

Effects of thermal acclimation and ecological background on the physiological responses of juvenile *Oreochromis esculentus* (Graham, 1928). Implications for the species adaptive capacity to global warming

Jane Kalule Yawe Nagayi¹  | John Joseph Kisakye¹ | Matthew Tenywa Mwanja² | Juliet Nattabi¹ | Alfonse Opiyo³

¹Makerere University, Kampala, Uganda

²Kajjansi Aquaculture Research and Development Centre, Jinja, Uganda

³Gulu University, Gulu, Uganda

Correspondence

Jane Kalule Yawe Nagayi, Makerere University, Kampala, Uganda.
Email: jnagayi@yahoo.ca

Funding information

The research was funded by the Swedish International Development Agency (SIDA) under Makerere University SIDA Grant Contribution No: 5180060 towards PhD studies Jane.

Abstract

Climate change is a recognized global concern in the 21st century. Water temperature elevation is likely to affect fish physiological and ethological function, causing great economic losses for fisheries resources and aquaculture production. Fish are particularly vulnerable to climate change as they are unable to control their body temperatures with changes in environmental temperature. This study assessed the effects of thermal acclimation in three juvenile populations of *Oreochromis esculentus* on the critical thermal maximum CT_{max} , cortisol, biochemical and haematological responses to acute temperature increase ($0.3^{\circ}Cmin^{-1}$). Fish from Senya farm, Lake Kacheera and Lake Nabisojjo were acclimated at $25^{\circ}C$, $28^{\circ}C$, $31^{\circ}C$ and $34^{\circ}C$ for 21 days at Kajjansi aquaculture research and development centre KARDC. The CT_{max} and blood cell counts increased, while plasma cortisol, glucose and proteins decreased at higher acclimation temperatures. Two-way ANOVA showed significant differences among treatments in CT_{max} , cortisol, proteins and erythrocytes indices, but not in glucose and leucocytes. However, significant variations among populations were only showed in CT_{max} . Overall, these results suggest that *O. esculentus* can respond to higher climate change induced temperatures, when allowed time for acclimation. Since temperature adaptation not only depends on the parameters assessed in this study, we recommend further investigations into metabolic rate and digestive enzymatic activity.

KEYWORDS

cortisol, haematological response, *Oreochromis esculentus*, thermal acclimation

1 | INTRODUCTION

Climatic variability and change present a major global challenge on the aquatic ectotherms (Burel et al., 1996) such as fish. Fish are highly vulnerable to the impacts of climatic variations because their body metabolism is mainly dependent on the environmental temperature (Fry, 1971), exacerbated by the blood water counter-current

flow in their gills during gaseous exchange (Beitinger et al., 2000; Schmidt-Nielsen, 1997). Hence, the predicted global warming may profoundly affect fish physiology and reduce their fitness (Crozier & Hutchings, 2014; Ficke et al., 2007). Like all organisms, fish lie within a limited thermal range ($CT_{min}-CT_{max}$) of body temperature due to optimized structural and kinetic coordination of molecular, cellular and systemic processes, which likely evolved to be as narrow as

possible to reduce energy costs (Pörtner & Farrell, 2008). The critical thermal minimum (CT_{min}) and critical thermal maximum (CT_{max}) illustrate the lower and upper limits of the environmental temperature within which the organisms exhibit normal life activities (Nyboer & Chapman, 2017) and are commonly used as indicators of thermal biology (Peng et al., 2014).

In order for fish to survive with global warming, they must migrate to cooler habitats or respond through phenotypic plasticity or genetic change (Rosset & Oertli, 2011). However, the tropical inland freshwater ecosystems are fragmented; therefore, fish relocation to cooler higher latitudes may not be possible (Walters et al., 2012); hence, the alternative is adaptation, lest they become extinct (Nyboer & Chapman, 2017; Rosset & Oertli, 2011). Furthermore, it is reported that since the tropical species evolved in warm environments with very narrow diel and seasonal thermal gradients, they may already be living close to their optimal or even above the optimum thermal range (Nyboer & Chapman, 2017; Somerao, 2010; Tewksbury et al., 2008) and may have limited performance change and narrow thermal tolerance range ($CT_{max} - CT_{min}$) as well (Huey et al., 2012). Moreover, their habitats already suffered intense interruptions due to anthropogenic disturbances (Cowx & Collares-Pereira, 2002). For example, pollution, habitat alteration, flow obstructions such as dams and water management schemes, introduction of alien species, over-exploitation, contaminants and nutrient additions (Schindler, 2001) have had serious negative impacts on the freshwater organisms. Already close to twenty per cent (20%) of the freshwater taxa are threatened, endangered or extinct (Cowx & Collares-Pereira, 2002; Revenga et al., 2000). Therefore, any additional interactions like the increasing global temperature may easily damage them completely, leading to serious ecological and economic consequences given the fact that the tropical freshwater species contribute significantly (almost 40%) to the overall global diversity of fish (Arthington et al., 2003). Furthermore, the freshwater fisheries are the main source of livelihood and animal proteins especially for the rural poor communities in the mostly low-income landlocked countries (FAO, 2009). Therefore, it is urgently required that the freshwater biodiversity be protected against the impacts of global warming and the predicted extreme events (Abell et al., 2002; Meehl & Tebaldi, 2004; Perkins et al., 2012).

Unfortunately, there is limited information concerning the impacts of increased temperature on the tropical freshwater fish species (Comte et al., 2013) to guide the formulation of more inclusive regulations, conservation actions and management interventions (Cooke et al., 2013). Most research has been biased towards temperate marine species and species of high economic or recreational value (Comte et al., 2013). Moreover, it is increasingly evident that adaptability and the extent of vulnerability to the effects of climatic warming vary greatly among species and among populations of the same species with regard to their physiological sensitivity, geographical location and evolutionary background (Deutsch et al., 2008; Diamond et al., 2018; FAO, 2012; Huey et al., 2012; Hulme et al., 2010; Portner, 2001). Therefore, more species-specific researches comparing species populations, especially for the tropical

freshwater fish species where information is least available, are required for predicting climate change effects on the organisms' phenotypic plasticity to inform climate change action.

Therefore, the two natural populations from a drier L. Kyoga basin and wetter L. Victoria basin were compared against a farmed population.

To date, most environmental adaptability and stress assessments for both marine and freshwater species have involved haematological comparisons mainly because blood constituents respond rapidly to any factor that affects homeostasis and get altered long before there is any physical mark of the condition (Adams, 1990; Fazio et al., 2018; Gabriel et al., 2011). For instance, the European sea bass *Dicentrarchus labrax* and Gilthead sea bream *Sparus aurata* (Fazio et al., 2018), the great sturgeon *Huso huso* (Zarejabad et al., 2009), the red spotted grouper *Epinephelus akaara* (Cho et al., 2015) and Nile tilapia *Oreochromis niloticus* (Barcellos et al., 1999; Delaney et al., 2005; Panase et al., 2018) have been studied using haematological assessments. Researchers conveniently categorized the homeostatic alterations as follows: Primary stage which involves neuroendocrine responses such as catecholamine release and activation of the corticotropin-interrenal axis. Secondary stage which involves physiological responses such as haematological, osmoregulatory, blood enzymatic and metabolic changes. Finally, the tertiary stage emanating from long-term exposure to source of stress which may lead to decreased growth and fecundity increased susceptibility to pathogens and behavioural changes (Kalamarz-Kubiak, 2018; Kindle & Whitmore, 1986; McCormick, 2001; Pankhurst, 2001). According to Kindle and Whitmore (1986), prolonged homeostatic perturbation may result into acclimation whereby an organism's tolerance to environmental changes is either reduced or enhanced. Acclimation allows the individual to compensate for the direct thermodynamic effects on metabolism and glucose utilization (Johnston & Dunn, 1987; Schulte et al., 2011). Hence, the attribute of thermal acclimation capacity is progressively being considered as a key feature in determining species' resilience to climate change (Franklin & Seebacher, 2009; Somero, 2010; Stillman, 2003), mainly monitored through haematological parameters. The species displaying the highest acclimation capacity are believed to have greater energetic and competitive advantage (Sandblom et al., 2014) over others.

