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Effect of environmental regulated water temperature variations on survival, growth performance and haematology of African catfish, *Clarias gariepinus*

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Abstract

A 56 day study was carried out to evaluate effect of temperature changes on survival, growth performance and haematology of *Clarias gariepinus* fingerlings fed housefly maggot meal (magmeal) diet. Ninety (90) *Clarias gariepinus* fingerlings, (initial mean weight 4.33±0.03g) were subjected to different environmental regulated temperature conditions in three locations (laboratory, outdoor, greenhouse). The observed average temperature was 26.53±0.01°C, 26.06±0.01°C and 31.52±0.00°C for laboratory, outdoor, greenhouse, respectively. Ten fingerlings stocked per experimental tank were fed in triplicates at 5% body weight in two portions per day. It was observed that different water temperatures affected fish growth. All experimental fish in the greenhouse died after 8 days of exposure. This happened around 14.00 hrs when water temperature reached 40°C. There was no significant difference ($P<0.5$) in final weight, weight gain, food conversion ratio and standard growth rate among the fish reared in the laboratory (26.53°C) and outside tanks (26.06°C), respectively. The initial carcass crude protein (Cp) value was 58.97%. At the end of the experiment fish reared in the laboratory had a crude protein value of 63.97±0.06% Cp and those reared outdoor (26.06°C) had 71.28±0.00% Cp. No significant difference in values of packed cell volume; white blood cell; haemoglobin and mean corpuscular haemoglobin concentration was found between fish reared in laboratory (26.53°C) and outside (26.06°C). However, the red blood cell (RBC); mean corpuscular haemoglobin and mean corpuscular volume showed significant difference ($P>0.05$). The result confirms that *Clarias gariepinus* fingerlings reared at a mean temperature of 26°C (within the recorded optimal temperature range for good growth) performed well. *Clarias gariepinus* fingerlings are not able to survive when water temperature reaches to 40°C.

Key words: Fish growth, Climate change, Temperature, Blood characteristics

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Introduction

Climate change refers to the variation in the earth's global climate or in regional climate over time. It describes changes in the variability or average state of the atmosphere over time scales ranging from decades to millions of years (Emmanuel, 2005). Ongoing climate change is predicted to affect individual organisms during

all life stages, thereby affecting populations of a species, communities and the functioning of ecosystems (Pörtner and Peck, 2010). Current rates of climate change are unprecedented, and biological responses to these changes have also been rapid at the levels of ecosystems, communities, and species (Heino *et al.,* 2009).

The effects of climate change according to Pörtner and Peck (2010) can be direct, through changing water temperatures and associated phenologies, the lengths and frequency of hypoxia events, through ongoing ocean acidification trends or through shifts in hydrodynamics and in sea level. Okorafor (2014) posits that change in climate affects aquatic species at various trophic levels, the physical and chemical environment that make up their habitat and the processes that act on and within freshwater ecosystems. Climate change directly affects a range of physical, chemical and biological processes in the aquatic systems. The extent and magnitude of the ecological consequences of climate change in freshwater ecosystems depends largely on temperature and alterations in water chemistry such as nutrient levels, dissolved organic carbon (DOC), dissolved oxygen (DO) and particulate organic matter (POM).

In fish as well as other such organisms, the body physiological and biochemical processes functions according to the dictates of prevailing water temperature (Holt and Jorgenson, 2015). Elevated temperatures positively alter the breathing rates, feed consumption, enzyme activities, oxygen consumption and feed metabolism thereby affecting growth (Magawata and Ipinjolu, 2014). The growth rate will determine how fast maturity size is attained, the fecundity, recruitment into the exploitable phase of the population and ultimately influence increase in population size (Koeypudsa and Jongjareanjai, 2010).

Fish exist closely with their surroundings. Hence, it is always helpless to physical or chemical fluctuations which possibly will in turn be manifested in the fish haematological characteristics (Wilson and Taylor, 1993). The study of fish blood has advanced rapidly in fish farming due to its relevance in checking the wellbeing of fishes (Hrubec *et al.,* 2000). Haematocrit (PCV), haemoglobin (Hb) concentration and red blood cell count (RBC) differ with the type of food the fish eats, temperature, time of the year and strain (Dienye and Olumuji, 2014).

