

Table 2:
Water Quality Parameters of Cultured Pond and Surface Water

Water quality parameters	**Cultured Pond**	**Surface Water**
Dissolved Oxygen	0.29 ± 0.19	5.61 ± 1.73
Temperature	29.34 ± 1.56	29.60 ± 1.44
Alkalinity	469.98 ± 34.03	130.64 ± 70.81
CO2	168.60 ± 37.19	65.00 ± 58.94
Chloride	185.34 ± 131.70	142.00 ± 47.64
pH	7.60 ± 0.41	7.70 ± 0.27
Total Hardness	246.24 ± 25.93	188.10 ± 93.66

Table 3:
Haematological Parameters of Male and Female Cultured and Feral *Clarias gariepinus*.

Hematological Parameters	Cultured Fish		Feral Fish	
	Male	**Female**	**Male**	**Female**
PCV	28.60 ± 6.26	28.40 ± 2.51	33.80 ± 13.23	39.00 ± 9.19
HB	8.92 ± 2.09	8.76 ± 0.76	10.50 ± 4.37	12.32 ± 3.12
RBC	2.51 ± 1.00	2.15 ± 0.58	3.03 ± 1.51	3.45±1.12
WBC	14860.00 ± 2476.48	13090.00 ± 5997.33	10250.00 ± 4194.63	15160.00 ± 7861.80
PLATELET	130400.00 ± 62616.29	128200.00 ± 52509.04	115300.00 ± 10945.31	130000.00 ± 23054.28
LYMPHOCYTE	48.00 ± 21.64	48.60 ± 17.05	47.00 ± 21.84	46.40 ± 20.72
HETEROPHIL	48.20 ± 22.43	48.20 ± 16.93	49.80 ± 22.17	50.00 ± 19.68
MONOCYTE	2.40 ± 0.54	2.00 ± 0.70	2.00 ± 0.00	2.40 ± 1.67
EOSINOPHIL	1.00 ± 0.70	2.00 ± 1.22	1.00 ± 0.70	1.60 ± 0.89
MCHC	3.10 ± 0.06	3.08 ± 0.09	3.07 ± 0.20	3.14 ± 0.10
MCH	380.06 ± 81.38	442.17 ± 172.94	369.08 ± 84.52	375.39 ± 88.76
MCV	122.66 ± 28.38	142.27 ± 50.82	121.73 ± 37.69	111.07 ± 27.37

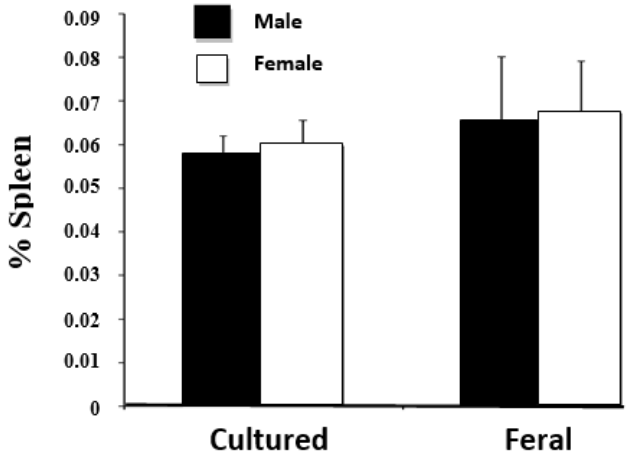

Figure 1
Comparative splenosomatic indices of male and female *Clarias gariepinus* from cultured and feral sources

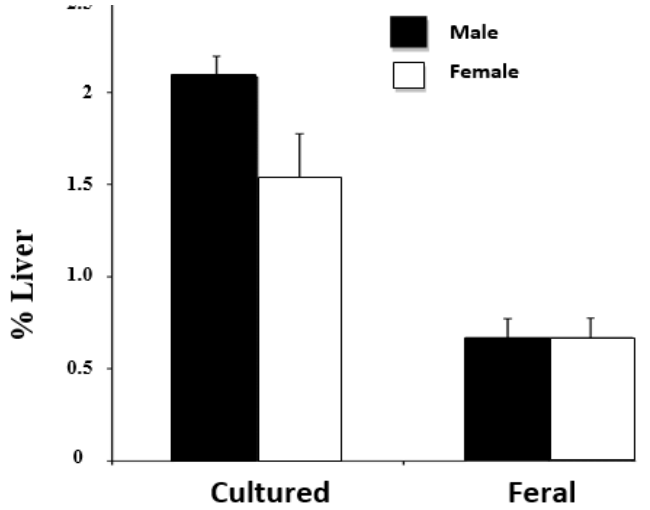

Fig 3:
Comparative hepatosomatic indices of male and female *Clarias gariepinus* from cultured and feral sources