This study hypothesized that thermal acclimation and species population significantly affect fish stress responses. The purpose of this study was to assess the effects of thermal acclimation and species population on physiological responses of juvenile *Oreochromis esculentus*. The main aim was to compare environmental responses among three populations of the species which we thought may differ due variations in their ecological background. Two are capture fish populations with differing ecological backgrounds that is one being from a drier L. Kyoga basin and another from wetter L. Victoria basin and a third domesticated population which has existed in captivity for three generations experiencing human influence in feeding and handling stress. We selected the juvenile stage because this is the most plastic and therefore can reveal the most dependable species' thermal tolerance limits and physiological processes

(Pörtner & Farrell, 2008). A wide range of stress responses to critical temperature increase in relation to thermal acclimation and species population were assessed. They ranged from the primary (cortisol), secondary (plasma glucose and proteins, plus whole blood cell components and indices) to tertiary (critical thermal maximum CT_{max}) acclimated at different temperature regimes. The range of temperatures selected in these trials was based on the average lake water temperatures of 25°C in the Lake Victoria basin (Witte & Van Densen, 1995) and the predicted range of temperature rise due to global warming of 1–7°C (IPCC, 2007).

Selection of *Oreochromis esculentus* as a subject for this study was based on the fragility of its existing populations despite the species' socio-economic importance due to its distinct preference among the attendant communities for its superior flesh (Graham, 1929; Mwanja et al., 2017; Trewavas, 1983). *O. esculentus* is a tropical freshwater tilapiine fish species and only endemic to the Victoria and Kyoga Lake basins (Graham, 1929; Ogutu-Ohwayo, 1993) located in Uganda, Kenya and Tanzania (East Africa). Although the species was plentiful throughout the first half of the 20th Century and it exclusively supported the commercial fishery of Lake Victoria (Ogutu-Ohwayo, 1993), it is currently non-existent in the main L. Victoria and L. Kyoga (Nagayi et al., 2006; Ogutu-Ohwayo, 1993) and it is critically endangered (IUCN, 2020). However, the surviving populations are seemingly vulnerable to the negative impacts of climatic warming given that their refugia habitats, the satellite lakes, are very small and shallow. Shallow lakes tend to heat fast due to their low heat storage capacity (Oswald & Rouse, 2004). Therefore, conservation action for the surviving populations of *O. esculentus* should be considered as critical with due consideration of the effects of climate change which this study aims to generate.

2 | MATERIALS AND METHODS

2.1 | Collection and acclimation of *Oreochromis esculentus*

Juveniles of *Oreochromis esculentus* were collected from Lake Kacheera (Lake Victoria basin), Lake Nabisojjo (Lake Kyoga basin) and a fish farm in Rakai District (Figure 1). The farmed population was a composite mixture from lakes Mburo, Kijanebalola and Kacheera and were the third generation. The fish were captured using boat seines and selected based on the maturity stage and closeness in size, since most ecological and physiological factors are size dependent in fish (Akinrotimi et al., 2010).

The choice of the fish sources was based on the fact that environmental adaptability may differ among populations of the same species (Diamond et al., 2018). Therefore, the two natural populations from a drier L. Kyoga basin and wetter L. Victoria basin were compared against a farmed population.

The fish were transported to Kajjansi Aquaculture Research and Development Center KARDC in a 500-L circular water tank, supplied with pure oxygen. They were kept in separate outdoor concrete

tanks (6 × 4 × 1 m), with a natural photo-period and partial natural food production to minimize fish stress ensuring that the Uganda National Council for Science and Technology UNCST's guidelines for the care and use of laboratory animals were followed.

The fish were left for a minimum of 14 days, which is presumably long enough to permit natural healing from capture and transportation injuries and acquainting with the artificial feed supplement.

2.2 | Acclimation experimental procedure

A temperature acclimation experiment was set up indoors, in 300 l circular plastic tanks. The fish were reared at different temperatures including a control ($C=25^{\circ}\text{C}$) and three treatments ($T_1 = 28 \times C$, $T_2 = 31 \times C$ and $T_3 = 34^{\circ}\text{C}$) for a total of 21 days, long enough to elicit adequate physiological responses in aquatic trials (Nyboer & Chapman, 2017). All the treatments and the control were replicated into sets of three tanks, thus adding up to a total of 12 tanks.

Each experimental tank was stocked with five (5) fish from each of the three fish populations to keep an optimum stock density that would minimize stress and support proper water quality management. The experiment was done twice in order to have enough specimens for the CT_{max} , cortisol and other haematological tests. For easy identification and follow-up, individuals of a given population were tagged with a visible implant elastomer tag of a unique colour. In order to minimize additional stress in the biological clock, the fish were subjected to an almost natural photo-period of ~12-h light and 12-h dark. The fish, ranging 11.4 cm to 16.9 cm total length, were fed to satiation, twice a day (8 am and 5 pm) on floating pellets of 1.5 mm (28% crude protein) to match their low protein herbivorous feeding in the wild, which takes place almost throughout the day but with peaks at dawn and dusk near the water surface.

Temperature in the experimental tanks was maintained using thermostat (W1209®) controlled immersion water heaters (within 0.5°C above or below). In order to maintain the desired water quality, experimental tanks were cleaned twice daily (morning and evening) mainly after feeding, by siphoning out feed remainders and solid wastes before ensuring a 10% water replacement. This was done in conjunction with continuous water aeration to support a high oxygen supply near to saturation and water quality standard. Any dead fish were removed immediately.

Water parameters were monitored daily (dissolved oxygen DO, Electrical Conductivity EC and pH) using a multi-probe water meter (H19146-Hana instruments®) and bi-weekly (ammonia and nitrite) according to APHA (1985) to ensure uniformity. It was important because haematological values of fish can be affected by extrinsic and intrinsic factors such as age, weight, sex, food, pathogens and water quality parameters including water temperature, salinity, oxygen availability and pH (Sandblom et al., 2014). The results of DO (4.48–7.8 mg/L), EC (180–250 µS/cm) and pH (7.1–7.8), ammonia (0.7–1.0 mg/L) and nitrite (0.0 mg/L) did not show significant differences among the treatments and were within acceptable range for fish rearing.