It has been observed (Cheung *et al.,* 2010), that biological, ecological and physical consequences of global warming on water bodies are becoming glaring. Fishes that attract high market values now migrates pole wards into deeper seas hastily thereby moving away from tropical seas. Holt and Jorgenson (2015) noted that poikilothermic organisms have been subjected to huge and often frequent alterations in the temperature of their habitats. The ultimate effect of temperature fluctuations may be felt on growth impairment, maturation, reproductive capacity and overall population size of fish stocks. This no doubt may present an economic issue for any country.

An alteration in the environmental temperature of a habitat may change the behaviour and haematological processes of fish (Mali and Chavan, 2014). Temperature therefore, is an indispensable and variable ecological issue affecting all living actions which brings about direct outstanding variations on haematological characteristics of organisms that live in water. It is equally acknowledged that temperature seriously affects reproduction, effective growth, immunological efficiency and activities of enzyme in fish (Tanck *et al.,* 2000).

Considering the serous effect of climate change on fish and fish culture, this study is intended to provide information on the effect of slight temperature changes on *Clarias gariepinus.* It will illuminate the sensitivity of *Clarias gariepinus* to temperature alterations in the light of climate change. The research is undertaken to determine the effect of environmental regulated temperature variations on the haematology of *Clarias gariepinus* fingerlings fed housefly maggot diet.

Materials and methods

Preparation of experimental diets

One experimental diet, with protein content of 41.97% dry matter, was prepared. Housefly maggot meal (50%) was used as the major dietary protein source in the diet. Other ingredients that were used in the formulation include soya bean meal (34%), maize (14.5%), fish oil (0.25%), Goundnut oil (0.25%) and vitamin premix (1%). The diets were pelleted using pelleting machine and the pellets were sundried. Magmeal was produced according to the description of Ogunji *et al*. (2008).

Vitamin premix supplying for each kilogram of food at 5 kg/tone had following

addition: 20,000 i.u, 30 mg Manganese, 4 mg Copper, 40 mg Zinc, 0.2 mg Selenium, 100 mg Lysine, 100 mg Methionine, 100 mg Anti-oxidant, Vitamin A, 2000 i.u, Vit. D3, 200 mg Vit E, 8 mg Vit K3, 20 mg Vit B1, 30 mg Vit B2, 12 mg Vit B6, 50 mg Pantothenic acid, 0.8 mg Biotin, 2.0 mg Cobalt, 40 mg Iron, 5.0 mg lodine, 150 mg Niacin, 0.05 mg Vit B12, 4.0 mg Folic acid, 500 mg Vit C, 600 mg Choline chloride, 200 mg Inositol, 200 mg Betaine.

Experimental fish

A total of ninety (90) *Clarias gariepinus* fingerlings (initial average weight 4.33 ± 0.03 g) were acclimatized for seven days. They were weighed and distributed among nine experimental tanks at a rate of ten fish per aquarium tank with 20 litres of water. Test diets were randomly assigned using completely randomized design to triplicate tanks located in laboratory, outdoor and greenhouse. The fish were fed a restricted ration of 5% body weight per day in two portions (by 9.00 hrs and 15.00 hrs) for 56 days in static water. Quantity of feed was adjusted forth nightly after batch-weighing of experimental fish. The aquaria were cleaned and water partly replaced by siphoning every three days to avoid fouling. Water temperature, dissolved oxygen, pH, nitrate, nitrite and ammonia were checked daily using mercury in glass bulb thermometer (Fresh Innovative Multitech, NIFFRI and water testing kit (Nice Chemicals India) respectively in the six treatment tanks. Water temperature in the experimental tanks was measured three times daily in the morning (6.00 hrs), afternoon (14.00 hrs) and evening (18.00 hrs).

