Sokoine University of Agriculture

PhD Thesis

Analysis of Diurnal and Seasonal Dynamics of Water Quality Parameters and their Implications on Metabolism and Physiology of Nile Tilapia (*Oreochromis niloticus*) Cultured in Pond

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ANALYSIS OF DIURNAL AND SEASONAL DYNAMICS OF WATER QUALITY PARAMETERS AND THEIR IMPLICATIONS ON METABOLISM AND PHYSIOLOGY OF NILE TILAPIA (*Oreochromis niloticus*) CULTURED IN POND

A Thesis Submitted in Fulfilment of the Requirements for the Degree of Doctor of Philosophy of Sokoine University of Agriculture

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EXTENDED ABSTRACT

Aquaculture production of Nile tilapia (*Oreochromis niloticus*) has attracted considerable attention around the world because of their fastest growth rate, good quality meat, and fascinating physiological characteristic that allow them to adopt and strive in a broad range of production systems. Because of these characteristics, Nile tilapia has been adopted as the key aquaculture species in Tanzania. Nile tilapia production in Tanzania takes place primarily in earthen ponds, mostly under a semi-intensive system without the use of water treatment technology. In Tanzania more than 80% of the aquaculture produced fish comes from Nile tilapia raised ponds. The production of Nile tilapia in ponds has for long time been unsuccessful due to poor growth performance. This is because, unlike in other production systems such as Recirculating Aquaculture Systems (RAS), in small water bodies such as earthen ponds water quality parameters vary considerably on a daily basis, depending on the equilibrium reached with the regular physical and chemical characteristics of the surrounding environment. These variation are likely to considerablly effect physiology and welfare of the cultured fish. Unfortunatly, the extent of diurnal, and seasonal variation in water quality parameters in the production ponds across the country are not yet known. This makes it difficult for estimating the influence of changes in fish ponds water quality influences on physiology and performance of cultured Nile tilapia.

This study was carried out to determine the current status and dynamics of the diurnal, and seasonal fishpond water quality parameters in Tanzania. It was also carried out to assess the effects of the observed water quality variation on the physiology, metabolism and performance of the pond-cultured Nile tilapia. To achieve the study objectives, a field survey of fish onds and three laboratory experiments were performed. The diurnal and seasonal trends in pond water quality parameters were documented and the effect of changes of some selected water

quality parameters on the Nile tilapia physiological characteristics and performances were reported.

Study 1. A field survey was conducted between June 2019 and April 2021 in Tanzania. Four agroecological zones (Easter, Southern Highland, Northern, and Lake Zones) were selected as study sites. A total of 120 fish farmers were interviewed using a well structured questionnaire to collect information on the current general farm characteristics and farming practices. In addition, a fish farm with identical ponds stocked with Nile tilapia was selected in each agro-ecological zone, and an *in-situ* measurement of the water parameters was carried out for one year, covering two seasons (the cold: June to September and the warm season November to January). Results revealed significantly difference in mean pond water temperature between the study zones ($p < 0.001$) with the highest in the Eastern and the lowest in the Southern Highland zones. Results show that the temperature in the Northern zone and Southern highlands during the cold season (June to September) was below the level required for Nile tilapia growth. Other water quality parameters (pH, DO, turbidity, and TDS) varied between seasons within zones, but, were within the range suitable for Nile tilapia growth. The annual fish yield was higher in the Lake zone (629 ±124 kg/ha/yr) suggesting a positive relationship between temperature and fish yield. High net primary production during the warm season in all zones indicates the need for good water quality management to prevent algae blooms, which may result in acute and prolonged stress from hypoxia and hypercapnia, which are the primary physiological stressors for fish. The available data from the survey study was used as a baseline in a series of experiments conducted in the lab at Denmark Technical University (DTU-aqua) to critically assess the effects of the variation in water parameters on the physiology and performance of the cultured Nile tilapia.

Experiment 1. High level s of dissolved carbon dioxide (CO_2) occurs nightly in earthen ponds characterized by high respiration

rates. Exposure to high CO_2 conditions (hypercapnia) leads to acidosis in fish, which can be compensated by an accumulation of HCO_3^- to recover intra - and extracellular pH levels, with a capacity that appears to be species-specific. For Nile tilapia, a freshwater tropical teleost traditionally produced in earthen ponds, little information is available on the tolerance to dissolved levels of CO_2 and associated acid-base disturbances. Here, we investigated first the effects of acute and progressively increasing CO_2, from normocapnic conditions to 60 mg CO_2 L^{-1}, on oxygen uptake rates (MO_2). This was followed by exposure to three concentrations of CO_2; 10, 30, and 60 mg L^{-1} (equivalent to pCO_2 of 5.4, 16.2, and 32.4 mmHg) against a normocapnic control (pCO_2 0.3 mmHg), to investigate acute (1h) or prolonged (24h) effects on standard (SMR) and maximum metabolic rate s (MMR), haematology, and extra - and intracellular acid-base status in adult Nile tilapia (mean BM 435 ± 16 g ±SE). Acute exposure to hypercapnia led to concentration-dependent decreases in both SMR and MMR. Fish were able to fully or partially recover MMR and metabolic scope (MS) after 24h, while depression of SMR persisted at all CO_2 levels. Acute exposure to CO_2 caused intra-and extracellular pH levels to decrease by up to 0.5 units in a concentration-dependent manner. Only the lowest hypercapnic treatment (pCO_2 5.4 mmHg) was able to fully recover within 24h. Changes in haematological variables appeared minor, being restricted to increasing haematocrit, haemoglobin concentration, and mean cell volume in the highest CO_2 treatments after 24h exposure. Although the Nile tilapia is generally considered a species able to tolerate poor water quality, the modest or slow acid-base regulation following hypercapnic exposure suggests sensitivity to hypercapnia.

Experiment 2. Dissolved gases (carbon dioxide and oxygen) in earthen ponds exert strong opposing effects due to photosynthesis and respiration. This leads to daily conditions that fluctuate between being hyperoxic and hypocapnic during the day and becoming severely hypoxic and hypercapnic at night. The interacting effects of hypoxia and hypercapnia and fluctuating

conditions on pond-farmed Nile tilapia have not received any attention. This study evaluated the effects of daily variations in dissolved O_2 and CO_2 on the appetite and feed utilization in Nile tilapia using groups reared under normoxic - normocapnic conditions (control, C), diurnal hypoxia (HO), diurnal hypercapnia (HC), or combined diurnal hypoxia and hypercapnia (HO × HC) in a digestibility system. Results show that hypoxia and hypercapnia exerted strong individual effects on appetite, specific growth rate, and feed conversion and that simultaneous hypoxia and hypercapnia amplified these effects. Appetite depression induced by nocturnal hypoxia or hypercapnia was restored at mid-day, but simultaneous exposure resulted in a day-long loss of appetite. Hypoxia and hypercapnia reduced specific growth rates by up to 40% and increased feed conversion ratio by up to 80%, while combined exposure to hypoxia and hypercapnia reduced specific growth rates by >60%. Surprisingly, the digestibility of dry matter, protein, and lipid was improved in groups exposed to single or combined diurnal variation in dissolved oxygen and carbon dioxide. This study concludes that nocturnal exposure to hypoxia or hypercapnia has adverse effects on the growth performance of Nile tilapia driven by a combination of reductions in appetite and poor feed utilization. The results emphasize that there is a need for careful management of dissolved gases in pond culture. Application of aeration technology, combined with management of the nutrient status of the pond and sludge accumulation to control oxygen consuming and CO_2 liberating processes, is likely able to greatly improve production performance.

Experiment 3. Temperature plays a significant role in controlling oxygen solubility in water and its availability to aquatic breathing species including fish. In fish ponds, the water temperature can increase gradually during the day resulting in elevated temperature and an extended period of low dissolved oxygen. In this study, the response of Nile tilapia (*Oreochromis niloticus*) interms of thermal tolerance limit (CT*max*), metabolic rate (SMR), Cardiac performance (f_H), and ventilation frequency (fv) to the acute increase in temperature upon changing dissolved oxygen

saturation was investigated. Nile tilapia were experimentally exposed to an increased temperature of 0.1 °C min^{-1} at 100% DO saturation (Normoxia), 70% (hypoxia), and 130% (hyperoxia) from 26 °C (Optimum) until the onset of loss of equilibrium (LOE). Oxygen consumption rate (MO_2), heartbeat, and opercula activity were averaged at each temperature increase for three DO saturation levels. Results show that high DO saturation (130%) increased Nile tilapia's Critical Thermal Maximum (CT*max*) by 10%. The Nile tilapia metabolic rate (SMR) varied significantly with the changing DO saturation at acute increased temperature levels (One-way ANOVA; F(2,44) = 4.596, P = 0.016). Temperature-DO-limited metabolic relationship affected cardiorespiratory activities and maximum thermal tolerance (CTmax) in Nile tilapia (one-way ANOVA; F (2,44) = 0.34, P = 0.713). Different levels of DO saturation were found to significantly affect Nile tilapia's respiration ability (Opercula beats min^{-1}) ($P < 0.001$).

This study is concluding that diurnal and seasonal pond water quality varies significantly between zones and between seasons within the investigated zones and affectsthe production performance of the pond-cultured Nile tilapia. Nile tilapia might only be stocked in the warm season in the Northern and southern highland zone if farmers are to achieve the best growth of Nile tilapia. Although the Nile tilapia is generally considered a species able to adapt to and tolerate poor water quality, the modest or slow acid-base regulation following hypercapnic exposure suggests that it is quite sensitive to CO_2 exposure. Although Nile tilapia is considered resilient to environmental stress, feeding and feed utilization, oxygen uptake, and Critical thermal maximum are strongly influenced by daily fluctuations in dissolved CO_2, O_2 and Temperature.

IKISIRI KUU

Muhtasari

Uzalishaji wa samaki aina ya Sato (Nile tilapia) umevutia watu wengi duniani kote kwa sababu ya kasi ya ukuaji wao, nyama bora, na sifa za kifiziolojia zinazowaruhusu kustahamili mifumo mbali mbali ya uzalishaji. Kwa sababu hizo, Samaki aina ya Sato wamekua moja kati ya aina kuu ya samaki wafugwao nchini Tanzania. Uzalishaji wa Sato nchini Tanzania hufanyika hasa katika mabwawa ya kuchimba, bila kutumia teknolojia ya kusafisha maji. Nchini Tanzania zaidi ya 80% ya samaki wanaozalishwa wanatoka kwenye mabwawa. Uzalishaji wa Sato katika mabwawa kwa muda mrefu haujafanya vizuri kutokana na ukuaji duni. Hii ni kwa sababu, tofauti na mifumo mingine ya uzalishaji kama vile sistimu ya maji ya mzunguko (Recirculating Aquaculture Systems (RAS), kwenye mabwawa, ubora wa maji hubadilika badilika kila siku, kulingana na hali ya hewa ya maeneo mabwawa yanakopatikana . Tofauti hizi zinaweza kuathiri sana fiziolojia na ustawi wa samaki waliofugwa. Kwa bahati mbaya, kiwango cha mabadiliko ya mchana, na msimu katika vigezo vya ubora wa maji katika mabwawa ya uzalishaji nchini kote bado hayajajulikana. Hii inafanya kuwa vigumu kukadiria athari za mabadiliko katika mabwawa ya Samaki. Hivyo utafiti huu umefanyika ili kubaini hali na mienendo ya sasa ya vigezo vya ubora wa maji ya mabwawa ya samaki ya kila siku na msimu nchini Tanzania. Pia utafiti huu uliangalia na kutathmini athari za mabadiliko ya ubora wa maji kwenye fiziolojia, kimetaboliki na utendaji wa Sato kwenye mabwawa. kufikia malengo ya utafiti, uchunguzi wa shamba wa samaki wa samaki na majaribio matatu ya maabara.

Utafiti wa 1. Utafiti ulifanyika kati ya Juni 2019 na Aprili 2021 katika kanda nne za kilimo-ikolojia (Nyanda za juu kusini, Kanda ya Kaskazini, na Kanda ya Ziwa na kanda ya mashariki) zilichaguliwa kama maeneo ya utafiti. Jumla ya wafugaji wa samaki 120 walihojiwa kwa kutumia dodoso lililoandaliwa vyema kukusanya taarifa kuhusu sifa za sasa za ufugaji na kanuni za

ufugaji. Katika kila kanda, mashamba matatu ya samaki yenye ukubwa unaofanana yalichaguliwa katika kila eneo la ikolojia ya kilimo, na vipimo vya maji vilifanyika kwa kwa masaa 24 ndani ya mwaka mmoja, ikijumuisha misimu miwili (baridi: Juni hadi Septemba na msimu wa joto kutoka Novemba hadi Januari). Matokeo yalionyesha tofauti kubwa katika wastani wa halijoto ya maji ya bwawa kati ya maeneo ya utafiti ($p < 0.001$). Kanda ya mashariki iliongoza kw akua na kiwango kikubwa cha halijoto na cha chini kabisa katika ukanda wa Nyanda za Juu Kusini. Matokeo yanaonyesha kuwa halijoto katika ukanda wa Kaskazini na nyanda za juu Kusini wakati wa msimu wa baridi (Juni hadi Septemba) ilikuwa chini ya kiwango kinachohitajika kwa ukuaji wa Sato. Vigezo vingine vya ubora wa maji (pH, DO, tope linaloelea kwenye maji) vilitofautiana kati ya misimu ndani ya kanda, lakini, vilikuwa ndani ya viwango vinavyofaa kwa ukuaji wa Sato. Mavuno ya samaki kwa mwaka yalikuwa ya juu zaidi katika ukanda wa Ziwa (629 ±124 kg/ha/mwaka) ikionyesha uhusiano mzuri kati ya joto na mavuno ya samaki. Uzalishaji wa juu wa chakula cha asili (algae) kwenye mabwawa unaonyesha kuwa kunahitajika jitihada zausimamizi mzuri wa ubora wa maji ili kuzuia ukuaji wa chakula cha asili uliozidi kiwango, ambayo yanaweza kusababisha ukosekanaji wa hewa kwa muda mrefu hivyo kuathiri fiziologia na ukuaji wa sato. Taarifa zilizopatikana kutoka kwa utafiti huu zilitumika katika mfululizo wa majaribio yaliyofanywa katika maabara za Chuo Kikuu cha Kiufundi cha Denmark (DTU-aqua) ili kutathmini kwa kina athari za kutofautiana kwa vigezo vya maji kwenye fiziolojia, ulaji na ukuaji wa Sato.

Jaribio la 1. Ongezeko la hewa ukaa (CO_2) ni jambo linalotokea kila siku katika mabwawa ya Samaki, lakini hakuna taarifa inayopatikana inayoonesha ustahamilivu wa Samaki aina ya Sato kwenye viwango vya juu vya´hewa ukaa (CO_2). Uwezo wa kimetaboliki wa sato na athari za CO_2 inayoongezeka kwa gafla na inayoongezeka kidogo kidogo kwa muda mrefu ulichunguzwa kutoka hali ya kawaida 0 hadi 60 mg CO_2 L^{-1}. Pia uchunguzi huu ulifanyika kutahmini athari za hewa ukaa kwa viwango vitatu vya

CO_2 (pCO_2 10, 30, na 60 mg L^{-1}) vikilinganishwa na kiwango cha kawaida (0), kwa muda mfupi (1h) na muda mrefu (24h) kwenye uwezo wa Kiwango cha kawaida cha metaboliki (SMR) na kiwango cha juu cha kimetaboliki (MMR), mabadiliko ya viambata vya damu, na tindikali ndani ya seli. Ongezeko la ghafla la hewa ukaa (hypercapnia) ulisababisha kupungua kwa SMR na MMR. Samaki waliweza kurejesha kikamilifu au kwa sehemu SMR na MMR baada ya 24h kwenye ongezeko la hewa ukaa la 10 mg L^{-1}, lakini viwango vya juu vya 30 na 60 mg L^{-1} CO_2 vilisababisha upotevu unaoendelea wa uwezo wa upeo wa kimetaboliki (MS). Kiwango cha tindikali (pH) ndani ya seli kilishuka hadi 0.5 uniti kulingana na kiwango cha hewa ukaa *p*CO2 iliyokuepo ndani ya muda mfupi wa saa moja (1h) lakini iliweza kurudishwa kikamilifu ndani ya masaa 24 kwenye kiwango 10 mg L^{-1}. Vigezo vyote vya viambata vya damu viliathiriwa na *p*CO2 iliyoongezeka ndani ya muda mfupi wa saa 1, lakini zilirudishwa kikamilifu au kwa kiasi baada ya masaa 24 kwenye 10 mg L^{-1}. Ingawa Sato kwa ujumla inachukuliwa kuwa aina ya Samaki anayeweza kuzoea na kustahimili ubora duni wa maji, lakini wastani wa uwezo wake wa kustahamili uwezo wa tindikali unaishia kwenye ukomo wa 10 mg L^{-1}.

Jaribio la 2. Mabadiliko ya gesi za hewa ukaa (CO_2) na oksijeni (DO) katika mabwawa zina madhara kwa samaki na uwepo wake kwenye mabwawa ni wa kupingana hii ni kutokana na photosynthesis na upumuaji wa viumbe kwenye maji. Hii husababisha mabadiliko ya hali ya hewa ndani ya maji ambayo yanasababisha uwepo wa kiwango kidogo cha oksijeni (hypoxia) na kiwawango kikubwa cha hewa ukaa (hypercapnia) Utafiti wa athari za kuingiliana kwa hypoxia na hypercapnia na hali ya kubadilika-badilika kwa Sato kwenye bwawa bado haijaripotiwa. Utafiti huu ulitathmini athari za tofauti za kila siku za kupungua kwa O_2 na kuongezeka CO_2 juu ya hamu ya kula, ukuaji na usagaji chakula. Samaki waligawanywa katika vikundi vya Vinne: hali ya kawaida (normoxic, control, C), mbadiliko ya hewa ya oksijeni kwa siku (diurnal hypoxia, HO), mabadiliko ya hewa ukaa kwa siku (diurnal hypercapnia (HC), na maingiliano ya hypoxia na hypercapnia (HO × HC). Matokeo yanaonyesha kuwa hypoxia na hypercapnia binafsi zilileta athari kubwa kwenye kupunguza hamu ya kula, kiwango cha ukuaji, na usagaji wa chakula, lakini athari kubwa zaidi zilionekana wakati hypoxia na hypercapnia zilipoungana kwa wakati mmoja. Upungufu wa hamu ya kula uliosababishwa na hypoxia au hypercapnia ulionekana kurudi katikati ya siku inayofuata, lakini hamu ya kula kwenye muunganiko wa hypoxia na hypecapnia ulisababisha kupotea kwa hamu ya kula kwa siku nzima. Hypoxia na hypercapnia ilipunguza viwango vya ukwaji wa sato kwa hadi asilimia 40 na kuliongeza uwiano wa kubadilisha chakula kua nyama kwa asilimia 80, wakati muumganiko wa pamoja wa hypoxia na hypercapnia ulipunguza viwango maalum vya ukuaji kwa zaidi ya asilimia 60. Jambo la kushangaza ni kwamba usagaji wa chakula hususa protini, na lipid uliongezeka kwenye hypoxia na hypercapnia. Utafiti huu unahitimisha kuwa mabadiliko ya siku ya hypoxia au hypercapnia ina athari mbaya katika utendaji wa ukuaji wa Sato.

Jaribio la 3. Ongezeko la ghafla la joto kwenye maji husababisha upungufu wa upatikanaji wa hewa ya oksijeni kwenye maji hivyo kuathiri upatikanaji wake kwa samaki. Katika mabwawa ya samaki, joto la maji linaweza kuongezeka polepole wakati wa mchana na kusababisha joto la juu na upungufu wa muda mrefu wa oksijeni. Katika utafiti huu, mwitikio wa Sato (Oreochromis niloticus) katika kustahimili joto (CTmax), uwezo wa kimetaboliki (SMR), utendaji wa moyo (fH), na mzunguko wa uingizaji hewa (fv) kwa ongezeko kubwa la joto wakati wa mabadiliko wa viwango vya oksijeni. Sato walijaribiwa kwa ongezeko la joto la 0.1 °C min-1 kwenye asilimia 100 DO (Normoxia), 70% (hypoxia), na 130% (hyperoxia) kutoka joto ridi la 26 °C (Optimum) hadi mwisho wa uwezo ustahimilivu wao (LOE). Kiwango cha matumizi ya oksijeni (MO_2) na mapigo ya moyo zilikadiriwa katika kila ongezeko la joto kwa viwango vitatu vya kueneza kwa DO. Matokeo yanaonyesha kuwa kueneza kwa kiwango cha juu cha DO (130%) kuliongeza upeo wa ustahimilivu wa joto kwa Sato (CTmax) kwa asilimia 10. Kiwango cha kimetaboliki cha sato (SMR) kilitofautiana kwa kiasi kikubwa na mabadiliko ya uwepo wa DO wakati wa kuongezeka kwa joto (ANOVA; F (2,44) = 4.596, P = 0.016). Uhusiano wa kimetaboliki na uwepo wa hewa ya oksijeni na joto vimeonesha kuathiri shughuli za upumuaji na mapigo ya moyo na ustahimilivu wa juu kifo (CTmax) (ANOVA, F (2,44) = 0.34, P = 0.713).

Utafiti huu unahitimisha kuwa ubora wa maji ya bwawa wakati wa mchana na misimu hutofautiana kwa kiasi kikubwa kati ya ikolojia za kilimo Tanzania. Sato wanaweza kufugwa katika msimu msimu wa joto katika ukanda wa Kaskazini na nyanda za juu Kusin. Ingawa Sato kwa ujumla inachukuliwa kuwa aina ya Samaki wanaoweza kuzoea na kustahimili ubora duni wa maji, lakini uwezo wao binafsi wa kuweza kustahimili na kuzoea ongezeko la hypercapnic unafikia kwenye kiwango cha chini kisichozidi 10 mg L^{-1}, hivyo wakulima wa Samaki kwenye mabwawa wanahitaji kuzingatia kwa umakini mabadiliko ya hali yam aji kwenye mabwawa.

DECLARATION

I, MUUMIN IDDI HAMAD**,** do hereby declare to the Senate of Sokoine University of Agriculture that this thesis is my original work done within the period of registration and has neither been submitted nor been concurrently submitted in any other institution.

Muumin Iddi Hamad Date
(Ph.D. Candidate)

The above declaration is confirmed by:

Dr. Heiromin Amon Lamtane Date
(1st Supervisor)

Dr. Renalda Nanziga Munubi Date
(2nd Supervisor)

Prof. Peter Vilhelm Skov Date
(3rd Supervisor)

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Last but not least, I want to express my gratitude to my extended family at home and my friends at the Sokoine University of Agriculture, particularly the department of Animal, Aquaculture, and Range Sciences (DAARS), for their unwavering support throughout my study period.

DEDICATION

I am dedicating this thesis to my Family. My beloved Mother Fatma (“Da Nchafu”), my Father Iddi, my wife Fatma (“Bit Shaa”), and my daughters Nafisa and Nairat (“Binas”), you have meant so much to me and still do. I learned the importance of hard work from your love and support.

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LIST OF PUBLISHED PAPERS AND MANUSCRIPTS

Paper 1: **Muumin Iddi Hamad,** Heiromin Amon Lamtane, and Nazael Amos Madalla (2022). An Overview of the Seasonal and Diurnal Rhythms in Pond Water Quality Parameters, Farming Characteristics and Yield of Pond Cultured Nile tilapia in Tanzania. **Status:** Submitted to the *Journal of Applied Aquaculture*.

Paper 2: **Muumin Iddi Hamad,** Christian Damsgaard, Renalda Nanziga Munubi and Peter Vilhelm Skov (2023). Acid-Base Disturbances and Effects on Oxygen Uptake in Nile Tilapia Following Acute and Prolonged Hypercapnic Exposure. **Status:** Published, *Aquaculture,(2024),*Elsevier
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Paper 3: **Muumin Iddi Hamad.,** Heiromin Amon Lamtane., Renalda Nanziga Munubi, and Peter Vilhelm Skov. (2023). Individual and combined effects of hypoxia and hypercapnia on feeding and feed utilization in Nile tilapia (Oreochromis niloticus). **Status:** Published, *Aquaculture,* 567(2023)739239.
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Paper 4: **Muumin Iddi Hamad,** Renalda N. Munubi, Peter Vilhelm Skov (2022). Metabolic rate, Cardio Ventilatory Response and Critical Thermal Limit (CT*max*) of Nile Tilapia (*Oreochromis niloticus*) exposed to Acute Temperature Elevation upon Oxygen Restriction. **Status:** Manuscript.

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LIST OF ABBREVIATIONS AND SYMBOLS

%	Percentage
°C	Degree centigrade
ANOVA	Analysis of Variance
$CaCO3^-$	Calcium Carbonate Ion
CL^-	Cloride Ion
CO_2	Carbon dioxide
CTmax	Thermal Tolerance Limit (maximum)
DO	Dissolve Oxygen
EC	Electric Conductivity
ECG	Electrocardiogram
FAO	Food and Agricultural Organization of the United Nations
f_H	Cardiac performanc
f_V	Ventilation Frequency
H^+	Hydrogen Ion
HBM	Heart Beat per Minute
$HCO3^-$	Hydrogen Carbonate Ion
LOE	Lose of Equilibrium
mg L^{-1}	Miligram per litre
MMR	Maximum Metabolic Rate
MO_2	Oxygen Consumption rate
MS	Aerobic Metabolic Scope
NH4-N	Ammonia
NO2	Nitrite
OPM	Oparcular Beat per Minute
PCO_2	Carbon dioxide parcial presure
P_{CRIT}	Critical Parcial presure
PO_2	Oxygen parcial presure
Q_{10}	Temperature coefficient
RAS	Recirculating Aquaculture System
SMR	Standard Metabolic Rate
T_{CRIT}	Critical Temperature
TDS	Total Dissolved Solid
TOPT	Temperature optimum

CHAPTER ONE

1.0 INTRODUCTION

1.1 Background information

As the world population rises, so does the demand for aquatic food products. Meanwhile, the dwindling fish supply from capture fisheries cannot meet the rising global demand for aquatic food. Therefore, Aquaculture has been heavily promoted as a potential alternative that can fill the gap of fish supply from the rising demand. As a result, aquaculture production has increased dramatically globally over the previous few decades, reaching 122.6 million tonnes in 2020 (FAO, 2022). Fish and other farmed aquatic animals make over 87% of aquaculture production and are directly consumed as food (FAO, 2022). Unquestionably, the rising global aquaculture production trend is anticipated to continue as aquatic food plays a more significant role in 21st century food security and nutrition (Naylor *et al.,* 2021).

In recent years, the increase of global aquaculture production has been contributed by a recent expansion of the inland freshwater fish production sector (Naylor *et al.,* 2021). According to FAO, (2022), inland aquaculture contributed 54.4% of total annual aquaculture-produced fish in 2020. Compared to coastal or marine water production, inland freshwater fish production supports a variety of species and offers a larger choice of farming systems (FAO, 2022). However, despite the great variety of species and farming structures that the inland farming industry cansupply, carp, tilapia, and catfish are the ony species that dominat this farming sector. Moreover, despite the availability of other farming techniques such as raceways, floating systems, cages, and net pens, inland farming has historically been dominated by pond farming systems.

Fish raised in inland waters dominate Tanzania's aquaculture fish production sector (Duijn et al., 2018). In 2019, fish produced from inland waters accounted for 90% of the total annual fish produced through aquaculture (Mbiru *et al*., 2020). Given the available variety of production inputs such as water (from lakes, reservoirs, and rivers), and thelarge number of potential farmed species, inland freshwater aquaculture production in Tanzania has a high potential for further development (Lugendo & Mwaijande, 2015; Respikius *et al.,* 2020). However, for many years, the inland fish production sector has relied solely on ponds and Nile tilapia (*O. niloticus*) as the main species (Rothuis *et al.,* 2014). There are currently over 26 400 small-scale ponds, with an average size of 300m^2 (Kajungiro et al., 2019a), and their number is expected to grow in response to the government agenda of increasing fish production from aquaculture to fill the gap caused by shortage supply from capture fisheries.

Pond farming has become increasingly popular across the country because of its ability to provide farmers with an array of farming practices, including intensive, semi-intensive, and excessive farming. Lower start-up costs, fewer management procedures, and decreased feeding expenses due to availability of natural food in the ponds produced by fertilizer application are among the factors that have led to pond system adoption (Respikius *et al.*, 2020). Among the limitations of adopting pond farming system in a tropical area such as Tanzania is its narrow room for the species that can be cultured as it is suitable to withstand a large range of fluctuations in environmental parameters. This is because, most water quality parameters in most tropical fish ponds, are greatly influenced by variations in the local climate variables of the surrounding areas and other factors such as fertilization, feeding and poor management practices (Shoko *et al.,* 2011; Tadesse *et al*., 2004).

In tropical areas, the changing climatic variables (microclimate) of the surrounding areas always have an impact on the pond environment (Emese, 2019). Photoperiod, temperature, and wind are the main environmental factors that determine the pond ecological system (Ahn *et al.*, 2019; Tadesse *et al*., 2004). Wheter individually or in combination, photoperiod, temperature, and wind can regulate pond ecological functions such as photosynthesis during the day and respiration rate at night, resulting in either supersaturated oxygen (hyperoxia) and low dissolved carbon dioxide (normocapnic) during the day and high carbon dioxide (hypercapnia) and low oxygen (hypoxia) at night (Gyamfi et al., 2022). As a result, fish raised in ponds are affected by these periodic changes in water quality parameters, and the majority of them are likely to have an impact on fish physiology and welfare. The impact of variations in water quality parameters in fish may include changes in cardiorespiratory functioning (Lee et al., 2022), reduced oxygen consumption (Bergstedt et al., 2021), poor feed intake (Magnoni *et al.*, 2018; Tran-Duy *et al.*, 2008), and acid base disturbances which leading to respiratory acidosis (Gam *et al.*, 2018; Michaelidis *et al.,* 2007). Therefore, to increase fish output from ponds in tropical countries like Tanzania, the farming environment must be controlled to allow for a stress-free environment that will optimize the growth performance of the cultured fish. Moreover, the management system must be significantly improved.

1.2 Problem Statement and Justification

In the last two decades, Tanzania has experienced a significant increase in the use of pond systems for culturing Nile tilapia (*O. niloticus*). Nile tilapia raised in earthen ponds account for more than 60% of total annual aquaculture fish production. In just a decade, the number of fish ponds has doubled, from 12 000 in 2011 to more than 26 400 in 2020. The number of fish ponds are expected to increase even further as more people adopt this simplest and least expensive farming system to support their livelihood and food security. For many years, pond farming of Nile tilapia has been practised under a semi-intensive system without any water treatment technology. In recent years, poor growth performance of pond-raised Nile tilapia have recently raised a tremendous concern.

Variations in water quality parameters during production cycle are among the causes of the poor growth performance of the pond cultured Nile tilapia. The majority of fish ponds used for production are constructed outdoors, where they are subjected to the direct influence of the changing localweather conditions. It is well known that the water quality parameters in small water bodies such as ponds, fluctuate on a daily basis depending on the equilibrium reached with the regular physical and chemical characteristics of the surrounding environment. Vatiation in temperature, wind, and photoperiod can directly influence the variation of water quality parameters in a fish pond. The variation may create acute or chronic stress which may directly affects the performance, physiology and welfare of the cultured fish. Although Tanzania is classified as a tropical country, it has a wide range of agro-ecological zones and there are no consistent environmental conditions. Local environmental conditions differ dramatically between agroecological zones and between seasons. However, no information is available about the extent to which variation in local weather condition changes the pond water quality parameters in these agroecological zones and how the variation affect pond-cultured Nile tilapia.