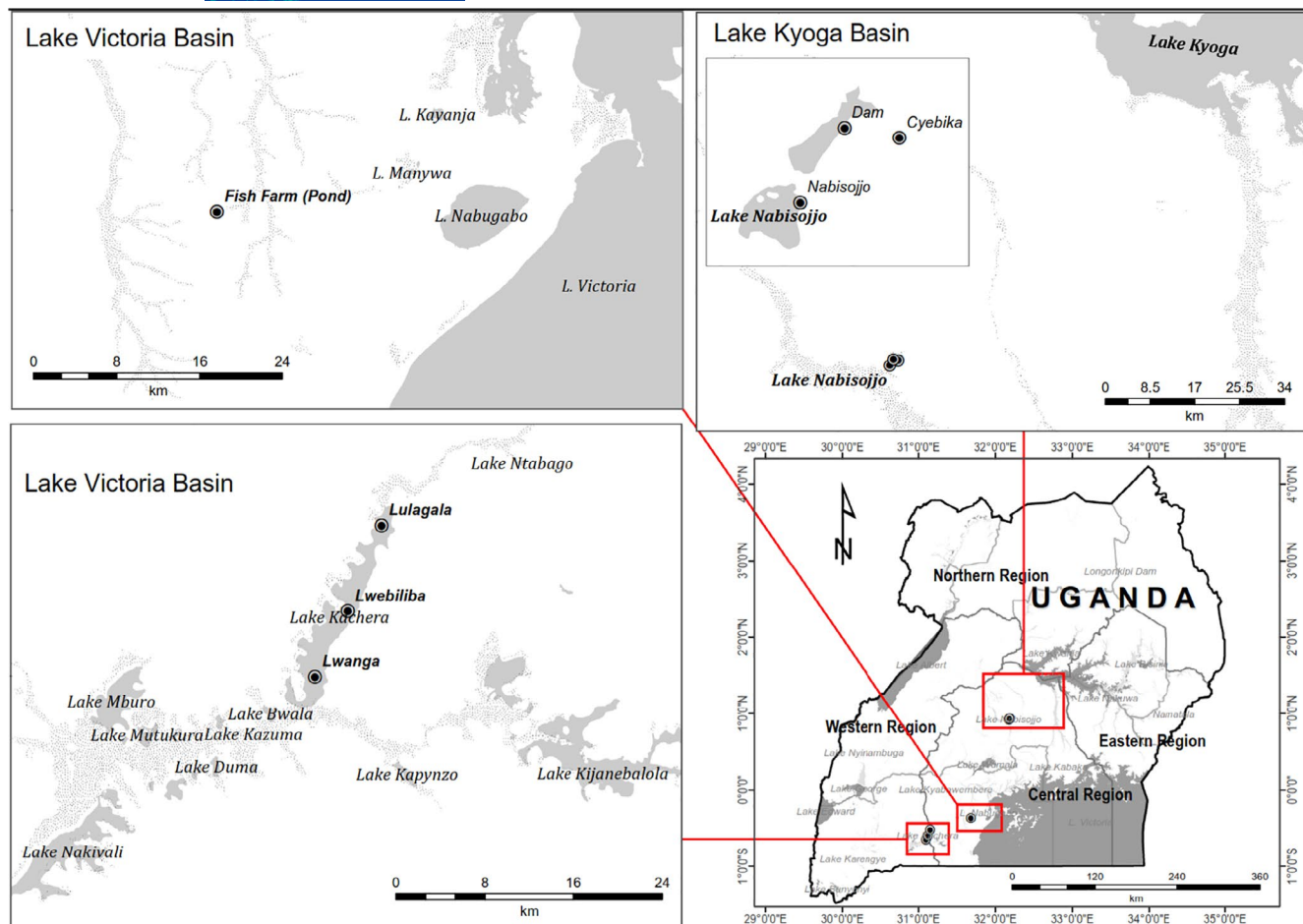


FIGURE 1 Location map of lakes and farm from where the experimental fish were collected in Uganda.

2.3 | Determination of the critical thermal maximum (CT_{max})

At the end of the acclimation period, fish were randomly selected from the same temperature treatment and quickly transferred to a thermos tank, ten (10) hours before the onset of the experiment, to recover from handling stress. A higher voltage water heater was used to increase the water temperature at a rate of $0.3^{\circ}\text{C min}^{-1}$ which is recognized as low enough to allow the fish body temperature to change, but fast enough to inhibit acclimation during the trial (Beitinger et al., 2000). The temperature at which the fish lost swimming stability for approximately five (5) seconds (Nyboer & Chapman, 2017) was recorded as the CT_{max} , and the fish were transferred to a recovery water tank with continuous aeration. We recorded almost 100% recovery rate.

2.4 | Determination of plasma cortisol

The fish used in the CT_{max} experimentation were allowed approximately one hour of rest before use in the cortisol tests. According to Kebus et al. (1992) and Kalamarz-Kubiak (2018), cortisol secretion

reaches the peak at least one hour after exposure to acute stress in most fish species. The same was reported in salmonids and in tilapia (Barcellos et al., 1999; Delaney et al., 2005).

The fish were anaesthetized with 40 ppm clove oil. Blood samples (1.0 ml) were collected from the individual fish by puncturing their caudal veins using a two-millilitre syringe rinsed with ethylenediaminetetraacetic acid (EDTA) to slow coagulation. The samples were transferred to heparin free Eppendorf tubes from which serum was extracted after centrifugation at $5400 \times g$ for 10 min and preserved in separate Eppendorf tubes at -20°C for cortisol analysis. The concentration of cortisol was tested by enzyme-linked immunosorbent assay according to Zarejabad et al. (2009).

2.5 | Determination of serum glucose and protein and haemogram profile

A second batch of acclimated fish were anaesthetized with 40 ppm clove oil. Blood samples (1.8 ml) were collected from the individual fish by puncturing their caudal veins using a two-millilitre syringe rinsed with ethylenediaminetetraacetic Acid

(EDTA) to slow coagulation. The blood was divided into two aliquots. The first aliquot was transferred to heparin free Eppendorf tubes and left to coagulate and the plasma used in glucose and protein analysis. The second aliquot was transferred to heparinized Eppendorf tubes to be used for whole blood cell determination. All samples were immediately transferred to Ebenezer Medical Laboratory limited for analysis. Serum glucose and serum protein were analysed by an analytical chemistry analyser (P400 and PC400, HORIBA, Japan).

2.6 | Haemogram profile determination

Using the second aliquot, the assessment and classification of the blood cellular components was done following the standard haematological methods described by Blaxhall and Daisley (1973) and Brown (1993). The red blood cell parameters, haemoglobin (Hb), packed cell volume (PCV) and platelets (PLT), were determined.

The data were used to calculate the mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentrations (MCHC) and mean corpuscular volume (MCV) based on Javed et al. (2016) as shown in Equations (1), (2) and (3) below respectively.

$$\text{MCH (pg)} = \frac{\text{Hb (g/dL)} \times 10}{\text{TEC} (10^6 \text{mm}^3)} \quad (1)$$

$$\text{MCHC (g/dL)} = \frac{\text{Hb (g/dL)} \times 100}{\text{PCV} (\%)} \quad (2)$$

$$\text{MCV} (\mu\text{m}^3) = \frac{\text{PCV} (\%) \times 10}{\text{TEC} (10^6 \text{mm}^3)} \quad (3)$$

To estimate the differential leukocyte counts, blood smears were air-dried, fixed in methanol and stained using May-Giemsa solution before being classified into lymphocytes, neutrophils, monocytes and eosinophils according to Blaxhall and Daisley (1973).

2.7 | Data analysis

Data obtained were corrected for normality and homogeneity of variance before analysis. All statistical analyses were carried out in R studio using R software. The study parameters were subjected to a two-way analysis of variance, ANOVA, to determine significant differences among the acclimation temperatures in the three fish populations and the interaction between the main factors. To investigate more into the differences among all groups, Tukey's tests were performed. Statistical tests were considered significant at more than 95%, that is $p < 0.05$. All results are expressed as mean $\pm$ standard deviation.

3 | RESULTS

Our results show significant effect of both species population (CT_{max}) and acclimation temperature (CT_{max} , cortisol, serum glucose, serum protein, Hct, PVC, MCH, MCHC and PLT) on the physiological responses to thermal stress in juvenile *O. esculentus*. However, there was significant interaction between species population and acclimation temperature in the CT_{max} and MCV (Table 1).

3.1 | Critical thermal maximum (CT_{max})

A total of 123 fish were used in the assessment of CT_{max} (Table 2).

We observed an average increment in CT_{max} ($^{\circ}\text{C}$) of 0.87°C for every 3°C rise in acclimation temperature. The lowest increment in CT_{max} (0.65°C) was recorded between 25 and 28°C acclimation temperatures, and the highest (1.15°C) recorded between 28 and 31°C , with a subsequent decline (0.82°C) CT at higher acclimation temperature, $31\text{--}34^{\circ}\text{C}$.

There was also a significant interaction between the effects of acclimation temperature and ecological background/population on CT_{max} [$F(3, 111) = 2.217, p = 0.0465$]. For instance, *L. Nabisojjo*, which recorded the lowest CT_{max} (40.56°C) at the lowest acclimation temperature (25°C), attained the highest CT_{max} (43.38°C) at the highest acclimation temperature 34°C compared with other populations. Conversely, *L. Kacheera* recorded the highest CT_{max} (40.72°C) at 25°C acclimation temperature, and its CT_{max} was the lowest (43.21°C) at 34°C among the three populations. However, the farm population with a medium CT_{max} record (40.63°C) at the lowest acclimation temperature of 25°C recorded the highest CT_{max} level (41.51°C) at 28°C in comparison with the other two populations and finally attained medium CT_{max} (42.48 and 43.23°C) at 31°C and 34°C acclimation temperatures respectively (Figure 2). Tukey's post hoc tests showed that all species populations and all treatments/acclimation temperatures were significantly different from one another.