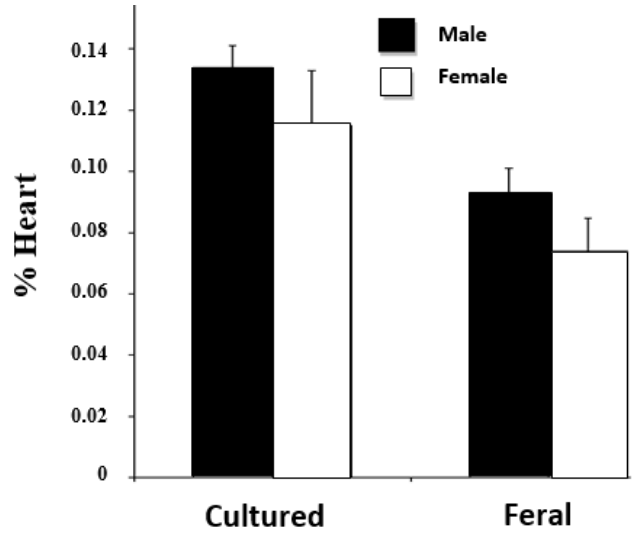

Fig 2:
Comparative cardiosomatic indices of male and female *Clarias gariepinus* from cultured and feral sources

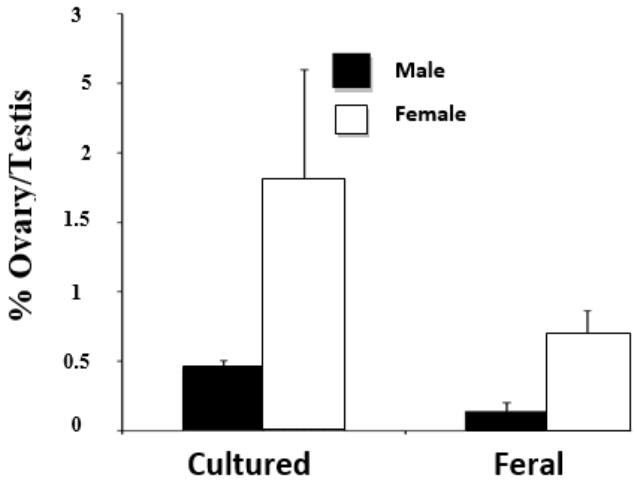

Fig 4:
Comparative gonadosomatic indices of male and female *Clarias gariepinus* from cultured and feral sources

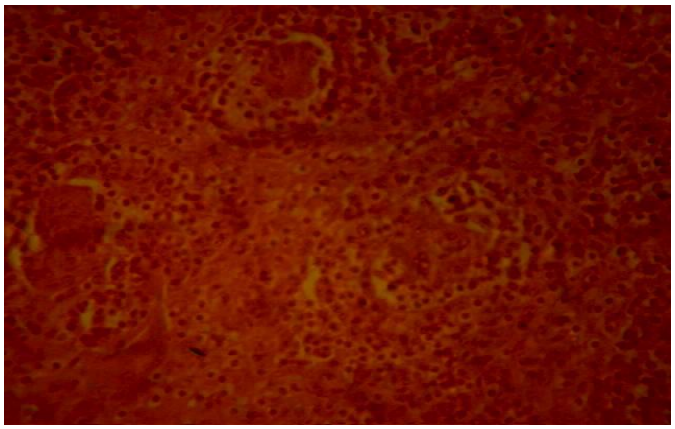

Plate 1
Photomicrograph of Normal Spleen in feral fish (H & E, X 1000). Scale bar = 20mm

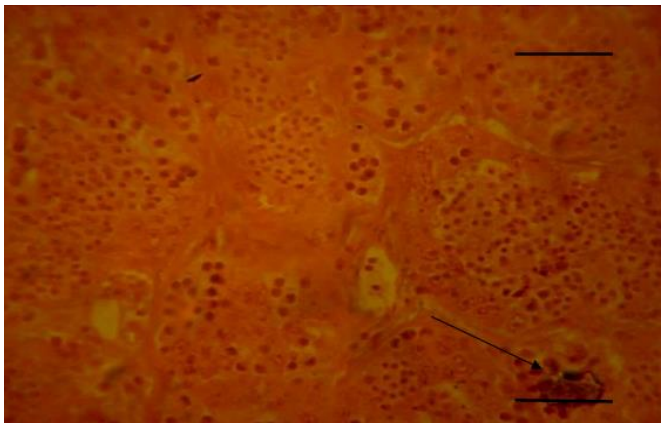

Plate 2
Photomicrograph of Macrophage Hyperplasia in Cultured Female Spleen (H & E, X 1000). Scale bar = 20mm