On the basis of the above-described context, and given that, it is crucial for farmers to fully understand the trends of water quality variations for them to create a stress-free environment that meets production standards and promotes optimal growth, there is a need to establish the diurnal and seasonal Variation of pond water quality parameters in Tanzania, Furthermore, it is important to provide information of how diurnal and seasonal variation in pond water quality parameters affect fish physiology and growth performance in order to establish a coping mechanism of fish production based on locality. Therefore, this study evaluated the current trend of water quality parameters in fish ponds in four different agroecological zones around Tanzania and the observed trends were experimentally tested to evaluate how they affect the physiology and performance of cultured Nile tilapia.

1.3 Objectives

1.3.1 General objective

The main aim of this study was to evaluate the current status of the diurnal and seasonal dynamics of pod water quality parameters in Tanzania and evaluate their implications on the physiology and metabolism of the pond-cultured Nile tilapia (*Oreochromis niloticus*).

1.3.2 Specific objectives

i. To assess the diurnal and seasonal trend of pond water quality parameters in four agroecological zones in Tanzania.
ii. To determine the metabolic and physiological response of Nile tilapia exposed to different levels of water pH and dissolved Carbon dioxide (pCO_2).
iii. To evaluate the effect of the diurnal interaction of dissolved oxygen (DO) and Carbon dioxide (CO_2) on the growth and feeding of Nile tilapia.
iv. To evaluate the metabolic and physiological responses of Nile tilapia exposed to acute changes in temperature and dissolved oxygen (DO) saturation.

1.4 Literature Review

1.4.1 An overview of aquaculture production in Tanzania

Aquaculture in Tanzania dated back to 1927 when Scottish trout were released for sport fishing in streams around the Kilimanjaro and Mbeya regions (Mmanda *et al.,* 2020). The foundation for actual aquaculture production was established in the 1950s when experimental ponds were stocked with tilapia fingerlings from the Congo, and Pangani rivers, and Lake Victoria (Mosha & Daudi, 2020). This was folloed by government effort to introduce tilapia fingerlings in public water reservoirs and commercial and establishment of public fish farms in the late 1970s (Rothuis *et al.*, 2014). Since the early time of aquaculture development, earthen ponds have been the primary production system (Duijn *et al.,* 2018).

Tanzania's Fisheries and Aquaculture Development Policy of 2015 recognizes the aquaculture sector as an important tool for socio-economic improvement, poverty alleviation, and potential alternative of the growing demand for fish protein which was previously supplied by stagnant capture fisheries (URT, 2015). The policy's primary objective is to improve aquaculture fish production (Figure 1) by encouraging the use of innovative production technology, improving small-scale farms, and promoting the establishment of large-scale production farms (URT, 2015).

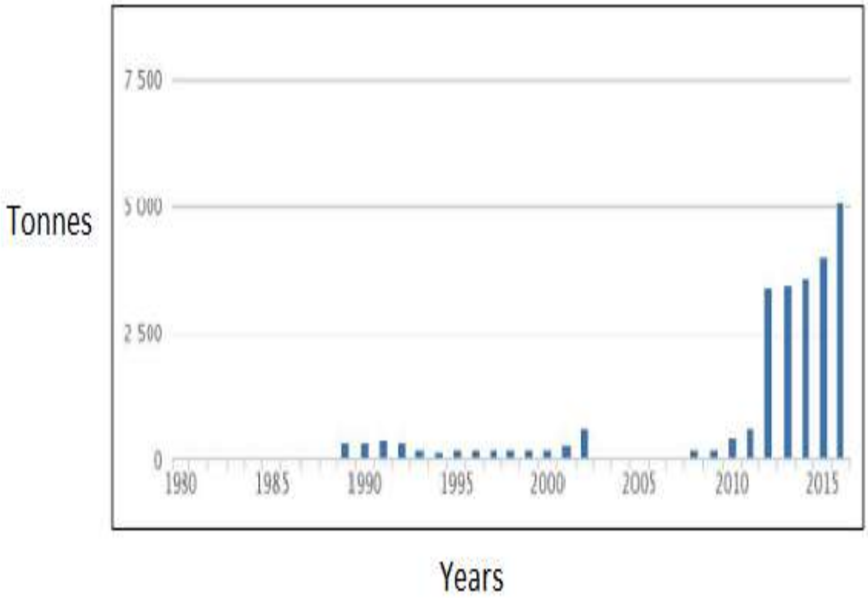


Figure 1: Tanzania aquaculture production Trend: Total annual production (Tonnes). The source (Mosha & Daudi, 2020).

However, despite continued efforts and promotion from the government, aquaculture in the country is still a modest industry (Rothuis *et al.*, 2014), which, at the current development rate, it cannot meet the rapid rise in demand for fish and fish products. Even though the aquaculture industry appeared to have the potential for future growth (Duijn *et al.*, 2018), significant efforts need to be taken to expedite and ease its production challenges.

At the moment, aquaculture production is primarily dominated by small-scale inland pond farming systems (Mbiru et al., 2020; Pius *et al.*, 2021; Respikius *et al.,* 2020). The number of fish ponds has expanded in the past ten years, from 13,011 in 2005 to 21,300 in 2015, with Nile tilapia contributing 90% to the total pond production (Rukanda, 2018), followed by catfish and a few saltwater species such as milkfish *Chanos chanos* and shrimps (Mosha & Daudi, 2020). The survey conducted in 2017 reported the number of inland freshwater fish farmers to be around 17 726, which was ten times higher than the marine counterparts, who were 1,545 (Rothuis *et al.,* 2014). In addition, in 2020, Tanzania has reported a dramatic increase in aquaculture production with 11 802 tonnes of fish (Mosha & Daudi, 2020), the amount which is three times larger compared to 3,407 tonnes reported just a decade earlier (Duijn *et al.,* 2018). However, even with this increased production status, aquaculture contribution is still less than 2% of the total annual fish production (Pius *et al.,* 2021).

1.4.2 Tilapia Production from aquaculture

Nile tilapia (*Oreochromis niloticus*) is the main species raised in Tanzania's aquaculture (Figure 2) (Kajungiro *et al.,* 2019b). It contributes about 90% of the aquaculture produced fish (Mbiru *et al.,* 2020). According to Kajungiro *et al.* (2019) and Rothuis *et al.* (2014) the tilapia is preferred due to its low production costs and resistance to the native tropical environment, and it has a high potential for future aquaculture production in the country. In 2019, it was estimated that 17,725 farmers, about 80% of all fish farmers in the country were directly engaged in the production of tilapia (Korving, 2019). Currently, there are about 20,235 ponds that exclusively grow tilapia. The production has doubled from 1522 tonnes in 2007 to 3,942 tonnes in 2014 (Korving, 2019). However, the majority of these farmers only produce at a relatively very low scale, mostly using poor production techniques (Duijn et al., 2018; Rukanda, 2018).

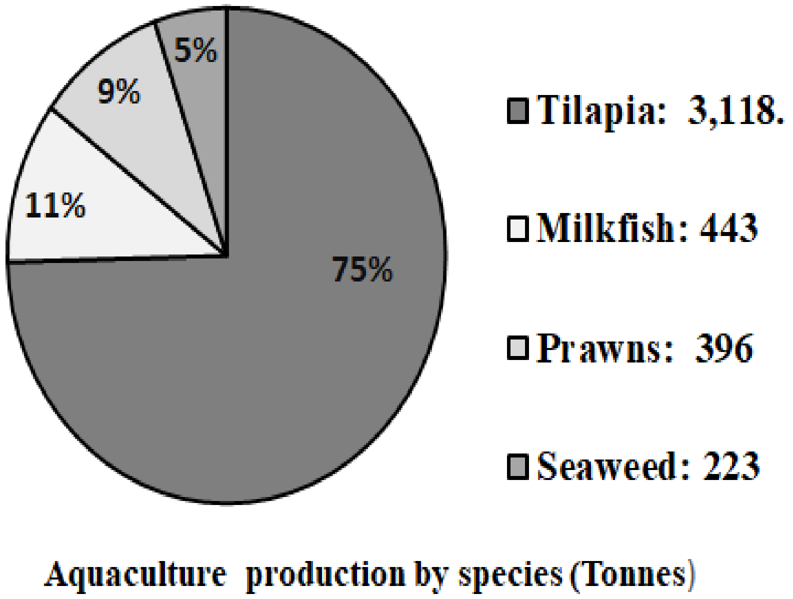


Figure 2: Total annual aquaculture production by species (Tonnes) in Tanzania. The source (Mosha & Daudi, 2020).

Freshwater tilapia farming is mainly practised in the Southern highland regions, (particularly Ruvuma, Njombe, and Iringa) (Pius *et al.,* 2021), the eastern zone (Dar es Salaam, Pwani and Morogoro) and around the Lake zone (Rukanda, 2018). In addition to Nile tilapia, other species such as Mozambique tilapia (*O. mossambicus* Peters 1852) and Zanzibar tilapia (*Tilapia hornorum* Trewavas 1966) are also produced in different parts of Tanzania, although their contribution to aquaculture fish production is still insignificant (Rukanda, 2018).

1.4.3 Water quality in aquaculture

Water quality is defined as the summation of all physical, chemical, and biological characteristics of water that influence its use (Boyd & Lichtkoppler, 1979; Ferdous *et al.*, 2014). Water quality in an aquaculture system involves a complex interaction of several parameters, each with a unique effect on fish physiology and well-being. Temperature, dissolved oxygen (DO), pH, carbon dioxide (CO_2), Nitrite (NO_2) and ammonia (NH4-N) are common variables that define the quality of the water in aquaculture systems (Makori *et al.,* 2017b). Variations in water quality parameters can have a direct impact on the biology and the rate of important physiological activities including feeding and growth (Mallya *et al.*, 2007). Thus; aquaculture fish producers must understand the chemical and physical quality of water throughout the production cycle in order to avoid physiological disturbance of the cultured fish, as this could result in slow growth (Adeosun *et al.*, 2019; Makori *et al.*, 2017b). Individual variations in water quality parameters can have an instant impact on fish. Therefore, maintaining a balance of levels of all water quality parameters in production structures is crucial because water quality parameters influence each other in complex and dynamic production systems such as earthen ponds (Boyd & Lichtkoppler, 1979).

Increased water temperature in aquaculture system can reduce the solubility of dissolved gasses, particularly dissolve oxygen and Carbon dioxide which may lead to hypoxic condition and causes death in fish (Mallekh & Lagardere, 2002). High levels of CO_2 can directly impact important physiological functions including cardiorespiratory function, digestion and swimming performance (Good *et al.,* 2010). Dissolved gasses are well known for their ability to control fish metabolic activities such as ability for oxygen uptake and feeding (Bergstedt *et al.,* 2021). Several studies have highlighted the importance of maintaining good water quality during production (Cho *et al.,* 2015; Ferdous *et al.,* 2014; Makori *et al.*, 2017b; Tumwesigye *et al.,* 2022).

1.4.4 Water quality Dynamic in Fish Pond

Water quality parameters in small bodies of water, such as earthen ponds, change on a daily basis, depending on the equilibrium reached with the regular chemical and physical characteristics of the surrounding environment (Figure 3) (Tadesse *et al.,* 2004). These changes can occur abruptly due to rapid changes in environmental factors (temperature, wind, and/or photoperiod) or gradually due to poor management practices (feeding, water exchange or fertilization). Photoperiod and temperature of the surrounding environment can cause fluctuations in water quality parameters and change the entire ecological function of a fish pond (Tadesse *et al.,* 2004). A long photoperiod, for example, can cause fluctuations in pond dissolved gasses (DO and CO_2) due to photosynthesis and respiration, resulting in hypoxia and hypercapnia periods (Boyd, 2008). Therefore, it is crucial for fish farmers to fully understand the trends in pond water quality during production in order to design a management practices which can create a stress-free environment that meets production standards and promotes optimal growth.

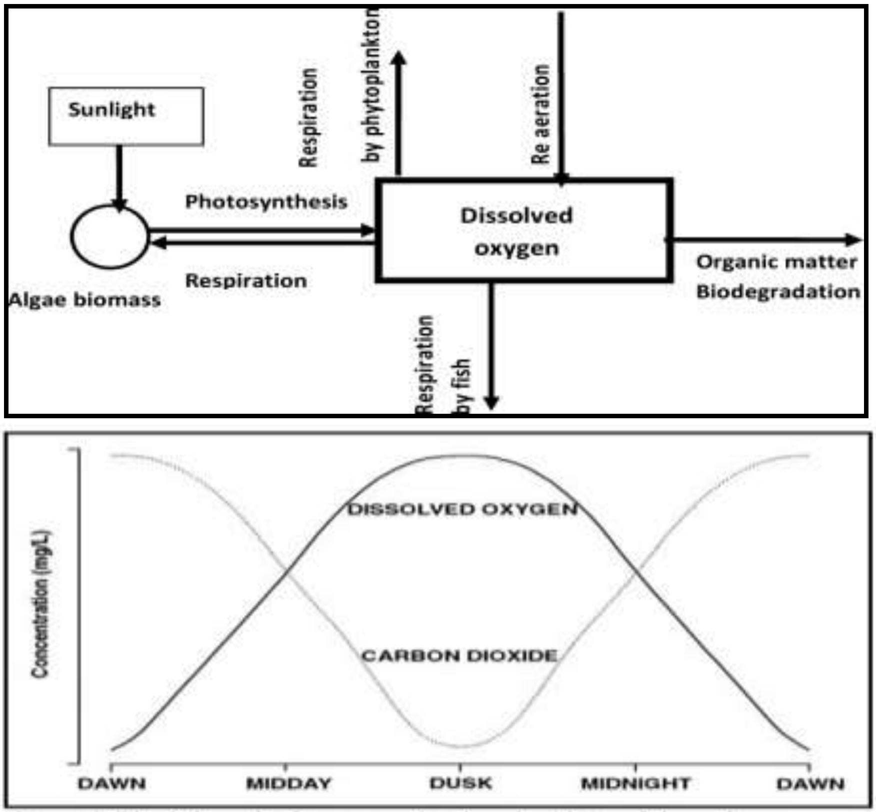


Figure 3: A schematic illustration of pond diurnal photoperiod effects on dissolved gasses (oxygen and Carbon Dioxide) through photosynthesis and respiration rate. Sources (Hargreaves & Brunson, 1996).

In a tropical fish pond, in the presence of sunlight during the day, pond plants (algae) fix CO_2 and produce oxygen. This process raises the DO levels above normoxia to high saturation (hyperoxia) (Jacobs *et al.,* 2008) (Figure 3). A DO level of 300% saturation has recently been reported in a fertilized earthen pond stocked with Nile tilapia in the afternoon (Gyamfi *et al.,* 2022). At night (dark period), all aquatic organisms such as bacteria, fish, and algae consume DO and release CO_2 as a byproduct of respiration, resulting in low dissolved oxygen levels (hypoxia) and elevated levels of CO_2 (hypercapnia) (Montgomery *et al.,* 2019).

The periodic changes in dissolved CO_2 in the fish pond due to the accumulation of metabolic carbon dioxide may lead to disturbance of the acid-base status of cultured fish and consequently changes fish physiology characteristics including growth (Brauner *et al.,* 2019; Claiborne *et al.,* 2002; Damsgaard *et al.,* 2015). Changes in pH caused by photosynthesis and respiration can lead to changes in the water's acidity and alkalinity, which can disrupt the ionic balance of fish and ultimately change the ecological cycle of the pond.

1.4.5 Influence of water temperature on Physiological parameters and metabolic rate

Temperature is defined as the “abiotic master factor” among all factors affecting fish physiology and metabolism (Panase et al., 2018). Given their ectothermic nature, fishes are very sensitive to changes in temperature (Frisk *et al.,* 2012), thus, all metabolic, chemical and biochemical reactions are reported to change upon temperature variation (Giacomin *et al.*, 2017; Volkoff & Rønnestad, 2020; Worden *et al.,* 2006). However, the metabolic and physiological response to temperature changes depends on the fish species, origin, intensity of exposure, and exposure time (Methling *et al.,* 2013). Metabolic functions such as oxygen consumption rate (MO_2), heart rate and gill ventilation react immediately upon acute temperature exposure while factors such as growth, feed utilization, gene expression and enzyme activities are affected by gradual (chronic) temperature exposure. Some fish species can cope with prolonged (chronic) temperature exposure and develop a tolerance mechanism which in some species may be passed on to the next generation (Methling *et al.,* 2013). The effect and intensity of temperature on fish metabolic process and the tolerance capacity of a particular specie can be quantified using question derived by van’t Hoff as a Q_{10} value.

$$Q_{10} = \left(\frac{R_2}{R_1}\right)^{\left(\frac{10}{T_2 - T_1}\right)}$$
…………………………………….. (1)

where R_2 is the rate of a physiological or biochemical process at a given temperature t_2, and R_1 is the rate of that process at a different temperature t_1. The Q_{10} factor represents the change in the rate of a process when the temperature is increased by 10 °C (Tirsgaard *et al.*, 2015).

With increase in temperature, fish often show an exponential or power function rise in metabolic rate (Hernández-sandoval *et al.*, 2018). The solubility of dissolved oxygen in water varies with temperature, which also influences the oxygen consumption rate in fish (MO_2) (Schulte, 2015). Oxygen consumption rate (MO_2) is the primary component determining fish Standard Metabolic Rate (SMR), which steadily increases when temperatures exceed the optimum species requirement. For example, the typical Q_{10} values for fish standard metabolic rate (SMR) is between 2 to 3 (Methling *et al*., 2013). Fish often increase their oxygen demand dramatically with temperature until the lethal temperature is reached (Figure 4) (Methling *et al.*, 2013).

Maximum Oxygen Consumption (also known as the Maximum Metabolic Rate, or MMR), also increase with increased temperature intensity (Figure 4A), up to a certain point, where rising temperatures make MMR increase more quickly and faster than SMR before it decline (Figure 4A). Aerobic metabolic scope (MS) (Figure 4B), which is the difference between SMR and MMR, often has a bell-shaped form when shown as a function of temperature (Min et al., 2019).

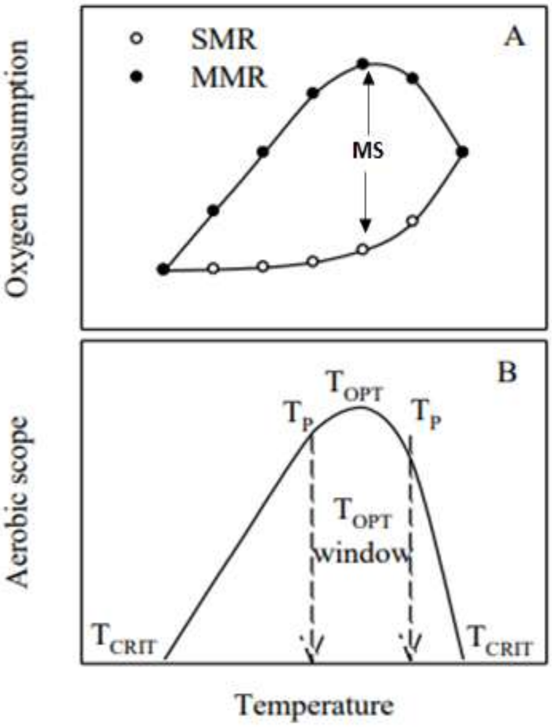


Figure 4: Graphical presentation of how metabolic rate changes with temperature. A: standard metabolic rate (SMR) and Maximum metabolic rate (MMR) with aerobic Metabolic scope (MS). B: metabolic scope as a function of temperature. TOPT; temperature optimum, T_{CRIT}; critical temperature (Tirsgaard *et al.*, 2015).

The aerobic metabolic scope defines the maximal oxygen supply that can be made available for functions other than maintenance, such as feeding, swimming, growth, and reproduction. The term "optimal temperature" (TOPT) refers to the temperature where aerobic metabolic capacity is at its highest (Figure 4B). This relationship between temperature and oxygen availability, and how it affects the general physiological performance of fish is highlighted in substantial number of scientific literature (Chatterjee *et al*., 2004; Cho *et al.,* 2015; Mottola *et al.,* 2020; Remen et al., 2015)

1.4.6 Effects of temperature on Cardiorespiratory functions and thermal tolerance limit (CT*max*)

The temperature tolerance ability of a certain fish species is the most crucial factor to consider when choosing a species for aquaculture, because the tolerance ability of fish to temperature variation depends on the species, amount of temperature exposure, and acclimatization duration (Das *et al.,* 2005). Fish increase their cardiorespiratory performance at high temperatures because high temperatures reduce oxygen solubility, limiting oxygen supply capacity, and resulting in critical oxygen tension which consequently decreases the rate of oxygen uptake in fish (MO_2) (Farrell *et al.,* 2009; Gilbert *et al.*, 2020; Nyboer & Chapman, 2018).

In order to assess the thermal tolerance of fish, it is essential to understand thermal acclimation and critical temperature (Majhi & Das, 2013). The critical thermal maxima (CT_{max}), which identifies the first temperature at which stress begins to occur, is considered as a useful measure of an organism's thermal tolerance (Pörtner *et al.,* 2004). In fish, the degree of vulnerability to thermal stress depends on the thermal tolerance limit (CT_{max}) of a certain species (Ern *et al.*, 2016). For a certain species to perform optimally, it must inhabit a specific thermal assortment. The critical thermal maximum (CT_{max}), is the determinant of the upper limit of a species' thermal position, or the temperature where most physiological functions of an animal tumble and the animal exhibit a loss of equilibrium (LOE) (Ern *et al.,* 2016).

1.4.7 Effects of temperature on feeding and energy utilization

The connection between temperature, feed efficiency and energy budget has been reported in several studies (Jobling, 1981; Volkoff & Rønnestad, 2020; Wang *et al.*, 2017; Xie, 1997). The energy budget describes the partitioning of food energy (FE), into excretory energy (including urinary energy and branchial energy), heat production (HE) and recovered energy (growth) (RE) (Hernández-sandoval *et al.,* 2018). dos Santos *et al.* (2019) and Xie, (1997) reported that increased water temperature affects feeding strategies, including feed intake and assimilation and nutrient metabolism. Pörtner *et al.* (2004) reported that temperature affects fish feed utilization through the partitioning of food energy into different components of energy budget, hence, determine the fate of growth. Pandit & Nakamura (2010) revealed that feed consumption and FCR in Nile tilapia is optimized at temperature between 27^{0}C and 32 ^{0}C.

1.4.8 Temperature optimum for Nile tilapia

The optimum temperature is defined as the temperature at which aerobic metabolism is at its maximum (T_{OPT}). In fish, T_{OPT} is related to species. For Nile tilapia, the preferred temperature range for optimum growth is 26 - 32 °C (Azaza et al., 2008; Pandit & Nakamura, 2010), its growth rate is reduced significantly at a temperature below 20 °C; and death will occur when the temperature drop below 10 °C (Malcolm, & Brendan, 2000). Feeding activity in Nile tilapia ceases when the water temperature is decreased to 16 °C (Malcolm, & Brendan, 2000), and FCR gradually decreasing when the temperature exceeds 35 °C (Azaza *et al*., 2008).

1.4.9 Dissolved Oxygen

Dissolved oxygen is crucial and limiting factor in fish growth and metabolism (Kolding *et al.,* 2008). In intensive modern fish farming aquaculture systems such as RAS, DO is directly supplied with cultured water or through dosing pure oxygen through oxygen pumps, whereas in ponds, oxygen enters the water through photosynthesis by aquatic plants, primarily phytoplankton, and by diffusion at the air-water interface or manual aeration (Gyamfi *et al.,* 2022; Obirikorang *et al.,* 2020). Pond water loses oxygen as a result of fish, plankton, and other organisms' respiration, as well as the aerobic decomposition of organic waste. In most tropical fish ponds, there are noticeable diurnal variations in oxygen content, with the lowest valuesexperienced immediately after daylight and the highest (sometimes above saturation) values during the day (Gyamfi *et al.,* 2022; Kayombo *et al.,* 2002; Shoko *et al.*, 2014).

1.4.10 Hypoxia

Hypoxia is the condition of low dissolved oxygen (DO) at which all oxygen-dependent physiological activities become suppressed (Mallya *et al.,* 2007). Fish suffer greatly from hypoxia in pond water when DO levels drop below 1-2 mg/L for a few hours. A long period of exposure to hypoxia can negatively impact fish growth or may result in fish mortality (Abdel-Tawwab *et al.,* 2019). In addition, hypoxia also suppress fish immunological systems, hence increasing fish susceptibility to disease (Abdel-Tawwab *et al*., 2019). Low levels of DO occur naturally in a majority of aquatic systems as a results of low photosynthesis rate or high respiration, especialy in natural shallow freshwater systems with high organic matter content, like lakes, streams, and ponds (Montgomery *et al*., 2019). In the aquaculture system, hypoxia may occur as a result of overstocking or decomposition of wastes and organic matter from fish waste or uneaten feed (Gyamfi *et al.,* 2022; Sriyasak *et al*., 2015).

1.4.11 Effect of Hypoxia on metabolic rate and Aerobic metabolic scope

In fish, all aerobic metabolism is oxygen-limited and requires a sufficient level of dissolved oxygen to be optimally performed (Bergstedt *et al.,* 2021; Chatterjee e*t al.*, 2004; Mallekh & Lagardere, 2002; Obirikorang *et al.,* 2020). Under the aquaculture environment, the oxygen partial pressure (PO_2) gradually decreases because of fish respiration (Methling *et al.,* 2013). The long-term hypoxia decreases the capacity of aerobic activity by lowering the maximum metabolic rate (MMR) (Figure 5) (Domenici *et al.*, 2017). The minimum rate of oxygen uptake required to support vital maintenance activities in ectotherms is called the standard metabolic rate (SMR) and the difference between MMR and SMR gives the aerobic metabolic scope (MS) (Rosewarne *et al.,* 2016). Other activities such as growth, reproduction, and swimming require an ability to increase oxygen uptake above the SMR and within the confined aerobic metabolic range (Domenici *et al.,* 2017; Rosewarne *et al.,* 2016). Therefore, under a hypoxic environment, fish maintain oxygen uptake at or slightly above SMR, therefore, limiting the aerobic metabolic scope necessary for activities other than basic maintenance such as growth, reproduction, and swimming (Methling *et al.,* 2013).

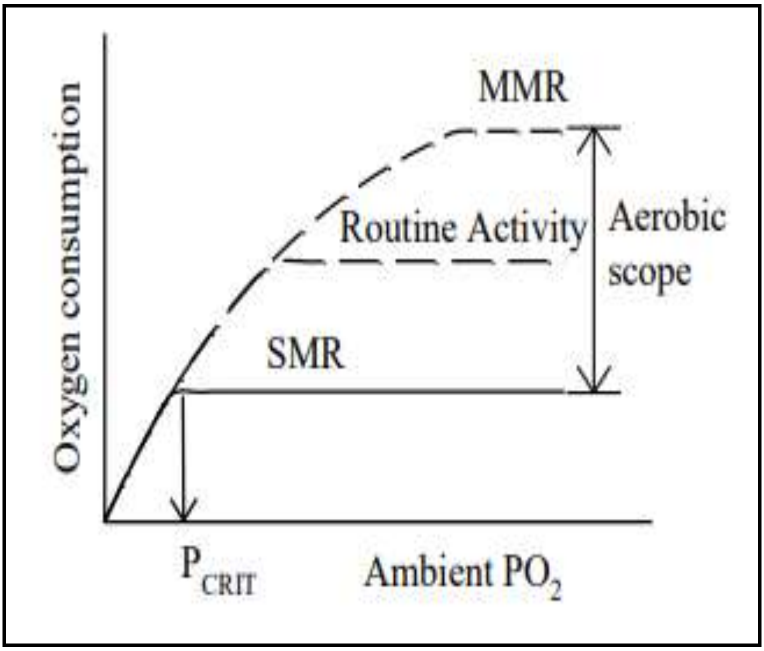


Figure 5: Illustration of metabolic rate (MO_2) as a function of dissolved oxygen level (PO_2) (Tirsgaard *et al.,* 2015).

The majority of fish species can cope with the level of oxygen available in water and control their oxygen absorption through making behavioural, ventilatory, and circulatory modifications. The fish with this oxygen compensation ability is known as "oxygen regulators". However, in this case, the minimal metabolic rates (SMR) can only be maintained at a certain oxygen level (Figure 5)

(Claireaux & Chabot, 2016; Methling *et al.,* 2013). The critical partial pressure of oxygen (P_{CRIT}) (Reemeyer & Rees, 2019) is the point below which all behavioural and physiological adaptations are exhausted and oxygen uptake exclusively dependent on PO_2. (Tirsgaard *et al.,* 2015).

Fish can increase gill ventilation and heart function to make compensation for oxygen intake to cope with oxygen shortage circumstances, however, the compensation mechanism relies on the species and the length of exposure (Chapman & McKenzie, 2009; Claireaux & Chabot, 2016). To conform to ambient PO_2 at higher levels, the P_{CRIT} for the maximum oxygen consumption rate (MMR) is significantly higher than for the SMR (Figure 5) (Eliason & Farrell, 2016). The justification for this is that since fish have already altered their ventilation and circulation, they have no further means of enhancing oxygen intake (Farrell *et al.,* 2009; Methling *et al.*, 2013). With decreasing PO_2, the scope of metabolism will eventually disappear at P_{CRIT} (Claireaux & Chabot, 2016; Min *et al*., 2019).

1.4.12 The effect on feeding and biochemistry

Changes in the biochemical and physiological status of cultured fish can be used to determine the overall health status during production. Changes in these parameters above or below the optimum level indicates the presence of stressors such as improper feed, overcrowding, toxic chemicals, excessive organic compounds, or even changes in routine aquaculture practices (Abdel-Tawwab *et al*., 2019). In many fish species, the hypoxia stress, cause a complicated cascade of neurological, behavioural, and physiological alterations (Abdel-Tawwab *et al.*, 2019; Gil Barcellos *et al*., 2004). Under the long exposure to hypoxia, fish undergo a variety of physiological and biochemical changes to help them adapt (Behrens *et al.,* 2012; Oikawa-cardoso *et al.,* 2021). These changes include decreased metabolic rate, increased ventilation and anaerobic respiration, and reduced hemoglobin oxygen binding (Hb-O_2) affinity (Behrens *et al.*, 2012), reduce oxygen consumption by slowing their activity and increasing their red blood cells (RBCs) and haemoglobin levels (Gil Barcellos *et al.*, 2004).

Nutritional metabolism is also impacted by hypoxia. Hypoxia effects on feeding and feed utilization have been reported for many species, including European sea bass (*Dicentrarchus labrax*) (Brest & Vivantes, 2001), common carp (Bernier *et al.,* 2012), Atlantic Cod, (*Gadus morhua*) (Behrens *et al.*, 2012) and Nile tilapia (Obirikorang *et al.,* 2020). Poor feed utilization was recorded in Nile tilapia *O. niloticus,* exposed to DO levels below 3.0 mg/L (Tran-Duy *et al.,* 2008). A study conducted by Magnoni et al., (2018) revealed that hypoxia affects all aspects of fish feeding, including energy metabolism. Anaerobic glycolysis produces less ATP than aerobic glycolysis, therefore substrates like glucose and glycogen are heavily utilized, which causes the building up of lactate (Muusze *et al.*, 1998; Oikawa-cardoso *et al.,* 2021; Thomas *et al.,* 2019). Poor glucose disposition and low aerobic glycolysis were observed in a study by Muusze *et al.* (1998), in freshwater fish exposed to long-term hypoxia stress.