4 | CORTISOL LEVELS

A total of 79 fish were assessed for serum cortisol level of which 25 (31.65%) were farmed, 28 (35.44%) *L. Kacheera* and 26 (32.91%) from *L. Nabisojjo*. Cortisol production was inversely correlated with acclimation temperature. That is, cortisol level was highest in the control group (25°C) and lowest in fish acclimated at 34°C . Although the level of cortisol was clearly highest in *L. Kacheera* and lowest in *L. Nabisojjo*, a two-way ANOVA test of cortisol secretion showed that the effect of population was not significant, but acclimation temperature had a significant effect on cortisol production [$F(3,67) = 4.839, p < 0.00416$]. There was no significant interaction between acclimation temperature and fish population on the cortisol level. Pairwise comparisons using Tukey's HSD test indicated that

TABLE 1 Summary of the two-way ANOVA test results for the various physiological parameters. Stars indicate significant effect ($p < 0.05$)

Parameter	Variable	F value	p value
CTmax (°C)	Population	36.881	5.23e-13***
	Treatment	489.531	<2e-16***
	Interaction	2.217	0.0465*
Cortisol (nmol/l)	Population	1.572	0.21524
	Treatment	4.839	0.00416**
	Interaction	0.827	0.55280
Serum glucose (g/dl)	Population	0.491	0.613
	Treatment	1.899	0.134
	Interaction	1.085	0.375
Serum protein (g/dl)	Population	0.128	0.880
	Treatment	8.925	2.63e-05***
	Interaction	1.500	0.186
Red blood cell total count (10^6cm^{-1})	Population	0.562	0.572
	Treatment	2.030	0.114
	Interaction	1.196	0.314
Haemoglobin (g/dl)	Population	1.879	0.158
	Treatment	0.474	0.701
	Interaction	1.700	0.128
Hct (%)	Population	1.227	0.297
	Treatment	9.620	1.11e-05***
	Interaction	1.606	0.152
PCV (%)	Population	2.740	0.069052
	Treatment	6.908	0.000268***
	Interaction	1.809	0.103956
MCV (fl)	Population	1.779	0.1738
	Treatment	1.322	0.2711
	Interaction	2.451	0.0292*
MCH (pg)	Population	0.128	0.880
	Treatment	7.805	9.17e-05***
	Interaction	0.547	0.771
MCHC (%)	Population	0.280	0.757
	Treatment	12.081	7.03e-07***
	Interaction	12.081	0.466
PLT (10^2cm^{-1})	Population	1.513	0.225
	Treatment	8.360	4.77e-05***
	Interaction	0.441	0.850
White Blood Cell total count (cm^3)	Population	1.677	0.192
	Treatment	0.701	0.553
	Interaction	0.444	0.848
Neutrophils (%)	Population	2.094	0.128
	Treatment	0.391	0.759
	Interaction	1.065	0.388
Lymphocytes (%)	Population	1.394	0.253
	Treatment	0.221	0.881
	Interaction	0.760	0.603

TABLE 1 (Continued)

Parameter	Variable	F value	p value
Monocytes (%)	Population	0.036	0.9649
	Treatment	1.226	0.3040
	Interaction	2.009	0.0705
Eosinophils (%)	Population	0.183	0.833
	Treatment	0.152	0.928
	Interaction	0.385	0.888

TABLE 2 Total number of fish used in CT_{max} assessments

Treatment	Farmed	Kacheera	Nabisojjo	Total
25	10	24	7	41
28	10	9	10	29
31	9	9	10	28
34	7	8	10	25
Total	36	50	37	123

the mean cortisol level at 34°C (254 ± 146) was significantly different from 25°C (488.20 ± 404) and 28°C (431.12 ± 186) but did not differ significantly between other pairs. (Figure 3).

5 | BIOCHEMICAL PARAMETERS

A total of 114 fish were assessed for serum protein level of which 37 (32.46%) were farmed, 37 (32.46%) L. Kacheera and 40 (35.08%) from L. Nabisojjo, while for glucose assessment a total of 131 fish, 42 (32.06%) farmed, 44 (33.59%) L. Kacheera and 45 (34.35%) L. Nabisojjo, were used. Both glucose and protein productions were highest in the control group (25°C), but declined at 28°C to the lowest level at 31°C, then rose again at 34°C. Two-way ANOVA of the glucose showed no significant differences among populations and acclimation temperatures, but the highest and lowest levels of glucose were recorded in farmed and L. Nabisojjo fish populations respectively.

Results of serum protein were analysed using a two-way ANOVA, and they showed that the differences among the populations were not significantly different. However, there was a significant effect of the acclimation temperature on the serum protein [$F(3,102) = 8.925$ $p < 2.63e-05$]. The analysis showed no interaction in the effects of acclimation temperature and fish population on the serum protein level. Pairwise comparisons using Tukey's HSD test indicated that the mean serum protein in the control group, 25°C (5.79 ± 1.05), was significantly different from 28°C (5.01 ± 0.61), 31°C (4.61 ± 0.84) and 34°C (4.87 ± 1.08) but did not differ significantly between other pairs. (Figure 4).

6 | THE HAEMOGRAM PROFILE

For the assessment of the whole blood cell profile, 150 fish were selected. However, some of the samples clotted leaving 120 fish for

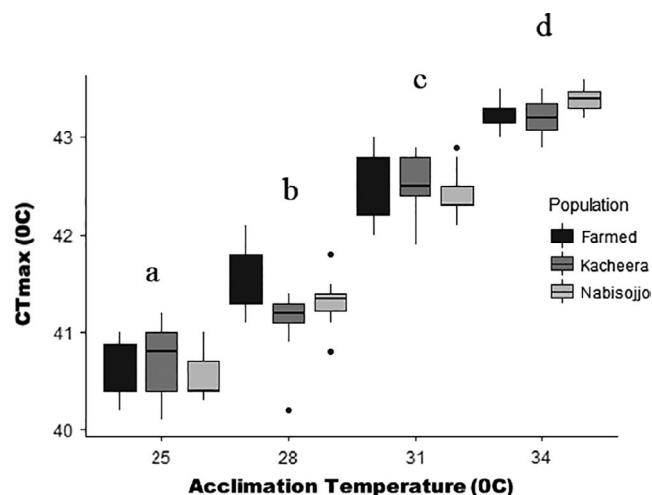


FIGURE 2 Comparing CT_{max} in three populations of *O. esculentus* acclimated at different temperatures. Different lowercase letters are ranked significantly different using Tukey's HSD tests ($p < 0.05$)

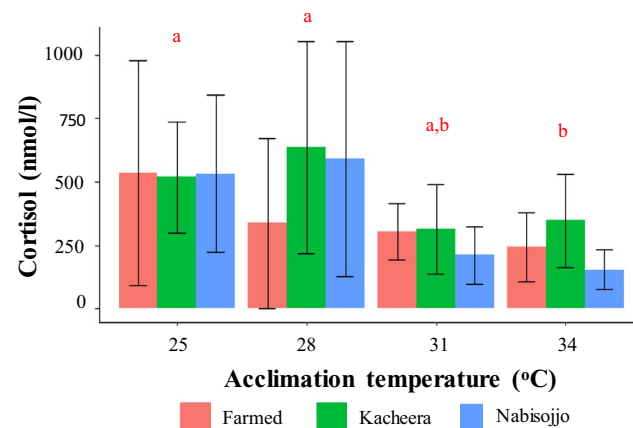


FIGURE 3 Cortisol production in juvenile *O. esculentus* in the three species populations acclimated at different temperatures. The results are expressed as mean $\pm$ SEM. Means with different lowercase letters in red font are ranked significantly different using Tukey's HSD tests ($p < 0.05$)

the haemogram profiling, comprising of farmed = 42 fish (34.17%), L. Kacheera = 40 fish (34.17%) and L. Nabisojjo = 38 fish (31.67%). Although there were observed variations in the haematological composition among the fish populations, none of them were statistically

significant based on the two-way ANOVA. Moreover, there was no significant interaction effect between the acclimation temperature and fish population on any of the tested haematological parameters. Hence, the table of results is only showing comparisons among acclimation temperatures.