Biochemical and haematological analysis

Blood samples were collected at the commencement and at the end of the experiment from the caudal vein into an EDTA litium tubes. The blood was analyzed to determine the packed cell value (PCV) with microhaematocrit using heparnized capillary tube (25 mm). Red blood cell (RBC) and white blood cell (WBC) counts were determined as described by Blaxhal and Diasley (1973). Hemoglobin (Hb) concentration was determined by the methods described by Wedemeyer and Yasutake (1977). Other haematological indices like mean cell haemoglobin (MCH), mean cell volume (MCV) and mean cell haemoglobin concentration (MCHC) were determined using the formula put forward by Dacie and Lewis (2001) thus:

MCH (pg) = [Hb (g dl^{-1}) x 10]/ RBC ($10^6 \mu l^{-1}$)
MCV (fl) = Hct/RBC (10^6 μl^{-1})
MCHC (g l^{-1}) = (Hb (g dl^{-1}) x 10]/Hct x 100

Protein (N x 6.25) was determined by the Kjeltec System (Tecator) and crude fat by Soxtec System HT (Tecator) using petroleum ether. Ash was determined by burning in a muffle furnace at 550°C for 10 hrs. Gross energy was calculated using the following factors: crude protein = 23.9 kJ/g, crude lipids = 39.8 kJ/g and NFE = 17.6 kJ/g (Schulz *et al*., 2005).

Statistical and growth analysis

At the end of the experiment, all the fish was weighed and data obtained from triplicate tanks were used to calculate weight gains, specific growth rate (SGR), feed conversion ratio (FCR) and percentage body weight.

Weight gain = final weight - initial weight,

$SGR = (LnW_2 - LnW_1) / (T_2 - T_1)100$
Where, W_1 and W_2 = initial and final weight of fish and T_1 and T_2 = time in days.

$$FCR = \frac{Feed\ \ fed}{live\ \ weight\ gain}$$

Protein efficiency ratio (PER) = live weight gain (g) / protein fed (g)
Where,
F1 = number of fish at the end of experiment,
F2 = number of fish at the beginning of experiment.

All growth and heamatological data were subjected to one way analysis of variance (ANOVA). The significance of difference between means was determined by Duncan's Multiple Range test ($p < 0.05$) using SPSS for windows (version 21). Values were expressed as means ± SE.

Results

Fish mortality was observed in the greenhouse treatment when water temperature reached 40°C. All the experimental fish died on the 8^{th} day of experimental exposure. Therefore there was no fish in this particular treatment for subsequent and final sample collection at the end of the experiment. Nevertheless, there was no significant mortality in the remaining treatments.

The mean value and standard error (SE) of all water quality parameters for each treatment group are summarized (Table 1). Temperature values differed significantly ($P < 0.05$) in the

three locations but did not differ significantly in the varying water levels (volumes). Highest temperature value was recorded in greenhouse tanks (MMG20).

Table 1. Water quality parameters in experimental tanks.

Parameter	MML20[1]	MMO20[2]	MMG20[3]
Temp (°C)	26.53±0.01[b]	26.06±0.01[a]	31.52±0.00[c]
DO (mg/l)	5.17±0.14[a]	5.33±0.17[a]	4.65±0.57[a]
pH	6.71±0.12[a]	6.72±0.12[a]	7.16±0.17[a]
Nitrate (mg/l)	1.96±0.05[a]	1.96±0.05[a]	1.62±1.11[a]
Nitrite (mg/l)	0.02±0.01[a]	0.02±0.01[a]	0.00±0.00[a]
Ammonia (mg/l)	2.56±0.72[a]	2.37±0.14[a]	3.00±0.00[a]

[1]MML20 (Laboratory Tank 20L Treatment), [2]MMO20 (Outdoor Tank 20L Treatment), [3]MMG 20 (Greenhouse Tank 20L Treatment)

Dissolved oxygen, pH, nitrate, nitrite and ammonia were observed not to be significantly different ($P < 0.05$) in all the treatment groups.

Proximate nutrient compositions of feed stuffs and experimental diet used in this trial are presented (Tables 2-3). The dietary crude protein composition was 41.97% while dietary crude fat was 3.56%.

Table 2. Proximate composition of maggot meal and soybean meal used for diet formulation.

Proximate components (%)	Maggot meal (MGM)	Soybean meal (SBM)
Crude protein	44.87	43.78
Crude fat	7.38	3.67
Crude fibre	6.88	6.48
Crude Ash	7.95	5.96
Moisture content	7.35	5.63
NFE	25.57	34.48

Table 3. Proximate composition (%) of the experimenttal diet.