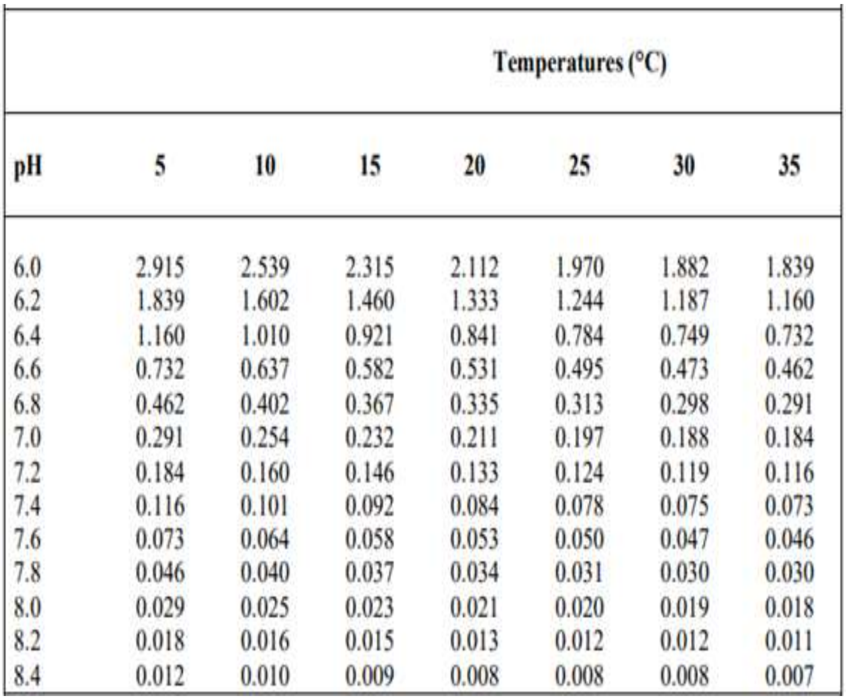
1.4.13 pH and Carbon dioxide

The concentration of H^+ in water determines pH (Wurt & Durborow, 1992). It is a numerical value that represents the acidity or alkalinity of water on a scale of 0 to 14, with 7 representing neutral. Acidic water has a pH of less than 7, while basic or alkaline water has a pH greater than 7 (Boyd, 2008). Pond water with a pH close to 7.4 is ideal because fish have an average blood pH of 7.4 (Makori *et al.,* 2017). In fish, a good pH has a range of 6.5 to 9.0. pH. A pH less than 4.0 or greater than 11.0 always results in death (Boyd & Lichtkoppler, 1979). In a fish pond, pH can change on a daily basis due to complex interactions between carbon dioxide, hardness, alkalinity, photosynthesis, and respiration (Bhatnagar & Devi, 2013). In a pond, pH is always low because in the nighttime increased carbon dioxide concentrations from respiration, which interact with water to produce carbonic acid (Obirikorang *et al.*, 2020).

The ability of water to resist changes in pH is known as alkalinity (Wurts & Durborow, 1992). Alkalinity is a measure of the total concentration of bases in pond water, which is contributed by alkalinity components such as carbonates, bicarbonates, hydroxides, phosphates, and borates. In ponds, these bases are responsible for neutralizing/buffering pH by reacting with CO_2. There is a strong relationship between water pH, CO_2, carbonates, and bicarbonates, however, in many culture systems dissolved CO_2 is rarely measured. For example, pH concentration in fish ponds decreases when the rate of CO_2 excretion exceeds removal (e.g., by photosynthesis or diffusion across the water surface) (Tadesse *et al.,* 2004).

Fish are believed to withstand carbon dioxide concentrations below 10 mg/l (Hargreaves & Brunson, 1996), although, the susceptibility to the dissolved CO_2 varies between species. High CO_2 concentrations or low water pH inhibit the rate at which CO_2 from the fish's own metabolism to be discharged from the blood through the gills, resulting in a higher concentration of CO_2 in the fish's blood (Hargreaves & Brunson, 1996). Elevated CO_2 directly interferes with the rate at which oxygen is consumed in fish even if the water has maximum dissolved oxygen saturation (Obirikorang *et al.*, 2020). Measuring CO_2 in water is complex and costful. This is the reason why despite of CO_2 having toxic effect in fish, most fish farmers are mainly concentrated in measuring dissolved oxygen level. However, the level of CO_2 in water can be extrapolated using the formulae developed by Wurts & Durborow, (1992) (Table 1).

Table 1. The relationship between alkalinity, pH, and water temperature on extrapolating the levels of dissolved CO_2 in the water.



	Temperatures (°C)						
pH	5	10	15	20	25	30	35
6.0	2.915	2.539	2.315	2.112	1.970	1.882	1.839
6.2	1.839	1.602	1.460	1.333	1.244	1.187	1.160
6.4	1.160	1.010	0.921	0.841	0.784	0.749	0.732
6.6	0.732	0.637	0.582	0.531	0.495	0.473	0.462
6.8	0.462	0.402	0.367	0.335	0.313	0.298	0.291
7.0	0.291	0.254	0.232	0.211	0.197	0.188	0.184
7.2	0.184	0.160	0.146	0.133	0.124	0.119	0.116
7.4	0.116	0.101	0.092	0.084	0.078	0.075	0.073
7.6	0.073	0.064	0.058	0.053	0.050	0.047	0.046
7.8	0.046	0.040	0.037	0.034	0.031	0.030	0.030
8.0	0.029	0.025	0.023	0.021	0.020	0.019	0.018
8.2	0.018	0.016	0.015	0.013	0.012	0.012	0.011
8.4	0.012	0.010	0.009	0.008	0.008	0.008	0.007

$[CO_2]$ in mg/L = (corresponding factor of pH and temperature) x water alkalinity in mg/L

1.4.14 Hypercapnia

In any natural aquatic system, if the total volume of water dissolved CO_2 is in equilibrium with atmospheric CO_2 (Tirsgaard *et al.*, 2015), then the increased partial pressure of CO_2 (pCO_2) is refered to Hypercapnia. Carbon dioxide (CO_2) tends to be ignored (is not often measured) in most aquaculture systems, although its elevated level can caurse critical effects on fish performance and well being (Skov, 2019). In the natural environment, fish will always avoid areas with high pCO_2 levels. However, under a confined environment, i.e. tank or pond system, fish can experience a high level of pCO_2 as a result of metabolic respiration and surface diffusion from the surrounding environment (Damsgaard *et al.,* 2015). Naturally, dissolved CO_2 undergo a chemical reaction quickly by reacting with water to form carbonic acid, which dissociates and produces bicarbonate while releasing H^+ leading to a decrease in water pH (Shartau *et al.*, 2019) i.e. CO_2 (gas) ↔ CO_2 (dissolved) + H_2O ↔ H_2CO_3 ↔ $HCO3^-$ + H^+ ↔ $CO3^-$ + $2H^+$

Hypacapnic water prevents ion exchange between fish gills and the surrounding environment. As a result, the metabolic CO_2 in the fish body accumulate and decreases the blood pH which disturbs the acid-base status, causing respiratory acidosis (Shartau *et al.*, 2016). In fish, gills are responsible for maintaining an osmotic balance between fish blood and surrounding water through an ion exchange system, however, under a high CO_2 environment, the ability of gills to buffer metabolic acid loads is highly reduced (Evans *et al.,* 2005).

1.4.15 The effect on physiology and metabolism

The main physiological effect of high CO_2 levels is a reduction in blood pH, which lowers hemoglobin affinity and oxygen-carrying ability (Montgomery *et al.,* 2019; Shartau *et al.,* 2019), which, in turn, impacts both metabolic activities and osmotic balance (Brauner *et al.,* 2019). The effect of elevated CO_2 has been observed even at level below 5 mg L^{-1} in some marine species, but in most fish species the metabolic rate and acid base status is impared whe pCO_2 level goes beyond 10 mg L^{-1}. For example, in Atlantic salmon, CO_2 has been reported to be a threat and prevent growth at higher levels between 15 and 20 mg/L (Fivelstad *et al.,* 2013). Standard metabolic rate (SMR), maximum metabolic rate (MMR) and aerobic metabolic scope (MS) are negatively affected by elevated level of CO_2 and high concentration of dissolved CO_2 above 30mg/l can directly affect fish physiological functions, including gill ion exchange and enzyme functions (Kaya *et al.,* 2016). In most cases, control measures to reduce pCO_2 in the culture system involve a chain of conversion process i.e. changing carbonic acid to HCO_3^-/Cl^-. These processes are always costly and takes some time (Fivelstad et al., 2007). In culture system, fish oppose extra H^+ and $HCO3^-$ through ion regulation mechanism, which involve gills, blood and muscle tissues. However, this buffering process prevents gills ion exchange and increases acid – base homeostasis and its maintenance demands a lot of energy and recovery time, hence, affect growth, metabolism, food conversion and even survival rate (Lefevre *et al.,* 2014).

High concentration of CO_2 also causes rapid dropping in blood pH which in turn reduces hemoglobin capacity for oxygen uptake (Kaya *et al.,* 2016). Fish can regulate and compensate for extra H+ (acidic condition) through an ion-exchange mechanism via gills epithelial or by an accumulation of $HCO3^-$ in exchange with Cl^- (Shartau *et al.,* 2020), Elevated CO_2 concentration can be high in a confined culture system such as tanks and recirculating systems (RAS) due to high stocking density than ponds or raceways where most carbon dioxide is continuously removed through photosynthethis by aquatic plants. In Fish, the response to an elevation plasma pCO_2 as a results of high CO_2 is the reduction in plasma pH, which significantly reduces oxygen transport (Eddy *et al.*, 1977). However, in some species, the plasma pH is restored close to normal values within 2–7 days as a result of this (Heisler, 1984, 1986). Other physiological effects on fish exposed to hypercapnia include hyperventilation (Janson and Randall, 1975; Smith and Jones, 1982; Fivelstad *et al.,* 1999; Hosfeld *et al.,* 2008), reduced branchial chloride influx rates (Perry *et al.,* 1986a; Goss et al., 1994) and reduced plasma chloride (Lloyd and White, 1967; Eddy *et al.,* 1977; Fivelstad *et al.,* 1999, 2003a,b). Reduced growth, reduced feed conversion efficiency and nephrocalcinosis are commonly observed effects of long-term freshwater exposures to carbon dioxide (Smart *et al.,* 1979; Smart, 1981; Fivelstad *et al.,* 1999, 2003a,b, 2007; Hosfeld *et al.,* 2008).

1.4.16 Hypercapnia effect on feeding

In addition to decreased feed intake, hypercapnia also has been reported to affect other parameters for feeding and energy metabolism in fish. Parameters such as excretion of waste products (i.e. faeces, urea and ammonia) and protein synthesis are also affected by hypercapnia. Recent studies on the European eel (*Anguilla anguilla*) (Methling *et al.,* 2013) and Atlantic cod have indicated that the reduced growth rate observed is caused by a prolonged digestion time. This may be the result of reduced feed intake, which have been observed in Atlantic salmon (Hosfeld *et al.,* 2008) and in other species e.g. sea bass (*Dicentrarchus labrax*) (Cecchini *et al.,* 2001) and spotted wolfish (Anarhichas minor) (Foss *et al.,* 2003). This suggests that eels exposed to hypercapnia absorbe less protein from their diet. Further it suggests that there is a greater faecal loss. Together, these observations indicate that hypercapnia can cause a disturbance in the protein metabolism of fish towards an increased catabolism (breakdown) and a decreased anabolism (synthesis). Moderate hypercapnia levels (< 10.000 µatm.) has been found to reduce growth in Atlantic salmon (*Salmo salar* L.) and the Atlantic cod (Hosfeld *et al.,* 2008; Moran and Støttrup, 2011) as a results of reduced feed intake (Cecchini *et al.,* 2001; Foss *et al.,* 2003).

Tilapia can tolerate a wide range of pH, estimated from 5 to 11, but optimum growth is achieved when cultured in water with a pH between 7.0 and 9.0. Growth in Nile tilapia is negatively affected in acidic waters below 3.7 pH (El-Sherif & El-Feky, 2009). Exposed tilapia to either a high acidic concentration (low pH) below 4 or an alkaline condition with high pH above 10 may cause imbalanced osmoregulation resulting in poor growth and if the condition persists for some time resulting to death (Wurts & Durborow, 1992). Nile tilapia are reported to be highly affected by alkalinity rather than acidic conditions (Rebouças *et al.*, 2016).

1.5 General Materials and methods

1.5.1 Study areas

This study was partly implemented in the United Republic of Tanzania, latitudes 1°S and 12°S and longitudes 29°E to 41°E. The first study was conducted in four selected local agro-ecological zones in Tanzania (Southern highland, Northern, Eastern, and Lake zones). Three experiments were conducted at Technical

University of Denmark (DTU), National Institute of Aquatic Resources, Section for Aquaculture, North Sea Science Park, DK-9850 Hirtshals, Denmark.

1.5.2 Study design

The main objective of this PhD thesis was to study the variation status of pond water quality parameters and evaluate the basic physiological functions of Nile tilapia exposed to the variation in water quality parameters. The first study (Paper I) which was conducted in Tanzania focused on studying the diurnal and seasonal changes in pond water quality variables. Study zones were purposely selected based on fish farming characteristics and availability of fish ponds, specifically Nile tilapia ponds.

Based on the information obtained from paper I, three experiments were designed and conducted at DTU. First experiment (Paper II) was conducted using respirometers to evaluate the effect of elevated dissolved CO_2 (PCO2) on three major physiological characteristic (i) whole animal oxygen uptake in order to estimate metabolic capacity and aerobic scope as a function of CO_2 (ii) hematological parameters and (iii) acid-base disturbance and regulation.

The second experiment (Paper III) examined feeding and feed utilization efficiency under three different diurnal interactions of dissolve oxygen (hypoxia) and carbon dioxide (hypercapnia) in Nile tilapia. The setup for diurnal interaction of hypoxia and hypercapnia was set to mimick the naturally outside pond environment.

The third experiment (Paper IV) focused on environmental temperature variation and dissolve oxygen saturation on cardio respiratory function and metabolic process in Nile tilapia The experiment used an optimum temperature at 26°C as a control, which represent normal optimum temperatures for Nile tilapia. Then temperature was gradually elevated by 0.1°C min^{-1} representing acutely increase in temperature in the fish pond if it happens during the day time in hot season. In addition, dissolved oxygen were kept at normoxia (100%), hypoxia (70%) and Hyperoxia (130%) saturation. During the study period, Critical thermal maximum (CTmax), Opercula movement (OBM), heart rate (HBM) and Lose of equilibrium (LOE) were estimated with respect to the changes in temperature upon changes in DO saturation

1.5.3 Thesis organization

This thesis is structured according to publishable manuscript format of the Sokoine University of Agriculture. This thesis has seven chapters starting with an extended abstract which summarises the objective and results of the study. General intoduction is presented in chapter one and provides an overview of the background information about aquaculture, pond fish production and water quality. Chapter one also include literature review section which presents aspects of Tilapia production in Tanzania, pond farming system and waterquality parameters and their effect on fish physiolgy and growth. Furthermore, chapter one has information about the general methodology, describing the study area, design and an overview of thesis structure.

Chaper two to five include four scientific papers which which present the experimental procedure and the findings. from studies and experimetal work that was conducted to answer the proposed specific objectives. The informetion in these chapters are presented as puplished or journal submited papers and manuscripts. Chapter six consist of general discusion of the main findings. Chapter seven consist of general conclusions and recommendations.

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CHAPTER TWO

PAPER ONE

Diurnal and Seasonal Rhythms in Pond Water Quality Parameters, Farming Characteristics and Pond Yield of Cultured Nile tilapia in Tanzania.

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Abstract

Over the last decade, pond farming of Nile tilapia (*Oreochromis niloticus*) gained increasing interest in Tanzania as a means to increase aquaculture fish production. Unfortunately, little is known about the current status of the farming practices, diurnal and seasonal fluctuation of ponds water quality and their effects on annual fish yield. This study was conducted in four agro-ecological zones with different climatological aspects. The main objectives were to describe the present diurnal and annual trends in pond water quality parameters and to assess the farming practices and management strategies used in Nile tilapia pond farming. Water quality parameters (Temperature, DO, pH, TDS and Conductivity), annual fish yield and pond primary productivity were assessed. Results revealed that temperature differs significantly between the study zones with the highest in the Eastern and the lowest

Southern Highland zones. Additionally, the temperature in the Northern zone and Southern highlands during the cold season (June to September) was below the level required for Nile tilapia growth suggesting that Nile tilapia might be stocked during the warm season to achieve the best growth. Other water quality parameters vary between seasons within zones and were within the range suitable for Nile tilapia growth. The annual fish yield was higher in the Lake zone and Eastern zone suggesting a positive relationship between temperature and fish yield. High net algal primary production during the warm season indicates the need for good water quality management to prevent algae blooming, which may result in acute and prolonged stress from hypoxia and hypercapnia, which are the primary physiological stressors for fish.

Keywords: Diurnal variation; Water quality; Nile tilapia; Fish Pond

1.0 INTRODUCTION

Nile tilapia raised in earthen ponds account for more than 90% of the Tanzania aquaculture produced fish (Mbiru *et al.*, 2020). The number of small-scale fish ponds has increased drastically across the country in the last decade, with over 20,000 ponds of 150 to 300 m^2 in size (Duijn *et al.*, 2018). The number of fish ponds is expected to increase even further in the coming years following the ongoing government agenda of increasing fish supply through aquaculture in order to meet the nation's rising demand for animal protein and to make up for the shortfall of fish protein previously supplied by the depleting capture fisheries. Indeed, given its financial advantages to disadvantaged rural populations, inland fish farming is also anticipated to expand further around the country in the near future (Kajungiro *et al.*, 2019a; Lugendo & Mwaijande, 2015). However, the expansion of fish production using ponds is jeopardized by the poor growth performance of cultured fish which is mainly reported to be attributed to the unavailability of quality feeds and seeds and lack of knowledge about the water use and water quality management of the water used for aquaculture purposes (Shoko *et al.,* 2014).

Water quality parameters in small water bodies such as earthen ponds varies on a daily basis, depending on the equilibrium reached with the regular physical and chemical characteristics of the surrounding environment (Sriyasak *et al.,* 2013). Water temperature, pH, ammonia, turbidity, total dissolved solids, and dissolved gasses including dissolved oxygen (DO) and carbon dioxide (CO_2) are among water parameters that are mostly constrained when climatic factors of the surrounding environment changes (Tadesse *et al.,* 2004). These water variables have a considerable impact on fish physiology and wellbeing, such that a minor change in certain compositions below or above the optimal level of a certain species can pose a significant physiological consequence (Ahn *et al.,* 2019; Majhi & Das, 2013).

In a fish ponds, water quality variables can change suddenly due to environmental occurrences (Temperature, wind and or photoperiod) or gradually as a result of ineffective production management (feeding, water exchange or fertilizing). Variation in temperature and or long hours of photoperiod for example may directly resulting into fluctuation in water temperature and other water quality parameters such as DO, CO_2 and pH in earthen ponds, which may cause an episode of either acute or chronic stress and consequently affects both the performance of the pond's biological communities such as bacteria and plankton (Kayombo *et al.,* 2002) as well as the physiology and welfare of the farmed fish (Shoko *et al.,* 2014; Tadesse *et al.,* 2004).

In temperate and tropical regions, temperature is a main variable controlling the ecology of the small water bodies such as ponds. Temperature is mainly referring to as abiotic master factor, as it is diurnally and seasonal fluctuate depending on the fluctuation of the temperature of the surrounding environment. Temperature play a significant role in affecting the physiology, metabolism and growth of fish. On the other hand, in addition to its impact on the living communities of the water, temperature can also control the magnitude of other water parameters like pH, ammonia, and the solubility of the dissolved gasses including dissolved oxygen (DO) and carbon dioxide (CO_2) among others. The optimum and tolerance limit to temperature vary by species and originality. For example, fish cultured in a pond in the temperate region are likely to be extremely affected by the temperature in the summer. For Nile tilapia, a tropical species, the optimum level is between 25 and 27°C (Pandit & Nakamura, 2010). For Nile tilapia, any temperature below 16°C will prohibit grow and their survival is limited at temperatures below 10°C and above 40°C (Azaza *et al.,* 2008).

The ecology of fish pond in the tropical area, in addition to its vulnerability to temperature, also controlled by photoperiod (12 hours dark and 12 hours light). In the presence of sunlight during the day, pond plants (algae) fix CO_2 and produce 0_2 through photosynthesis. This process increases the DO levels leading to high oxygen saturation (hyperoxia) (Jacobs *et al.,* 2008). For example, a DO level of 300% saturation have recently been reported in a fertilized earthen pond stocked with Nile tilapia in the afternoon (Gyamfi *et al.,* 2022). The reversible reaction is anticipated to occur at night (dark period) when all aquatic organisms such as bacteria, fish, and algae consume DO and release CO_2 as a byproduct of breathing, resulting into low dissolved oxygen level (hypoxia) and elevated level of CO_2 (hypercapnia) (Montgomery *et al.,* 2019).

The elevated CO_2 level and low oxygen in the in the pond during night hours, apart from posing stress to fish can also resulted into fluctuations in the acidity and alkalinity of the water, which may cause stress and ultimately alter the pond's entire ecological cycle. Dissolved oxygen is an essential part of the aerobic process in fish (Abdel-Tawwab *et al.,* 2015). Low DO (Hypoxia) affect most physiological processes, especially growth and feed consumption (Bernier *et al.,* 2012). A recent study by Obirikorang *et al*. (2020) has revealed a diurnal drop in DO in a fish pond at a level below 3 mg L^{-1} in the late-night hours. A study by Bergstedt *et al.* (2021) showed that a dissolved oxygen saturation of fewer than 3 mgL-1 may result in metabolic collapse, low oxygen consumption and loss of equilibrium. In contrast, dissolved carbon dioxide (CO_2) is rarely measured during fish production. However, in most cases, its level is normally extrapolated from the level of water pH and bicarbonate (Wurt & Durborow, 1992). High [CO_2] above 15mgL-1 (hypercapnic), become toxic, lowers the water pH and prevents ion exchange between the fish and the water environment resulting in CO2 accumulation in the fish's blood and causing respiratory acidosis (Brauner *et al.,* 2019), primarily affecting the fish's capacity to oxygen consumption (Methling *et al.*, 2013), perform cardiorespiratory functions (Kohin *et al.*, 1999), and feeding characteristics (Bernier & Randall, 1998).

In Tanzania, pond production of Nile tilapia is mostly a semi-intensive system whereby farmers fertilize their ponds and feed supplementary diets (Pius *et al.,* 2021), without the use of water treatment technologies (Shoko *et al.*, 2014). On the other hand, pond farming of Nile tilapia is practiced in the context that Tanzania is a tropical country with uniform environmental conditions. However, the country has a wide range of different agroecological zones and no consistent environmental conditions (Odiyo *et al.*, 1999). Temperature, wind, and photoperiod differ dramatically between zones and seasons (Luhunga *et al.*, 2018) which are likely to lead to differences in local climatic conditions which may affect the water quality parameters in fish ponds. Given that, the pond environment is unstable, it is crucial for fish farmers to fully understand the diurnal and seasonal trends of water quality parameters for them to create a stress-free environment that meets production standards and promotes optimal fish growth.

Currently, information about Nile tilapia pond farming practices and water quality management during production in unavailable. Similarly, being a tropical country with different agroecological zones, information about local climate variation between different zones and how it affects water quality in the tilapia production ponds is not yet documented. Therefore, this study aims to gather complete data on the current Nile tilapia pond production methods and how water source and water management techniques affect pond fish production. The study attempted to document daily and seasonal trends of pond water quality parameters in different agroecological zones of Tanzania.

2.0 MATERIALS AND METHODS

2.1 Study Sites

Tanzania, lies between latitudes 1°S and 12°S and longitudes 29°E to 41°E. As a tropical County, Tanzania has several physical features that contribute to its local climate variabilities. This study was conducted in four selected local agro-ecological zones (Southern highland, Northern, Eastern, and Lake zone), Figure 1. Detailed local meteorological, geographical locations and characteristics of the selected study sites are presented in table 1.

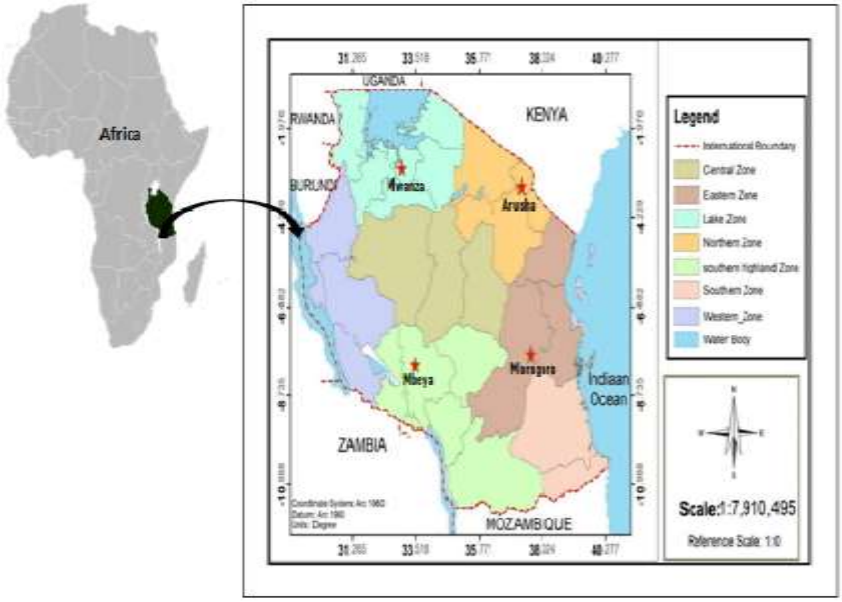


Figure 1: Map of Tanzania showing agroecological zones, and stars pointing to the four selected study sites.

Table 1: A detailed description, geographical locations and general climatic characteristics of the selected experimental farms (Study sites).

Sampling location	**Longitude (degrees)**	**Latitude (degrees)**	**Geographical characteristic**
Eastern Zone (Morogor)	-685255	37.64938	Eastern zone: Tropical climates, with yearly temperatures ranging from 19 °C to 32 °C with a mean of 27 °C. The coldest month (June-September) have temperatures ranging from 19 °C to 26 °C, while the warmest months (October-February) have temperatures ranging from 25 °C to 32 °C. The average annual precipitation is 1120 mm. The highest wet month is April, which has mean precipitation of 359mm. The average humidity is 65%.
Southern highland Zone (Mbeya)	-9.16870	33.55825	The southern high land zone: The healthy climate of the semi-temperate highlands, with an average annual temperature of 17.7 °C and a maximum temperature of 23.79 °C. In the cold season (June - September), temperatures range from 10 °C to 21 °C, whereas in the warm season (November - January) temperature range from 20.1 °C to 26.8 °C. With mean annual precipitation of 1118 mm and high humidity of 55%, January experiences high precipitation of 196.1 mm.
Northern Zone (Arusha)	-3.32359	37.297854	Northern zone: The range of the daily average temperature is 15 °C to 25.5 °C. In the warm season (November to January),

			the average temperature varies between 19 °C and 25 °C, while the coldest season (June - September) has a temperature range of 14.5 °C to 20.5 °C. Has average annual precipitation of 1125 mm, with April being the wettest month with 340 mm of precipitation.
Lake Zone (Mwanza)	-2.53832	32.83015	Lake zone: Litle seasonal temperature variation. With a mean temperature of 23.2 °C and an annual temperature ranging from 16.4 °C to 28.8 °C. 16.4 °C to 27.2 °C is the temperature range during the cold season (June - September), and 19.1 °C to 28.8 °C is the range during the summer season. The highly moist lake regions, experience humidity ranging from 55 to 68% and a mean annual rainfall of 1050 mm.

Note: (-ve) degrees means below the equator.

2.2 Sampling Procedures

2.2.1 Water Quality Measurement

Pond water quality assessment was done between June 2019 and April 2021. At each zone, three ponds of size range between 150 and 300 m^2 and depth between 1.0 and 2 m were selected and used as experimental ponds for seasonal water quality measurements. Ponds selected were categorized as small-scale semi-intensive ponds and selection was based on their similarities in physical features such as dimension (size, depth) and management practices (stocking density, fertilization and feeding style). Water sampling was performed in two seasons, the cold season (June - August) and the warm/hot season (November-January). Sampling involved insitu measurement of water quality parameters whereby a multiparameter water quality meter probes (Hanna field instrument, model HI98194, USA) was used to record water temperature (°C), dissolved oxygen (mg L^{-1}), pH, total dissolved solids (ppm) and electric conductivity (µS cm^{-1}). Every month of the sampling season measurement was done daily one week four times a day Morning, (06:00 am, Afternoon (2:00 pm), Evening (18:00 pm) and Mid-night (00.00 am). In addition, three days in every sampling month a 24 hours sampling at one-hour interval was done for the assessment of diurnal trends of pond water quality parameters.

2.2.2 Farming Practices and Estimation of Annual Fish Yield

To get information on the current Nile tilapia pond fish farming characteristics and farmers' practices, interviews and a focus group discussion were adopted. A survey was conducted from June 2019 to March 2020. Sampling size involved active pond fish farmers from the selected surveyed agro ecological zones. The interviewed personnel were randomly selected and the desirable number of respondents of a particular zone was calculated using the formular by Yamane 1987.

$$n = N/1+N(e)^2$$

where n represents sample size (the number of assessed farmers), N is the total number of pond fish farmers in the zone, and (e) is the level of precision (0.05).

A set of questionnaires with semi-structured questions was used to obtain both quantitative and qualitative information that addressed the objectives and hypothesis of the study. The questionnaire was pre-tested to assess its flexibility and validity. To evaluate annual pond yield (production) across selected agro-ecological zones, production data were collected through field surveys using a questionnaire, the following information were collected annual production output, pond size, stocking density and production cycle.

2.2.3 Primary Productivity Assessment

Primary productivity assessment was done twice at each site during hot and cold seasons. Using the Light and Dark bottle oxygen method (Silva & Silva, 1979), a pair of 1-litre glass BOD bottles were used in this experiment where one of the paired bottles was black to exclude light and the other was clear to admit light and allow photosynthesis. These pairs of bottles were replicated three times. At each site, the initial time and oxygen concentration were recorded by using oxygen probe (Hanna, multi-parameter water quality meter, USA). The average pond depth was 1.5 m, therefore, during experimentation (around 11 am to 2 pm), water sample for primary productivity was collected between 0.5 and 0.75 m and then suspended to the same depth. Initial oxygen concentration was recorded at the beginning (0 min) of the incubation and final at the end of the incubation (30 min) for both light and dark chambers. At the end of the experiment, the production rate was determined as follows:

$$\text{Respiration rate (mg } O_2\text{/L/hr)} = \frac{\text{(Final dark DO} - \text{initial DO)}}{\text{Time}}$$

$$\text{Gross productivity (mg } O_2\text{/L/hr)} = \frac{\text{Light DO} - \text{dark DO}}{\text{Time}}$$

$$\text{Net Productivity (mg } O_2\text{/L/hr)} = \frac{\text{Light DO} - \text{initial DO}}{\text{Time}}$$

2.3 Data Analysis

Statistical analysis was performed using SigmaPlot, v. 14.5 (Systat Software, Inc., Chicago, IL, USA). Data were tested for Normality using the Shapiro-Wilk test and the likeness of variances was assessed using Bartlett’s test. Diurnal and seasonal water quality parameters and primary productivity are presented as means ±SD. Paired T-test was used to test differences in productivity between seasons within different zones. All tests were applied at $p < 0.05$ significant levels.