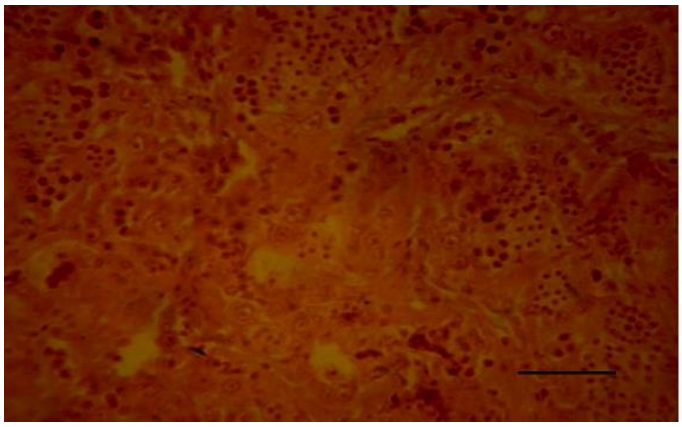

Plate 3
Photomicrograph of Lymphoid Depletion in the Spleen of Cultured Female fish (H & E, X 1000).Scale bar = 20mm

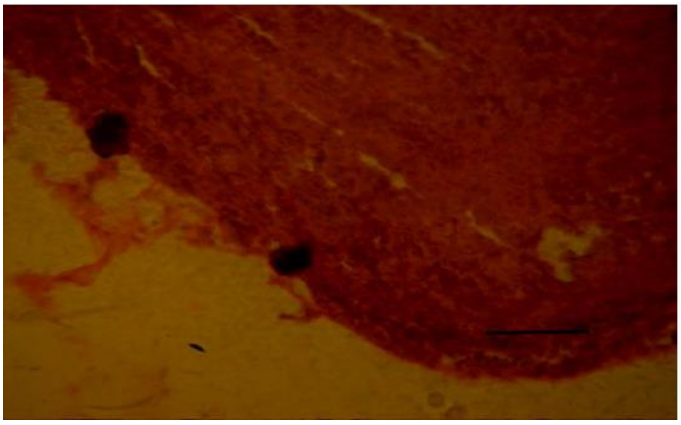

Plate 4
Photomicrograph of Haemosiderosis as observed in Spleen from feral source (H & E, X 1000). Scale bar = 20mm

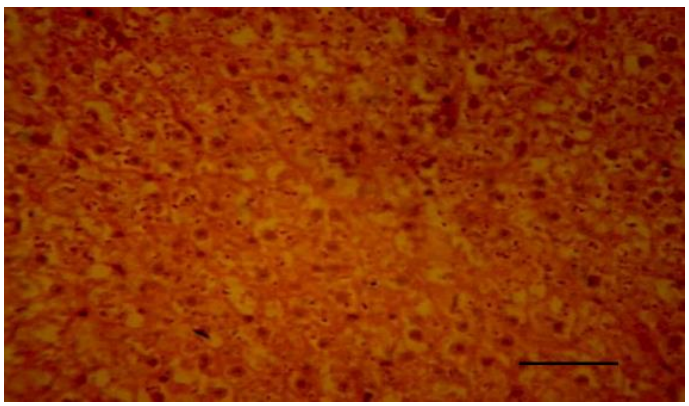

Plate 5
Photomicrograph of Lymphoid Depletion in the Spleen of Cultured Female fish (H & E, X 1000).Scale bar = 20mm

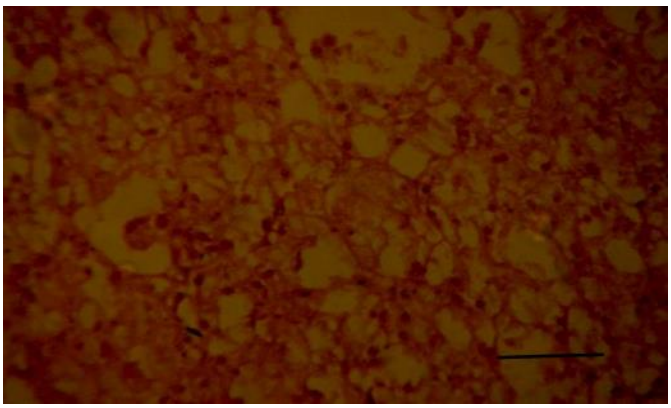

Plate 6
Photomicrograph of Haemosiderosis as observed in Spleen from feral source (H & E, X 1000). Scale bar = 20mm

