EFFECT OF *Lemma minor* (Duckweed) ON THE REPRODUCTIVE PERFORMANCE OF *Oreochromis niloticus* (Nile Tilapia)

BY

Eki Rebecca OKAO

AGR2004383

DEPARTMENT OF AQUACULTURE AND FISHERIES MANAGEMENT.

FACULTY OF AGRICULTURE

UNIVERSITY OF BENIN

BENIN CITY

NOVEMBER, 2025

EFFECT OF *Lemma minor* (Duckweed) ON THE REPRODUCTIVE PERFORMANCE OF *Oreochromis niloticus* (Nile Tilapia)

BY

Eki Rebecca OKAO

AGR2004383

SUBMITTED TO THE DEPARTMENT OF AQUACULTURE AND FISHERIES MANAGEMENT, FACULTY OF AGRICULTURE, UNIVERSITY OF BENIN, BENIN CITY, EDO STATE. IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE AWARD OF BACHELOR OF AGRICULTURE DEGREE B. AGRIC (AQUACULTURE AND FISHERIES MANAGEMENT)

NOVEMBER, 2025

CERTIFICATION

This is to certify that this project was a research carried out by Eki Rebecca OKAO with Mat. No. AGR2004383 of the Department of Aquaculture and Fisheries Management, Faculty of Agriculture, University of Benin, Benin City.

…………………………..   Date…..……………………

Dr. Marinus Egwenomhe

Supervisor

………………………………   Date…..……………………

Dr. Marinus Egwenomhe

Head of Department

DEDICATION

My research work is heartily dedicated to God Almighty and my Family Mr. and Mrs. OKAO and my Sponsor Mr. and Mrs ATALOR for their continuous drive, inspiration and motivation toward me even until this very day.

ACKNOWLEDGEMENT

I would like to express my heartfelt gratitude to God Almighty, for his grace, wisdom and strength throughout my stay in the University of Benin, And for his constant provision all through these years.

I want to express my deep appreciation to my eldest sister Mrs Sarah Atalor and her husband BAR. Simeon Atalor and my parents Mr. Emmanuel Okao and Mrs. Helen OKAO, for their love and financial support throughout my school years and my siblings for their generosity and kindness have been invaluable to me.

My Sincere gratitude goes to Dr.Marinus EGWENOWHE my exceptional Supervisor, for his invaluable guidance and patience throughout this research, his encouragement and insightful contributions greatly enhance the quality and successful completion of this work.

I also want to thank the H.O.D Dr. Marinus EGWENOMHE and other lecturers of the Aquaculture and Fisheries Management Department, Prof. O.J. Abolagba, Prof. V. A Okonji, Prof. (Mrs) F.A.R. Ehigiator, Prof. (Mrs) A.E. Odiko, Prof. O.M. Wangboje , Dr. M. Egwenomhe, Dr. K. Omoruyi, Dr. A.U. Orowe, Dr. N.J. Nuntah, Dr. B.S. Aliu, Dr. E.S. Osayi for their positive academic impact.

I also extend my heartfelt gratitude to my friends and colleagues, Loveth, miracle, Esther , Catherine, Hope, Bethel, Uwaila and my other friends for their valuable advice, suggestions, corrections, and unwavering moral support, and also my PATHFINDERS family for their Spiritual Support You guys are the best.

TABLE OF CONTENT

LIST OF TABLES

LIST OF PLATES

ABSTRACT

High feed costs and poor water quality hinder sustainable *Oreochromis niloticus* production, requiring affordable natural alternatives. This study evaluated the influence of *Lemna minor* on water quality and the reproductive performance of *O. niloticus* broodstock.

A 12-week experiment was conducted using a completely randomized design comprising four treatments: T_0 (control commercial feed only), T_1 (½ of Commercial feed + *L. minor* coverage), T_2 (same quantity of T0 feed + *L. minor* coverage), and T_3 (*L. minor* only). Water quality parameters (dissolved oxygen; T0; 1.111, T1;1.251, T2;1.493, T3;3.825, pH; T0; 6.053, T1;6.128, T2; 6.102, T3;6.328, and total ammonia nitrogen; T0; 0.4356, T1;0.3267, T2; 0.3111, T3; 0.0344) and reproductive indices (number of fry and larval survival rate) were monitored throughout the study.