Parameter	Diet
Dry Matter	91.95
Crude protein	41.97
Crude fat	3.56
Crude fibre	2.54
Crude Ash	7.69
Moisture content	8.05
NFE	36.19

The Food Conversion Ratio (FCR), Standard Growth Rate (SGR) and other growth performance parameters of *Clarias gariepinus* fingerlings fed experimental diet are presented (Table 4). At the ending of the experimentation, the fingerlings increased from an initial mean body weight of 4.33 g to represent a final mean body weight 13.10 g. Highest mean weight gain MWG and SGR were observed in *Clarias gariepinus* reared in laboratory (MML20), followed by fish in outdoor (MMO20). However, mean weight gain was highest in treatment laboratory (MML20). PER shows no significant difference between laboratory (MML20) and outdoor (MMO20).

Table 4. Growth performance of *Clarias gariepinus* fingerlings fed experimental diet*

Parameters	MML20	MMO20
Initial Weight (g)	4.34±0.26[a]	4.33±0.03[a]
Final Weight (g)	13.10±1.47[c]	11.17±1.93[c]
Weight Gain (g)	8.76±1.21[c]	6.83±1.94[c]
FCR[1]	1.61±0.10[b]	1.67±0.73[b]
SGR[2]	1.96±0.10[c]	1.64±0.30[c]
PER[3]	0.21±0.03[c]	0.16±0.05[c]

*All values are mean of triplicate feeding groups and values in the same row with different superscripts are significantly different ($P < 0.05$); [1]Food conversion ratio = food fed (g) / live weight gain (g); [2]Specific growth rate (% / d) = (InW2 - InW1 / T2 - T1) × 100; [3]Protein efficiency ratio = live weight gain (g) / protein fed (g),

The body proximate composition of *Clarias gariepinus* fed experimental diet is presented (Table 5). At all phases of the experiment, it was noticed that the dietary protein increased in all the treatments when compared with the initial status. However, fish in outdoor tank (MMO20) accumulated more body protein and was significantly different ($P < 0.05$) from other treatments. Crude ash, crude fibre, crude fat and moisture were significantly decreased ($P < 0.05$) from the initial status. Values of greenhouse tanks (MMG20) are not shown because the experimental fish in the treatment died before the end of experiment.

Table 5. Initial and final carcass composition of *C. gariepinus* fingerlings fed experimental diet (%)*

Components (%)	Initial status	MML20	MMO20
Crude protein	58.97±0.00[a]	63.97±0.06[b]	71.28±0.00[e]
Crude fat	8.25±0.01[d]	5.87±0.06[a]	6.89±0.00[c]
Crude ash	12.49±0.01[d]	7.58±0.06[a]	9.07±0.00[b]
Moisture	8.4±0.01[e]	6.68±0.06[c]	5.25±0.00[a]
NFE[1]	11.71±0.01[c]	15.90±0.14[d]	7.51±0.00[a]

*All values are mean of triplicate feeding groups and values in the same row with different superscripts are significantly different ($P<0.05$);[1]Nitrogen free extract + fibre, (NFE) = 100 - (% protein + % fat + % ash).

Haematological indices of *Clarias gariepinus* fingerlings fed experimental diet and exposed to varying temperatures are presented

(Table 6). The packed cell volume results showed that the initial PCV count was significantly decreased ($P < 0.05$) in all treatments. The results obtained for haemoglobin Hb showed that the initial status was significantly different in all the treatments. However, other treatments were significantly decreased ($P < 0.05$) from the initial status. With regards to WBC, all the treatment groups did not differ significantly. RBC values in all treatment groups significantly decreased ($P < 0.05$) from the initial status.

Table 6. Haematological Indices of *Clarias gariepinus* fingerlings fed experimental diet and exposed to varying temperatures*

Parameters	Initial	MML20	MMO20
PCV (%)	26.00 ±0.58^{b}	20.00 ±1.54^{a}	20.00 ±0.58^{a}
Hb (g /100 ml)	8.70 ±0.58^{a}	6.60 ±0.23^{a}	6.60 ±0.17^{a}
WBC (10^3) mm^{-3}	78.00 ±57.74^{ab}	57.00 ±230.94^{a}	58.00 ±173.20^{a}
RBC (10^3) mm^{-3}	6.40 ±0.12^{a}	3.80 ±0.17^{a}	6.90 ±0.14^{a}
MCHC (%)	30.56 ±3.13^{a}	33.09 ±0.76^{a}	33.01 ±0.09^{a}
MCH (pg)	7.36 ±0.18^{a}	17.93 ±0.71^{c}	9.58 ±0.41^{b}
MCV (fl)	40.62 ±0.17^{b}	52.57 ±0.65^{c}	29.00 ±1.34^{a}