3.0 RESULTS

3.1 Annual, Diurnal and Seasonal Variation in Water Physico-chemical parameters

3.1.1 Annual

Table 2 displays the findings of the average annual pond water physico-chemical parameters of the sites that were investigated. The annual average temperature differs significantly between zones (Table 2), with the highest value recorded in the Eastern zones (Dar es Salaam) at 26.79 ±2.53 ^{0}C and the lowest in the Southern highland (Mbeya) at 22.16 ±3.50 ^{0}C. The average annual pond DO saturation (mg L^{-1}) were higher in the Eastern and Northern zones 6.05 ±1.48 mg L^{-1} and 6.02 ±0.88 mg L^{-1} respectively than in the southern highland and lake zone. Ponds in the Lake zone and Southern highland had mean annual pH values of 8.81 ±0.75 SD and 8.71 ±0.75 SD, respectively, and were basic with high pH all year round, while the water in the ponds in the Eastern zone was rather acidic with an annual mean pH of 6.85 ±1.07 SD. The average annual Total Dissolved Solids (ppm) in the ponds of the lake zones was higher (413.83 ±6.28 SD ppm) than the average annual Total Dissolved Solids (ppm) in the ponds of the eastern zones (81.83 ±5.52 SD). Similar to TDS, results also revealed higher conductivity in the lake zones (881.75 ±71.04 SD µS cm-1), than the rest of the zones (Table 2).

Table 2: Mean ±SD of annual water physical-chemical parameters of ponds cultured with Nile tilapia in four agro-ecological zones recorded from June 2019 to April 2021.

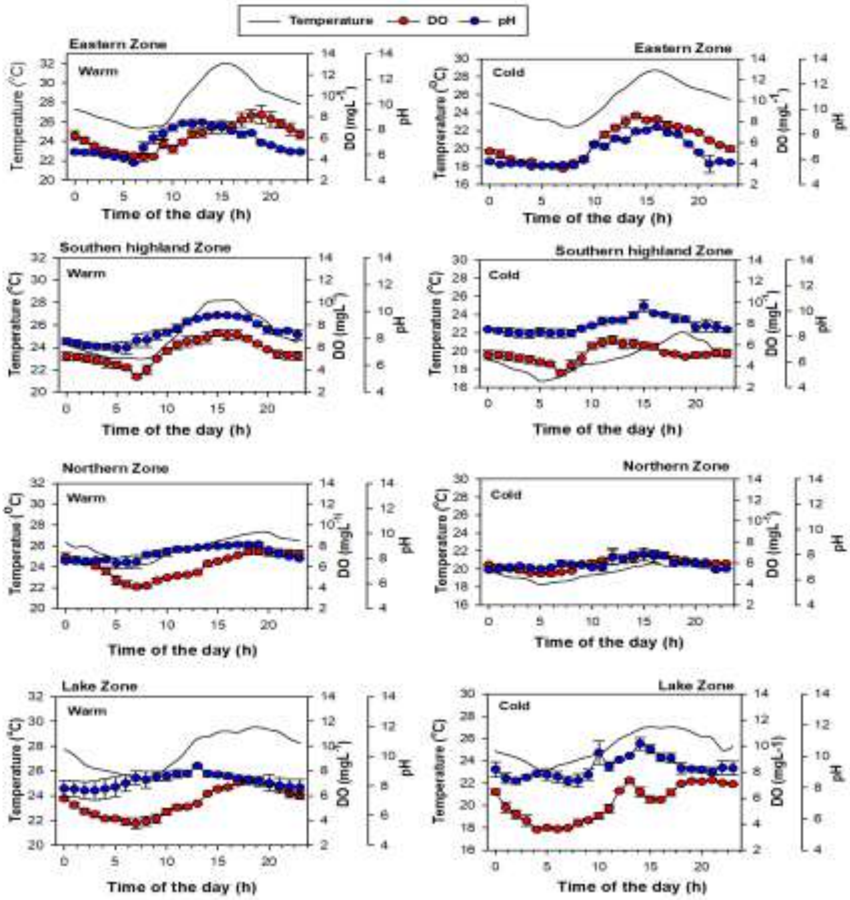
	Study Sites			
Parameters	**Dar-Es Salaam**	**Mbeya**	**Moshi**	**Mwanza**
Temperature (ºC)	26.79 ±2.53^{a}	22.16 ±3.50^{b}	22.71 ±3.31^{b}	26.42 ±1.84^{a}
Dissolved Oxygen (mg L^{-1})	6.05 ±1.48	5.39 ±0.94	6.02 ±0.88	5.58 ±1.27
pH	6.85 ±1.07	8.71 ±0.75	7.83 ±0.73	8.81 ±0.75
Total Dissolved Solids (ppm)	81.83 ±5.52	180.21 ±6.67	130.79 ±39.44	413.83 ±6.28
Electrical Conductivity (µS cm^{-1})	164.13 ±16.96	361.04 ±16.94	216.13 ±1.26	881.75 ±71.04

Note: Dar Es Salaam (Eastern), Mbeya (Southern Highlands), Arusha (Northern) and Mwanza (Lake zone)

3.1.2 Diurnal variation

A summary of diurnal trend in the water temperature, DO, and pH of the experimented ponds recorded during the cold season (June to September) and the warm season (November - January) are shown in Figure 2. The diurnal variation pattern of pH and DO followed the trend of temperature variation (Figure 2), with the average hourly water temperature being higher during the day and lower at night time. Diurnal temperature, DO and pH were higher in the warm season than in the call season in all zone. However, in the Eastern zone the maximum DO recorded in the warm season was higher by 5% (8.2 mg L^{-1}) than the highest DO recorded in the cold season. In the southern highland zone pond water reached a diurnal maximum DO concentration of 7.12 mg L^{-1} in the warm season, while in the cold season highest DO concentration was 6.5 mg L^{-1}. Ponds in the Northern zone had DO concentration value higher than 7.51 mg L^{-1} in the warm season and the lowest value of 4.08 mg L^{-1} while in the cold season the highest recorded diurnal

DO value was 6.70 mg L^{-1} and the lowest was 4.99 mg L^{-1}. The highest diurnal DO value in the Lake zone ponds was 7.16 mg L^{-1} in the warm season and 7.37 mg L^{-1} in the cold season and the minimum values were 3.75 mg L^{-1} in the warm season and 3.60 mg L^{-1} in the cold season.



Figures 2: Daily variation of pond water temperature (°C), Dissolved oxygen (mg/l) and pH recorded in the warm season (November – January) and cold season (June - September).

Pond water pH also shows variation with season among zones. Ponds in the Eastern zone were basic in the afternoon and late evening with maximum pH of 8.6 in the warm season while acidic in the nighttime and early morning hours with mean pH of 5.7, correspondingly, in the cold season water remains slightly alkaline during the day with pH 8.6 and acidic in the night with a pH value of 5.5. Ponds in the Southern highland zone were basic during the day with a pH of 9.7 and neutral at night pH 7.3 during the warm season while throughout the day during the cold season pH levels were higher between 8.2 and 10. Northern zone water was basic during the day hours pH of 9.1 and slightly neutral at night and early morning with a pH of 7.7 in the warm season while in the cold season water is acidic during the day pH 6.8 to high acidic in the night hours with pH of 5. In the warm season, ponds in the Lake zone were basic during the day and neutral at night with the highest recorded pH of 9.3 and 7.7 respectively while in the cold season maximum water pH was 10.4 in the day and the lowest 8.4 at night.

3.1.3 Seasonal variation between zones

The seasonal variations in the physical-chemical characteristics of the investigated ponds across the research zones are summarized in Table 3. A paired T-test revealed that all study zones' mean seasonal temperatures and pH varies significantly between warm and cold seasons (Table 3). The only region where DO concentration varied significantly across seasons was the southern highland zone ($p = 0.002$).

Table 3: Seasonal changes in ponds water physico-chemical parameter of the study zones

		Descriptive statistics						
		Warm		Cold		Paired T-test statistics		
Parameters	Zone	Mean	Range	Mean	Range	*df*	*t*	*p-value*
Temperature (°C)	Dar Es Salaam	28.14	25.3-32.0	25.37	22.5-28.7	23	27.62	< 0.001
	Mbeya	25.15	22.9-28.2	19.2	16.7-22.1	23	26.28	< 0.001
	Arusha	25.89	24.1-27.3	19.6	18.2-20.6	23	71.86	< 0.001
	Mwanza	27.57	25.4-29.5	25.24	23.1-27.0	23	18.42	< 0.001
Dissolved Oxygen DO (mg L^{-1})	Dar Es Salaam	6.1	4.14-8.23	5.92	3.53-8.56	23	0.902	0.376
	Mbeya	5.64	3.38-7.27	5.15	3.36-6.47	22	3.474	0.002
	Arusha	6.05	4.08-7.51	5.92	5.00-6.80	23	0.636	0.531
	Mwanza	5.46	3.75-7.16	5.63	3.58-7.38	23	-1.231	0.231
pH	Dar Es Salaam	7.06	5.4-8.6	6.58	5.4-8.6	23	3.598	0.002
	Mbeya	8.48	7.3-9.7	8.89	8.2-10.4	23	-5.579	< 0.001
	Arusha	8.38	7.6-9.1	7.28	6.8-8.1	23	15.835	< 0.001
	Mwanza	8.32	7.7-9.4	9.27	8.4-10.8	23	-9.246	< 0.001
Total Dissolved Solids (TDS) (ppm)	Dar Es Salaam	81.3	76-84	82.3	75-100	11	-0.475	0.644
	Mbeya	183.3	179-187	177.2	166-193	11	2.05	0.065
	Arusha	117.8	106-128	143.8	107-217	11	-1.916	0.082
	Mwanza	419.5	416-424	408.2	404-413	11	12.608	< 0.001
EC (µS cm^{-1})	Dar Es Salaam	159	151-175	169	146-200	11	-1.631	0.131
	Mbeya	377	369-385	345	340-355	11	23.675	< 0.001
	Arusha	215	214-216	217	216-218	11	-5.702	< 0.001
	Mwanza	946	900-1002	817	806-836	11	12.465	< 0.001

Note: Dar Es Salaam (Eastern), Mbeya (Southern Highlands), Arusha (Northern) and Mwanza (Lake zone)

While there is no difference in mean TDS between seasons in the other examined zones, the quantity of total dissolved solids in the lake zone varies dramatically between seasons (Table 3). In the Southern, Northern, and Lake zones, the mean seasonal conductivity was substantially higher during the warm season than during the cold season (Table 3).

3.2 Characteristics of Nile Tilapia Pond Farming

3.2.1 Demographic Characteristics of Pond Tilapia Farmers.

Table 4 presents the findings for the socioeconomic characteristics of the surveyed Nile tilapia pond farmers. The Chi-square test of correlation between farmer demographic characteristics and zonation reveals that there is no significant relationship between farmer socioeconomic variables and zonation. Results indicate that 83.3% of the farmers who work in pond Nile tilapia farming are men, while 16.7 are female. Level of education of the surveyed Nile tilapia farmers varies greatly among zones, generally, majority had college education (37.5%). The lowest level of education was observed in the Southern highland zone where majority had primary education (40%). Results also indicates that 92.5% of the farmers received fish farming management training.

Table 4: Social Economic Characteristics of Tilapia Farmers in Four Agro Ecological **Zones**

Parameters		**Study sites**												
		Dar es Salam		**Mbeya**		**Arusha**		**Mwanza**		**Overall**		**Chi square test**		
		No.	**%**	**No.**	**%**	**No.**	**%**	**No.**	**%**	**No.**	**%**	X^2	***df***	***P-value***
Gender	Male	24	80	24	80	27	90	25	83	100	83.3			
	Female	6	20	6	20	3	10	5	16	20	16.7	1.44	3	0.69
Education	Non-Formal	1	3.3	0	0	1	3.3	2	6.7	4	3.3			
	Primary	4	13.3	12	40	7	23.3	4	13.3	27	22.5			
	Technical	0	0	1	3.3	0	0	2	6.7	3	2.5			
	Secondary	12	40	9	30	12	40	8	26.7	41	34.2	15.22	12	0.23
	University	13	43.3	8	26.7	10	33.3	14	46.7	45	37.5			
Farming purpose	Business	10	33.3	7	23.3	8	26.7	8	26.7	33	27.5			
	Food	3	10	0	0	1	3.3	1	3.3	5	4.2	5.3	6	0.51
	Both	17	56.7	23	76.7	21	70	21	70	82	68.3			
Aquaculture Farming knowledge	Yes	26	86.7	28	93.3	27	90	30	100	111	92.5			
	No	4	13.3	2	6.7	3	10	0	0	9	7.5	4.2	3	0.24

Note: Dar Es Salaam (Eastern), Mbeya (Southern Highlands), Arusha (Northern) and Mwanza (Lake zone)

3.2.2 Farms and farming characteristics

Figure 3 A – D, summarizes the distribution of farms and farming characteristics among zones. Results revealed that there was no statistical significance ($p>0.05$) in the distribution of farming experience, pond number, and pond size among zones. Farmers have an average of 1 - 16 years of experience in fish farming, with farmers in the Northern zone having more experience than farmers in the other surveyed zones. The findings on pond ownership indicate that the vast majority of tilapia farmers own between one and four ponds, with farmers in the Lake zone having a higher number of fish ponds (3.7) than the remainder of the surveyed zone (Figure 3B). The average farm size varied significantly ($p<0.05$) among zones (Figure 3C). In general, the average pond size was 294.3 ± 22.98 m2 with the Lake Zone having a bigger average size (365.2±60.6 m2). Farmers in the lake zone have also invested lager areas for fish farming than farmers in the other zones (Figure 3D).

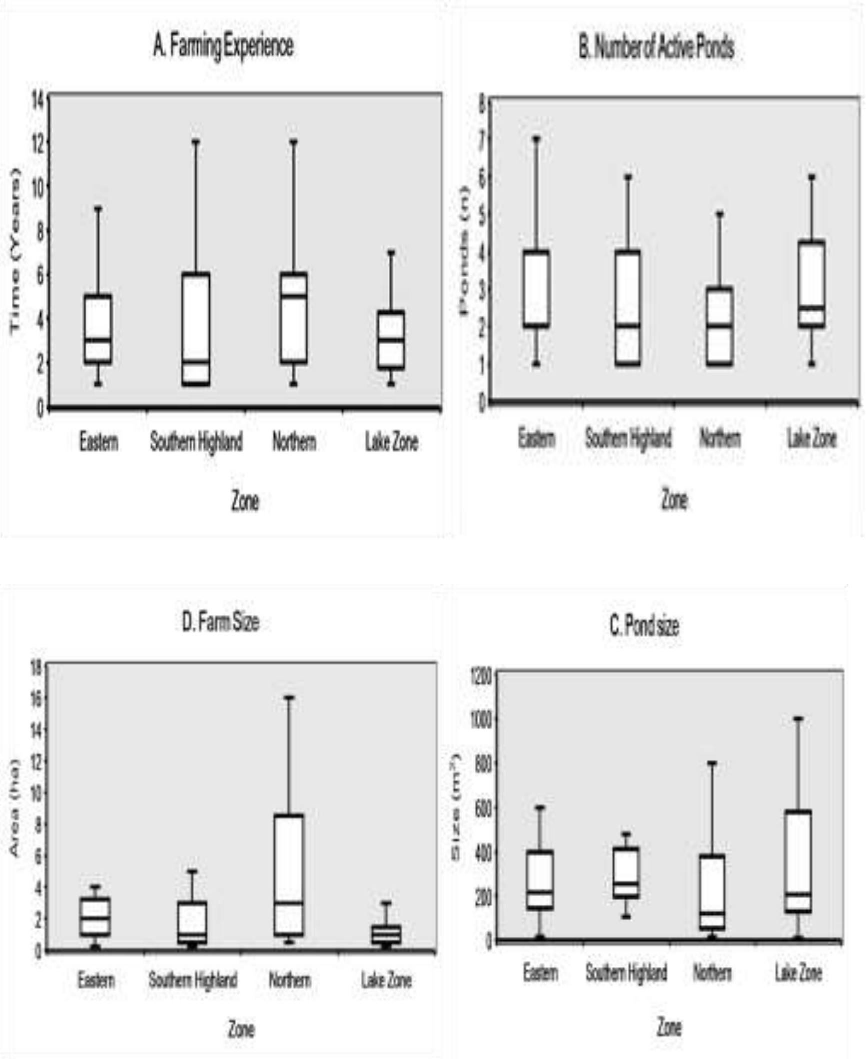


Figure 3: Distribution of farm characteristics: Farmers experience (A); Number of production Ponds (B); Size of pond used (C) and Farm size (D).

3.2.3 Feed and feeding practices

Fish yield in Tanzania is affected by factors such as feed quality and feeding procedures. Table 5 summarizes the results for feeds and feeding techniques. Chi square test for the feeding practices among farmers in all zones was done to find a relationship between feeding practices and fish yield, results show that there is a significance relationship ($p < 0.05$) between frequency of feeding, form of feed and type of fertilizer used among zones. According to the findings, 85.8% of tilapia farmers fertilize their ponds before stoking, with cow dung being the most commonly used fertilizer, where Southern highland zone was the leading (70%). Chicken manure was also found to be widely used (56.7%) in the Lake zone. All farmers stated that they supplemented their tilapia with artificial feeds, with 45% producing their own feeds from locally available ingredients and 29.2% importing feed from outside Tanzania.

One of the most important parts of fish farming is feeding frequency since it ensures that cultured fish receive an adequate distribution of food. According to this study, the majority of farmers in all zones (60%), with the Northern Zone leading with 76.7%, feed their fish twice daily.

Table 5: Types of manure, feeds and feeding practices for each study zone.

Parameters		Study Sites												
		Dar es Salam		**Mbeya**		**Arusha**		**Mwanza**		**Overall**		**Chi-square test**		
		No.	%	No	%	No.	%	No.	%	No.	%	*X^2*	*df*	*P–value*
Fertilizer	Yes	25	83.3	28	93.3	24	80	26	86.7	103	85.8			
	No	5	16.7	2	6.7	6	20	4	13.3	17	14.2	2.3	3	0.49
Type of manure used	Cow dang	13	43.3	21	70	20	66	9	30	63	52			
	Chicken	10	33.3	6	20	3	10	17	56.7	36	30	22.5	9	0.01
	Others	7	23.4	3	10	7	23.3	4	13.3	21	27.5			
Source of feed	Imported	10	33.3	11	36.7	4	13.3	10	33.3	35	29.2			
	Local made	12	40	15	50	17	56.7	10	33.3	54	45	8.3	6	0.21
	Both	8	26.7	4	13.3	9	30	10	33.3	31	25.8			
Feeding frequency per day	Once	5	16.7	11	36.7	3	10	1	3.3	20	16.7			
	Twice	20	66.7	16	53.3	23	76.7	13	43.3	72	60			
	Thrice	5	16.7	3	10	4	13.3	15	50	27	22.5	13.2	9	0.000
	Above three times	0	0	0	0	0	0	1	3.3	1	0.8			

Note: Dar Es Salaam (Eastern), Mbeya (Southern Highlands), Arusha (Northern) and Mwanza (Lake zone)

3.2.4 Water source and Water quality management practices.

Table 6 summarizes the findings on the sources of water, their availability, and their management during production. The Chi-squire result revealed that there was a significant difference ($p < 0.05$) in sources of water, water ownership and water re-use between zones. Generally, rivers account for 42% of the water used by pond fish producers, except in the lake zone, where most tilapia farmers (50%) rely on lake water, underground water contributes approximately 63.3% of all water utilized for fish farming in the Eastern zone. Results also show that 93.3% of the respondents claimed that accessibility of water for aquaculture is permanently available throughout the year.

The quality of water used for fish pond farming is very crucial in determining fish productivity and health, our study revealed that 82.5% of farmers in all zones are not doing any water quality assessment before filling and during production seasons. Additionally, most farmers (84.2%) discharge used pond water freely or return it back to the water source without any treatment. Only a few (15.8%) treat and re-use the water from tilapia ponds.

Table 6: Sources, availability and quality management of water used for fish culture

Parameters		Study Sites												
		Dar es Salam		Mbeya		Arusha		Mwanza		General		Chi-square test		
		No	%	No.	%	No.	%	No.	%	No.	%	X^2	df	*P - Value*
Source of water	River	4	13.3	26	86.7	19	63.3	2	6.7	51	42.5	125.902	15	0.0001
	Well	19	63.3	3	10	1	3.3	2	6.7	25	20.8			
	Borehole	6	20	0	0	9	30	9	30	24	20			
	Dam	1	3.3	1	3.3	0	0	2	6.7	4	3.3			
	Lake	0	0	0	0	0	0	15	50	15	12.5			
	Others (specify)	0	0	0	0	1	3.3	0	0	1	0.8			
Water availability	Seasonal	3	10	3	10	0	0	2	6.7	8	6.7			
	Permanent	27	90	27	90	30	100	28	93.3	112	93.3	3.2	3	0.36
Ownership	Own	20	66.7	4	13.3	8	26.7	4	13.3	36	30			
	Shared	10	33.3	26	86.7	22	73.3	26	86.7	84	70	27.3	3	0.0001
Water assessment	Yes	2	6.7	4	13.3	7	23.3	8	26.7	21	17.5			
	No	28	93.3	26	86.7	23	76.7	22	73.3	99	82.5	5.3	3	0.154
Water re-used	Yes	2	6.7	1	3.3	9	30	7	23.3	19	15.8			
	No	28	93.3	29	96.7	21	70	23	76.7	101	84.2	11.2	3	0.011

Note: Dar Es Salaam (Eastern), Mbeya (Southern Highlands), Arusha (Northern) and Mwanza (Lake zone).

3.3 Annual fish yield

Results of the total annual fish yield (kg/ha/yr) from the four agro-ecological zones assessed are presented in figure 4. The Lake zone had higher annual mean (±se) fish yield of 629 ±124 (kg/ha/yr) and the lowest mean fish yield was recorded in the Northern zone 247 ±45.4 (kg/ha/yr).

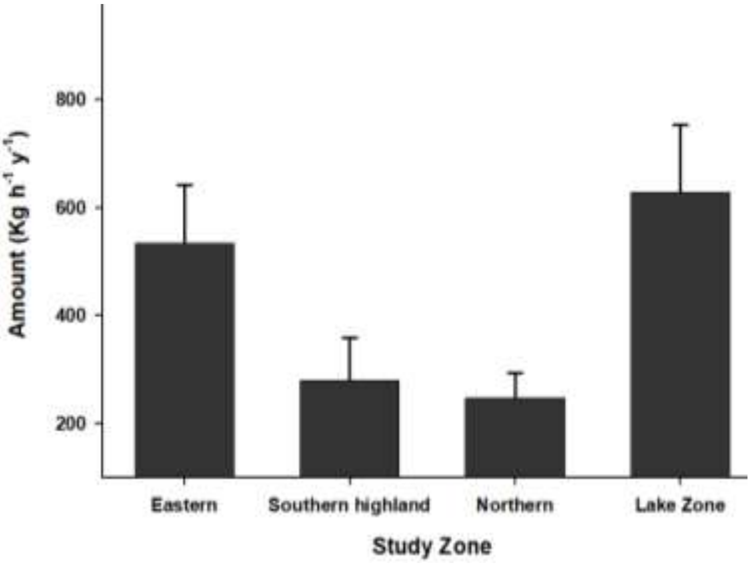


Figure 4: Mean (±SE) annual fish yield (Kg/ha/y) from four surveyed agro-ecological zones (N = 120), data collected between 2019 and 2021. *Note: Dar Es Salaam (Eastern), Mbeya (Southern Highlands), Arusha (Northern) and Mwanza (Lake zone)*

3.4 Seasonal pond primary productivity for the four ecological zones

The mean seasonal primary production (mg O_2 /L/h), both Net and Gross are summarized in table 6. Results shows that There were no significant differences in Gross primary productivity and Net production between seasons among investigated zones (Table 7). In the southern highland zone, respiration rates differ significantly between seasons (p = 0.04).

Table 7: Mean maximum and minimum gross primary productivity, net production and respiration rate (mg O_2/ L/ hr), of the investigated fish ponds during the warm and cold seasons, with paired T-test statistics.

		Descriptive Statistics						
		Warm		**Cold**		**Paired T-test statistics**		
Parameters	**Sites**	**Mean**	**Range**	**Mean**	**Range**	***df***	***t***	***P-value***
Respiration Rate (mg O_2/ L/ hr)	Dar Es Salaam	0.29	0.27- 0.33	0.51	0.44 - 0.64	2	-2.91	0.10
	Mbeya	0.41	0.35- 0.46	0.56	0.46 - 0.63	2	-4.61	0.04
	Arusha	0.87	0.61- 1.34	0.46	0.23 - 0.73	2	1.11	0.38
	Mwanza	0.63	0.54- 0.74	0.58	0.34 - 0.74	2	0.26	0.82
Gross Primary Productivity (mg O_2/ L/ hr)	Dar Es Salaam	1.33	0.84-1.79	1.24	0.95 - 1.72	2	0.18	0.88
	Mbeya	1.24	0.92- 1.74	1.34	1.05 - 1.67	2	-0.70	0.56
	Arusha	1.79	1.39- 2.18	1.19	0.77 - 1.82	2	1.42	0.29
	Mwanza	2.1	1.62- 2.59	1.47	1.05 - 1.76	2	1.28	0.33
Net Primary Production (mg O_2/ L/ hr)	Dar Es Salaam	1.04	0.59- 1.46	0.73	0.51 - 1.08	2	0.72	0.55
	Mbeya	0.83	0.5- 1.28	0.78	0.59 - 1.09	2	0.47	0.69
	Arusha	0.92	0.73- 1.2	0.73	0.54 - 1.09	2	3.45	0.08
	Mwanza	1.47	1.08- 1.85	0.89	0.71 - 1.02	2	1.86	0.21

Note: Dar Es Salaam (Eastern), Mbeya (Southern Highlands), Arusha (Northern) and Mwanza (Lake zone)

4.0 DISCUSSION

4.1 Annual, Diurnal and Seasonal Variation in Water physico-chemical parameters

Given that variations in water quality parameters below or above the optimum required level can have a major impact on fish physiological processes, including growth, the water quality must be given special consideration during aquaculture production (Pandit & Nakamura, 2010). Majority of Nile tilapia production ponds lack water treatment technologies and are thus exposed to the outside environment, the general trends in water quality metrics are constantly changing depending on the state of the local climate (Tadesse *et al.,* 2004), which presents a challenge to the cultured animal, particularly fish. Understanding the trends and variations in pond water quality is essential for determining the current physicochemical and biological status of the water, which will aid in the planning and production management.

Changes in water variables can affect fish growth in small bodies of water like ponds, but they can also interact with one another (Sriyasak *et al.,* 2015). Temperature is generally known for the abiotic power to control the DO and pH of water as well as the growth rate of fish (Pandit & Nakamura, 2010). In this study, in all investigated zones, diurnal and seasonal pond water temperature significantly varied with time of the day and seasons, and the trend of variation significantly differed between zones, this temperature variance may be the cause of the noted large differences in the zones' diurnal and seasonal mean DO and pH, as well as the low annual fish yield in the Southern high-land and Northern zones (Table 2) and (Figure 3). Nile tilapia are known to attain their optimum growth at a temperature range between 25 and 27 °C (Pandit & Nakamura, 2010), on the other hand, Sriyasak *et al.,* (2013) described the ideal temperature for tilapia's normal development, growth and reproduction as between 20 °C to 32 °C. The mean annual temperature observed in the Southern highland and Northern zone was slightly below the accepted range (25°C –

32 °C) for Nile tilapia to attain optimum growth (Pandit & Nakamura, 2010) however, the temperature observed in the two zones were within the range Nile tilapia survival (Pandit & Nakamura, 2010).

The optimum DO for tilapia growth should be above 5 mg/l (Abdel - Tawwab *et al.*, 2015; Sriyasak *et al.,* 2015) and the minimum level should not be below 3 mg/l (Makori *et al.,* 2017a). This study revealed that despite low annual mean water temperatures in the Southern highlands and Northern zone and high variation in diurnal or seasonal levels among zones, the mean annual DO and pH were within the ideal range appropriate for Nile tilapia growth (Makori *et al.,* 2017). Based on temperatures below the optimum requirement for growth observed in the Southern highlands and Northern zone (June-September), Nile tilapia farming in ponds can only be profitable and practical with the installation of additional farming structures that allow for temperature control, particularly during the cold season. Fish yield is always higher in ponds with higher DO concentrations. The low observed seasonal and daily variation in DO in the Southern highland zone in the warm and cold season compared to other zones, could be a reason for the poor annual fish yield reported for the Southern highland zone.

The mean pH measured between the warm season (June to September) and the cold season (November to January) in all analyzed zones (Figure 2 and Table 3) were within the ideal range (6.5 to 9) for Nile tilapia growth as advised by (Makori et al., 2017). The pH trends observed in all zones during warm and cold seasons could explain the high primary productivity reported in table 5, which suggest a high photosynthesis rate during the day, which increases dissolved Oxygen (O2) and decreases dissolved Carbon dioxide (CO2) gas, which is the main regulator of water pH. The optimum Total dissolved solids (TDS) should not exceed 1000 (ppm) (Ezeanya *et al.,* 2015) (Orobator *et al.*, 2020) while the optimum conductivity should range between 150 and 500 (µS cm^{-1}) (Makori *et al.*, 2017). Except for ponds in the Lake zone, TDS and electric conductivity did not vary between seasons within zones, still the observed results during warm and cold seasons were within the optimum range recommended for fish farming.

4.2 Characteristics of Nile Tilapia Pond Farming

The demographic distribution of Nile tilapia pond producers confirms the findings of Chenyambuga *et al.* (2014) and Mulokozi *et al.* (2020), who reported 84% and 83% men engaged in aquaculture production, respectively. According to FAO (2014), females account for only approximately 20% of African fish producers. Education is crucial factor while considering production and is expected that high level of knowledge increases level of decision making hence play an important role in the production process. In contrast to the findings of Mulokozi *et al.* (2020), who showed that only 45% of farmers had a general understanding of fish farming, this study found that a significant percentage of farmers (92%) were receiving training in aquaculture. This might be as a result of the study's inclusion of all four zones. The responses to water treatment and water reuse during production are consistent with the long-term experience of farmers in the Northern zones. This result agreed with the results by Milstein *et al.* (2002) and Mulokozi *et al,* (2020) explaining that, long term experience reduce management risk in fish production. Likewise, large pond size and high number ponds in the Lake zones suggest increased awareness on the economic potential of fish farming for farmers and high investment in fish farming (Rothuis et al. 2014; FAO, 2012; Mwaijande and Lugendo, 2015).

Feed constitute between 40 to 60% of the total aquaculture production costs. Different studies revealed that most farmers use locally available feed ingredient to reduce the cost of production. A study by Mmanda *et al.* (2020) reported 80% of fish farmers relay on the local available material, while Mwaijande and Lugendo, (2015) reported 66%. Low percentage of farmers using locally available material as feed ingredient observed in this study (45%) implies shortage of ingredients to be used as fish feed (Mmanda *et* *al.,* 2020) and high importation rates of commercial fish feeds from outside (Shoko *et al*., 2016).

Water quality management is important criteria of fish productivity (Shoko *et al.,* 2016). The study revealed that sources of water used for fish farming depend on the location. For example (Table 6), due to the availability of many rivers in the Northern and Southern Highland zones, the mail source of water was rivers, and Lake Victoria was the main source of water for the farmer in the Lake zone, while in the Eastern Zone underground water was the nail source. A study conducted by Chenyambuga *et al.* (2014), in the Southern highland zone revealed that 63% out fish farmers use river water for aquaculture.

4.3 Primary productivity for the four ecological zones

Temperature, pH, DO, and photosynthesis all have a significant impact on primary productivity in fish ponds. Due to photoperiod, primary productivity is predicted to be higher during the day and lower at night due to insufficient light. Algae (natural food) in fish ponds are a good predictor of primary productivity since they reflect high DO during the day due to high photosynthetic rates and low DO at night due to respiration. In all zones, high net primary productivity is seen during the warm seasons, and these values are consistent with the availability of high temperature and DO readings.