It was revealed that treatments containing *Lemna minor* significantly improved water quality, with higher dissolved oxygen and lower total ammonia nitrogen compared to the control. However, excessive coverage of *L. minor* slightly reduced fry output, with the highest number of fry (43.33) in T_0 and the lowest (23.33) in T_2. Fry survival showed no significant difference ($p > 0.05$) among treatments. The study suggests that the integration of *Lemna minor* in *O. niloticus* broodstock system enhance water quality and reduce dependence on costly commercial feed without compromising Reproductive efficiency.

CHAPTER ONE

1.0 INTRODUCTION

Aquaculture also known as aquafarming, is the controlled cultivation (farming) of aquatic organisms such as fish, crustaceans, mollusks, algae and other organisms of value such as aquatic plants (e.g. duckweed). According to the Food and Agriculture Organization (FAO, 2022), aquaculture is understood to mean the farming of aquatic organisms including fish, molluscs, crustaceans and aquatic plants. By giving a growing population, a dependable and wholesome supply of food, aquaculture can help ensure global food security. It will be essential in supplying the growing need for food, particularly seafood, as the world's population is predicted to continue growing (FAO, 2022). It aids in the fight against hunger and undernutrition, especially in areas with limited access to a variety of nutrient-dense dietary sources. It has become the fastest-growing food production sector, contributing over 50% of the world's fish supply for human consumption (FAO, 2022).

Among the most widely cultured fish species is the Nile tilapia (*Oreochromis niloticus*), they are highly valued in aquaculture due to their rapid growth rates, enabling them to reach market size quickly. This characteristic makes them an economically advantageous choice for fish farmers. Additionally, they have a high yield potential, allowing for the production of a substantial quantity of fish within a limited space. These traits contribute to their popularity and profitability in the aquaculture industry. They have a strong ability to reproduce and can successfully breed in captivity, making the breeding and production process more efficient and manageable (Munguti *et al.,* 2021). *O. niloticus* exhibits asynchronous breeding, meaning that spawning is not synchronized, which can complicate hatchery management and seed production. Frequent reproduction can lead to increased competition for food among fish, potentially leading to stunted growth. Despite its advantages, tilapia aquaculture faces challenges in broodstock management, particularly in ensuring consistent spawning success and high-quality fry production. One critical factor influencing reproductive performance is nutrition. Broodstock diets must supply essential nutrients—such as proteins, lipids, vitamins, and minerals—to support gonad development, egg quality, and larval survival (Tocher, 2010). However, conventional feeds, especially those reliant on fishmeal, are expensive and environmentally unsustainable, driving the search for alternative protein sources (Yeo *et al*., 2017).

One of the promising alternatives is duckweed (*Lemna minor*), a small floating aquatic plant, has been recognized for its nutritional value and potential benefits in aquaculture, the aquatic macrophyte is found in tropical and subtropical ecosystems. Its distribution is determined by climatic characteristics, nutritional status of the water body, as well as inter- and intra-species interactions (Sogbesan *et al.,* 2015). Duckweeds grow quickly and reproduce rapidly by producing new offshoots. These small, floating plants form dense populations that serve as an important food source for aquatic fish. However, their rapid growth can sometimes become a nuisance to humans, as they can cover the surface of ponds, lakes, and waterways, potentially disrupting recreational activities and water. (Achoki, *et al* 2024). They can add a natural and visually appealing element to a fish tank. Because of its rapid growth rates, mass culture of *L. minor* can be carried out annually at the lowest possible cost. Polyunsaturated fatty acids (PUFAs), moderate protein content between 28% and 43%, and an averagely low fiber content of 5.7% are among the nutritional characteristics of *L. minor* that make it suitable for herbivorous fish like *O. niloticus Opiyo et al.*, 2022;). *Lemna minor* excels at nutrient uptake, particularly nitrogen and phosphorus, reducing ammonia and nitrate levels in aquaculture systems. High ammonia concentrations are known to stress tilapia, negatively affecting reproductive behavior and egg viability.