The lowest total count of the white blood cells (leucocyte) was 42.91 ± 9.35 at 34°C , while the highest was 43.46 ± 8.26 at 31°C . The lowest percentage of neutrophils was 46.26 ± 0.57 at 28°C , and the highest was 47.00 ± 0.68 at acclimation temperature

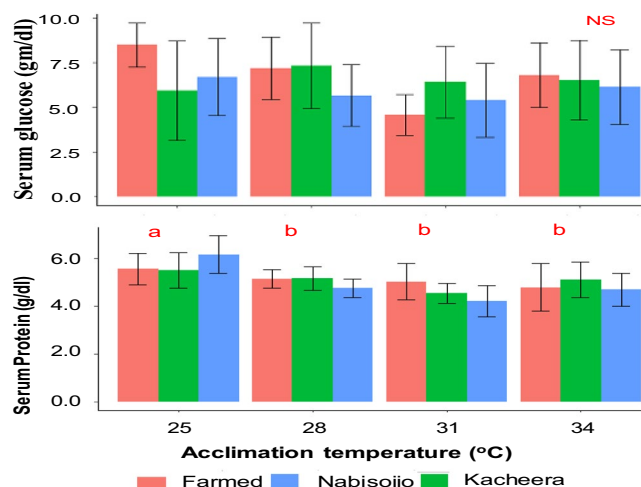


FIGURE 4 Serum glucose and protein production in juvenile *O. esculentus* acclimated at different temperatures. The graph compares mean $\pm$ SEM of three populations. Means with different lowercase letters in red font are ranked significantly different using Tukey's HSD tests, while NS means no significant difference ($p < 0.05$)

34°C . The lowest percentage of lymphocytes was 47.47 ± 0.53 at 34°C , and the highest was 47.91 ± 0.51 at 31°C . The lowest percentage of monocytes was 4.59 ± 0.21 at 34°C , and the highest was 5.11 ± 0.22 at 28°C . The lowest percentage of the eosinophils was 0.85 ± 0.13 at 25°C , and the highest was 0.96 ± 0.16 at 28°C (Table 1).

The highest levels of the total white blood cell (leucocyte) count ($44.83 \pm 1.199\%$) were at 25°C in *L. Nabisojjo* fish and lowest ($39.88 \pm 2.91\%$) at acclimation temperature 34°C in farmed fish population. However, a two-way analysis of variance, ANOVA, for all the leucocytes did not show a significant difference among the acclimation temperatures (Table 3).

Results show that the total red blood cell/erythrocyte count RBC was lowest 1.26 ± 0.06 ($\times 10^6/\text{cm}$) in the control group (25°C) and highest ($1.79 \pm 0.31 \times 10^6/\text{cm}$) at 28°C . Although the results of the erythrocyte total count, RBC, were plainly highest in *L. Kacheera* population, statistical test using a two-way ANOVA indicated no significant differences in RBC among populations and the acclimation temperatures.

Components of the erythrocytes Hct, MCV, PCV and Hb were positively correlated with acclimation temperature. The lowest levels were recorded at 25°C , but their peaks varied. While the MCH, MCHC and PLT were negatively correlated with acclimation temperature, the highest levels were recorded at 25°C and the lowest varied for each.

A two-way ANOVA indicated significant differences in Hct, PCV, MCH, MCHC and PLT levels at different acclimation temperatures but not among the species populations. However, the effect of acclimation temperatures on the levels of the different components of the erythrocytes did not significantly interact with the effect of

Parameter	Treatments/Acclimation temperature ($^{\circ}\text{C}$)			
	25 (n=28)	28 (n=26)	31 (n=34)	34 (n=32)
WBC total (cm^3)	42.91 ± 9.35	43.00 ± 8.95	43.46 ± 8.26	41.49 ± 13.07
Neutrophils (%)	46.85 ± 0.54	46.26 ± 0.57	46.50 ± 0.54	47.00 ± 0.68
Lymphocytes (%)	47.52 ± 0.55	47.78 ± 0.53	47.91 ± 0.51	47.47 ± 0.53
Monocytes (%)	4.96 ± 0.20	5.11 ± 0.22	4.76 ± 0.19	4.59 ± 0.21
Eosinophils (%)	0.85 ± 0.13	0.96 ± 0.16	0.94 ± 0.13	0.88 ± 0.13
RBC ($10^6/\text{cm}^3$)	1.26 ± 0.06	1.79 ± 0.31	1.58 ± 0.06	1.58 ± 0.06
PCV (%)	19.44 ± 1.00^a	22.58 ± 1.28^a	25.30 ± 1.26^b	25.37 ± 0.94^c
MCV (fl)	153.49 ± 7.44	152.76 ± 7.66	167.70 ± 5.20	158.67 ± 3.38
MCH (pg)	75.16 ± 3.16^a	66.33 ± 3.23^a	60.35 ± 1.60^b	59.55 ± 2.27^c
MCHC (%)	49.40 ± 2.16^a	44.46 ± 1.82^b	37.21 ± 1.55^c	38.05 ± 1.08^d
PLT ($10^2/\text{cm}^3$)	5.62 ± 0.58^a	4.38 ± 0.69^a	2.61 ± 0.40^b	2.54 ± 0.37^c
Hb (g/dl)	9.10 ± 0.29	9.45 ± 0.33	9.38 ± 0.24	9.57 ± 0.29
Hct (%)	19.25 ± 1.00^a	22.22 ± 1.29^b	26.23 ± 1.09^c	25.49 ± 0.92^d

Notes: The results are expressed as mean $\pm$ standard errors; sample size in brackets. RBC, red blood cell total count; PCV, packed cell volume; MCV, mean corpuscular volume; MCH, mean corpuscular haemoglobin; MCHC, mean corpuscular haemoglobin concentration; PLT, Platelets; Hb, haemoglobin; Hct, Haematocrit. Values in the same row having different small letters (a, b, c and d) are significantly different ($p < 0.05$, Tukey's HSD test).

TABLE 3 Effect of temperature on the leucocytes and erythrocytes components in juvenile *O. esculentus*

fish background/population. Tukey's HSD tests were carried out to determine which pairs of the acclimation temperatures were significantly different and the results are shown in Table 1.

7 | DISCUSSIONS AND CONCLUSIONS

Water temperature is a strong abiotic factor influencing biology of fish (Beitinger & Fitzpatrick, 1979), especially due to the close contact between their bodies and the water. Therefore, many studies aimed at understanding the relationship between water temperature and animal physiology have used fish as a model animal (Crockett & Londraville, 2006). When faced with altered water temperatures away from optimum levels, fish shows altered physiological and ethological functions (Barton & Iwama, 1991), collectively known as stress (Zarejabad et al., 2009). They are governed by the neuroendocrine responses such as corticosteroid–catecholamine responses, metabolic changes, and haematological, osmoregulatory and immunological responses (Barton 2002; Mazeaud et al., 1977).

The results of this study showed significant increase in the CT_{max} with temperature and significant variation among the three populations of *Oreochromis esculentus*. Therefore, it is likely that juvenile *O. esculentus* can reach a higher CT_{max} if acclimated to higher temperatures. However, as indicated by McDonnell and Chapman (2015), this is only possible as long as temperatures lie within the species' critical thermal range. It is also important to note that the average level ($0.87^{\circ}C$) of CT_{max} rise in the present study was lower than the $3^{\circ}C$ increase in the acclimation temperature, indicating that thermal acclimation response does not keep pace with the absolute change in acclimation temperature. Our findings conform to the study of Nile perch *Lates niloticus* by Chrétien and Chapman, (2016), where CT_{max} was $1.5^{\circ}C$ higher in the group acclimated at higher temperature ($29.5^{\circ}C$) compared with the group acclimated at lower ($25.5^{\circ}C$). Similar adjustments in CT_{max} (1.2 – $1.7^{\circ}C$) were recorded by Lapointe et al. (2018) for every $4^{\circ}C$ increase in acclimation temperature in six fish species including *Oreochromis niloticus* and *Labeo victorianus* from Uganda, *Brycon amazonicus* and *Colossoma macropomum* from Brazil and *Barbonymus gonionotus* and *Labeo pierrei* from Cambodia. Similarly, McDonnell and Chapman (2015) recorded significant CT_{max} increase with each increase in acclimation temperature in *Pseudocrenilabrus multicolor victoriae*. Therefore, the significant CT_{max} increase with each increase in acclimation temperature recorded in fish suggests that there is a degree of thermal compensation induced by short-term exposure to higher temperatures. However, the significant variations in CT_{max} among the populations may imply that there is genetic variation; hence, further studies for genetic assessments are required.