4.4 Conclusion

The objective of this study were to investigate the present Nile tilapia farming practices and the annual trend of fish pond water quality indicators in four agro-ecological zones of Tanzanian. The findings of this study suggest that the trend of climatic fluctuation, in particular, has a substantial impact on changes in the parameters governing pond water quality in a given zone. Ponds in the Eastern and Lake zones had year-round ideal water temperatures required for fish culture, and other water quality parameters (DO and pH) were kept at a sufficient level throughout the production cycle, leading to a satisfactory yearly fish output (Kg/ha/yr). Additionally, the temperature in the Northern zone and Southern highlands varies greatly throughout the year and was below the level required for Nile tilapia growth during the cold season (June to September), suggesting that Nile tilapia might be stocked during the warm season to achieve the best growth. High net production during the warm season indicates the need for good water quality management to prevent algae blooming, which could deteriorate water quality and result in acute and prolonged stress from hypoxia and hypercapnia, which are the primary physiological stressors for fish.

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Declaration of Competing Interest

The authors declare no conflict of interest.

Authorship contribution statement

Muumin Iddi Hamad: Conceptualization, Methodology, Formal analysis, Investigation, Writing – original draft, Visualization. **Heiromin Amon Lamtane:** Formal analysis Writing – review & editing. **Nazael A. Madalla:** Formal analysis, Data curation, Supervision, Writing – review & editing.

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CHAPTER THREE

PAPER TWO

Journal Pre-proof

Aquaculture

Acid-base disturbances and effects on oxygen uptake rates in Nile tilapia (Oreochromis niloticus) following acute and prolonged CO2 exposure

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Acid-base disturbances and effects on oxygen uptake in Nile tilapia (*Oreochromis niloticus*) following acute and prolonged CO_2 exposure

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Abstract

High level s of dissolved carbon dioxide (CO_2) occurs nightly in earthen ponds characterized by high respiration rates. Exposure to high CO_2 conditions (hypercapnia) leads to acidosis in fish, which can be compensated by an accumulation of HCO_3^- to recover intra - and extracellular pH levels, with a capacity that appears to be species-specific. For Nile tilapia, a freshwater tropical teleost traditionally produced in earthen ponds, little information is available on the tolerance to dissolved levels of CO_2 and associated acid-base disturbances. Here, we investigated first the effects of acute and progressively increasing CO_2, from normocapnic conditions to 60 mg CO_2 L^{-1}, on oxygen uptake rates (MO_2). This was followed by exposure to three concentrations of CO_2; 10, 30, and 60 mg L^{-1} (equivalent to pCO_2 of 5.4, 16.2, and 32.4 mmHg) against a normocapnic control (pCO_2 0.3 mmHg), to investigate acute (1h) or prolonged (24h) effects on standard (SMR) and maximum metabolic rate s (MMR), haematology, and extra - and intracellular acid-base status in adult Nile tilapia (mean

BM 435 ± 16 g ±SE). Acute exposure to hypercapnia led to concentration-dependent decreases in both SMR and MMR. Fish were able to fully or partially recover MMR and metabolic scope (MS) after 24h, while depression of SMR persisted at all CO_2 levels. Acute exposure to CO_2 caused intra-and extracellular pH levels to decrease by up to 0.5 units in a concentration-dependent manner. Only the lowest hypercapnic treatment (pCO_2 5.4 mmHg) was able to fully recover within 24h. Changes in haematological variables appeared minor, being restricted to increasing haematocrit, haemoglobin concentration, and mean cell volume in the highest CO_2 treatments after 24h exposure. Although the Nile tilapia is generally considered a species able to tolerate poor water quality, the modest or slow acid-base regulation following hypercapnic exposure suggests sensitivity to hypercapnia.

Keywords: Hypercapnia; Nile Tilapia; Metabolism; Haematology; Acid-base.

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1. Introduction

Global aquaculture production of Nile tilapia *(Oreochromis niloticus)* predominantly occurs in earthen ponds (Pimolrat et al., 2013) without water treatment. Unlike in recirculating aquaculture systems (RAS), water quality parameters in earthen ponds are allowed to fluctuate daily, which is likely to affect the physiological performance of the cultured fish (Frimpong et al., 2014; Pandit and Nakamura, 2010). In nutrient-rich ponds with high chlorophyll concentrations, the photoperiod creates an intermittent fluctuation of dissolved gases (oxygen and carbon dioxide), resulting in acutely occurring episodes of hypoxia and hypercapnia and carbonate associated pH fluctuations that may last several hours. Dissolved oxygen in the surface layers can reach extreme levels of supersaturation in the middle of the afternoon, while the whole pond approaches anoxic conditions during the night due to the respiration of fish, phytoplankton, and bacteria (Gyamfi et al., 2022;

Obirikorang et al., 2020). Dissolved levels of CO_2 have rarely been measured in earthen ponds. However, the CO_2 concentration increases as the rate of CO_2 excretion exceeds the rate of removal by photosynthesis and diffusion across the water surface (Tadesse et al., 2004). As such, the concentration of dissolved CO_2 has been approximated to range from 10 to 15 mg L^{-1} in the early morning (Hargreaves and Brunson, 1996) or above 20 mg L^{-1} in ponds with high feed loading (Boyd, 2008). Recent measurements of water pH and alkalinity in a fertilized fish pond suggest that dissolved carbon dioxide levels could reach concentrations of up to 50 mg L^{-1} (Hamad, 2023).

Increasing levels of dissolved CO_2 in the water reduce the pCO_2 diffusion gradient across the gill epithelium, leading to an internal accumulation of CO_2 (Brauner et al., 2019). As hydrated CO_2 (carbonic acid) equilibrates with bicarbonate and H^+ (Truchot, 1994), pH decreases in both the intra- and extracellular compartments, causing a widespread acid-base disturbance (Evans et al., 2005; Shartau et al., 2019). Most fish species can buffer the extra proton load through alterations in ion exchange across the gill epithelium (Shartau et al., 2020), where HCO_3^- is accumulated in exchange with Cl^- at the gills (Brauner et al., 2004). However, these physiological mechanisms are energy-demanding, and compensation may be time-sensitive (Claiborne and Heisler, 1986) and limited by the capacity to accumulate sufficient bicarbonate (Brauner and Baker, 2009; Fivelstad, 2013; Shartau et al., 2019) and environmental ion concentration (Larsen and Jensen, 1997). Therefore, pH compensation may increase the total energy expenditure for acid-base regulation, making less energy available for other metabolic processes such as growth.

The temporal and spatial capacity for pH regulation in fish tends to vary considerably across species (Hayashi et al., 2004; Kaya et al., 2016; Petochi et al., 2011; Ross et al., 2001; Shartau et al., 2019), environment (freshwater vs. marine) (Claiborne et al., 2002), temperature (Shartau et al., 2020), and life stage (Kikkawa et al., 2004; Shartau et al., 2016). It appears to be a normal response among most fish species to employ a strategy of coupled pH regulation, in which intracellular (pHi) recovery occurs faster but cannot be fully compensated until extracellular (pHe) is more than 50% recovered (Shartau et al., 2016).

Multispecies studies show that the time required to compensate for CO_2-induced pH disturbances is species-specific, ranging from hours to days (Brauner et al., 2004; Claiborne and Heisler, 1986; Damsgaard et al., 2015; Heuer and Grosell, 2014) The rate of compensation also depends on the environmental ion concentrations (Brauner et al., 2004; Claiborne and Heisler, 1986), while the intra- and extracellular compartments also show differences in the recovery kinetics (Brauner et al., 2019; Shartau et al., 2019; Shartau et al., 2020). Currently, no information is available regarding the degree to which the intra- and extracellular acid-base status in Nile tilapia is impacted by increasing environmental CO_2 concentrations, nor is the time required or capacity to compensate for the acidification.

In addition to its effect on acid-base balance, CO_2 has a wide range of effects on other physiological aspects of fish (Cupp et al., 2017; Good et al., 2010; Heuer and Grosell, 2014; Ross et al., 2001). Neto and Steffensen (1997) reported decreased oxygen uptake (MO_2) in freshwater European eel, *Anguilla anguilla*, subjected to water pCO_2 of 4.0 kPa. Reduced oxygen consumption upon exposure to increased CO_2 has been hypothesized to contribute to the re-distribution of metabolic capacity to facilitate ion regulation (Baker and Brauner, 2012; Claiborne et al., 2002), where both standard (SMR) and maximum metabolic rate (MMR) are impaired and the capacity for aerobic metabolic scope become reduced. This CO_2-induced reduction in metabolic scope demonstrates the reduced ability of fish to consume oxygen that can be made available for activities above those considered maintenance (feeding, swimming, growth, and reproduction). CO_2 can also simultaneously induce changes at the intra and extracellular levels through changes in HCO_3^-, pH, Cl^- and pCO_2 (Montgomery et al., 2019). To some degree, the acid-base disturbance in fish is buffered by internal buffer systems in the blood and extracellular space, serving as an initial line of defense against acidosis (Munday et al., 2016; Shartau et al., 2019).

In aquaculture production, increased CO_2 levels may be problematic and impact the physiology, welfare, and growth performance of captive species when CO_2 content fluctuates during production due to excessive stocking density or poor management (Skov, 2019). The effects of increased acidification and hypercapnia is likely to be highly species specific. Understanding the significance of this and determining the capacity and mechanisms of individual species in aquaculture are very important in optimizing production.

The objective of this study was to evaluate the response of Nile tilapia aerobic metabolism (MO_2), haematological characteristics, and cellular acid-base regulation process when exposed to elevated CO_2 in the short and long term. This study contributes to determining CO_2 threshold levels necessary to ensure good welfare by providing fresh information on the physiological adaptation mechanisms and stress response of Nile tilapia reared under increasing hypercapnia.

2. Materials and methods

2.1. Experimental fish

All-male Nile tilapia (*Oreocromis niloticus*), were acquired from a laboratory stock originally procured as larvae from a commercial supplier (Til-Aqua International, Velden, Netherlands). Fish were maintained in 500 L fiberglass tanks connected to a recirculation system. Water temperature was maintained at 26°C, and oxygen saturation levels were maintained above 80% by direct aeration in tanks. Approximately 10% of the water was replaced daily. Fish were hand-fed every morning using a commercial diet (Efico Cromis832F, BioMar A/S, Denmark). Tanks were regularly cleaned and the photoperiod regime was maintained at 12h light: 12h dark.

2.2. Effect of progressively increasing [CO_2] on metabolic rate.

Mass-specific oxygen consumption (MO_2) was measured in 12 Nile tilapia with an average mass of 435 ± 16g (S.E.) in response to progressive increases in [CO_2]. Oxygen consumption in fish (MO_2) was determined by intermittent flow through respirometry (Steffensen, 1989) using AutoResp software (Loligo Systems, Viborg, Denmark)(Bergstedt et al., 2021) .Tilapia were fasted for 24 hours before being transferred to a respirometer with a volume of 5.6 L, where they were allowed to acclimate overnight prior to experimentation. The following morning, fish were exposed to increasing CO_2 concentrations from normocapnic (< 1 mg L^{-1}) to severely hypercapnic (60 mg CO_2 L^{-1}) by ramping CO_2 concentrations at 5 mg L^{-1} h^{-1} over 12 hours. CO_2 levels were controlled as previously described (Hamad et al., 2023). In brief, pH was used as a proxy for CO_2 concentration based on a standard curve for the correlation between pH and dissolved CO_2 specific for the water used (ca. 4 mEq alkalinity L^{-1}). pH was monitored on a pH meter (Radiometer PHM220, Copenhagen, Denmark), with a voltage output collected via a digital acquisition device (U6, LabJack, USA) to a PC running a custom script (DAQFactory Express, AzeoTech, USA). The desired CO_2 levels were defined in the script using extrapolation between time-points to achieve a steadily increasing CO_2 concentration, rather than a step-like ramping. Dissolved CO_2 levels were monitored using a handheld CO_2 meter (Oxyguard, Farum, Denmark) and logged on a PC.

Oxygen saturation in the respirometers was measured using oxygen sensors connected to an OXY-4 mini oxygen meter (PreSens, Regensburg, Germany). For each exposure level, the average of the last 3 consecutive MO_2 measurements was used. Each measurement loop lasted 8 min, consisting of a 3-min flush, a 1-min wait, and a 4-min measurement period. Experiments were conducted on 4 fish simultaneously, using identical respirometers submerged in an external reservoir with a total volume of 250L. A small recirculation pump ensured continuous water mixing in the reservoir tank. Oxygen saturation was maintained at 100% by bubbling pure oxygen controlled by a solenoid valve connected to a programmable relay (PR Electronics, Rønde, Denmark) with a galvanic oxygen sensor (Handy, Oxyguard).

2.3. Effect of CO_2 exposure concentration and time on standard (SMR) and maximum metabolic rate (MMR).

SMR and MMR measurements were performed to evaluate the response in oxygen consumption rate following acute (1h) and prolonged (24h) exposure to four CO_2 concentrations (0, 10, 30, or 60 mg CO_2 L^{-1}). Compared to control and exposure time, at each CO_2 concentration, SMR and MMR were measured in individual fish with average body mass 397.3 ± 15 g (n = 8). During the experiment, CO_2 concentration was controlled in a similar way as described in section 2.2, with the modification that during exposure to a certain CO_2 concentration, once the target CO_2 exposure concentration was reached, the concentration was computer monitored and maintained for the duration of the evaluation. During the acute and prolonged exposure time MO_2 was recorded, SMR was determined as a mean mass specific oxygen consumption (MO_2) of individual fish at a specific CO_2 concentration while the MMR was evaluated by removing the fish from an exposed concentration of CO_2 at a certain exposure time from the respirometers chambers and subjected to aerial exposure for 4 min.after four min of air exposure (exhaustion), fish were returned back in to the respirometer chamber and their MO_2 was recorded. MO_2 readings with a regression coefficient not lower than 0.95 recorded during the CO_2 exposure experiment were used as standard metabolic rate (SMR), while MMR is presented by the first MO_2 reading of individual fish recorded after fish exhaustion.

2.4. Effects of exposure level and duration on haematology and acid-base status.

Eight fish with average body mass 246.7 ± 6.3 g were exposed to one of three elevated CO_2 concentrations described above (N = 24), during which fish were individually confined in non-transparent PVC tubes (L 500 × Ø 105 mm). Fish were transferred after 24 h fasting and allowed to recover for a further 24 h before the onset of experiments. At the desired time point, fish were gently but quickly anesthetized in a buffered solution containing 0.1 g L^{-1} benzocaine (ethyl-p-aminobenzoate). When fish became unresponsive to tactile stimulation, they were euthanized by pithing, and a 2 mL blood sample was taken from a caudal vessel using a heparinized syringe. Blood samples were divided into 2 aliquots. One aliquot was centrifuged at 13000*g* for 5 min, plasma was transferred to a new aliquot, and the plasma and red blood cell (RBC) fractions were frozen on dry ice. A sample of white muscle (~1g) was taken from the left dorsal aspect of the fish at the level of the dorsal fin and frozen on dry ice. Plasma, RBCs, and muscle samples were transferred to -80℃ until analysis. Whole blood pH was measured in the second aliquot using a pH microelectrode (MI-410, Microelectrodes, Inc., USA) attached to a multimeter (HI 98185, Hanna Instruments, Sweden). Haematocrit was determined in duplicate using wax-sealed micro-capillary centrifuged at 8700*g* for 5 min. Plasma chloride was determined using a chloride analyser (M926, Sherwood Scientific, Cambridge, UK). Muscle pH was measured using a glass pH electrode from a homogenised 1 g of fish muscles minced with 1 ml of distilled water (Lima dos santos et al., 1981; Vyncke, 1981) for one min at room temperature using Ultra turrax homogenizer. RBC pH was determined on thawed RBC at room temperature using a glass pH electrode. Haemoglobin concentration was determined using the cyanmethemoglobin method (Drabkin's). RBC counts were determined using a Neubauer haemocytometer (C-Chip, NanoEntek, Massachusetts, USA) loaded with 10 µl blood diluted 200 × with Ringer solution (containing in mmol 124 NaCl, 3 KCl, 0.75 $CaCl_2$, 1.30 $MgSO_4$, and 12 $NaHCO_3$)..

Total plasma CO_2 concentration was measured on a custom-built CO_2 setup, inspired from Lee et al. (2018). The CO_2 setup consisted of a custom-made 6 ml gas-sparging column containing 2 ml 10 mmol l^{-1} HCl, that converted the plasma HCO_3^- into CO_2.

The gas-sparging column was continuously bubbled with pure N_2 gas at a flow of 40 ml min^{-1} to release the generated CO_2 into the gas phase. The outflowing gas was dehydrated in a column of $CaCl_{2,}$ and CO_2 gas content was measured on a LiCOR LI-7000 gas-analyser using LiCOR software (LI-COR, Cambridge, UK). To measure total plasma carbon dioxide concentration ($[CO_2]$), plasma samples were first thawed on ice. Then, 5 µl plasma sample was injected into the gas-sparging column followed by a 5 µl injection of freshly made 20 mmol l^{-1} NaHCO3 solution. The volume of expelled CO_2 was calculated by integrating the CO_2 signals of the two injections, and the $[CO_2]$ was calculated as the ratio between the two integrations multiplied by 20 mmol l^{-1}.

The partial pressure of blood CO_2 was calculated from measured CO_2 and pH values using the Henderson-Hasselbalch equation, plasma CO_2 solubility constant, and pK´ from Siggaard-Andersen (1976). HCO_3^- was then subtracted from the same equation using the actual value of plasma CO_2 with the same pK´.

2.5. Analytical methods and Data analysis

Data were verified for normality using the Shapiro-Wilk test, and for equal variance using the Brown-Forsythe test. Changes in oxygen uptake rates during progressive increases in dissolved CO_2 and effects of short and prolonged exposure on SMR and MMR was tested using one-way ANOVA and all pairwise multiple comparison (Holm-Sidak method). Changes in haematological parameters and plasma chemistry during short (1 h) and long-term (24 h) exposure to hypercapnia were tested using two-way ANOVA with $[CO_2]$ and time considered as factors, with multiple comparison against the control group (Holm-Sidak). For all statistical tests, a probability below 0.05 was considered significant. All data are presented as mean ± standard error of the mean. Statistics and plots were produced using SigmaPlot (v. 14.5 Systat Software, Inc.).

3. Results

3.1. Changes in metabolic rate in response to progressive increases in CO_2.

Exposure to progressively increasing [CO_2] resulted in a concentration-dependent reduction in oxygen uptake in Nile tilapia (Figure 1, $p < 0.001$). The mean absolute value oxygen consumption (MO_2) of Nile tilapia was 87.02±0.27 mg O_2 kg^{-1} h^{-1} under normocapnic control conditions (~ 1 mg L^{-1} CO_2). MO_2 immediately declined by 10% when [CO_2] increased to 5 mg L^{-1}, and with increasing CO_2 concentrations, MO_2 continued to decrease linearly by an average of 1.4 mg O_2 kg^{-1} h^{-1} (1.6%) for every 5 mg L^{-1} CO_2 increase. At the highest tested CO_2 concentration, the mean MO_2 had declined by 30% to 61.65 ± 0.91 $mgO_2kg^{-1}h^{-1}$ compared to the control condition.

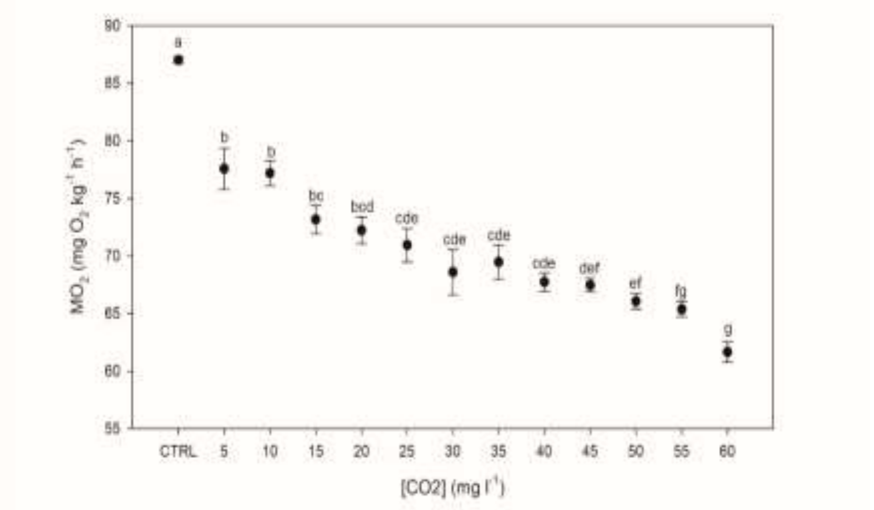

Figure 1: Changes in mean oxygen uptake rate (MO_2) in Nile tilapia during exposure to hypercapnia, with dissolved CO_2 levels progressively increasing at a rate of 5 mg L^{-1} h^{-1}. Each data point represents the mean value (± S.E.) from a group of fish (n=12). Differences in superscript letters denote significant changes in MO_2 at the different CO_2 concentrations.

3.2. Effects of acute and long-term CO_2 exposure on MO_2

Exposing Nile tilapia to different CO_2 concentrations acutely (1h) or prolonged (24h) significantly affected SMR and MMR. Following 1h exposure to CO_2, all concentrations showed a significant decrease in by 11.22 and 29% in the 10, 30, and 60 mg L^{-1} CO_2 treatments compared to the normocapnic (control) group respectively, while only the 30 and 60 mg L^{-1} treatments showed significant reductions in MMR. SMR showed little signs of recovery in any of the CO_2 treatment groups, as all remained significantly decreased by 10, 16, and 28%, respectively. In a similar manner, exposure to 30 and 60 mg L^{-1} CO_2 (Figure 2B) for 1 h also resulted in significant decreases in MMR by 30 and 31%, respectively, compared to normocapnic control conditions. After 24 h, all CO_2 treatments had recovered their MMR to an extent that there were no longer significant differences from control values (Figure 2B). Consequently, MS was significantly reduced after 1 h exposure to CO_2 concentrations of 30 and 60 mg L^{-1}, but were recovered after 24 h (Figure 2C).

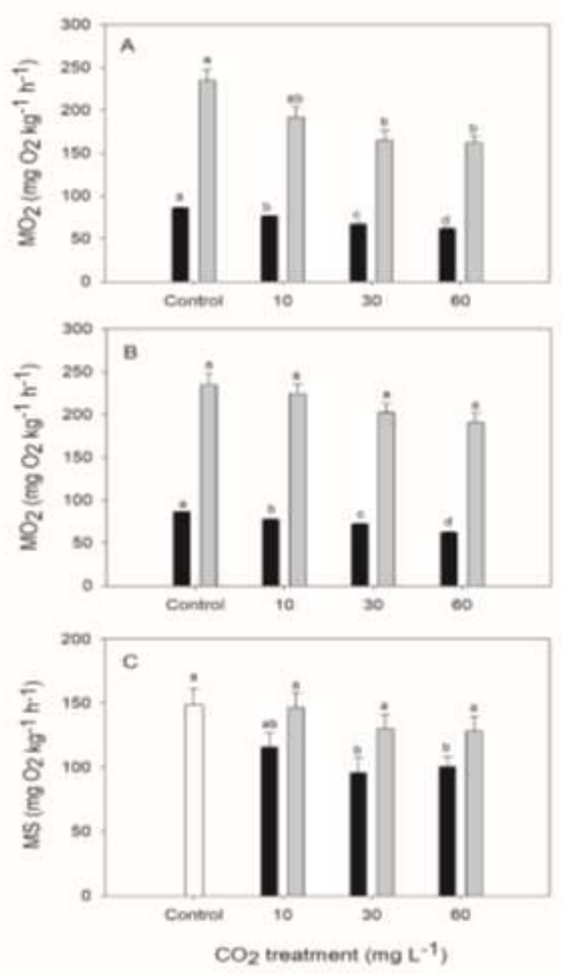

Figure 2: (A) SMR (black bars) and MMR (grey bars) of tilapia exposed to 10, 30 and 60 mg L^{-1} CO_2 for 1h (n=8, N=32). (B) SMR and MMR of tilapia exposed to a different level of CO_2 for 24h (n=8, N=32), (C) Metabolic scope (MS) of tilapia exposed to a different level of CO_2 for 1h (black bars) and 24h (grey bars) and normocapnic control fish (no fill). Different letters on the bars indicate significant differences between treatments at $p < 0.05$.

3.3 Haematological parameters

The haematological parameters at 1 h and 24 h following exposure to different CO_2 concentrations are shown in Table 1. Overall, significant changes were observed for several haematological parameters in response to CO_2 concentration during both acute and prolonged exposure. There were no effects of time, nor were there interacting effects between [CO2] and time. There was a dose dependent increase in haematocrit and haemoglobin concentration. No changes were observed in RBC count (RBCc) or mean cell volume (MCV). Because haematocrit and [Hb] increased proportionally, there was no overall effect of mean cell haemoglobin concentration (MCHC).

Table 1: Effect of CO_2 (Normocapnic control, 10, 30, and 60 mg L^{-1}) and exposure duration on blood parameters of Nile Tilapia. Data expressed as mean ±SE (n=8, N=56), different superscripts in each row indicates a significant effect of CO_2 concentration for the given time of exposure. Haemoglobin concentration ([Hb]) is expressed in g dL^{-1}, red blood cell count (RBCc) is in millions of cells per mL, mean cell volume (MCV) is expressed in femtoliters (fL), while mean cellular haemoglobin concentration (MCHC) is expressed in g L^{-1}. Calculations were performed as described in Wells and Baldwin (1990).

	Time		$[CO_2]$			*P*-value		
Parameter	(h)	Control	10 mg L^{-1}	30 mg L^{-1}	60 mg L^{-1}	$[CO_2]$	Time	$[CO_2]$ × Time
Haematocrit (%)	1	17.9 ± 1.6^{a}	19.5 ± 1.4^{a}	20.1 ± 1.6^{a}	21.4 ± 1.1^{a}	0.002	0.418	0.277
	24		17.5 ± 1.7^{a}	21.7 ± 1.1^{ab}	25.2 ± 1.6^{b}			
[Hb] (g dL^{-1})	1	$6.87\ 0.57^{a}$	7.70 ± 0.76^{a}	8.94 ± 0.66^{a}	9.36 ± 0.35^{b}	<0.001	0.898	0.568
	24		6.80 ± 0.77^{a}	8.70 ± 0.59^{ab}	10.28 ± 0.68^{b}			
RBCc (10^6 µL^{-1})	1	2.53 ± 0.22^{ab}	1.99 ± 0.14^{a}	1.93 ± 0.11^{a}	2.67 ± 0.20^{b}	0.013	0.859	0.239
	24		2.21 ± 0.18^{a}	2.19 ± 0.13^{a}	2.28 ± 0.17^{a}			
MCV (fL)	1	74.7 ± 9.3^{a}	99.4 ± 6.7^{a}	107.2 ± 10.9^{a}	81.5 ± 5.2^{a}	0.014	0.666	0.085
	24		82.5 ± 12.1^{ab}	103.2 ± 11.0^{ab}	114.0 ± 9.9^{b}			
MCHC (g L^{-1})	1	392 ± 27	397 ± 27	447 ± 23	445 ± 27	0.280	0.159	0.655
	24		390 ± 23	398 ± 10	408 ± 7			

3.4 Blood and muscle pH

Changes in intracellular and extracellular pH at different CO_2 concentrations and exposure times are shown in Fig. 3. Extracellular pH showed a progressive decline in proportion to increasing CO_2 concentration at 1h of exposure, decreasing by up to 0.52 units in the 60 mg CO_2 L^{-1} treatment. All treatment groups showed an ability to partially, but significantly, restore extracellular pH after 24h exposure, but all groups remained significantly lower than the normocapnic control group (Figure 3A). Intracellular pH measured in RBCs decreased significantly in all treatment groups during short term exposure by 0.21, 0.41, and 0.55 units in the 10, 30, and 60 mg L^{-1} CO_2 concentration, respectively, from the normocapnic control (Figure 3B). Following 24h of exposure, the 10 mg L^{-1} CO_2 group had recovered RBC pHi. While RBC pH remained significantly lower than the control group at 24h in the 30 and 60 mg CO_2 L^{-1} treatments, they were significantly increased from 1h exposure. Muscular pHi followed this trend of recovery at 10 mg CO_2 L^{-1}, and while pHi in the 30 mg CO_2 L^{-1} treatment was significantly increased at 24h compared to 1h, the 60 mg CO_2 L^{-1} showed no signs of recovery (Figure 3C).

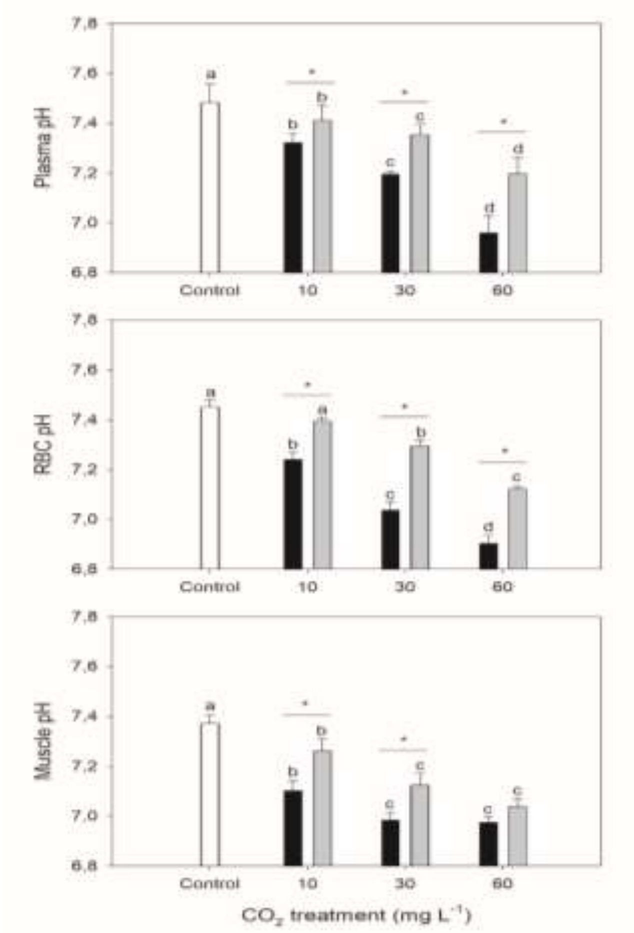

Figure 3: Changes in extracellular pH measured in plasma (A), and intracellular pH measured in lysed red blood cells (RBC) (B) and homogenised skeletal muscle (C) in response to increasing dissolved CO$_2$ concentrations, after 1 hour (black bars) and 24 hours (grey bars), and control values for normocapnic conditions (white bars). Values are given as means ±SE (n=8, N=56). Different superscripts indicate significant differences between treatment groups within each exposure time. Differences between exposure times, within each concentration, are indicated by an asterisk.

3.5. Plasma carbon dioxide partial pressure and chloride shift

Changes in plasma *p*CO_2 and differences in Cl^- and HCO_3^- between acute (1h) and prolonged (24h) exposure to CO_2 are presented in Figure 4. In comparison to the normocapnic plasma *p*CO_2 of 12.1 mmHg, 1 h exposure caused a dose dependent increase in *p*CO_2 in the 10, 30 and 60 mg CO_2 L^{-1} treatments (Figure 4A). Following 24 hr of exposure, Nile tilapia were able to compensate the increased pHi in the 10 mg L^{-1} CO_2 group, while the concentration remained the same in 30 mg L^{-1} CO_2 group, and continued to increase in the 60 mg L^{-1} group. Nile tilapia responded to an increased CO_2 concentration by a significant decrease in plasma HCO_3^- following 1h exposure (Figure 4C), which was fully recovered following 24h CO_2 exposure. Mean plasma Cl^- was not significantly different between CO_2 exposure groups (10, 30, and 60 mg L^{-1}) following 1h exposure, but all showed a significant decrease in after 24 hr of exposure compared to control values (Figure 4B).