There were no significant differences in the haematological parameters (Table 3). Cultured fish had non-significantly higher hepatosomatic, cardiosomatic, and gonadosomatic indices compared to the feral, while the splenosomatic index was higher in the feral (Figures 1-4). Compared to the normal spleen (Plate 1), histological assessment revealed macrophage hyperplasia in cultured fish (Plate 2).Another abnormality observed was lymphoid depletion in the spleen of cultured female (Plate 3). Haemosiderosis was also observed in the spleen of feral fishes (Plate 4). Compared to a normal liver (Plate 5), severe fatty degeneration and sinusoid dilation was observed in cultured fish (Plates 6 and 7).

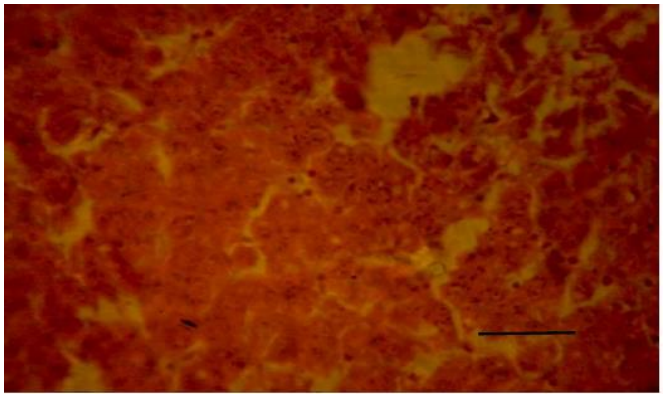

Plate 7
Photomicrograph of Sinusoid Dilation in Liver of Feral Female (H & E, X 1000). Scale bar = 20mm

DISCUSSION

The generally higher values recorded for organosomatic indices in cultured fish can be adduced to supplementary diet fed to cultured fishes. This is in line with the report that there is an increased level of macrophage markers in adipose tissues of leptin-deficient (Lepob/ob), leptin receptor-deficient (LepRdb/db), and diet-induced obese (DIO) mice compared with lean controls (Xu et al., 2003 and Weisberg et al., 2003). These reports have subsequently been confirmed and expanded on by other researchers (Christiansen et al., 2003; Bruun et al., 2003; Chen et al., 2005; Cinti et al., 2005; Weiberg et al., 2006; Cancello et al., 2006 and Aprovian et al., 2008). Xu et al., 2003, demonstrated that macrophage infiltration into adipose tissues temporally preceded elevations in plasma insulin levels. Intensified lymphoid depletion has been speculated to be associated with viral load in post-weaning multisystemic wasting syndrome (PMWS) in nursery and fattening pigs (Liu et al., 2000; Rovira et al., 2002) and in human immunodeficiency virus (HIV) patients (O'Brien, 1998; Hay and Kannourakis, 2002).

Haemosiderosis is common, if excess iron is absorbed or if much is released from RBC during haemolysis. The pigmented macrophage aggregates is a very important tool for monitoring stress which is evident in latest efforts morphometrically quantifying these aggregates in histologic sections (Russo et al. 2007, Jordanova et al.2008).

Fatty degeneration observed in cultured fish could be due to high protein content in the feed. Cloudy swelling, bile stagnation, focal necrosis, atropy and vacuolization have been reported in the Corydoras paleatus exposed to methyl parathion (Fanta et al., 2003). Hyperplasia, vacuolation, disintegrated blood vessels, disrupted hepatocytes, focal coagulative necrosis, disorganized hepatic canaliculi, increase of Kupffer cells, fatty degeneration, nuclear pycnosis and narrowing of sinusoids were observed by (Sarkar et al. 2005 and Cengiz and Unlu, 2006). Hepatocellular vacuolation is therefore more commonly associated with over nutrition from the compounded diet or toxicity (Wolf and Wolfe 2005).

In conclusion, this study indicated that histological changes integrate the impact of a variety of stressors including pathogens, harmful compounds or adverse nutritional and temperature conditions and for this are valuable markers of environmental stress (Schmidt-Posthaus et al., 2001; Au, 2004). Additionally, cultured fishes have a higher turnover rate in terms of organs and gonads and this could be attributed to supplementary feeding which is absent in feral fishes. The pond in a cultured system requires proper management and constant changing due to fast depletion of oxygen content. Also, it is easier for feral fishes to contact infection from the water due to various anthropogenic impacts in the rivers where these fishes live, while the cultured pond has less contact with human activities, their own problem being nutritional deficiencies.

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