*All values are mean of triplicate feeding groups and values in the same row with different superscripts are significantly different ($P < 0.05$) PCV, Packed cell volume; WBC, white blood cell; RBC, red blood cell; Hb, haemoglobin; MCHC, mean corpuscular haemoglobin concentration; MCH, mean corpuscular haemoglobin; MCV, mean corpuscular volume.

Discussion

Temperature of water based ecosystem is critical for guaranteeing the existence, production and adequate metabolic activities in fish. Inability to get used to temperature variations may result in fish mortality (Singh *et al.,* 2013). In this experiment all the experimental fish in the greenhouse died after 8 days of exposure. This happened around 1400 hrs when the temperature in the water reached 40°C. According to Cnaani (2006) temperature beyond optimum limits negatively impacts the wellbeing of fish as a result of metabolic strain which ultimately hinders immunity levels, reproductive capacity and growth. The normal range of temperature tolerance in the tropics to which fish are adapted is 22-35ºC (Howerton, 2001). This concurs with the work of Adeyemo *et al.* (2003) who found out that during temperature extremes *Clarias gariepinus* fails to adapt and will be unable to respond physiologically. This results in mortality which is caused by changes in the metabolic pathways and collapse in osmoregulatory functions.

One of the most critical abiotic factors impacting oxygen levels in water based ecosystems is the temperature of water. There is an inverse relationship between temperature increase and oxygen level. The solubility of oxygen decreases as water temperature increases (NOAA, 2008). The result of the present study shows that mean temperature values differed significantly in all the treatment groups. MML20 and MMO20 recorded mean temperatures of 26.53 ± 0.01°C and 26.06 ± 0.01°C respectively. Kausar and Salim (2006) established that the most favourable temperature for superlative growth of European catfish *silirus glanis* was between 25°C and 28°C. Most excellent outcomes were recorded at 27°C.

Greenhouse treatment recorded the highest value 31.52 ± 0.00 (Table 1). This can be attributed to the realities that for the period of sunlight, all the energy from the sunrays are taken up by greenhouse covering. Some of the energy is reflected outwards while a larger percentage is soaked up and conveyed within the greenhouse rooftops and walls (Ani-sabwa *et al.,* 2014). A huge quantity of this conveyed energy is taken up by water thereby helping to raise water temperature within the greenhouse. Once water is extremely warmed, vapour, oxygen and a large amount of energy is emitted into the atmosphere. The water is then highly concentrated with carbon dioxide thereby extremely increasing its acid content.

Dissolved oxygen and pH values for the treatment groups were within the range suitable for rearing fish (Dienye and Olumuji, 2014). Research has proved that fish feeding, growth and reproduction is enhanced at DO level of 5mg/l and above. Fish will feed poorly and starve at low concentration of DO that is below 3 mg/l (NAERLS, 1996). NAERLS (1996) further posits that pH is a key chemical factor to contemplate because it influences metabolic activities and other body processes of aquacultural animals. An acceptable pH range (6.8-8.7) ought to be sustained for satisfactory growth efficiency and production capacity.

Fish growth performance was good as evidenced by an upsurge in body mass and growth of the experimental fishes. By the end of

the experimentation, the fishes developed from an original body mass of 4.33 g to a finishing average body weight of 13.10 g. Growth performance of *Clarias gariepinus* was highest in laboratory treatment tanks (MML 20) having final mean weight of 13.10 ± 1.47 g whereas outdoor treatment tank 20 (MMO 20) was 11.17 ± 1.93 g (Table 4).