Another stress parameter cortisol serves as an indicator of physiological disturbances in fish (Applebaum et al., 2010). Cortisol levels in this study decreased at higher temperatures compared with the control group. This may indicate that the fish were adapting to the thermal stress. Our results conform to those in channel catfish *Ictalurus punctatus* by Strange (1980), where plasma resting

cortisol levels also decreased with increasing acclimation temperature. Another study by Zarejabad et al. (2009) in juvenile great sturgeon (*Huso huso* L.) also reported decreased cortisol at higher temperatures. This may suggest that long-term exposure to climatic warming may lead to better adaptation to the predicted heat waves. However, our results differed from the expected trend of higher temperatures resulting into higher cortisol levels as was reported in the closely related species, *O. niloticus* (Delaney et al., 2005; Panase et al., 2018) and in the red hybrid tilapia (Musa et al., 2017) as well as in brook trout (Chadwick et al., 2015). Since stress is an energy demanding process, it requires the animal to mobilize energy substrates to supply tissues such as the brain, gills and muscles, in order to cope with the increased energy demand (Vijayan et al., 1997). The plasma glucose results in this study were highest in the control group and lowest at $31^{\circ}C$ which may suggest that it is the temperature at which the fish were stressed the most. Similar results were reported in *Ictalurus melas* where the glucose concentration was lowest in summer ($29^{\circ}C$, 39.7 mol/L), higher in spring/autumn ($24^{\circ}C$, 42.1 mol/L) and highest in winter ($18^{\circ}C$, 46.5 mol/L) (Ottolenghi et al., 1995) and in *Huso huso*, where highest glucose (62 mg/dl) was recorded at lower temperature (15 – $20^{\circ}C$) compared with (58 mg/dl) glucose recorded at higher temperature (21 – $26^{\circ}C$) (Zaejabad et al., 2009). It implies that as metabolism increases with temperature, glucose reserves in the blood reduces, and vice versa. However, the high glucose at low temperature may indicate retarded metabolism and could be an index of sub-lethal stress (Connors et al., 1978). Similarly, the reduction in serum protein level from the control to the minimum at $31^{\circ}C$ and rising again at $34^{\circ}C$ implies cellular restoration and protection as well as increased stressor tolerance (Wendelaar Bonga, 1997).

Finally, the haemogram profile, in particular the leucocyte counts, is known to denote stimulation of the cellular immune system (Tavares-Dias & Moraes, 2007). Their values are used to guide the postulation of the health state of the species in relation to escalating temperatures and guide in the prophylactic management of infections. Our results of the leucocytes components did not differ significantly at different acclimation temperatures and among species populations showing that they were temperature-independent and thus indicative of the species' high adaptive capacity. Additionally, the RBC and Hb were almost similar at all acclimation temperatures and could be attributed to the high dissolved oxygen concentration maintained at almost saturation by use of aerators. However, the higher Hct at higher acclimation temperatures which indicates increased percentage of red blood cells is deemed as a compensation for the decreased amount of haemoglobin per blood volume which is indicated by lower MCHC at higher acclimation temperatures, implying that increased acclimation temperature caused further improvement in oxygen transportation mechanism. Therefore, we accept the null hypothesis that thermal acclimation and species population significantly affect fish stress responses.

In conclusion, results of this study indicate that CT_{max} increased and cortisol level decreased at higher acclimation temperatures while the leucocyte counts were temperature-independent; therefore,

Oreochromis esculentus responds better to higher temperatures after acclimation. Therefore, the species possesses the capacity to survive under the predicted global warming.

ETHICS APPROVAL STATEMENT

The authors confirm that the ethical policies of the journal, as noted on the journal's author guidelines page, have been adhered to and the appropriate ethical review committee approval has been received from the Uganda National Council for Science and Technology, UNCST Ref No. NS103ES. All the UNCST's guidelines for the Care and Use of Laboratory Animals were followed.

ACKNOWLEDGEMENTS

We are grateful to the Swedish International Development Agency (SIDA) and Makerere University under SIDA Grant Contribution No: 5180060 for funding the PhD studies for the first author. The data were collected during the PhD study entitled Physiological response of *Oreochromis esculentus* (Graham, 1929) to different temperature regimes. The authors are also indebted to the management of Gulu University, the employing University of the first author for granting her study leave during her PhD studies at the Department of Zoology, Entomology and Fisheries Sciences, School of Biological Sciences, College of Natural Sciences, Makerere University, Uganda. We are also thankful to the management and staff of Kajjansi Aquaculture Research and Development Centre KARDC where the experiment was conducted, for their support, advice and assistance, special appreciations go to Michael for his tireless assistance in the laboratory set up and maintenance, Ms. Ganda E., Mr. Kityo the farm manager and to Mr. Okello. We are highly indebted to the director and staff of Ebenezer Medical Laboratory limited for the skilful guidance and assistance in sample analysis.

CONFLICT OF INTEREST

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could cause any competing interests.

AUTHOR CONTRIBUTIONS

Jane, Matthew, Joseph and Juliet conceptualized the study and designed the methodology. Jane, Matthew and Alfonse validated the study and edited the draft. Jane involved in data analysis, writing original draft and funding acquisition. Matthew and Joseph supervised the study.

DATA AVAILABILITY STATEMENT

The data used in the development of the manuscript entitled "Effects of thermal acclimation and ecological background on the physiological responses of juvenile *Oreochromis esculentus* (Graham, 1928). Implications for the species adaptive capacity to global warming" are uploaded by Nagayi, Jane on Mendeley Data, V1, <https://doi.org/10.17632/h9s85rsfw9.1> can be reached at <http://dx.doi.org/10.17632/h9s85rsfw9.1>

ORCID

Jane Kalule Yawe Nagayi  <https://orcid.org/0000-0003-1000-4374>

REFERENCES

- Abell, R., Thieme, M., & Lehner, B. (2002). *Ecoregion conservation for freshwater systems, with a focus on large rivers*. World Wildlife Fund.
- Adams, S. M. (1990). *Biological Indicators of stress in Fish* (pp. 1–8). American Fisheries Society.
- Akinrotimi, O. A., Uedeme-Naa, B., & Agokei, E. O. (2010). Effects of acclimation on haematological parameters of *Tilapia guineensis* (Bleeker, 1862). *Science World Journal*, 5(4), 1–4.
- APHA (1985). *Standards Methods for the Examination of Water and Waste Waters*, 16th ed. (p. 360). American Public Health Association.
- Applebaum, S. L., Wilson, C. A., Holt, G. J., & Nunez, B. S. (2010). The onset of cortisol synthesis and the stress response is independent of changes in CYP11B or CYP21 mRNA levels in larval red drum (*Sciaenops ocellatus*). *General and Comparative Endocrinology*, 165, 269–276.
- Arthington, A. H., Lorenzen, K., Pusey, B. J., Abell, R., Halls, A., Winemiller, K. O., Arrington, D. A., & Baran, E. (2003). River fisheries: ecological basis for management and conservation. In *Proceedings of the 2nd International Large Rivers Symposium*, FAO.
- Barcellos, L. J. G., Nicolaiewsky, S., de Souza, S. M. G., & Lulhier, F. (1999). Plasmatic levels of cortisol in the response to acute stress in Nile tilapia, *Oreochromis niloticus* (L.), previously exposed to chronic stress. *Aquaculture Research*, 30, 437–444. <https://doi.org/10.1046/j.1365-2109.1999.00348.x>
- Barton, A. B., & Iwama, K. G. (1991). Physiological changes in fish from stress in aquaculture with emphasis on the response and effects of corticosteroids. *Annual Review of Fish Diseases*, 3–26. [https://doi.org/10.1016/0959-8030\(91\)90019-G](https://doi.org/10.1016/0959-8030(91)90019-G).
- Beitinger, T. L., Bennett, W. A., & McCauley, R. W. (2000). Temperature tolerances of North American freshwater fishes exposed to dynamic changes in temperature. *Environmental Biology of Fishes*, 58, 237–275.
- Beitinger, T. L., & Fitzpatrick, L. C. (1979). Physiological and ecological correlates of preferred temperature in fish. *American Zoologist*, 19, 319–329. <https://doi.org/10.1093/icb/19.1.319>
- Blaxhall, P. C., & Daisley, K. W. (1973). Routine hematological methods for use with fish blood. *Journal of Fish Biology*, 5, 771–781.
- Brown, B. A. (1993). *Haematology: principles and procedures* (p. 405). Williams and Wilkins.
- Burel, C., Ruvet, J. P., Gaumet, F., Roux, A. L., Sévère, A., & Bouf, G. (1996). Effects of temperature on growth and metabolism in juvenile turbot. *Journal of Fish Biology*, 49, 678–692. <https://doi.org/10.1111/j.1095-8649.1996.tb00064.x>
- Chadwick, J. G., Nislow, K. H., & McCormick, S. D. (2015). Thermal onset of cellular and endocrine stress responses corresponds to ecological limits in brook trout, an iconic cold-water fish. *Conservation and Physiology*, 3, cov017.
- Cho, H. C., Kim, J. E., Kim, H. B., & Baek, H. J. (2015). Effects of water temperature change on the hematological responses and plasma cortisol levels in growing of red spotted grouper, *Epinephelus akaara*. *Development & Reproduction*, 19(1), 19–24. <https://doi.org/10.12717/DR.2015.19.1.019>
- Chrétien, E., & Chapman, L. J. (2016). Tropical fish in a warming world: thermal tolerance of Nile perch *Lates niloticus* (L.) in Lake Nabugabo, Uganda. *Conservation Physiology*, 4, 13. <https://doi.org/10.1093/conphys/cow062>
- Comte, L., Buisson, L. T., Daufresne, M., & Grenouillet, G. L. (2013). Climate-induced changes in the distribution of freshwater fish: observed and predicted trends. Special review. *Freshwater Biology*, 58, 625–639. <https://doi.org/10.1111/fwb.12081>