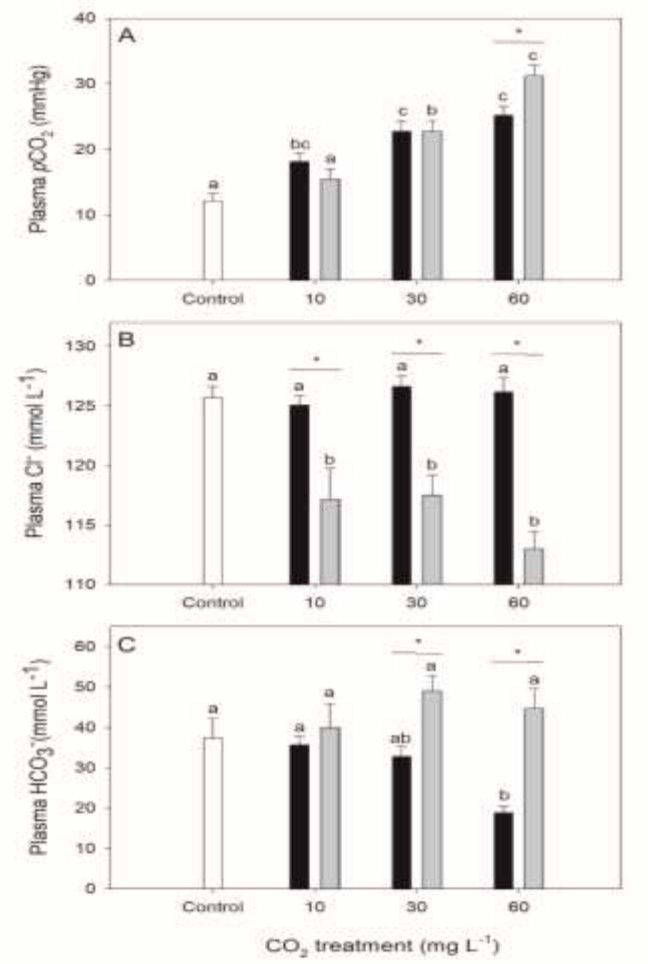

Figure 4: Changes in plasma pCO_2 (A), chloride (B), and bicarbonate (C) in response to increasing dissolved CO_2 concentrations, after 1 hour (black bars) and 24 hours (grey bars), and control values for normocapnic conditions (white bars). Values are given as means ±SE (n=8, N=56). Different superscripts indicate significant differences between treatment groups within each duration of exposure. Differences between exposure times within a concentration are indicated by an asterisk.

4. Discussion

Elevated levels of dissolved carbon dioxide induce changes in the ion and acid-base balance in water breathing fish (Brauner et al., 2019; Ern and Esbaugh, 2016; Michaelidis et al., 2007; Shartau et al., 2020). While fish are able to regulate the pH of their intra- and extracellular compartments, presumably this comes at an energetic cost that could otherwise have been allocated towards somatic growth (Baker and Brauner, 2012; Hayashi et al., 2004). The temporal and spatial capacity for acid-base regulation and ion compensation is species-specific (Melzner et al., 2313), ranging from a few hours to several days, and is affected, among other things, by the chemical composition of the water (Larsen and Jensen, 1997; Hayashi et al., 2004). Collectively, all studies report similar findings that elevated CO_2 levels in the rearing environment of fish affect their metabolism, mainly reducing oxygen consumption rate and growth performance (Ishimatsu et al., 2004; Skov, 2019). This has also been shown for Nile tilapia experiencing nocturnal hypercapnic events where specific growth rate and appetite were significantly depressed (Hamad et al., 2023).

In the present study, acute exposure of Nile tilapia to increasing levels of CO_2 caused a depression in resting metabolic rate (SMR) in a direct and dose-dependent manner. This response differs from observations on Atlantic salmon (*Salmo salar*), where MO2 is maintained until a critical CO2 concentration of 25 mg L^{-1}, beyond which reductions in MO_2 are observed (Khan et al., 2019). However, the response to CO_2 is similar to the decrease in MO_2 observed for Nile tilapia exposed to severe hypoxia (Bergstedt et al., 2021). Such reductions in aerobic metabolism are typically attributed to a reduction in the capacity to do mechanical work or to extract oxygen from the water. This does not appear to be the case in the present study. Mechanical work for oxygen uptake is comprised of ventilation and cardiac work, and although cardiac performance has been shown to decrease in response to extracellular acidosis for several fish species (Farrell et al., 1986, 1983) Nile tilapia maintain a capacity to increase their MO_2, as evident from the MMR determinations. However, rather than maintaining SMR, they allow it to drop, also during prolonged exposure. The mechanisms and pathways that trigger this response, and the underlying strategy, are not clear.

The rate of oxygen consumption (MO_2) is often used to evaluate metabolic performance and indirectly reflects how much energy fish spend to maintain normal physiological functions (Jobling, 1981). In the present study, a significant reduction in metabolic rate (MO_2) of more than 10% was observed immediately upon acute exposure to even modest CO_2 concentrations (5 mg L^{-1}). It appears that Nile tilapia quickly sense changes in the external CO_2 concentration and respond by a depression in their MO_2, probably in advance of any extracellular acidification. Reducing metabolic rate in the face of increasing environmental CO_2 could be a protective, although short-lived, mechanism aimed at limiting CO_2 influx, which persisted over time (to 24h).

Increasing environmental CO_2 induces changes in fish red blood cell number, haemoglobin concentration, and haematocrit (Kaya et al., 2016) that directly affect blood gas transport capacity (Brauner et al., 2004). In the present study, Nile tilapia exposed to acute (1h) increased CO_2 significantly shifted the status of their haematological parameters (decreased RBCc, increasing Hb concentration, and haematocrit). *Oreochromis mossambicus* exposed to a maximum of 14 mg L^{-1} CO_2 at different temperatures (Kaya et al., 2016) showed a significant decline in RBC and Hct after 7 days of exposure to CO_2. In the present study, Nile tilapia shows a rapid increase in Hct following 1h exposure to $CO_{2,}$ which recovered to control values in the 10 mg L^{-1} group after 24h, but further increased in the 30 and 60 mg L^{-1} treatment groups. This suggests that hypercapnia induces the swelling of the red blood cells, which is typical for adrenergically stimulated fish blood (Nikinmaa, 1982). It could also indicate the release of red blood cells into the circulation, as also occurs during stress in fish (Fänge and Nilsson, 1985). Together, these data suggest an adrenergic stimulation of the red blood cells and spleen, which, in combination, increases the capacity for blood oxygen transport.

The extent and timing of pH compensation and recovery in fish following exposure to elevated levels of CO_2 vary greatly depending on the regulatory capacity (Brauner et al., 2019; Shartau et al., 2019) and exposure concentration (Claiborne and Heisler, 1986; Shartau et al., 2020). In the present study, Nile tilapia was also able to compensate pH changes in a concentration-dependent manner where pH was partially compensated for at the high CO_2 concentrations of 30 and 60 mg CO_2 L^{-1} and it was fully compensated at 10 mg CO_2 L^{-1}. The majority of studies that have examined fish's responses to elevated levels of CO_2 have found that the initial acidosis is rapid, arterial pCO_2 equilibrates with water pCO_2 in a matter of moments, and the pH of the blood (pHe) and tissues (pHi) decreases as a function of both the newly equilibrated CO_2 tension and the non-bicarbonate (i.e., intrinsic) buffer capacity of the respective compartment (Shartau et al., 2016). Additionally, most of these studies noted that the pH recovery took between 24 and 96 hours and is driven by transferring acid-base-relevant ions with the surrounding water primarily at the gills (Claiborne and Heisler, 1986; Petochi et al., 2011). Nile tilapia showed a similar response to hypercapnia with blood pH reduced in proportion to the increase in pCO_2, where a respiratory acidosis was fully or partially compensated for to control values within 24 hours, with subsequent accumulation of plasma HCO_3^- in exchange for Cl^- (Figure 4).

The pH compensation during hypercapnic acidosis in fish follows a coupled pH regulation pattern where blood pH (pHe) is regulated, at least in part, to muscle pH (pHi) (Claiborne et al., 2002; Claiborne and Heisler, 1986; Damsgaard et al., 2015; Munday et al., 2016) or can be preferential (maintaining constant pHi in the face of lowered pHe) (Baker and Brauner, 2012; Shartau et al., 2019). According to Brauner et al. (2019), fish must regulate pHi to maintain normal cellular functions regardless of the status of pHe. In the present study, Nile tilapia showed a higher capacity for regulating pHi with increased exposure concentration than for pHe, which suggests that regulating CO_2 induced pH disturbance in Nile tilapia is preferential (Baker and Brauner, 2012). The compensation of pH at higher CO_2 by elevating plasma HCO_3^- in exchange for Cl^- both intra and extracellularly can increase the energy demands for ion-exchanging processes to maintain HCO_3^- at a higher level (Strobel et al., 2012). In the present study, Nile tilapia were tested to a maximum hypercapnia tolerance of a direct increased dissolved CO_2 of 60 mg L^{-1}, during which blood HCO_3^- levels reach almost 50 mM, with an equivalent decrease in plasma Cl^-. This compensation limits plasma [HCO_3^-] elevation of Nile tilapia observed during hypercapnia exposure appears to be the highest above the theoretical threshold level of [HCO_3^-] 30 mM proposed by (Heisler 1982; 1984). It can be suggested from this threshold capacity that the intracellular compartment in Nile tilapia has a higher buffer capacity than the extracellular compartment. Thus, preferential pHi regulation may allow intracellular acid-base balance to be achieved due to the reduced amount of HCO_3^-.

4.1 Conclusion

Increasing levels of environmental CO_2 significantly impacted the ion and acid-base regulation in Nile tilapia. In this study, Nile tilapia responded to ambient hypercapnia up to 10 mg L^{-1} with quick and effective acid-based compensation mechanisms within 24h. During exposure to CO_2, no mortality, or any sign of loss of equilibrium (LOE), was observed. However, the ultimate CO_2 concentration employed in our investigation was 60 mg L^{-1} was greater than the concentration characterized as lethal by Boyd, (2008). This concludes that Nile tilapia have a high tolerance to high CO_2 levels, which may be related to the ecological characteristics of this freshwater species, which enable them to cope with different environmental conditions, including critical levels of dissolved gasses. The acute (1h) and prolonged (24h) exposure above 30 CO_2 mgL^{-1} was potentially stressful and detrimental for this species, as evident by the consistent and significant decreases in both intra- and extracellular pH, and an apparent inability to accumulate bicarbonate above ~50 mmol L^{-1}. While fish could potentially have recovered further given time, the study was undertaken to assess the daily fluctuations that occur in earthen ponds. The Nile tilapia is generally considered a species that tolerate poor oxygen conditions, the pattern of acid-base regulation following hypercapnic exposure implies some sensitivity to CO_2 exposure which should be considered in pond management.

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Authorship contribution statement

Muumin Iddi Hamad: Conceptualization, Methodology, Software, Validation, Formal analysis, Investigation, Data curation, Writing – original draft, Visualization. Christian Damsgaard: Formal analysis, Writing – review & editing. Renalda Nanziga Munubi: Formal analysis, Data curation, Supervision, Writing – review & editing. Peter Vilhelm Skov: Conceptualization, Methodology, Software, Resources, Supervision, Writing – original draft, Writing – review & editing

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CHAPTER FOUR

PAPER THREE

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Individual and combined effects of hypoxia and hypercapnia on feeding and feed utilization in Nile tilapia (*Oreochromis niloticus*)

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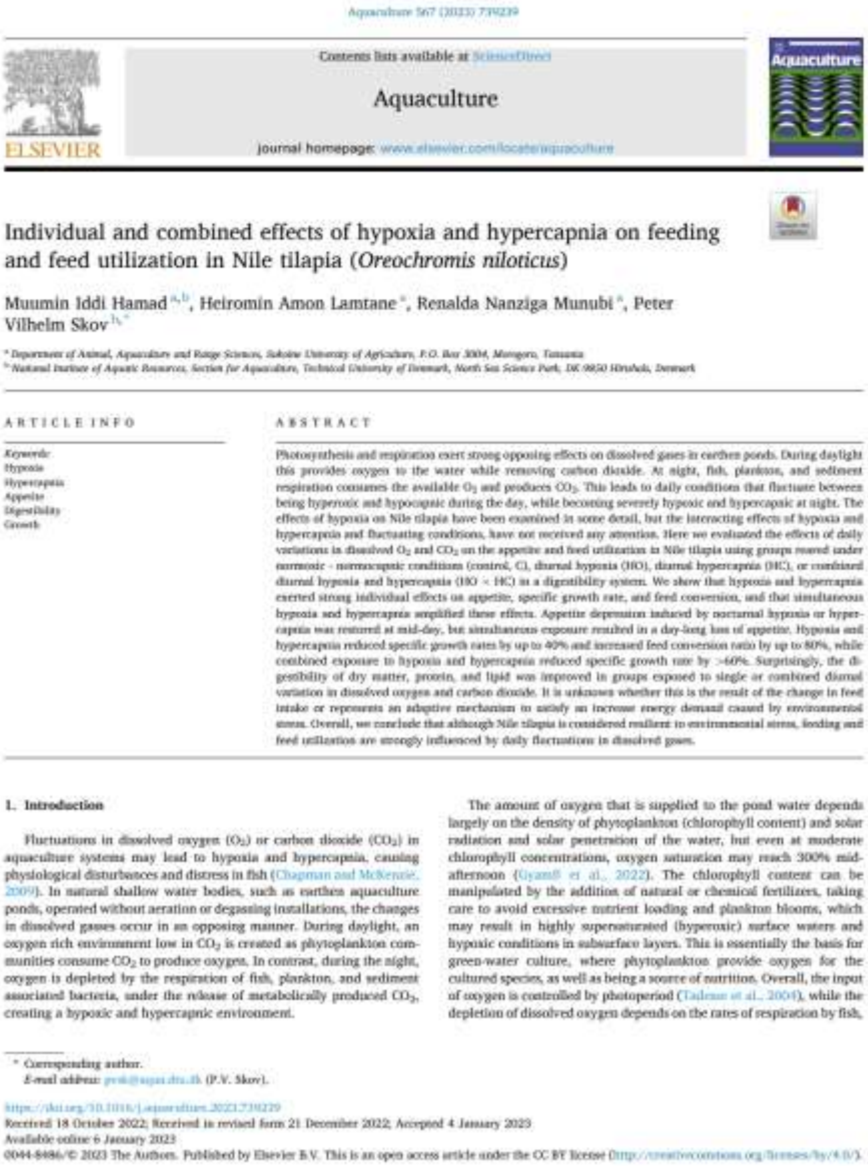

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A R T I C L E I N F O

Keywords:
Hypoxia
Hypercapnia
Appetite
Digestibility
Growth

A B S T R A C T

Photosynthesis and respiration exert strong opposing effects on dissolved gases in earthen ponds. During daylight this provides oxygen to the water while removing carbon dioxide. At night, fish, plankton, and sediment respiration consumes the available O_2 and produces CO_2. This leads to daily conditions that fluctuate between being hyperoxic and hypocapnic during the day, while becoming severely hypoxic and hypercapnic at night. The effects of hypoxia on Nile tilapia have been examined in some detail, but the interacting effects of hypoxia and hypercapnia and fluctuating conditions, have not received any attention. Here we evaluated the effects of daily variations in dissolved O_2 and CO_2 on the appetite and feed utilization in Nile tilapia using groups reared under normoxic - normocapnic conditions (control, C), diurnal hypoxia (HO), diurnal hypercapnia (HC), or combined diurnal hypoxia and hypercapnia (HO × HC) in a digestibility system. We show that hypoxia and hypercapnia exerted strong individual effects on appetite, specific growth rate, and feed conversion, and that simultaneous hypoxia and hypercapnia amplified these effects. Appetite depression induced by nocturnal hypoxia or hypercapnia was restored at mid-day, but simultaneous exposure resulted in a day-long loss of appetite. Hypoxia and hypercapnia reduced specific growth rates by up to 40% and increased feed conversion ratio by up to 80%, while combined exposure to hypoxia and hypercapnia reduced specific growth rate by >60%. Surprisingly, the digestibility of dry matter, protein, and lipid was improved in groups exposed to single or combined diurnal variation in dissolved oxygen and carbon dioxide. It is unknown whether this is the result of the change in feed intake or represents an adaptive mechanism to satisfy an increase energy demand caused by environmental stress. Overall, we conclude that although Nile tilapia is considered resilient to environmental stress, feeding and feed utilization are strongly influenced by daily fluctuations in dissolved gases.

1. Introduction

Fluctuations in dissolved oxygen (O_2) or carbon dioxide (CO_2) in aquaculture systems may lead to hypoxia and hypercapnia, causing physiological disturbances and distress in fish (Chapman and McKenzie, 2009). In natural shallow water bodies, such as earthen aquaculture ponds, operated without aeration or degassing installations, the changes in dissolved gases occur in an opposing manner. During daylight, an oxygen rich environment low in CO_2 is created as phytoplankton communities consume CO_2 to produce oxygen. In contrast, during the night, oxygen is depleted by the respiration of fish, plankton, and sediment associated bacteria, under the release of metabolically produced CO_2, creating a hypoxic and hypercapnic environment.

The amount of oxygen that is supplied to the pond water depends largely on the density of phytoplankton (chlorophyll content) and solar radiation and solar penetration of the water, but even at moderate chlorophyll concentrations, oxygen saturation may reach 300% mid-afternoon (Gyamfi et al., 2022). The chlorophyll content can be manipulated by the addition of natural or chemical fertilizers, taking care to avoid excessive nutrient loading and plankton blooms, which may result in highly supersaturated (hyperoxic) surface waters and hypoxic conditions in subsurface layers. This is essentially the basis for green-water culture, where phytoplankton provide oxygen for the cultured species, as well as being a source of nutrition. Overall, the input of oxygen is controlled by photoperiod (Tadesse et al., 2004), while the depletion of dissolved oxygen depends on the rates of respiration by fish,

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phytoplankton, and bacteria (Kramer, 1987; Montgomery et al., 2019). At night, as photosynthesis ceases, oxygen is progressively consumed, and an equimolar quantity of CO_2 is produced. As a result, nocturnal hypoxic conditions are accompanied by hypercapnic conditions. As the release of CO_2 to the water also results in acidification, some bicarbonate may also be dehydrated to become free CO_2. The magnitude of hypercapnia that occurs in earthen ponds has not been widely examined, but will in theory depend largely on the oxygen available for respiration and the alkalinity of the water (Boyd and Lichtkoppler, 1979; Hargreaves and Brunson, 1996; Wurt and Durborow, 1992).

Fish react to hypoxic incidents in their environment through a suite of physiological and behavioural changes, such as changes in swimming activity (Chapman and McKenzie, 2009) thermal tolerance (OCLTT) (Pörtner and Peck, 2010), feeding activity (Magnoni et al., 2018), and bioenergetic capacity (Claireaux and Chabot, 2016). Nile tilapia respond to hypoxia through changes in feeding activity (Obirikorang et al., 2020), as well as growth and feed utilization (Abdel-Tawwab et al., 2019; Kulding et al., 2008) but are able to compensate for and survive low oxygen levels through combinations of aquatic surface respiration (Chapman et al., 1995; Obirikorang et al., 2020; Verheyen et al., 1994), cardio-ventilatory adjustments (Leonard and Skov, 2022; Obirikorang et al., 2020) or even metabolic depression (Bergstedt et al., 2021). As a consequence, defining the tolerance thresholds for reductions in DO of a specific species not only from a survival perspective (e.g. critical saturation levels), but also those that impact the performance of cultured fish, is of great importance.

While there is no information available on the effects of hypercapnia on Nile tilapia, the effects of high levels of CO_2 have been reported for several fish species, such as Atlantic salmon (Khan et al., 2018; Mota et al., 2019), European eel (Methling et al., 2013), sea bass (Petochi et al., 2011) carp (Claiborne and Heisler, 1986) and Mozambique tilapia (Kaya et al., 2016). Generally, elevated CO_2 levels are associated with reduced feed intake and an increased cost of growth (Khan et al., 2018; Skov, 2019), disturbances in the acid-base balance (Brauner et al., 2004; Hayashi et al., 2004), and impaired oxygen uptake (Fivelstad et al., 1998; Methling et al., 2013; Khan et al., 2018).

To date, the majority of studies on the effects of hypoxia and hypercapnia on fish have focused on either hypoxia or hypercapnia, and typically where dissolved O_2 and CO_2 levels are maintained at constant levels. Presently, there is no information available on the synergistic effects of hypoxia and hypercapnia, either under constant or fluctuating conditions. Daily fluctuations in dissolved oxygen levels and the occurrence of nocturnal hypoxic or anoxic events are well-documented for earthen ponds (Lefevre et al., 2011; Mengistu et al., 2020; Obirikorang et al., 2020; Gyamfi et al., 2022), while reports on CO_2 levels are less frequent. However, Boyd (2008) reported nocturnal hypercapnic events with dissolved CO_2 exceeding 20 mg L^{-1}, and more recently, based on pH and alkalinity measurements from a fertilized Nile tilapia pond, we estimate that CO_2 levels may exceed 50 mg L^{-1}.

The growth of fish is a complex interaction between the intake, digestibility, assimilation, and excretion of nutrients. The appetite signals that control feed intake are influenced, among other things, by water quality conditions (Azaza et al., 2008; Jobling, 1981). As a result, when water parameters of the surrounding environment change, fish adjust their feeding behaviour and digestive processes. Jobling (1981) demonstrated how oxygen availability acts as a regulating mechanism for feed intake in fish, either because oxygen limitations reduce the available metabolic scope to fuel digestive processes, or because it prolongs the digestive process and delays further feed intake. Prolonged digestive processes caused by low oxygen availability may also increase the energy required for absorption and storage of ingested nutrients and result in poor body growth (Kramer, 1987; Nelson, 2016).

The central feeding system of the brain controls the intake-appetite mechanism in fish, where hunger and satiation signals are transmitted via a complex network of the peripheral neural system, with the help of blood metabolites, hormones, and some gastro-intestinal protein molecules that are switched on and off to regulate feed intake in fish (Volkoff and Rønnestad, 2020). Feed utilization can be improved, and feed loss can be avoided by adjusting meal sizes according to observed water quality parameters. However, a past experience in fluctuating water quality may have delayed effects on appetite. Re-establishing normoxic and normocapnic conditions may not result in the immediate recovery of appetite, as has previously been shown for nocturnal hypoxic events, where a delayed recovery of appetite occurred in the morning. Whether nocturnal hypercapnia exerts similar effects on feeding behaviour of Nile tilapia is currently unknown, as are the potential interacting effects of hypoxia and hypercapnia.

In the present study we aimed to investigate the effects of the diurnal occurrence of hypoxia, hypercapnia and combined hypoxia and hypercapnia on the production performance of Nile tilapia, through assessments of appetite, growth performance, feed conversion, and nutrient digestibility.

2. Material and methods

2.1. Experimental animals and rearing condition

Experiments were conducted at the Technical University of Denmark, using all male Nile tilapia, with an average body mass (±SD) of 114.5 ± 26.9 g (n = 160). Fish were originally obtained as juveniles from a commercial supplier (Til-Aqua International, Velden, Netherland) and reared in 400 L tanks supplied from a re-circulation aquaculture system. Water temperature was maintained at 26 ± 1 °C and oxygen saturation above 80%, under a 12 h:12 h light-dark cycle to mimic the light cycle that fish would encounter in their natural setting. Fish were reared on a commercial tilapia diet (Efico, Cromis832F, BioMar, France), consisting of floating pellets with a size of 4.5 mm, and containing 35% crude protein, 6% crude lipid, and 38.6% nitrogen free extract (NFE). Digestible energy content of the diet was 18.2 MJ kg^{-1}. All experiments were carried out in accordance with Danish and EU legislation (Directive 2010/63/EU).

2.2. Experimental procedure

Nile tilapia were randomly distributed among 120 L cylindric-conical digestibility tanks (Fig. 1), in one of 4 different treatment groups; a normoxic (>90% O_2 sat) and normocapnic (<3 mg CO_2 L^{-1}) control group (C); a diurnal hypoxia group (HO) where dissolved O_2 levels varied between normoxic (>90% O_2 sat) at mid-afternoon to hypoxic (~4% O_2 sat) in the night, while CO_2 levels were maintained ~3 mg L^{-1}; a diurnal hypercapnia group (HC) where carbon dioxide varied between normocapnic at mid-afternoon to hypercapnic (30 mg L^{-1} CO_2) in the night, while O_2 was kept at 100% saturation; and lastly a diurnal hypoxia and hypercapnia interaction group (HO × HC) where O_2 and CO_2 varied diurnally simultaneously as described for the HO and HC treatments (Fig. 2). All treatment group consisted of 4 experimental tanks each stocked with 10 fish. Each tank received a water flow of 40 L h^{-1} from a reservoir. Oxygen saturation levels were maintained as described by Skov et al. (2013). In brief, oxygen levels in the reservoir supplying the tanks were measured continuously using a galvanic oxygen probe connected to a custom oxygen meter that delivered a voltage output to a PC running DAQFactory Express (AzeoTech, USA) via a digital acquisition device (U6, LabJack, USA). A script was created for DAQFactory where O_2 levels fluctuated on an hourly basis, and the script extrapolated between setpoints to ensure a smooth O_2 profile. If oxygen levels exceeded the setpoint, the script would trigger a solenoid valve, whereby nitrogen gas would be added to the reservoir via a diffusor. Once the desired oxygen setpoint was achieved, the PC would shut off the nitrogen gas. If O_2 saturation was below the setpoint, the script would open the air supply from a blower to aerate the reservoir with atmospheric air.

CO_2 levels were maintained using the principle described by Khan

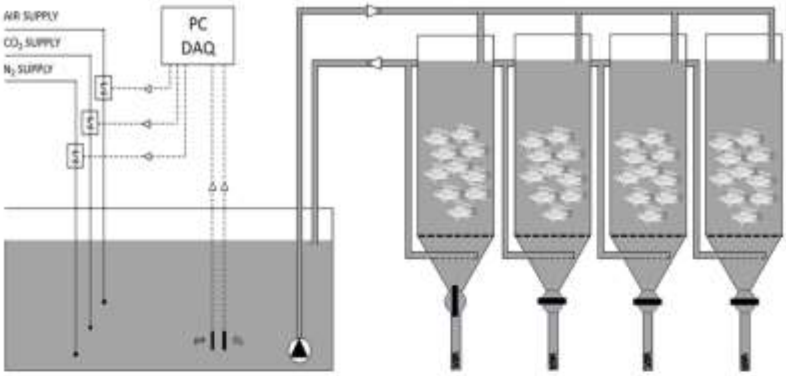

Fig. 1. Schematic diagram of the experimental setup showing one experimental condition. pH and oxygen were monitored in the supply reservoir via a PC interfaced with a digital acquisition device (DAQ). pH served as a proxy for CO_2. Solenoid valves (nominally closed) to control the inflow of air, carbon dioxide (CO_2), or nitrogen gas (N_2) were triggered by the PC to achieve pre-programmed desired levels of O_2 and CO_2. Fish were reared in Guelph-type digestibility tanks in quadruplicate.

et al. (2018). However, because CO_2 levels were dynamic, the dosing system was controlled via a PC in a similar manner as described for O_2. pH levels were used as a proxy for CO_2 levels in the reservoir by creating a calibration curve for the relationship between pH and dissolved CO_2 and desired CO_2 levels were then set based on pH. pH values were transferred to a PC based on a voltage output from the pH meter (Radiometer PHM220, Copenhagen, Denmark). CO_2 levels in the reservoir tank were checked daily using a handheld infra-red CO_2 analyser (Oxyguard, Farum, Denmark).

2.3. *Growth performance*

Fish were individually weighed to the nearest 0.1 g at day 0, and were fed on a daily ration corresponding to 2% of their biomass (Efico, Cromis832F, BioMar, France), where half of the ration was offered to the fish in the morning (10:00) and the other half in the afternoon (16.00). Changes in biomass were evaluated by weighing the fish every 7 days for five weeks, and the 2% feed ration was adjusted accordingly based on the fish weight. Feeding was withheld for 24 h prior to weight measurements to allow for gastric evacuation. To avoid feed wastage, feeding was done slowly by hand over a period of 15 min, and feeding was stopped when fish appeared satiated. All tanks were cleaned daily prior to the first feeding using magnetic cleaners to remove sedimented faecal materials, and were cleaned thoroughly fortnightly by scrubbing. Feed intake (FI) was determined by subtracting the weight of uneaten feed, from the feed given per day.

2.4. *Digestibility assessment*

The apparent digestibility coefficient (ADC) of nutrients was assessed over a 6-day period as described by Skov et al. (2013) and Ohirikorang et al. (2020), using the same feeding regime as described above. Faeces collectors were submerged into an ice-slurry (0 °C) to minimize bacterial degradation during collection. A ball valve at the bottom of the tank was closed during feeding to avoid collecting uneaten feed. Faeces were collected daily each morning prior to feeding and frozen (−20 °C) for later chemical analysis. Faecal samples from each tank were pooled into two 3-day periods, resulting in 8 samples per treatment group. Samples of feed and faeces were homogenised using a T18 Ultra Turrax homogenizer (IKA, Germany). Dry matter (DM) was determined by drying to constant weight at 105 °C. Crude protein was determined by the Kjeldahl method as 6.25 × N (ISO 5983-2), and crude fat by the method of Bligh and Dyer (1959). Ash was determined gravimetrically following incineration at 550 °C (Kolar, 1992). Nitrogen-free extract (NFE) was calculated as DM less the sum of crude protein, crude lipid, and ash. The calculation of apparent digestibility coefficients (ADC) were performed as described by Jobling (1994).

2.5. *Assessment of appetite recovery*

To quantify the effects of the nocturnal exposure to hypoxia and/or hypercapnia on appetite a two-hour interval feeding regime (8:00, 10:00, 12:00, 14:00, and 16:00 h) was established over a 6-day period following the growth and digestibility trials, during which maximum feed intake was quantified at each time point. On the day before the trial, fish were fed to satiation at 16.00 h. The next day each tank was fed to satiation at 8:00 h, and the feed intake was quantified. The remaining ration was delivered to the fish at 16.00 h until satiation. On day two, fish were fed to satiation at 10.00 h, and the remaining ration was delivered at 16.00 h, as above. The first feeding time was delayed 2 h every day so that on day 5, the first feeding occurred at 16.00 h. Feed intake was calculated for each time point and expressed as per cent body mass ingested.

2.6. *Data analysis*

Statistical analyses were performed using SigmaPlot (Systat Software Inc. v. 14.5). All data were verified for homogeneity and normality using the Kolmogorov-Smirnov test. One-way analysis of variance (ANOVA) was used to test and compare measured parameters for differences among tested treatments. Duncan's post-hoc test was used to show the comparison among treatments when one-way ANOVA showed significant differences among means. Results are reported as significant when $p < 0.05$.