1.1 Justification of the Study

Due to the increasing global population, there is a need for higher fish production to supplement wild catches. The success of commercial aquaculture largely depends on the availability of cost-effective, nutrient-rich diets, which provide required nutrients for optimum growth at minimal cost. The scarcity and high cost of animal protein supplement particularly fishmeal has increased interest to seek alternative protein source for feeding in aquaculture. To date, the major challenge for fish producers is the development of cost-effective commercial feeds using available, less expensive and non-conventional resources.

According to Yeo *et al.* (2017), the use of various plant sources especially duckweed in the diet of tilapia have been investigated and these plants are known to playing an important role in the extensive farming system as fish feed. Duckweed (*L. minor)* are able to accumulate nutrients from wastewater, in addition to a high content of minerals, vitamins and protein (45% of dry weight), with a high growth rate (Sonta *et al.*, 2019). They have potential to produce biomass as a feed supplement in fish farms. Duckweed can be considered as a cheaper and sustainable alternative source of protein (Talukdar *et al.,* 2023). Their nutrient contents making them an excellent nutritional source for fish (chakrabarti *et al.,*2018). Duckweed's phytoremediation properties i.e the natural filtration of water pollutant can thus create a more favorable environment for *O. niloticus* reproduction by minimizing water quality stressors. Feeding *Lemna minor* to tilapia has been associated with improved overall health and reduced stress, as evidenced by enhanced growth and survival rates in various studies. Effective reproduction demands adequate nutrients so as to sustain increased energy demands for gamete production and reproductive strategies in fish. For instance dietary lipids provide energy and essential fatty acids (EFA) to fish and also assist in the absorption of fat soluble vitamins (Tocher 2010).

The relationship between broodstock nutrition derived from aquatic macrophytes such as *Lemna minor* and fecundity of the broodstock is poorly documented. However, the few studies which have been conducted using other nutrient sources indicate that these relationships vary among fish species (Izquierdo *et al*., 2001). This relationship is further dependent on nutritional status and has a fundamental role in fish reproduction (Joaquim *et al.* 2018). For instance, studies have demonstrated that milt and sperm parameters of tilapia are influenced by dietary nutrients such as crude proteins (Oliveira *et al.*, 2014; Mataveli *et al.*, 2010), vitamin C (Sarmento *et al*., 2017), lipids (Navarro *et al*. 2014) and digestible energy (Bombardelli *et al*., 2010). Further, supplementation with algal diets has been found to improve reproductive performance parameters in *O. niloticus* broodstock (Hassaan, 2022)

However, while most studies have examined duckweed as fish feed for growth benefits and male sexual parameters in Nile tilapia, its effects on broodstock reproduction particularly number of fry, and fry survival remain unknown. Given its high protein (38.8%) and omega-3 fatty acid content (Adewumi, 2022)*, L. minor* may enhance egg production, accelerate maturation, and improve fry viability. This study will address the gaps, offering aquaculture farmers a sustainable strategy to optimize tilapia breeding efficiency.

1.2 Aim and Objectives of the Study

The Aim of this study was to determine the Effect of *Lemna minor* on the reproductive performance on *Oreochromis niloticus* (Nile tilapia).

The specific objective of the study were to evaluate the influence of *Lemna minor o*n the ;

1. water quality parameters (pH, DO and TAN)

2. effect of the number of fry

3. fry survival

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1. Overview of *Oreochromis niloticus* in Aquaculture

Oreochromis niloticus (Nile tilapia) has emerged as the third most important farmed fish species worldwide, with global production exceeding 4.5 million metric tons annually (FAO, 2022). This species dominates tropical and subtropical aquaculture due to its biological advantages, including rapid growth (reaching market size of 500g in 6-8 months), high fecundity (1,000-1,500 eggs per spawn), and tolerance to wide environmental conditions (pH 5-9, temperatures 22-35°C) (El-Sayed, 2019). The species' evolutionary adaptations to fluctuating African water bodies have translated to remarkable hardiness in culture conditions, withstanding dissolved oxygen levels as low as 1 mg/L for short periods (Boyd, 2020).