Feed conversion ratio (FCR) is a significant indicator of the excellence of fish feed. A lower FCR indicate improved use of the fish feed (Mungo-Bundi *et al.,* 2013). In this experiment, FCR presented no significant difference in all the treatments. However, the best FCR (1.61 ± 0.73) was observed in fish reared in laboratory tank (MML20) which had mean temperature of 26.53 ± 0.01°C. This was followed by (1.67 ± 0.10) for fish reared in outdoor tank (MMO20) with mean temperature of 26.06 ± 0.01°C. These outcomes are in agreement with the results of Kausar and Salim (2006). They reported that *Labeo rohita*, fingerlings reared at a temperature range of 24-26°C registered improvement in FCR. However, FCR values of less than 1 have been documented even though 1.2 - 1.5 is the normal range for fish reared with properly compounded diet (Ogunji *et al.,* 2008). The low FCR of 1.61 - 1.67 exhibited by fish in all the treatment groups is an indicator that the fish utilized the feed well.

The proximate composition of carcass in this study indicated that all experimental groups recorded higher levels of crude protein than the initial value. This shows that the magmeal experimental diet was well accepted and utilised by *Clarias gariepinus*. Several authors have reported same previously (Ogunji *et al.,* 2007; Idowu and Afolayan, 2013).

Study of haematological parameters is important for the diagnoses of health condition of fish under different stress situations (Adhikari *et al*., 2004). The constituents of blood are very susceptible to temperature and if there is a variation physiologically, it will be manifested in the standards of some blood characteristics (Ramesh *et al.,* 2009). This might influence the normal body process of procreation and overall amount of fish stock. Variation in the blood parameters of catfish as result of stresses brought about by changes in the temperature of water have been investigated by several persons (Adhikari *et al*., 2004; Mali and Chavan, 2014).

Every single one of the blood characteristics determined in this experiment fell within the authorized body limits documented for *Clarias gariepinus*. Haematocrit (PCV) limits 20 - 26% recorded in this investigation are within the limits of 20 - 50% documented by Dienye and Olumuji (2014). However, values above 50% are rarely reported (Etim *et al.,* 1999). A decrease in PCV values were observed in all treatments of this study, respect to initial status. A decrease in the amount of the PCV in the blood normally is an indication that there is a shortfall in the expected level of red blood cells. Causes of low haematocrit include anemia, trauma, and damage of red blood cells or decreased production of red blood cells (Oyawoye and Ogunkunle, 1998). A rise in temperature brings about a great reduction in the ability of blood to carry oxygen and this causes an upsurge in red blood cells in the blood (Holt and Jorgerson, 2015).

The haemoglobin result showed a decrease from the initial value. The haemoglobin (Hb) range 5.30 - 8.70 g/100ml (Table 6) recorded in this study is similar to 8.7 g/100ml for *Clarias gariepinus* as reported by Sowunmi, (2003). These records were as well more than 4.46 g/100ml as reported by Fagbenro *et al.* (2000) for *Heterotis niloticus*. In this study however, the decrease in haemoglobin from the initial value does not constitute any problem since it is within the required standards.

Also, a decrease in the erythrocytes (RBC) was detected in the treatment groups except outdoor tank. RBC for MMO20 (outdoor tank) was higher than the initial value. It is acknowledged that a low quality and small amounts of red blood cells as well as low level of haemoglobin leads to a decline in the availability of oxygen in the body. Apart from transportation of oxygen, red blood cells perform other important functions in the body and inadequate amounts and quality of red blood cells would have a chain of consequences on metabolic activities other than just the provision of oxygen for respiration (Gross *et al.,* 1996).

Conclusion

Findings from this study suggest that temperature changes affected the growth and body composition of *Clarias gariepinus.* Fish reared in MML20 (laboratory tank) did better than other treatments. Haematological characteristics of all treatment groups responded negatively to different temperature exposures. This is evidenc-

ed by the mortality witnessed in the greenhouse treatment and low blood characteristics values obtained from the experiment.

Nevertheless, the experimental fish showed good results in growth performance and body composition. The mean weight gain MWG, FCR, SGR and PER showed significant difference between the treatment groups. However, fish reared under laboratory ambient temperature conditions performed better than others.

Water temperature is known to influence the haematological parameters of fish. When blood parameters are affected negatively, it further impinges on growth presentation and overall wellbeing of fishes. The result of this investigation demonstrates that 25 - 28°C is a suitable temperature range that is appropriate for growth and wellbeing of *Clarias gariepinus*. However temperature exceeding 40°C can be very lethal to the fish. Finally, more detailed research is needed to authenticate this result.

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