3. Results

3.1. *Growth performance and feed utilization*

Results on feed utilization and growth performance are summarized in Table 1. No mortality occurred in any treatment groups. Overall, the control and HO groups consumed their full daily ration, the HC group

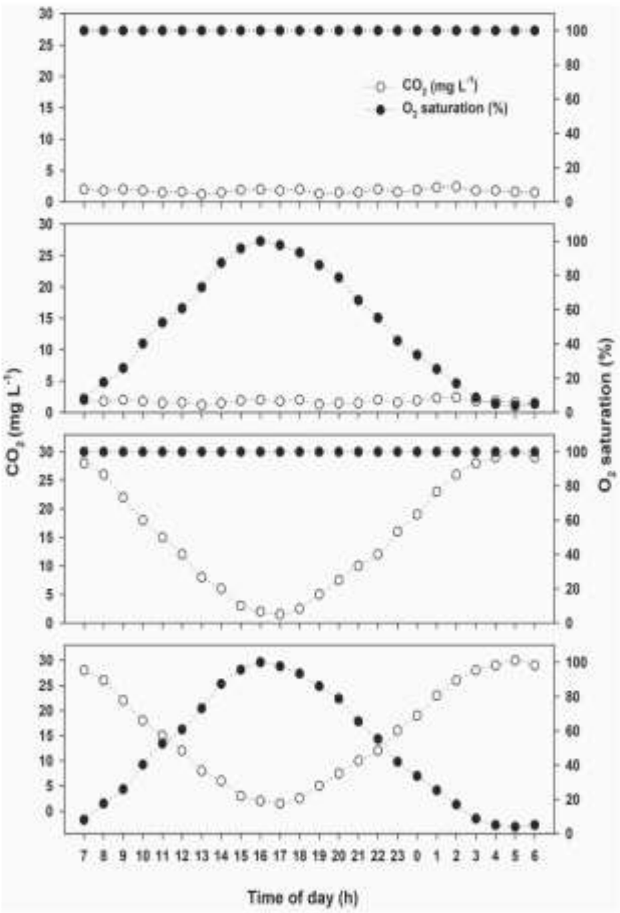

Fig. 2. Graphical illustration of the hourly interaction of dissolved levels of O_2 and CO_2 (treatments) employed during experimentation. (A) a control group (C) with oxygen levels at saturated (O_2 sat) and CO_2 levels below 3 mg L^{-1}. (B) a diurnal hypoxia group (HO) with DO levels between normoxic at mid-afternoon to hypoxic in the night, while CO_2 levels were maintained at ~3 mg L^{-1}; (C) a diurnal hypercapnia group (HC) with CO_2 levels between normocapnic at mid-afternoon to hypercapnic (30 mg L^{-1} CO_2) in the night while O_2 was kept at saturation (100%); and (D) a combined hypoxia-hypercapnia group (HO × HC) in which O_2 and CO_2 varied diurnally simultaneously as in B and C.

Table 1

Effects of dissolved gas treatment on daily feed intake, specific growth rate (SGR), feed conversion ratio (FCR), and survival. HO, hypoxia, HC, hypercapnia, HO × HC, combined hypoxia and hypercapnia. Values in a row not sharing a common superscript letter are significantly different. Values are shown as mean ± SE (n = 4).

	Treatment			
Parameters	Control	HO	HC	HO × HC
Daily feed intake (% body mass)	2.00 ± 0.00^a	1.98 ± 0.02^{ab}	1.61 ± 0.03^c	1.12 ± 0.06^d
SGR (% d^{-1})	1.65 ± 0.13^a	1.19 ± 0.08^b	1.02 ± 0.08^{bc}	0.64 ± 0.09^c
FCR (g feed g^{-1}gain)	1.24 ± 0.10^a	1.68 ± 0.10^b	1.61 ± 0.10^{ab}	1.77 ± 0.31^b
Survival (%)	100	100	100	100

showed a moderate (−20%) but significant reduction in feed intake, while feed intake in the HO × HC group was nearly halved. FCR in the HO and HO × HC groups was significantly higher compared to the control group, but only moderately elevated in the HC group. Specific growth rates (SGR) progressively declined through the HO, HC, and HO × HC treatments.

3.2. *Nutrient digestibility*

The digestibility of crude protein, lipid, nitrogen free extract, dry matter, and ash for each of the experimental groups are presented in Table 2. Digestibility of protein showed a moderate but significant increase in response to the HO, HC, and HO × HC treatments, while lipid digestibility also increased slightly for the HO and HC groups. Digestibility of nitrogen free extract was not affected in any of the experimental groups.

Table 2
Effects of dissolved gas treatment regime on ADC (%) of protein, lipid, NFE, dry matter, and ash. ADC, apparent nutrient digestibility, NFE, nitrogen free extract including fibre, (calculated as dry matter – protein – lipid – ash), HO, hypoxia, HC, hypercapnia, HO × HC, combined hypoxia and hypercapnia. Values in a row not sharing a common superscript letter are significantly different. Values are shown as mean ± SE. (n = 4, except for HO × HC where n = 2).

	Treatment			
Parameters	Control	HO	HC	HO × HC
Protein (%)	87.3 ± 0.3^{a}	91.0 ± 0.3^{b}	93.9 ± 0.5^{c}	91.4 ± 1.0^{b}
Lipid (%)	93.0 ± 0.1^{a}	94.8 ± 0.2^{b}	96.4 ± 0.4^{c}	93.5 ± 1.3^{ab}
NFE (%)	72.2 ± 0.4	68.7 ± 0.9	72.9 ± 2.6	68.6 ± 1.9
Dry matter (%)	80.5 ± 0.3^{a}	82.6 ± 0.5^{ab}	85.9 ± 1.3^{b}	82.2 ± 1.3^{ab}
Ash (%)	53.9 ± 0.6^{a}	59.7 ± 1.2^{ab}	64.9 ± 3.2^{b}	54.5 ± 0.6^{a}

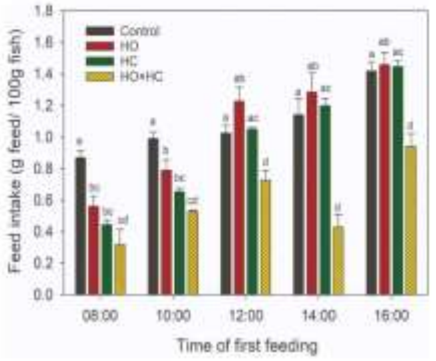

Fig. 3. Feed intake during a 15 min feeding in relation to time of first feeding of Nile tilapia after nocturnal exposure to hypoxia (HO), hypercapnia (HC), or combined hypoxia and hypercapnia (HO × HC). Values are means ± SE, n = 4. Differences in superscripts indicate significant differences in feed intake between treatments at the given feeding time.

3.3. *Appetite assessment*

The results for feed intake during appetite assessment are shown in Fig. 3. Nile tilapia in the control group were able to ingest significantly more feed than the other treatment groups during the morning feedings at 8.00 and 10.00 h. This persisted through the 10.00 am feeding, whereas when the first feeding occurred at midday, the hypoxia and hypercapnia treatment groups had recovered their appetite and ingested the same amount of feed as the control group. Fish cultured under combined nocturnal hypoxia and hypercapnia never fully recovered their appetite and were not able to ingest a ration greater than ca. 1% of their body mass.

4. Discussion

Fish cultured in earthen ponds are subject to nocturnal hypoxic and hypercapnic events, which are presumed to affect physiological processes such as appetite, feed intake and feed utilization efficiency. Our understanding of the effects of hypoxia and hypercapnia in fish is mainly based on studies examining their individual effects, typically under chronic conditions. In a pond environment, low dissolved oxygen, and high carbon dioxide occur simultaneously and fluctuate daily. We hypothesised that combined exposure to hypercapnia and hypoxia would induce a bigger physiological disturbance and that fluctuating conditions might be more difficult to cope with than a suboptimal steady state. The present data provides insight into how DO and CO_2 exert synergistic and interacting effects on growth, feeding characteristics and feed utilization in Nile tilapia.

The ability of Nile tilapia to tolerate extremely low levels of dissolved oxygen, through behavioural strategies such as aquatic surface respiration (Obirikorang et al., 2020) and the physiological ability to enter into a state of metabolic depression (Bergstedt et al., 2021), has been documented. In the present study we demonstrate that Nile tilapia also appears to be physiologically resilient to hypercapnia as well as combined hypoxia and hypercapnia, but that from an aquaculture perspective this has negative effects on production performance. The culture of Nile tilapia under hypercapnia and combined hypoxia and hypercapnia were also observed to engage more frequently in aquatic surface respiration, although this was not systematically quantified.

Low levels of dissolved oxygen (Abdel-Tawwab et al., 2019; Bergstedt et al., 2021; Obirikorang et al., 2020) and high levels of CO_2 (Fivelstad et al., 1998; Methling et al., 2013; Mota et al., 2019) individually affect a number of behavioural and physiological traits in fish (Pollock et al., 2007; Lefevre, 2019). Short-term acute exposure to hypoxia or hypercapnia have been shown to reduce resting as well as maximum metabolic rates in numerous aquaculture species (Beamish, 1964; Neto and Steffensen, 1997; Khan et al., 2018), including Mozambique tilapia (Van Dijk et al., 1993). The resultant loss of metabolic scope leads to a depression of appetite and feed intake, probably because maximum feed intake is adjusted to avoid that the energy requirement for digestion exceeds availability (Jobling, 1981; Mallekh and Lagardère, 2002). In the present study, hypoxia resulted in only a negligible reduction in appetite during the morning feeding, but no significant change in daily feed intake, compared to the control group that consumed the daily 2% ration. Reductions in feed intake became more apparent for the HC group, consuming on average 20% less than the control group, while the HO × HC group reduced their daily feed intake by nearly half. These results demonstrate that nocturnal hypercapnia exerts a depression in appetite that does not occur as a result of severe hypoxia, but that the combination of hypoxia and hypercapnia exerts a negative synergistic effect on feeding in Nile tilapia. It appears that while fish are able to recover from an oxygen debt incurred during a nocturnal hypoxic event when it occurs on its own but are unable to do so when it occurs in combination with hypercapnia (or vice versa, that the recovery from hypercapnia is prolonged in combination with hypoxia). In the present study, the metabolic responses of the different experimental groups, or any physiological changes that occurred over time, were not quantified. However, it is reasonable to assume that the overnight exposure to high CO_2 led to a lasting acidification of the blood and a resultant reduced oxygen affinity and carrying capacity of haemoglobin (Herbert et al., 2006), as well as an increased energy allocation towards acid-base regulation (Claiborne and Heisler, 1986; Brauner et al., 2004; Shartau et al., 2019). These represent metabolic costs that must be covered before energy can be allocated to support maximal feeding and warrant further research. Furthermore, fish were still experiencing moderate hypoxia or hypercapnia during morning hours, and could still be experiencing a reduced metabolic scope due to a persistent depression in maximum oxygen uptake capacity and/or an ongoing repayment of oxygen debt (Plambech et al., 2013; Svendsen et al., 2012).

In addition to feed intake, feed utilization is an essential factor for predicting fish growth. Feed utilization is affected first by how efficiently fish digest protein and energy, and secondly by how efficiently they can deposit digested protein as new muscle. In the present study, no negative effects from previous exposure to hypoxia or hypercapnia were observed in relation to digestibility of nutrients. On the contrary, the ADC for protein and lipid showed a slight but significant increase in response to diurnal fluctuations in DO and CO_2. The most likely explanation for this relate to feeding time and feed intake. Fish in the HC and HO × HC groups generally ate more of their ration during a limited time span in the afternoon and may have had smaller endogenous losses. This fits particularly well for the HC group that ate the bulk of their ration in the afternoon and had the highest ADC for protein and lipid. The HO × HC group also ate most of their feed in the afternoon but had a smaller increase in ADC compared to the control group, probably because they generally ate less.

Despite that ADC of nutrients were either unaffected or even improved in the HO, HC, and HO × HC groups, feed utilization was negatively affected in all treatments. The clearest example is from the HO group that ingested the same daily amount of feed as the control group yet had significantly poorer growth and a higher FCR. It seems most likely then that the poor growth is the result of an increase in metabolism driving fish to use a larger fraction of the ingested feed for energy production rather than growth. A similar picture emerges from the HC treatment, although here the decrease in SGR is the combined result of a decrease in feed intake and poor feed conversion. Finally in the HO × HC group the SGR bottomed out at 0.64% d^{-1} based on ingested daily rations of 1.12% of their body mass. We have shown that feed intake affects FCR in Nile tilapia, and that as daily feed intake declines towards 1% this approaches the maintenance ration where gains and losses equal out, and feed utilization efficiency rapidly worsens (Skov et al., 2017). In the present study, FCR values were still higher than expected for such a ration size illustrating that the negative effects of co-occurring hypoxia and hypercapnia on growth and feed utilization are a combined result dominated by a decreased feed intake but worsened by fish having to spend more energy on maintaining homeostasis and recovery.

From the assessment of appetite recovery during the day (Fig. 3) it was apparent that all experimental treatments resulted in a depression of appetite in the morning. It was quite remarkable that the HO was willing to feed at 8:00 h, where, despite an oxygen saturation near 20%, fish still ingested 0.5% of their body mass in feed. The HO group recovered their appetite faster than other experimental groups, and even had an appetite that was marginally greater than the control fish when first feeding occurred at noon, in accordance with previous observations (Obirikorang et al., 2020). A similar pattern emerged from the hypercapnic treatment group; fish were still willing to ingest reduced amounts of feed in the morning despite persistent hypercapnia (25 mg CO_2 L^{-1} at 8:00 h) and fully recovered their appetite by midday. Remarkably, fish in the HO × HC group were unable to fully recover their appetite, not even at the last time point (16:00 h) where water was normoxic and normocapnic. The explanation for this is not immediately obvious, but it is well known that hypoxia induces long-lasting effects on appetite through an upregulation in appetite depressant hormones (Bernier and Craig, 2005; Chu et al., 2010) Despite numerous efforts to determine the effects of CO_2 on fish feeding and appetite, detailed investigations into how and at which concentrations it affects appetite regulating hormones have yet to be conducted.

5. Conclusion

Nocturnal exposure to hypoxia or hypercapnia has adverse effects on the growth performance of Nile tilapia driven by a combination of reductions in appetite and poor feed utilization. Combined exposure to hypoxia and hypercapnia exacerbates these effects, particularly with respect to feed intake. Based on the calculated FCR values, nocturnal exposure to hypoxia, hypercapnia, or combinations may result in 30–40% increases in feed costs, and lead to increased excretion of dissolved nitrogen. While digestibility of nutrients was not negatively affected, reduced appetite may cause considerable feed waste if feeding regimes are not adjusted, although for the combined hypoxia and hypercapnia, this may not be possible due to a day-long loss of appetite. The results emphasize that there is a need for careful management of dissolved gases in pond culture. Application of aeration technology, combined with management of the nutrient status of the pond and sludge accumulation to control oxygen consuming and CO_2 liberating processes, is likely able to greatly improve production performance.

CRediT authorship contribution statement

Muumin Iddi Hamad: Conceptualization, Methodology, Software, Validation, Formal analysis, Investigation, Data curation, Writing – original draft, Visualization. **Heiromin Amon Lamtane:** Formal analysis, Writing – review & editing. **Renalda Nanziga Munubi:** Formal analysis, Data curation, Supervision, Writing – review & editing. **Peter Vilhelm Skov:** Conceptualization, Methodology, Software, Resources, Supervision, Writing – original draft, Writing – review & editing.

Declaration of Competing Interest

The authors declare no conflict of interest.

Data availability

Data will be made available on request.

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CHAPTER FIVE

PAPER FOUR

Critical Thermal Maximum (CT_{max}), Metabolic and Cardiorespiratory Response of Nile Tilapia (*Oreochromis niloticus)* exposed to Acute Temperature Elevation upon Oxygen saturation

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Abstract

Temperature plays significant role in controlling oxygen solubility in water and its availability to aquatic breathing species including fish. In tropical small water bodies such as ponds, the water temperature can increases gradually during the day resulting in elevated temperature and an extended period of low dissolved oxygen, which interferes with important physiological aspects including fish oxygen demand. In this study, we investigated the response of Nile tilapia (*Oreochromis niloticus*) thermal tolerance limit (CT_{max}), metabolic rate (SMR), Cardiac performance (f_H), and ventilation frequency (f_V) to the acute increase in temperature upon changing dissolved oxygen saturation. Nile tilapia were experimentally exposed to an increased temperature of 0.1 °C min^{-1} at 100% DO saturation (Normoxia), 70% (hypoxia), and 130% (hyperoxia) from 26 °C (Optimum) until the onset of loss of equilibrium (LOE). Oxygen consumption rate (MO_2), heartbeat, and opercula activity were averaged at each temperature increased upon three DO saturation levels. Results showed that high DO saturation (130%) increases Nile tilapia Critical Thermal Maximum (CT*max*) by 10%. The Nile tilapia metabolic rate (SMR) varied significantly with the changing DO saturation upon acute increased temperature levels (One-way ANOVA; $F_{(2,44)} = 4.596$, $P = 0.016$). Temperature-DO-limited metabolic relationship affects cardiorespiratory activities and maximum thermal tolerance (CTmax) in Nile tilapia (one-way ANOVA; $F_{(2,44)} = 0.34$, $P = 0.713$). Different levels of DO saturation were found to significantly affect Nile tilapia's respiration ability (Opercula beats min^{-1}) $P < 0.001$.

Keywords
Cardiorespiratory, CT_{max}, Nile tilapia, Metabolic rate, Earthen pond.

Status: Manuscript.

1.0 Introduction

Fish Environmental temperature is known for its affect on the biochemical and physiological activities of fish (Cooper et al., 2021; Giacomin et al., 2017; Majhi & Das, 2013; Turko et al., 2020). A small variation in temperature or a slight change above the ideal bounds of a certain species can lead to variation in routine metabolism and affect important physiological performance including oxygen consumption (Fernandes & Rantin, 1994). Temperature distribution in the natural aquatic environment can describe the thermal tolerance limit of a certain species and can ideally moderate the habitat range, growth performance, or even feeding habits. On the contrary, in an aquaculture system where fish are confined in an unnaturally small volume of water, the temperature has to be controlled more often to an ambient level to promote high fish growth. However, in an earthen pond, for example, the water temperature can acutely vary within a day, between seasons or between areas, and most of the time to a level above the thermal maximum limit of cultured fish as a result, cultured fish are forced to change or perhaps regulate their metabolic activities which may jeopardize their growth rate (Jaxion-

Harm & Ladich, 2014). Fish are known to have evolved an outstanding physiological mechanism to mitigate the adverse effects of thermal stress (Inoue et al., 2008) which include reduced rate of oxygen consumption (Barrionuevo & Burggren, 2022), increased ventilation (Ern et al., 2016) and excelling in cardiac output (Gilbert et al., 2020). However, a clear relationship underlying the mechanisms that fish use to mitigate thermal stress has not yet been demonstrated.

In fish, the degree of vulnerability to thermal stress depends on the thermal tolerance limit (CT_{max}) of a certain species (Ern et al., 2016), such that, for a certain species to perform optimally must inhabit a specific thermal assortment. The critical thermal maximum (CT_{max}), is the determinant of the upper limit of a species' thermal position, or the temperature where most physiological functions of an animal tumble and the animal exhibit a loss of equilibrium (LOE) (Ern et al., 2015, 2016). Temperature is critically known to affect oxygen solubility in the water and hence its consumption (MO_2) in fish (Eliason & Farrell, 2016; Remen et al., 2015). It is also been documented that temperature is the main modulator of intrinsic heart rate (Q). Therefore, temperature creates a strong relationship between oxygen supply capacity (the ability of blood to carry oxygen) and the cardiorespiratory system (ventilation rate, rate of cardiac output) in fish (Farrell et al., 2009).

Physiologically, at high water temperature fish reduce their cardiorespiratory performance and consequently limits oxygen supply capacity causing critical oxygen tension and resulting in decreasing the rate of oxygen consumption. Therefore, the main reason why fish are unable to maintain sufficient oxygen supply to support basic metabolic functions at high temperature or a temperature close to the maximum thermal limit (CT_{max}), is associated with the direct interference of temperature with a cardiorespiratory system which limits blood oxygen carrying capacity. This relationship between temperature and oxygen availability, and how it affects the general physiological performance of fish is highlighted in a substantial volume of scientific literature (Chatterjee et al., 2004; Cho et al., 2015; Cooper et al., 2021; Das et al., 2005; Dowd et al., 2015; Gilbert et al., 2020; Joyce et al., 2018; Mottola et al., 2020; Pandit & Nakamura, 2010; Remen et al., 2015). In some fish species, the thermal tolerance limit can vary depending on the performance of both rate of temperature change and duration of exposure to a new temperature, therefore, CTmax can preferably be used to evaluate the thermal tolerance limit of certain animals living in an acute temperature increasing environment such as shallow streams or earthen ponds.

Nile tilapia (*Oreochromis niloticus*) is the world's most pond-cultured species (Sriyasak et al., 2015). Its tropical and subtropical origin imitate their distribution and thermal preferences (Pandit & Nakamura, 2010). Despite their wide range of temperature preferences (Musa et al., 2017), Nile tilapia are reported to not grow well below 16°C and survivability is limited at a temperature just below 10°C, however, they can remarkably survive up to 40°C (Azaza et al., 2008; Nyanti et al., 2018; Pandit & Nakamura, 2010; Soltan et al., 2015). Although Nile tilapia’s preferable thermal limit is known, farming this species in the earthen pond represents a big challenge considering that temperature in most tropical ponds can acutely vary during the daytime to a level above the maximum thermal tolerance limit of the fish, hence, induce challenges to both fish physiological activities and dissolved oxygen (DO) solubility in the water. There is little information describing the response of physiological activities of Nile tilapia's when the temperature is abruptly increased while DO concentration in the water varies.

2.0 Material and Methods

2.1 Ethics statement

Experimental procedures were conducted under the protocol approved for animal experimentation by Denmark Technical University. All procedures and laboratory facilities were in accordance with the ethical guidelines of Directive 2010/63/EU.

2.2 Fish collection, acclimation and Experimental design

All experimental activities were done at the National Institute of Aquatic Resources, of the Technical University of Denmark, Hirtshals, Denmark. Fish were collected from a commercial fish farm (Til-Aqua International, Velden, Netherlands). The fish were acclimated in-stream tanks (tanks with continuous water flow) at approximately 25±1 °C at 12:12-hours, light: dark-cycle. Tested fish were all-male Nile tilapia (*Orechromis niloticus*) [N = 18, Total length (L_T) =28.27± 0.62 cm, Body mass (M_g) = 455.333±25.034 g]. During acclimation, fish were fed with the commercial diet 39% CP (Efico, Cromis832F, BioMar, Denmark) twice a day.

The experiment was executed using a custom-made 10L transparent polyethene respirometer chamber, immersed in a (100L) tank filled with 50L of water. Water in the experimental tank was continuously replaced with fresh water at a flowing rate of 1.5 $Lmin^{-1}$ during the experimental period. The main idea of the experiment was to acutely increase water temperature by 0.1°C per minute from 26°C to 40°C at a constant dissolved oxygen level of 100% (Normoxia), 70% (Hypoxia) and 130% (Hyperoxia). This was achieved by, inserting a submersible pump inside the main experimental tank to create a continuous circulation of the water aimed at maintaining an even distribution of temperature and dissolved oxygen during the experimental period. The respirometer chamber was also attached with two submersible pumps, one for controlling the internal water circulation during measurement and another was used for water flushing and introduction of new water into the chamber.

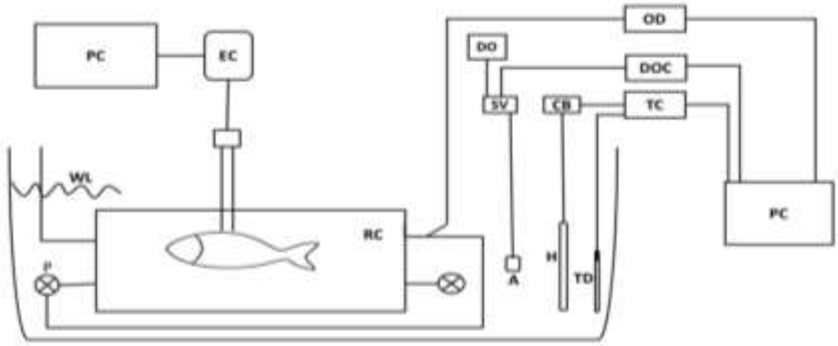

Figure 1: Illustration of the experimental set up used to assess the effect of acute temperature exposure against different levels of oxygen saturation. (OD: Oxygen detector, DOC: DO controller, CB: Regulator box, H: Heater, TD. Temperature detector, A: aerator, SV: solenoid valve, RC: respirometer chamber, EC: electro cardiometry, P: water pump).

The oxygen sensor was mounted beside the respirometer chamber (recirculation loop) and connected to an OXY- 4 mini (PreSens, Regensburg, Germany) and used to measure levels of oxygen concentration in the respirometer chamber during the oxygen evaluation period. The required level of oxygen saturation was regulated and kept at pre-determined levels by bubbling pure oxygen gas from an oxygen tank using air stones which were attached to a PC running a script in DAQFactory (AzeoTech, Inc., OR, USA). The oxygen degassing was automatically controlled by turning off the oxygen supply with the help of an O_2 electrode transferred to the PC via a digital acquisition device (U6 DAQ, LabJack, Inc., CO, USA). The temperature was maintained and acutely increased from the acclimated level of 26 °C to a required level over time using 1000W heaters immersed in the experimental tank which was controlled by a programable relay of (PR-5714, PR Electronics, Denmark) temperature script controlled by a solenoid valve.

2.3 Oxygen Consumption (MO_2) and Metabolic rate (SMR)

The metabolic rate (SMR) of Nile tilapia reared at an acutely increased water temperature of 0.1°C per minute from 26°C to 40 °C at an oxygen saturation of 100%, 70% and 130% was estimated by measuring the rate of oxygen consumption (MO_2) using Intermittent flow respirometry. Detailed experimental set-up as described in section 2.1 above. SMR was determined in 18 fish (6 fish per level of O_2 exposure). Fish were collected from an acclimation tank where they were previously starved for 24h before experimentation and inserted in a respirometer chamber later allowed to recover their metabolic rate from handling stress overnight before MO_2 measurements. During experimentation temperature control system which was running the script using a PC running software (section 2.1) was allowed to acutely increase water temperature at 0.1°C /min (6°C/hr) and at the same time, the required level of dissolved oxygen was maintained using the procedure described in section 2.1. Individual MO_2 at each level of temperature increase and required level of dissolved oxygen were recorded directly from the respirometer chamber via a fibre optic oxygen sensor attached to a PC running software (Loligo Systems, Viborg, Denmark) and its reading was expressed as mgO_2 kg^{-1} h^{-1}.

2.4 Critical Thermal Maximum (CTmax) and Lose of Equilibrium (LOE)

The critical thermal maximum (CTmax) of Nile Tilapia (N=6) was measured at three levels of oxygen availability (Normoxia 100%, Hypoxia 70% and Hyperoxia 130%) at a continuous temperature increase of 0.1°C min^{-1}. The fish were placed inside respirometry chambers during measurement and left overnight to recover from handling stress. The dissolved oxygen level was set at the required level of exposure (treatment). At the time of experimentation, a respective DO level was controlled and maintained following the procedures described in section 2.1. After maintaining the required level of dissolved oxygen, the water temperature was gradually increased at a rate of 0.1°C min^{-1} until the fish displayed LOE, which was chosen as the critical thermal maxima (CTmax). LOE was defined as the time when fish fell over and could no longer right themselves. Once CTmax was reached for individual fish, they were removed from their chambers and returned to a recovery tank.

2.5 Temperature and Dissolved Oxygen Effects on Heart Rate

The effect of acutely elevated water temperature at different oxygen saturation on heart rate (f_H) of Nile tilapia was evaluated on 18 fish (6 fish for every oxygen exposure group.) using a computer-controlled electrocardiogram (ECG) instrument, LabPro (Vernier, USA) electrodes (30 gauge insulated wire). During measurement, fish were collected from the acclimation tank and sedated with benzocaine solution (2 mils in 5 L) at 26 °C until ventilation stopped. Fish were then weighed and transferred to a Styrofoam board where their gills were irrigated with a pump. Stainless steel electrodes were inserted into the skin over the heart on the right side of the ventral midline and just posterior to the left pectoral fin. Once the electrodes were fixed fish were transferred to the respirometer chamber and allowed to recover at normoxia water at 26 °C overnight. This procedure was completed within 1–3 min. The electrocardiogram (ECG) was recorded and the ECG signal was recorded with BioPac MP100 data acquisition system (BIOPAC System, Inc., Santa Barbara, California)

2.6 Temperature and Dissolved Oxygen Effect on Ventilation Frequency

The effect of acutely increased temperature and different oxygen saturation levels of 100, 70, and 130 per cent saturation on Nile tilapia was measured simultaneously during LOE assessment. Ventilation activity was observed as the amplitude of opercula beats (OB) counted within 30 seconds of temperature exposure at a respective level of dissolved oxygen saturation and were presented as opercular beat per minute ($OBmin^{-1}$).

2.7 Data analysis

Statistical analyses were performed using SigmaPlot v. 14.5 (Systat Software, Inc., Chicago, IL, USA). The parametric assumption of normality of data was tested using Shapiro–Wilk normality tests, and the likeness of variances was assessed using Bartlett's test. One-Way Analysis of Variance ($P < 0.05$) was used to test the effect of elevated temperature and level of dissolved oxygen on SMR, CTmax, Heartrate and ventilation frequency, respectively. Where significant differences were found, in post hoc for multiple comparison analysis, Duncan's multiple range test was used to differentiate statistically distinct groups.

3.0 Results

3.1 Oxygen Consumption and SMR

The overnight MO_2 consumption of the recovered Nile tilapia after handling stress at different dissolved oxygen levels (100, 70, and 130%) under the acclimation temperature of 26°C is presented in figure 1. Results showed that dissolved oxygen saturation has a significant impact on Nile tilapia oxygen uptake rate (MO_2) upon recovery from handling stress ($P = 0.020$).

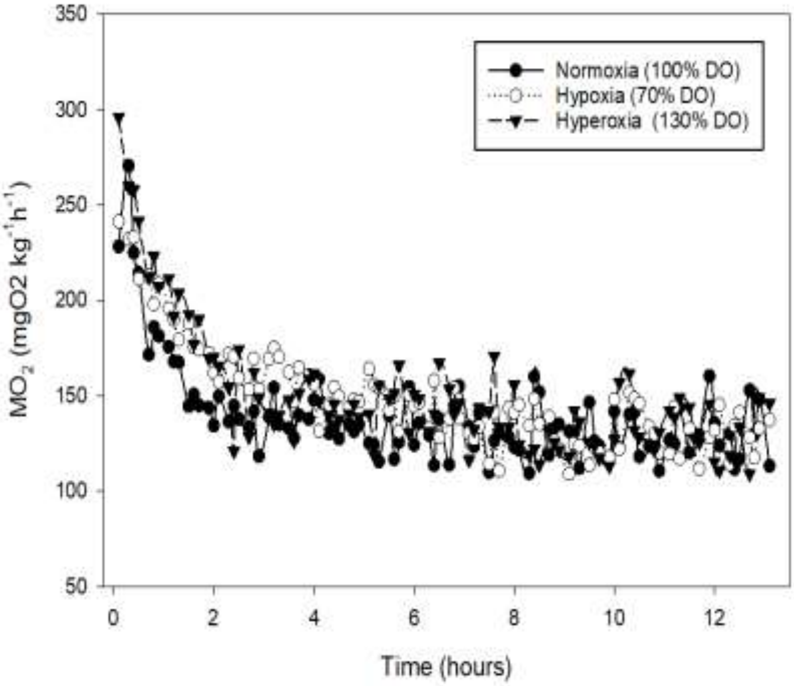

Figure 2: Oxygen consumption (MO_2) mgO2 kg^{-1} h^{-1} was recorded during Nile tilapia recovery from handling stress at 26 °C under different levels of DO saturation (n = 6, for each, DO level).

Nile tilapia showed, higher MO_2 in all dissolved oxygen groups immediately after handling stress, then returned to a basal level depending on the respective dissolve oxygen saturation exposure group, and fish in all groups stabilise MO_2 after 6 to 7 hours, with a mean SMR of respectively dissolved oxygen saturation (100, 70, and 130 per cent) of 139.17± 2.59, 149.05± 2.78 and 149.64±3.41 (mgO_2 kg^{-1} h^{-1}, ±SE) respectively. Few miscellaneous spots showed interactions from spontaneous activities that cause a brief rise in MO_2 (Figure 1).