- Connors, T. T., Schneider, M. J., Genoway, R. G., & Barraclough, S. A. (1978). Effect of acclimation temperature on plasma levels of glucose and lactate in rainbow trout, *Salmo gairdneri*. *Journal of Experimental Zoology*, 206, 443–449. <https://doi.org/10.1002/jez.1402060313>.
- Cooke, S. J., Sack, L., Franklin, C. E., Farrell, A. P., Beardall, J., Wikelski, M., & Chown, S. L. (2013). What is conservation physiology? Perspectives on an increasingly integrated and essential science. *Conserv. Physiol.*, 1(1), cot001. <https://doi.org/10.1093/conphys/cot001>
- Cowx, I. G., & Collares-Pereira, M. J. (2002). *Conservation of Freshwater Fishes: Options for the Future Freshwater Fish Conservation: Options for the Future*. *csa.com* (pp. 443–452).
- Crockett, E. L., & Londraville, R. L. (2006). Temperature. In *The physiology of fishes*, 3rd ed. (pp. 231–269). CRC Press.
- Crozier, L. G., & Hutchings, J. A. (2014). Plastic and evolutionary responses to climate change in fish. *Evolutionary Applications*, 7, 68–87. <https://doi.org/10.1111/eva.12135>.
- Delaney, M. A., Klesius, P. H., & Shelby, R. A. (2005). Cortisol response in Nile tilapia *Oreochromis niloticus* (L.) to temperature changes. *Journal of Applied Aquaculture*, 16(3), 95–104. https://doi.org/10.1300/J028v16n03_06
- Deutsch, C. A., Tewksbury, J. J., Huey, R. B., Sheldon, K. S., Ghalambor, C. K., Haak, D. C., & Martin, P. R. (2008). Impacts of climate warming on terrestrial ectotherms across latitude. *Proceedings of the National Academy of Sciences of the United States of America*, 105, 6668–6672. <https://doi.org/10.1073/pnas.0709472105>
- Diamond, S. E., Chick, L. D., Perez, A., Strickler, S. A., & Martin, R. A. (2018). Evolution of thermal tolerance and its fitness consequences: parallel and non-parallel responses to urban heat islands across three cities. *Proceedings of the Royal Society B*, 285, 20180036. <https://doi.org/10.1098/rspb.2018.0036>
- FAO (2009). *Climate change implications for fisheries and aquaculture. Overview of current scientific knowledge* (p. 530). Technical paper.
- FAO (2012). *The State of World Fisheries and Aquaculture*. FAO.
- Fazio, F., Ferrantelli, V., Piccione, G., Saoca, C., Levanti, M., & Mucciardi, M. (2018). Biochemical and hematological parameters in European sea bass (*Dicentrarchus labrax* Linnaeus, 1758) and Gilthead sea bream (*Sparus aurata* Linnaeus, 1758) in relation to temperature. *Veterinarski Arhiv*, 88(3), 397–411. <https://doi.org/10.24099/vet.arhiv.170406c>
- Ficke, A. A., Myrick, C. A., & Hansen, L. J. (2007). Potential impacts of global climate change on freshwater fisheries. *Reviews in Fish Biology and Fisheries*, 17, 581–613. <https://doi.org/10.1007/s11160-007-9059-5>
- Franklin, C. E., & Seebacher, F. (2009). Adapting to climate change. *Science*, 323, 876–877. <https://doi.org/10.1126/science.323.5916.876b>
- Fry, F. E. J. (1971). The effect of environmental factors on the physiology of fish. In W. S. Hoar, & D. J. Randall (Eds.), *Fish physiology*, Vol. 6 (pp. 1–98). Academic Press.
- Gabriel, U. U., Akinrotimi, O. A., & Esemokumo, F. (2011). Haematological responses of wild Nile Tilapia *Oreochromis niloticus* after acclimation to captivity. *Jordan Journal of Biological Sciences*, 4(4), 225–230.
- Graham, M. (1929). *The Victoria Nyanza, and its Fisheries. A Report on the Fishing Survey of Lake Victoria 1927–1928, and Appendices* (p. 255). Crown Agents for Colonies.
- Huey, R. B., Kearney, M. R., Krockenberger, A., Holtum, J. A. M., Jess, M., & Williams, S. E. (2012). Predicting organismal vulnerability to climate warming: Roles of behaviour, physiology and adaptation. *Philosophical Transactions of the Royal Society B: Biological Sciences*, 367, 1665–1679. <https://doi.org/10.1098/rstb.2012.0005>
- Hulme, M., Doherty, R., Ngara, T., New, M., & Lister, D. (2010). Africa climate change: 1900–2010. *Climate Research*, 17, 145–168.
- IPCC (2007). *Climate change 2007-synthesis report: the fourth assessment report of the intergovernmental panel on climate change*. IPCC. http://www.ipcc.ch/pdf/assessmentreport/ar4/syr/ar4_syr_cn.pdf
- IUCN (2020–2023). The IUCN Red List of Threatened species. Categories & Criteria (version 3.1). <https://www.iucnredlist.org/search?query=oreochromis%20esculentus&searchType=species>
- Javed, M., Ahmad, I., Ahmad, A., Usmani, N., & Ahmad, M. (2016). Studies on the alterations in haematological indices, micronuclei induction and pathological marker enzyme activities in *Channa punctatus* (spotted snakehead) perciformes, channidae exposed to thermal power plant effluent. *SpringerPlus*, 5(761), 9. <https://doi.org/10.1186/s40064-016-2478-9>.
- Johnston, I. A., & Dunn, J. (1987). Temperature acclimation and metabolism in ectotherms with particular reference to teleost fish. *Symposia of the Society for Experimental Biology*, 41, 67–93.
- Kalamarz-Kubiak, H. (2018). Cortisol in correlation with other indicators of fish welfare. Chapter 7. <https://doi.org/10.5772/intechopen.72392>
- Kebus, M. J., Collins, M. T., Brownfield, M. S., Amundson, C. H., Dayes, T. B., & Malison, J. A. (1992). Effects of rearing density on stress response and growth of rainbow trout. *Journal of Aquatic Animal Health*, 4, 1–6.
- Kindle, K. R., & Whitmore, D. H. (1986). Biochemical indicators of thermal stress in *Tilapia aurca* (Steindachner). *Journal of Fish Biology*, 29, 243–255.
- Lapointe, D., Cooperman, M. S., Chapman, L. J., Clark, T. D., Val, A. L., Ferreira, M. S., Balirwa, J. S., Mbabazi, D., Mwanja, M., Chhom, L., Hannah, L., Kaufman, L., Farrell, A. P., & Cooke, S. J. (2018). Predicted impacts of climate warming on aerobic performance and upper thermal tolerance of six tropical freshwater fishes spanning three continents. *Conservation Physiology*, 6(1), coy056. <https://doi.org/10.1093/conphys/coy056>
- Mazeaud, M. M., Mazeaud, F., & Donaldson, E. M. (1977). Primary and secondary effects of stress in fish. *Transactions of the American Fisheries Society*, 106, 201–212.
- McCormick, S. D. (2001). Endocrine control of osmoregulation in teleost fish. *American Zoologist*, 41, 781–794.
- McDonnell, L. H., & Chapman, L. J. (2015). At the edge of the thermal window: effects of elevated temperature on the resting metabolism, hypoxia tolerance and upper critical thermal limit of a widespread African cichlid. *Conservation Physiology*, 3, 13. <https://doi.org/10.1093/conphys/cov050>
- Meehl, G. A., & Tebaldi, C. (2004). More intense, more frequent, and longer lasting heat waves in the 21st century. *Science*, 305, 994–997. <https://doi.org/10.1126/science.1098704>
- Musa, N., Ramly, H. R., Manaf, M. T. A., Razzak, L. A., & Musa, N. (2017). High water temperature impairs physiological responses in red hybrid tilapia: effects on cortisol and its regulation. *AACL Bioflux*, 10(5), 1297–1308.
- Mwanja, M. T., Owani, S., Nadiope, E., Kwiri, N., Kudeeba, R., & Mwanja, W. W. (2017). Feeding regimes for Singida tilapia (*Oreochromis esculentus*, (Graham 1928) under controlled conditions: acceptance and utilization of natural feeds compared to dry rations. *International Journal of Fisheries and Aquatic Studies*, 5(2), 420–424.
- Nagayi, Y. K. J., Ogutu-Ohwayo, R., Kizito, Y. S., & Balirwa, J. S. (2006). Population characteristics of *Oreochromis esculentus* in the Victoria and Kyoga lake basins. Implications for conservation and improvement of the stocks. Blackwell Publishing Ltd. *African Journal of Ecology*, 44, 423–430.
- Nyboer, E. A., & Chapman, L. J. (2017). Elevated temperature and acclimation time affect metabolic performance in the heavily exploited Nile perch of Lake Victoria. *Journal of Experimental Biology*, 220, 3782–3793. <https://doi.org/10.1242/jeb.163022>
- Ogutu-Ohwayo, R. (1993). A paper on the effects of predation by Nile perch, *Lates niloticus* L. on the fish of Lake Nabugabo with suggestions for conservation of endangered endemic cichlids. *Conservation Biology*, 7, 701–711.
- Oswald, C. J., & Rouse, W. R. (2004). Thermal characteristics and energy balance of various-size Canadian Shield lakes in the Mackenzie