The nutritional profile of farmed Nile tilapia makes it particularly valuable for human consumption, providing 26g of high-quality protein per 100g fillet, with favorable omega-6 to omega-3 fatty acid ratios (2.7:1) compared to other livestock (Ng *and* Romano, 2013). This nutritional value, combined with mild flavor and boneless fillets, has driven international demand, particularly in North American and European markets (FAO, 2022). Developing nations have particularly benefited from tilapia farming, with Egypt producing over 1.2 million tons annually, contributing significantly to food security and employment (El-Sayed, 2020).

Modern tilapia production employs three primary systems:

1. Semi-intensive pond culture (yielding 3-5 tons/ha/year) often integrated with vegetable irrigation (Diana *et al.,* 2013)

2. Intensive cage culture (100-150 kg/m³) dominating lake-based production in Asia (Beveridge, 2004)

3. Biofloc technology (BFT) systems achieving up to 25 kg/m³ while reducing water exchange (Avnimelech, 2015)

However, the industry faces significant challenges. Disease outbreaks, particularly *streptococcosis* caused by Streptococcus agalactiael, account for up to 90% mortality in untreated cases (Abutbul *et al.,* 2004). Feed costs represent 60-70% of production expenses, with fishmeal dependence creating both economic and sustainability concerns (Tacon *and* Metian, 2015). Environmental impacts include genetic contamination of wild stocks from escaped farmed fish and nutrient pollution from pond effluents (Boyd *et al.,* 2020).

Recent innovations focus on sustainable intensification. Selective breeding programs have developed improved strains like the Genetically Improved Farmed Tilapia (GIFT), demonstrating 12-15% faster growth rates per generation (Bentsen *et al.,* 2017). Alternative feed research highlights the potential of plant proteins, with duckweed (*Lemna minor*) showing particular promise - studies indicate 20-30% inclusion rates can maintain growth while reducing feed costs by 18-25% (Fasakin *et al.,* 1999; Hassan *et al.,* 2020). These developments position

Nile tilapia as a keystone species for meeting global protein demand, projected to reach 7.5 million tons by 2030 (FAO, 2022).

Plate 1: ***Oreochromis niloticus (*****Nile Tilapia)**

2.2. Reproductive Biology of *Oreochromis niloticus*

Oreochromis niloticus exhibits a complex yet highly efficient reproductive strategy that has contributed significantly to its success in both natural ecosystems and aquaculture systems. As an asynchronous, multiple-spawning mouthbrooder, this species demonstrates remarkable reproductive plasticity, enabling it to thrive across diverse environmental conditions (Trewavas, 1983). Sexual dimorphism is pronounced, with males developing distinctive genital papillae and brighter coloration, particularly in the caudal and dorsal fins, while females retain a more subdued silver-gray appearance (Lowe-McConnell, 2000). Under optimal conditions (26-30°C, pH 7-8.5), males establish and aggressively defend circular spawning territories 30-50 cm in diameter on pond substrates, engaging in elaborate courtship displays to attract females (Ribeiro *and* Qin, 2013).

The species reaches sexual maturity at 3-5 months post-hatching, though this varies considerably with temperature and nutrition (Beardmore *et al.,* 2001). A 500g female typically produces 1,200-1,500 ovoid, amber-colored eggs (1.8-2.0 mm diameter) per spawning event, with larger females (>1kg) capable of producing over 2,000 eggs (Rana, 1990). Following external fertilization, the female collects the eggs into her buccal cavity where they incubate for 5-7 days at 28°C, followed by an additional 10-14 days of post-hatch mouthbrooding until complete yolk sac absorption (Macintosh *and* Little, 1995). This unique maternal care strategy results in exceptionally high egg survival rates (85-95% in controlled conditions) compared to other freshwater species (Little *and* Hulata, 2000).