DO saturation has a significant effect on Nile tilapia metabolic rate (SMR) when temperature increased acutely as represented in

Figure 2, ($F_{(2, 44)}$ = 4.596, P = 0.016). SMR in the 100% and 70% DO saturation groups increased exponentially with an increased temperature ($y = 30.311e^{0.0586x}$; R^2 = 0.93 and $y = 20.415e^{0.0609x}$; R^2 = 0.89) respectively, while at super saturated DO group (130%), SMR linearly increased with an increase in levels of temperature ($y = 15.255x - 291.74$; R^2 = 0.97) with the temperature coefficient (Q_{10}) of 1.87, 1.36 and 1.98 at 100, 70, and 130% DO respectively (Figure 2). Differences in DO saturation also showed variation in the thermal work limit (TWL) of metabolism (SMR_{TWL}) in Nile tilapia (100% DO saturation 331.18±32.96, 70% DO saturation 232.38±24.09, and 130% DO saturation 333.17±32.49. Mean ±SE), however, the difference was not statistically deferent (P > 0.05).

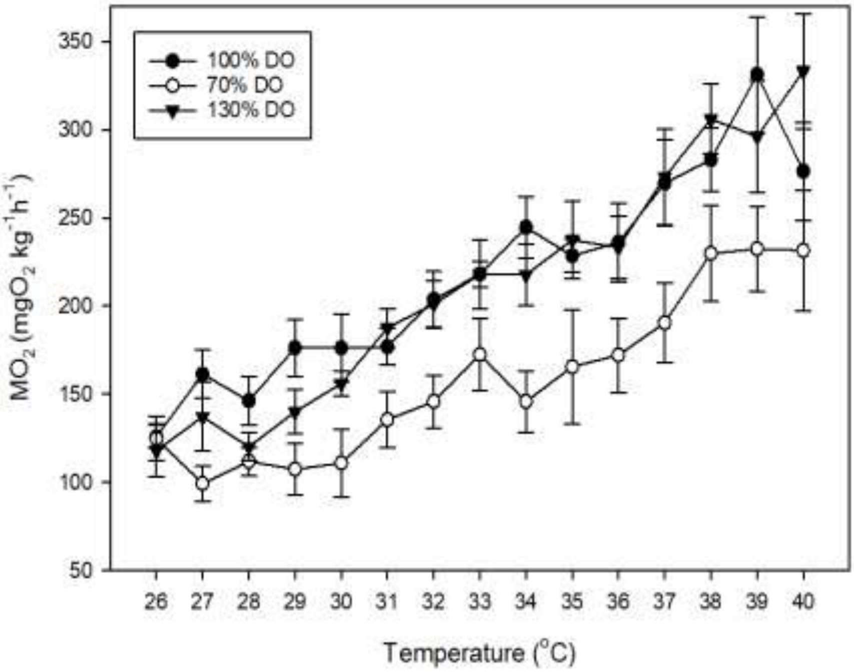

Figure 3: Metabolic rate (SMR) of Nile tilapia (Mean ±SEM) treated with water at different levels of DO saturation at progressively increased temperature (N = 18 per DO exposure group).

3.2 Critical Thermal Maximum (CTmax)

CTmax significantly increased with an increase in DO saturation (Figure 3; *P* = 0.011). CTmax increase by 0.61 °C (1.6%) in fish exposed to the high DO saturation (130% DO) and reduce by 0.24 (0.7%) at 70% DO exposed group compared to Normoxia (100% DO) group. A significant positive relationship was also observed between CTmax and the level of DO saturation. Not all fish survived to CTmax during the LOE test, two fish (33%) in the hypoxia group (70% DO) died while recovering after LOE. Levels of DO saturation have an impact on Nile tilapia recovery time from LOE ($F_{(2,14)}$ = 10.07, *P* = 0.001). Recovery times after LOE were recorded for each level of DO saturation and results showed that Nile tilapia treated with a high level of DO saturation had low mean recovery time (8.11± 0.36 mins) than those treated at 100% and 70% and their recovery time was almost similar (10.33±0.34 and 10.75±0.47 mins) respectively.

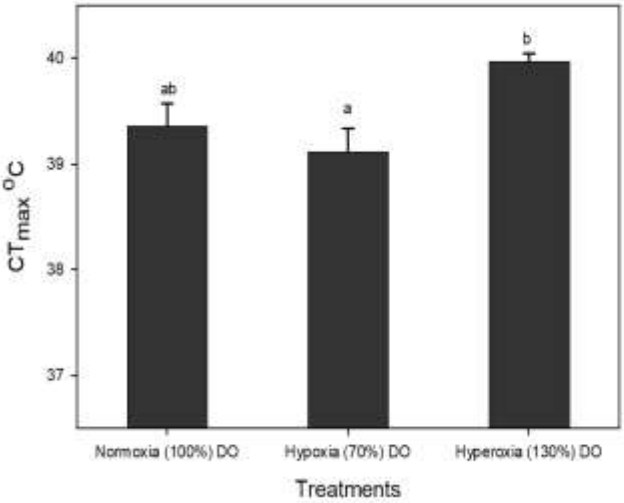

Figure 4: Critical thermal maximum, CTmax (°C) for Nile tilapia (n = 6 for each DO level), measured at a different level of DO saturation at an increased temperature of 0.1 min^{-1}. Means ±SEM.

3.3 Ventilation frequency

The mean dissolved oxygen saturation was found to significantly ($P < 0.001$) affect Nile tilapia's respiration ability (Figure 4). Results show a reduction in opercula beats in tilapia exposed to high DO water (130% DO saturation) than those cultured at 100 and 70 % DO. Regression analysis also shows a positive relationship between respiration rate and dissolved oxygen saturation when Nile tilapia exposed to an acutely increased temperature ($F_{(3,14)} = 177.8$, $P = 0.001$; $R^2 = 0.979$, n = 6) with Q_{10} of 1.91, 1.64, and 1.53 at 100, 70, and 130% DO respectively, Figure 4.

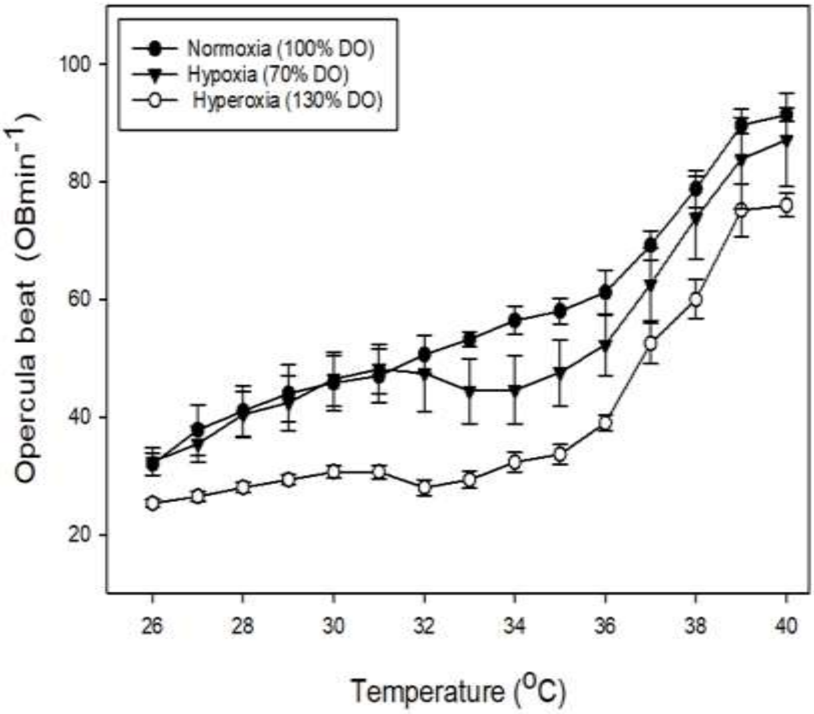

Figure 5: Effect of DO saturation and elevated level of temperature on Nile tilapia's opercula activities (OB min^{-1}). Data presented as mean ±SEM, n = 6.

3.4 Heartbeats variability

Heart rate (beats min^{-1}) increased with increasing temperature Figure 5, however, one-way ANOVA revealed that the levels of DO saturation in heart rate did not differ significantly ($F_{(2,44)} = 0.34$, $P = 0.713$). between the definite level of temperature. Heat beat increase linearly with increased temperature in all DO saturation levels, there was a strong relationship between temperature and Nile tilapia heartbeats ($R^2 = 0.992$, $F_{(2,14)} = 510.16$, $P = 0.001$) with Q_{10} of 1.75, 2.06 and 2.94 at 100%, 70% and 130% DO saturation respectively. A very low heartbeat was observed at 130% DO saturation (Cardiogram, Figure 6) at an optimum temperature (26 °C) compared to 100 and 70% however, there was a synchronization in heartbeats in all three DO saturation groups at a temperature of 34 °C. Results also showed that Nile tilapia exposed to a high level of DO saturation (130% DO) reduce their heartbeats by 39.6% at an optimum temperature of 26 °C, but increased by 5% at a high temperature of 35 - 38°C compared to Normoxia (100% DO).

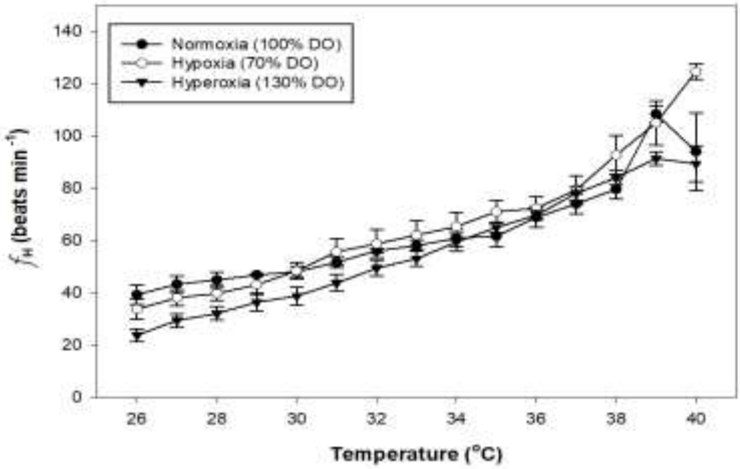

Figure 6: Effect of DO saturation and elevated temperature on Nile tilapia heart rate of (beats min^{-1}). Data presented as mean ±SE, n = 6.

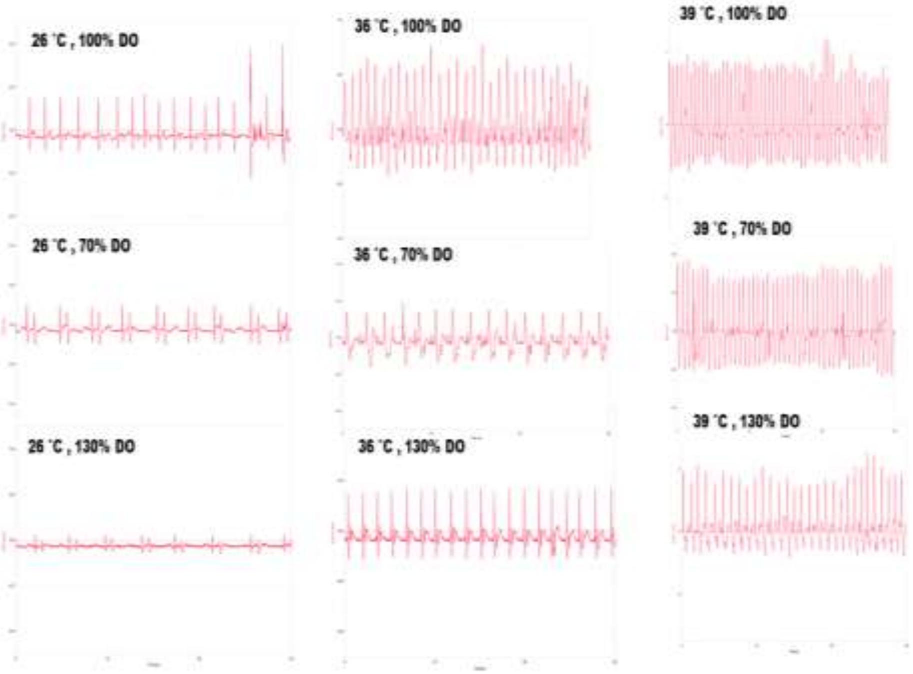

Figure 7: A cardiogram performance of Nile tilapia exposed to an increased temperature at a different level of DO saturation (100, 70 and 130% DO) at an optimum temperature of 26°C, at the time of coefficient (Q_{10}) 36 °C and temperature 39 °C before the LOE.

4.0 Discussion

In fish, temperature is critical for growth, biochemical responses, and physiology (Pandit & Nakamura, 2010; Singh et al., 2013). It controls both the solubility of oxygen in the water and the intake of oxygen by fish. DO, on the other hand, acts as a final electron acceptor in the generation of ATP and is a direct regulator of the aerobic process in fish (Pörtner et al., 2004). In this study, we evaluated the idea that DO saturation in the water limits the influence of temperature on the upper thermal tolerance limit (CTmax) and physiological processes (Oxygen intake, Ventilation frequency, and Heart rate) of Nile tilapia. Oxygen consumption in fish is frequently used as a measure of a metabolic index

(Claësson et al., 2016; Ern et al., 2015; Fernandes & Rantin, 1994), and has been highly associated with changes in the surrounding environment, such as temperature (Remen et al., 2015). Results from this study showed a significant association between DO saturation and increasing water temperature on metabolic rate (SMR), (Figure 2). A high rate of oxygen consumption (MO_2) in Nile tilapia exposed to high DO saturation (130%) suggests the need to consider solubility levels of DO when determining the temperature threshold of physiological activities in Nile tilapia.

The MO_2 results from this study also agreed with the findings observed for other fish species (Barrionuevo & Burggren, 2022; Remen et al., 2015). A very low rate of oxygen consumption (MO_2) in Nile tilapia exposed to 70 % DO group compared to the 100 % DO (normoxia) group, in combination with the temperature coefficient (Q_{10}) values for the standard metabolic rate (SMR), suggests that, standard metabolic function in Nile tilapia is directly correlated with both oxygen supply and temperature and entirely depend on DO availability. The results also showed that Nile tilapia exposed to 70% DO achieve their LOE earlier than the other DO exposed groups, this indicates that when DO is low, high temperatures may limit the supply of oxygen in the tissues and resulted in the quick metabolic collapse.

CTmax is a piece of useful information which provide early signs of a reference point for the physiological performance of a certain animal at an increased temperature (Rahman et al., 2021). In the current study, high DO exposure (130%) increased Nile tilapia thermal tolerance limit (CTmax) with a corresponding increase in metabolic rate SMR. At acute thermal stress, fish develop a physiological mechanism to increase tolerance (Nyboer & Chapman, 2018), however, most of these temperature tolerance mechanisms (production of heat shock protein, increasing membrane permeability and increasing number of mitochondria in the cell) enquire supply of DO. This could be the reason for higher

CTmax in the 130% DO exposure group and also the reason why Nile tilapia in the Low DO saturation (70%) showed earlier loss of equilibrium (figure 3) in comparison to those exposed to Normoxia. There are no similar study reporting related information that can be used to compare with our findings, however, literally with this result it can be concluded that DO is the fundamental factor for temperature tolerance in Nile tilapia.

According to the findings from figures 4 and 5), increased temperature of the surrounding environment has a direct effect on Nile tilapia respiratory functions including oxygen consumption, heart rate, and ventilation frequency. Many studies have highlighted the cardiorespiratory relationship in fish (Farrell et al., 2009; Min et al., 2019; Nyboer & Chapman, 2018). In this study, we observed low heartbeat and ventilation frequency in the high DO exposure group (130%) compare to the hypoxia group with 70% DO (Figures 4 and 5) which indicates a definite link between the circulatory and respiratory systems in acquiring and delivering oxygen to cells throughout the fish's body. Low oxygen solubility as a result of increased temperature in the Low DO exposure group increases ventilation rate (Figure 5) indicating, the low ability of Nile tilapia to oxygen uptake in a hypoxic environment. In the Hyperoxia (130 %) DO group, the Q10 values for breathing frequency and heart rate were generally higher than in the normoxia (100 % DO) group. This indicates that the oxygen supply in Nile tilapia is dependent on the capacity of the internal transport network, and the effect of external factors such as temperature and oxygen availability.

5.0 Conclusion

The temperature-oxygen-limited metabolic relationship can be used to describe the effects of water temperature and dissolved oxygen on the metabolic functions of aquatic organisms, and can also be used to evaluate the role of metabolic activities in influencing ecologically relevant performance characters (e.g. growth, swimming performance, feeding and behaviour). In this study, we evaluated the temperature-oxygen-limited metabolic functions and the relationship between the capacity for aerobic metabolism and cardiorespiratory performance in Nile tilapia. We have shown that DO saturation causes variation in Nile tilapia metabolic functions and Cardiorespiratory activities when temperature increases at an acute rate. It can therefore, be concluded that, although Nile tilapia is known as stress resistance species, their physiological characteristics can be easily affected when DO level is low and the temperature is high.

Acknowledgment

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Data availability statement

The data for this study findings are available upon request from co-author.

Authors contribution

Muumin Iddi Hamada and Peter Vilhelm Skov: Conceptualization and experimentation. Muumin Iddi Hamad: Manuscript drafting and Data analysis. Heiromin Amon Lamtanea and Renalda N. Munubi: reviewed and edited the manuscript.

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CHAPTER SIX

6.0 GENERAL DISCUSSION

6.1 Variation in pond water quality parameters among selected agro-ecological zones in Tanzania

Water quality management has become a vital part of effective aquaculture production. A minor variation in water quality parameters below or above the optimum required level for a particular species during any aquaculture operation can have a substantial impact on the physiological and metabolic processes of the cultured organism and result into poor growth (Brauner *et al.,* 2019; Claireaux & Chabot, 2016; El-khaldi, 2010). In a fish pond, water quality parameters may change suddenly due to environmental manifestations or gradually as a result of ineffective production management practices (feeding and fertilizing). Changes in the local climate variables (temperature and photoperiod) of the surrounding environment may influence changes in diurnal or seasonal fluctuation in water quality parameters specifically dissolved gasses of outdoor small water bodies such as ponds. Variations in pond water parameters may result in an episode of acute and chronic stress that may affect both the performance of the pond's biological communities (bacteria and plankton) and cultured fish (Kayombo *et al.,* 2002).

Results of the first study (Paper I), confirmed a significant correlation between variation in local climatic patterns (temperature, rainfall, humidity and photoperiod) of the surrounding environment with the variation in water quality parameters in the outdoor fish ponds in different agro-ecological zones in Tanzania. A significant difference ($p < 0.001$) in the diurnal and seasonal pond water temperature observed among the zones in this study corresponds with the results from a study conducted by Tadesse *et al.* (2004) and Jacobs *et al.* (2008) who also reported that differences in local climate variation can differently impact physiochemical characteristics of shallow water bodies of the surrounding areas. The accepted temperature range for optimum tilapia performance is between 25°C and 32 °C (Pandit & Nakamura, 2010). In the present study, the mean annual temperature observed in the Southern highland zone and Northern zone were slightly below this recommended level. This implies that, the current diurnal and seasonal water quality varables in these zones are not conducive for Nile tilapia farming unless some measures has to be tacan to improve the quality. Water temperature is known to regulate other water physicochemical parameters in the culture system (Boyd & Lichtkoppler, 1979). This study also revealed that there were variations in water temperature between seasons within the same zones and this in turn lead to seasonal variation in other water quality parameters such as DO and pH. Seasonal variation of pond water parameters observed might be caused by variation in local weather contions as it was revealed to have a grater impact on small water bodies such as ponds (Tadesse *et al.,* 2004). This study revealed that mean diurnal and seasonal water physicochemical parameters, DO, pH, TDS and Electric conductivity were recorded within the ideal range appropriate for Nile tilapia growth. The DO for optimum tilapia growth should be above 5 mg L^{-1} (Abdel-Tawwab *et al.,* 2019; El-khateeb, 2017) and the minimum level should not be below 3 mg L^{-1} on average (Makori *et al.*, 2017a). The preferred pH for tilapia culture is the level between 6.5 and 8.3 (Makori et al., 2017a). Total dissolved solids (TDS) should not be above 1000 (ppm) (Ezeanya *et al.,* 2015) and the Conductivity optimum range for fish farming of 150 to 500 (µS cm^{-1}) (Makori *et al.,* 2017a). Except for the temperature that was observed to vary below the optimum in the Northern and southern highland zones, in the present study, the means for all other measured variables were at an optimum rage for nile tilapia growth.

Optimum temperature and high DO concentrations promote higher fish yield (Shoko *et al.,* 2014) and pond Net and Gross primary productivity (Obirikorang *et al.,* 2020). This study revealed significant variation in mean diurnal and seasonal water temperature and DO in all zones between the warm and cold seasons with high temperature being observed in the warm season and a low in the cold season. Low DO and low temperature observed in the Southern highland and Northern zones corresponded with the low fish yield and low net productivity during the cold season.

6.2 The effect of elevated dissolved CO_2 (PCO_2) on metabolic rate and acid base regulation

In a fish pond, dissolved gasses (CO_2 and DO) are likely to vary on a daily bases depending on the diurnal photosynthesis and respiration rate. An elevated level of CO_2 (PCO_2) can increase to a hypercapnic level (above 10 mg L^{-1}) (Boyd, 2008) and pose a significant physiological and metabolic effect on cultured fish. The commonly known effect of elevated PCO_2 in fish is the disturbance of fish acid-base status which leads to respiratory acidosis (Baker & Brauner, 2012; Hayashi et al., 2004; Zimmer & Perry, 2022). The ability to regulate and compensate for extra acidic load from respiratory acidosis differs between fish species. This study (Paper II) evaluated the acid-base regulation ability of Nile tilapia upon exposure to an elevated level of CO_2 concentration and revealed that Nile tilapia has a strong acid compensating ability of > 25% within 24 hours of exposure to CO_2 at 10 mgL^{-1} CO_2. However, the results demonstrated that Nile tilapia s ability to compensate and regulate the extra acid load induced by elevated CO_2 depends on the CO_2 concentration and time of exposure.

The results (Paper II) also showed a relationship between Nile tilapia metabolic rate and CO_2 concentration. SMR and MMR were significantly affected by CO_2 exposure, specifically within the first hour of exposure. MMR and aerobic metabolic scope (MS) at 10mg L-1 exposure concentration were recovered to a level close to normocapnic concentration within 24 hours. However, it is also known that fish exposed to chronic hypercapnia do not generally have increased oxygen consumption at rest (SMR) (Ishimatsu *et al.*, 2005). An increase in metabolic activities above standard metabolism (SMR) during hypercapnia exposure lead to a further increase in blood PCO_2 from respiring tissues, proton load, and increased use of the buffering capacity (Claiborne *et al.,* 2002).

In this study, Nile tilapia haematological parameters (RBCc, Haematocrit, and haeboglobin (Hb)) were affected by elevated CO_2 concentration. However, similar to metabolic rate, Nile tilapia were able to fully recover variation in haematological parameters within 24 hours only in the 10 mg L^{-1} CO_2 exposure concentration. The CO_2 excreted from cellular respiration is transported as bicarbonate ($HCO3^-$) inside the red blood cells (Brauner *et al.,* 2019). At the gills, the release of bicarbonate depends on the anion exchange with Cl^- however, in this study Nile tilapia showed a potential limitation in the CO_2 compensation of only ability to compensate the rising acidosis at CO_2 concentration of 10 mg L^{-1} and not above within 24 hours of exposure.

6.3 The effect of Diurnal hypoxia and Hypacapnia interaction on feeding and feed utilization

In natural earthen ponds the concentration of dissolved gases (CO_2 and DO) shows opposite relationship to each other depending on photosynthesis and respiration. During the day oxygen is produced in the water and carbon dioxide is removed through photosynthesis while the respiration, fish, plankton, and sediment in the night consume the O_2 and release CO_2. This leads to daily conditions that fluctuate between being hyperoxic and hypercapnic during the day while becoming severely hypoxic and hypercapnic at night (Hamad *et al.,* 2023). The effects of hypoxia on Nile tilapia have been examined in some detail (Bergstedt *et al.*, 2021), but the interacting effects of hypoxia and hypercapnia and the fluctuating condition are not yet understood clearly. In this study (Paper III) Nile tilapia feeding response following exposure to the interactive effect of daily variations in dissolved O_2 and CO_2 was evaluated. In the present study, the interaction of hypoxia and hypercapnia resulted in a reduction in feed intake for an average of 50% less than the control group. In ddition, this study revealed a loss of metabolic scope in Nile tilapia during exposure to the interaction of diurnal hypoxia and hypercapnia. This condition leads to depression of appetite and feed intake, possibly because during exposure the maximum feed intake was adjusted to avoid exceeding the energy requirement for digestion (Jobling, 1981).

This study (Paper III) showed that, in addition, to feed intake, the ADC for protein and lipid were slight but significant increases in response to diurnal fluctuations in DO and CO_2. This study also revealed that Nile tilapia exposed to diurnal interaction of hypoxia and hypercapnia were unable to fully recover their appetite, not even at the last time point (16:00h) when water was normoxic and normocapnic. These results agreed with Chu et al., (2010) who demonstrated that hypoxia induces long-lasting effects on appetite through the upregulation of appetite depressant hormones.

6.4 Effects of Temperature Variation on Metabolic rate and Cardiorespiratory Characteristics

Temperature affects dissolve oxygen solubility in water and disturbs aerobic metabolic processes in fish, including the rate of oxygen consumption (MO_2). Temperature also affects cardiorespiratory activities and leads to reduction of thermal tolerance (CTmax) in fish. In fish, the degree of vulnerability to thermal stress depends on the thermal tolerance limit (CTmax) of a certain species (Ern *et al.,* 2016), thus, for a particular species to perform optimally, it must inhabit a specific thermal range. The critical thermal maximum (CTmax), is the determinant of the upper limit of a species’ thermal position, or the temperature where most physiological functions of an animal tumble and the animal exhibit a loss of equilibrium (LOE) (Ern *et al.,* 2015, 2016)

In a fish pond, especially in tropical areas where there is a long period of high temperature during the day, its water temperature acutely rises to a level above the fish's maximum tolerance limit and negatively affects the fish's physiological and metabolic process (Singh *et al.,* 2013). In his study (Paper IV), the response of Nile tilapia metabolic rate, cardiac performance and respiratory physiology were evaluated upon exposure to different DO saturation as acute temperature increased. Results proved that in Nile tilapia the upper thermal tolerance limit (CTmax) and physiological processes (oxygen intake, ventilation frequency, and heart rate) depend significantly on dissolved oxygen saturation when the temperature is acutely increased. According to Ern *et al.* (2015), the oxygen consumption rate in fish can be used as a measure of a metabolic index and there is a strong association between oxygen uptake rate with changes in the surrounding temperature (Remen *et al.,* 2015). This study confirmed a significant association between DO saturation and increasing water temperature on Nile tilapia metabolic rate (SMR), heartbeat (HBM) and ventilation frequency (opercular beat per minute). Temperature coefficient (Q10) values for the standard metabolic rate (SMR), Heartbeat and gill ventilation suggests that standard metabolic function in Nile tilapia is directly correlated with both oxygen supply and temperature and entirely depends on DO availability. This indicates that the oxygen supply in Nile tilapia is dependent on the capacity of the internal transport network, and the effect of external factors such as temperature and oxygen availability (Rahman *et al.,* 2021).

CHAPTER SEVEN

7.0. CONCLUSIONS AND RECOMMENDATIONS

This thesis presents the results from four studies which assessd the current status of the diurnal and seasonal dynamics in pond water quality parameters in fouragroecological zones of Tanzania and the implications of the variations in water quality parameters on the physiology and metabolism of the pond-cultured Nile tilapia (*Oreochromis niloticus*). Based on the findings from these studies the following conclusions were made:

7.1. Paper I: Evaluation of the current status of diurnal and seasonal variation in pond water quality parameters in Tanzania

i. The mean diurnal and seasonal pond water temperature varies significantly among agroecological zones. Mean diurnal and seasonal pond water temperature is higher in the Eastern and Lake zones and ideal for tilapia farming while in the northern and southern highland zones, both diurnal and seasonal mean temperature are only in the optimum range for Nile tilapia farming during the warm season (October to January).

ii. Despite low pond water temperature in the Northern and the Southern highland zones, generally, the mean diurnal and seasonal variation of other pond water quality parameters (DO, pH, total dissolved solid and electric conductivity) are within the optimum range for Nile tilapia farming, implying that Nile tilapia can be grown successfully during the warm season in the Northern and Southern highland to achieve the best growth.

7.2. Paper II: The effect of elevated diurnal CO_2 on Nile tilapia metabolism and acid-base regulation response

i. Nile tilapia's metabolic rate is highly affected by increased CO_2 concentration and the ability of Nile tilapia to recover metabolic loss is determined by the exposure concentration and time. In this case, Nile tilapia can completely recover aerobic metabolic scope (MS) at a CO_2 concentration of not above 10 mg L^{-1} within 24 hours.

ii. Hypercapnic conditions induce respiratory acidosis in Nile tilapia, and the buffering capacity of the metabolic acid load is indicated by an accumulation of $HCO3^-$ and exchange of CL^- however with high metabolic cost (reduced MMR with CO_2 concentration). Thus. this study concluded that the modest or slow acid-base regulation following hypercapnic exposure suggests that it is quite sensitive to CO_2 exposure. Therefore, CO_2 concentration in the Nile tilapia pond should not increase above 10 mg L^{-1}.

7.3. Paper III: the effect of diurnal interaction of hypoxia and hypercapnia on feeding and feed utilization

i. Feeding and feed utilization are strongly influenced by daily fluctuations in dissolved gases (CO_2 and DO). In addition, to feed and feed utilization, Nile tilapia growth is adversely affected by the combined effects of the interaction of diurnal hypoxia and hypercapnia rather than exposure to hypoxia or hypercapnia individually.

ii. The combination of hypoxia and hypercapnia did not affect the digestibility of nutrients in Nile tilapia but considerably reduced appetite and the FCR which according to results may result in 30-40% increases in feed costs or may lead to the increase excretion of dissolved nitrogen which may causes severe deterioration in water quality.

7.4. Paper IV: the effect of acute temperature increase and oxygen saturation.

i. This study, Confirmed that Nile tilapia has a wide range of thermal maximum limits (CTmax) as the Q_{10} was almost constant between 26 °C to 36 °C. However, other physiological characteristics can be easily affected when the temperature is acutely elevated and DO level is reduced.

ii. The result concluded that the acute increase in temperature under different oxygen saturation affects basal metabolic functions and disturbs the performance/relationship between the capacity for aerobic metabolism and cardiorespiratory functions in Nile tilapia. The DO saturation causes variation in Nile tilapia metabolic functions and Cardiorespiratory activities when temperature increases at an acute rate.

RECOMMENDATIONS

Based on the conclusions drawn from investigations and experiments carried out to address the study's objectives, the following recommendations are made:

i. In the Northern and southern highland zones, Nile tilapia should be stocked in ponds during the warm season, which starts from early October and ends in February. In this period temperatures are optimum for Nile tilapia growth.

ii. There is a need for careful management of dissolved gases, specifically those associated with photosynthesis and respiration (CO_2 and O_2) in ponds with Nile tilapia, either by application of aeration technology or careful management of the nutrient status of the pond and sludge accumulation.

iii. Pond fish farmers should follow the recommended pond construction procedure and follow the proper management practices such as proper required pond depth, proper fertilization and regular water exchange to avoid diurnal depletion of dissolved oxygen which lead to an unpredicted period of water quality deterioration which causes acute or chronic stress.

iv. Pond fish farmers in Tanzania should adjust their feeding regimes to avoid feed waste considering that the diurnal hypoxia and hypercapnia may cause short or even a day-long loss of appetite.

1) This study recommends further investigation on the effect of water quality parameters on other important fish physiological aspects such as health and reproduction.

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