- River Basin. *Journal of Hydrometeorology*, 5, 129–144. [https://doi.org/10.1175/1525-7541\(2004\)005<0129:TCAEBO>2.0.CO;2](https://doi.org/10.1175/1525-7541(2004)005<0129:TCAEBO>2.0.CO;2).
- Ottolenghi, C., Puviani, A. C., Ricci, D., Brighenti, L., & Morsiani, E. (1995). The effect of high temperature on blood glucose level in two teleost fish *Ictalurus melas* and *Ictalurus punctatus*. *Comparative Biochemistry and Physiology*, 111A, 229–235. [https://doi.org/10.1016/0300-9629\(95\)00009-V](https://doi.org/10.1016/0300-9629(95)00009-V)
- Panase, P., Saenphet, S., Saenphet, K., Pathike, P., & Thainum, R. (2018). Biochemical and physiological responses of Nile tilapia (*Oreochromis niloticus* Linn.) subjected to rapid increases of water temperature. *Comparative Clinical Pathology*, <https://doi.org/10.1007/s00580-018-2872-5>
- Pankhurst, N. W. (2001). Stress inhibition of reproductive endocrine processes in a natural population of the spiny damselfish *Acanthochromis polyacanthus*. *Marine and Freshwater Res*, 52, 753–761.
- Peng, J., Cao, Z. D., & Fu, S. J. (2014). The effects of constant and diel-fluctuating temperature acclimation on the thermal tolerance, swimming capacity, specific dynamic action and growth performance of juvenile Chinese bream. *Comparative Biochemistry and Physiology & Part A*, 176, 32–40. <https://doi.org/10.1016/j.cbpa.2014.07.005>
- Perkins, S. E., Alexander, L. V., & Nairn, J. R. (2012). Increasing frequency, intensity and duration of observed global heatwaves and warm spells. *Geophysical Research Letters*, 39, L20714. <https://doi.org/10.1029/2012GL053361>
- Portner, H. O. (2001). Climate change and temperature dependent biogeography: oxygen limitation of thermal tolerance in animals. *Naturwissenschaften*, 88, 137–146. <https://doi.org/10.1007/s001140100216>
- Pörtner, H. O., & Farrell, A. P. (2008). Ecology. Physiology and climate change. *Science*, 322, 690–692.
- Revenga, C., Brunner, J., Henninger, N., Kassem, K., & Payne, R. (2000). *Pilot analysis of global ecosystems: freshwater systems*. World Resources Institute.
- Rosset, V., & Oertli, B. (2011). Freshwater biodiversity under climate warming pressure: identifying the winners and losers in temperate standing waterbodies. *Biological Conservation*, 144, 2311–2319. <https://doi.org/10.1016/j.biocon.2011.06.009>
- Sandblom, E., Gräns, A., Axelsson, M., & Seth, H. (2014). Temperature acclimation rate of aerobic scope and feeding metabolism in fishes: implications in a thermally extreme future. *Proceedings of the Royal Society B*, 2014(281), 20141490. <https://doi.org/10.1098/rspb.2014.1490>
- Schindler, D. W. (2001). The cumulative effects of climate warming and other human stresses on Canadian freshwaters in the new millennium. *Canadian Journal of Fisheries and Aquatic Science*, 58, 18–29.
- Schmidt-Nielsen, K. (1997). *Animal physiology adaptation and environment*, 5th ed. (pp. 13–16). Cambridge University Press.
- Schulte, P. M., Healy, T. M., & Fangue, N. A. (2011). Thermal performance curves, phenotypic plasticity, and the time scales of temperature exposure. *Integrative and Comparative Biology*, 51, 691–702. <https://doi.org/10.1093/icb/acr097>
- Somero, G. N. (2010). The physiology of climate change: how potentials for acclimatization and genetic adaptation will determine 'winners' and 'losers'. *Journal of Experimental Biology*, 213, 912–920. <https://doi.org/10.1242/jeb.037473>
- Stillman, J. H. (2003). Acclimation capacity underlies susceptibility to climate change. *Science*, 301(5629), 65. <https://doi.org/10.1126/science.1083073>
- Strange, R. J. (1980). Acclimation temperature influences cortisol and glucose concentrations in stressed channel catfish. *Transactions of the American Fisheries Society*, 109, 298–303. [https://doi.org/10.1577/1548-8659\(1980\)109<298:ATCAG>2.0.CO;2](https://doi.org/10.1577/1548-8659(1980)109<298:ATCAG>2.0.CO;2)
- Tavares-Dias, M., & Moraes, F. R. (2007). Haematological and biochemical reference intervals for farmed channel catfish. *Journal of Fish Biology*, 71, 383–388.
- Trewavas, E. (1983). *Tilapiine fishes of the genera Sarotherodon, Oreochromis and Danakilia* (p. 583). British Mus. Nat. Hist.
- Vijayan, M. M., Pereira, C., Grau, E. G., & Iwana, G. K. (1997). Metabolic responses associated with confinement stress in Tilapia: the role of cortisol. *Comparative Biochemistry and Physiology*, 116, 89–95.
- Walters, J. R., Blanckenhorn, W. U., & Berger, D. (2012). Forecasting extinction risk of ectotherms under climate warming: an evolutionary perspective. *Review of Functional Ecology*, 26, 1324–1338. <https://doi.org/10.1111/j.1365-2435.2012.02045.x>
- Wendelaar Bonga, S. E. (1997). The stress response in fish. *Physiological Reviews*, 77, 591–625.
- Witte, F., & Van Densen, W. L. T. (1995). *Fish stock and fisheries of Lake Victoria, a hand book for field observations*. Samara Publishing Limited.
- Zarejabad, A. M., Sudagar, M., Pouralimotlagh, S., & Bastami, K. D. (2009). Effects of rearing temperature on hematological and biochemical parameters of great sturgeon (*Huso huso* Linnaeus, 1758) juvenile. *Comparative Clinical Pathology*, <https://doi.org/10.1007/s00580-009-0880-1>

How to cite this article: Nagayi, J. K. Y., Kisakye, J. J., Mwanja, M. T., Nattabi, J., & Opio, A. (2022). Effects of thermal acclimation and ecological background on the physiological responses of juvenile *Oreochromis esculentus* (Graham, 1928). Implications for the species adaptive capacity to global warming. *Aquaculture Research*, 53, 989–1000. <https://doi.org/10.1111/are.15640>