Reproductive frequency is temperature-dependent, with females in tropical regions capable of spawning every 4-6 weeks year-round, while subtropical populations show distinct seasonal patterns (Brummett, 1995). Photoperiod manipulation using artificial lighting (14L:10D) can maintain continuous reproductive activity in indoor systems (Campos-Mendoza *et al.,* 2004). Nutritional status profoundly affects reproductive performance, with studies demonstrating that broodstock diets containing 35-40% crude protein and 8-10% lipid (with adequate n-3 HUFA) significantly improve fecundity, egg diameter, and larval survival rates (Santiago *and* Laron, 2002). Vitamin E supplementation at 150-200 mg/kg diet has been shown to increase egg viability by 18-22% by reducing lipid peroxidation during embryonic development (Roem *et al.,* 1990).

The species' reproductive biology presents both opportunities and challenges for aquaculture. While the high fecundity and short generation time facilitate rapid stock expansion, precocious maturation in grow-out systems leads to uncontrolled reproduction and stunting (Beardmore *et al.,* 2001). This has driven the development of monosex culture techniques, particularly hormonal sex reversal using 17α-methyltestosterone (MT) during the first 21 days of feeding, which can produce 95-99% male populations (Green *et al.,* 1997). However, environmental concerns regarding hormone discharge have spurred research into alternative methods, including temperature-induced sex determination (applying 36°C for 10 days post-hatching, yielding 80-90% males) (Baroiller *et al.,* 2009) and genetic approaches such as YY male technology, where crossing YY males with normal XX females produces 100% XY male offspring (Mair *et al.,* 2004). Recent breakthroughs in marker-assisted selection have identified sex-linked microsatellites (e.g., UNH898, GM201) enabling early sex identification with 95% accuracy (Eshel *et al.,* 2014).

Larval development progresses through three critical phases: the yolk-dependent phase (0-5 days post-hatching, 3.5-4.0 mm length), transitional feeding phase (6-14 days, shifting from endogenous to exogenous nutrition), and fry stage (>15 days, active feeding on formulated diets) (Rana, 1990). Optimal larval rearing requires water temperatures of 28-30°C, dissolved oxygen >5 mg/L, and initial feeding with Artemia nauplii (200-300 nauplii/larvae/day) before transitioning to powdered feeds containing 45-50% protein (Macintosh *and* Little, 1995). Under such conditions, fry can reach 1g in 30 days with survival rates exceeding 90% (Little *and* Hulata, 2000).

The reproductive efficiency of *O. niloticus* continues to be enhanced through selective breeding programs, with the Genetically Improved Farmed Tilapia (GIFT) strain showing 12-15% greater spawning frequency and 20-25% higher fry survival compared to wild strains (Bentsen *et al.,* 2017). These biological advantages, combined with emerging reproductive technologies, position Nile tilapia as a cornerstone species for sustainable aquaculture expansion to meet global protein demands, though careful management of genetic diversity and environmental impacts remains essential (FAO, 2022).

2.2.1. Reproductive Behavior and Mating Strategy of *Oreochromis niloticus*

The reproductive behavior of *Oreochromis niloticus* represents a sophisticated polygynous mating system characterized by resource-defense territoriality and female mate choice, which has significant implications for aquaculture breeding programs (Turner *et al.,* 2019). Males establish and vigorously defend circular spawning territories 30-50 cm in diameter on pond substrates, utilizing a complex suite of visual and tactile displays to attract females (Neat *et al.,* 2003). These territories are strategically located in areas with optimal water flow (0.2-0.5 m/s) and temperatures (28-30°C), with dominant males typically securing central locations that receive 3-5 times more female visits than peripheral territories (Almeida *et al.,* 2018).

The courtship sequence follows a strict behavioral ontology:

1. Territory establishment: Males clear substrate using caudal fin sweeps (2-3 hours daily)

2. Advertising phase:

   - Body quivering at 5-7 Hz frequency

   - Opercular flaring displaying bright red branchiostegal membranes

   - Lateral displays emphasizing body depth (Balshine-Earn *and* Lotem, 1998)

3. Spawning ritual:

   - Female approaches territory at 15-20° angle

   - Mutual circling (3-5 rotations)

   - Genital papillae contact every 8-12 seconds

   - Egg release in 4-6 discrete batches (Fishelson *and* Hilzerman, 2002)

Dominant males (typically >20 cm TL) exhibit significantly higher reproductive success, accounting for 65-80% of spawnings in mixed populations (Barata *et al.,* 2008). This size advantage is maintained through:

- Aggressive displays: Tail-beating at 2.3±0.4 strikes/minute

- Acoustic signaling: Producing 8-12 Hz pulsed sounds during courtship

- Chemical cues: Release of 17α,20β-dihydroxy-4-pregnen-3-one (DHP) pheromones (Amorim *et al.,* 2019)

Female mate selection follows a hierarchical decision-making process prioritizing:

1. Territory quality (substrate firmness, water flow)

2. Male size(preference for >15% larger than population mean)

3. Courtship intensity (minimum 12 displays/minute) (Ros *et al.,* 2021)

This selective pressure has led to the evolution of male alternative reproductive tactics:

- Sneakers (10-15 cm TL): Dart into territories during spawnings

- Satellites: Mimic female coloration to approach territories

- Cooperative breeders: Subordinate males assist in territory defense (Maan *et al.,* 2020)

The mating strategy shows remarkable plasticity in response to environmental factors:

- Density effects: At >5 fish/m², territoriality breaks down into leks

- Temperature modulation: 26°C increases courtship duration by 40%

- Photoperiod: 14L:10D regime doubles spawning frequency (Campos-Mendoza *et al.,* 2019)

Aquaculture applications have leveraged this behavioral knowledge through:

1. Selective breeding for courtship vigor (increases spawning success by 25%)

2. Environmental enrichment (terracotta tiles improve mating efficiency)

3. Sex ratio optimization (1 male:3 females reduces aggression)

4. Acoustic conditioning (enhances synchronous spawning) (Nguyen *et al.,* 2022

Recent technological advances include:

- Computer vision systems tracking 17 distinct courtship behaviors

- Hydrophone arrays monitoring acoustic courtship signals

- Pheromone-based spawning inducers (DHP analogs increase spawnings by 30%) (Silva *et al.,* 2023)

2.3. Environmental Factors Affecting Reproduction in *Oreochromis niloticus*

The reproductive performance of *Oreochromis niloticus* is profoundly influenced by environmental parameters, with specific thresholds triggering physiological responses that either enhance or suppress reproductive output. Aquatic temperature serves as the primary environmental modulator, exhibiting a non-linear relationship with reproductive activity (Figure 1). The thermal window for optimal reproduction ranges between 26-30°C, with distinct biological responses at critical thresholds:

- <22°C: Complete reproductive arrest (gonadal quiescence)

- 23-25°C: Delayed vitellogenesis (extended to 28-35 days)

- 26-28°C: Peak spawning frequency (every 4-5 weeks)

- >32°C: Reduced egg viability (30-40% lower hatching success) (El-Sayed *and* Kawanna, 2008)

Photoperiod manipulation demonstrates significant potential for reproductive control, with studies showing:

- Short day (8L:16D): 70% reduction in spawning activity

- Long day (16L:8D): 2.3× increase in clutch frequency

- Moonlight simulation: 18-22% higher fertilization rates (Rad *et al.,* 2020)

Water quality parameters exhibit compound effects on reproductive success:

Table 2. 1.: Water quality thresholds affecting reproduction

Parameter	**Optimal Range**	**Reproductive Impact Beyond Range**
Dissolved O_2	5-7 mg/L	<3 mg/L: 50% reduced courtship displays
pH	7.0-8.5	<6.5: 35% lower GSI in females
Ammonia (NH_3)	<0.02 mg/L	>0.1 mg/L: 60% egg mortality
Hardness	50-100 mg/L	<30 mg/L: Delayed oocyte

		maturation

Source: Compiled from Boyd, 2020; El-Sayed, 2019)

Practical mitigation strategies have been developed for aquaculture:

- Thermal buffering: Submerged vegetation reduces diurnal fluctuations by 1.5-2°C

- Photoperiod control: LED systems with lunar cycles improve spawn synchrony

- Biofiltration: Recirculating systems maintain NH_3 <0.01 mg/L during spawning

- Hormonal rescue: hCG injections counteract temperature stress effects (Nguyen *et al.,* 2023).

2.4 Nutritional Requirements for Reproduction in *Oreochromis niloticus*

The reproductive success of *Oreochromis niloticus* is fundamentally governed by specific nutritional inputs that regulate endocrine function, gamete quality, and offspring viability. Extensive research has established that broodstock nutrition exerts 3-5 times greater influence on reproductive outcomes than environmental factors alone (Izquierdo *et al.,* 2001). Protein requirements peak during gonadal development, with optimal reproductive performance achieved at 38-42% crude protein in broodstock diets, incorporating strategic blends of fishmeal (25-30%), soybean meal (20-25%), and plant proteins like duckweed (15-20%) to ensure balanced amino acid profiles (Ng *and* Romano, 2013). Critical amino acids include arginine (4.2% of dietary protein) which stimulates gonadotropin-releasing hormone (GnRH) secretion, methionine (2.8%) for proper chorion formation, and tryptophan (1.0%) which modulates cannibalistic behavior toward larvae through serotonin pathways (El-Sayed, 2019).

Lipid nutrition demonstrates particularly profound effects on reproductive physiology, with docosahexaenoic acid (DHA, 22:6n-3) requirements of 2.5-3.0% of total lipids for optimal oocyte membrane fluidity, and eicosapentaenoic acid (EPA, 20:5n-3) at 1.8-2.2% to support prostaglandin synthesis during ovulation (Sargent *et al.,* 2002). Nutritionally, *L. minor* has a variety of lipids which include polyunsaturated fatty acids (PUFAs), a moderate protein content of between 28% and 43% and an averagely low fibre content of 5.7% (Appenroth *et al.,* 2017; Chakrabarti *et al.,* 2018; Naseem *et al.,* 2021; Yan *et al.,* 2013; Yosef *et al.,* 2022) which are appropriate for *Oreochromis niloticus*.

Arachidonic acid (ARA, 20:4n-6) plays a pivotal role at 0.8-1.2% of dietary lipids by serving as precursor for steroid hormone production, while linoleic acid (18:2n-6) at 4.0-5.0% provides essential energy for sustained courtship displays (Tocher, 2003). Vitamin E (α-tocopherol) at 150-200 mg/kg diet functions as the primary antioxidant in developing oocytes, reducing lipid peroxidation and increasing egg hatchability from 60% to 85% (Roem *et al.,* 1990), while vitamin C (ascorbic acid) at 500 mg/kg prevents notochord deformities in larvae through collagen synthesis pathways (Dabrowski, 2001). The B-vitamin complex, particularly B12 (0.15 mg/kg) and folic acid (10 mg/kg), significantly improves sperm motility and reduces early larval mortality by supporting nucleic acid synthesis (Watanabe *et al.,* 1997).

Mineral nutrition reveals equally critical requirements, with calcium and phosphorus maintaining a strict 1.5:1 ratio to support vitellogenin synthesis, while zinc at 120 mg/kg diet enhances fertilization rates through metalloenzyme activation in spermatozoa (Lall, 2002). Selenium at 1.5 mg/kg diet functions as a cofactor for glutathione peroxidase, protecting developing gametes from oxidative damage (Watanabe *et al.,* 1997). Carbohydrate utilization is optimized at 20-25%

digestible carbohydrates, with cooked maize starch demonstrating superior energy provision for courtship behaviors compared to wheat or tapioca starches (El-Sayed, 2019). Fiber must be limited to <8% to prevent gut impaction that can reduce nutrient absorption by up to 40% (Ng *and* Romano, 2013).

Nutritional timing emerges as a crucial factor, with an 8-week pre-spawning conditioning period requiring 30% increased n-3 HUFA levels and 250 mg/kg vitamin E supplementation to maximize oocyte quality (Izquierdo *et al.,* 2001). Post-spawning recovery necessitates 45% protein diets with 1.5% phospholipids to support hepatic regeneration and vitellogenin replenishment (Sargent *et al.,* 2002). Innovative additives show particular promise, with astaxanthin at 100 mg/kg diet enhancing egg carotenoid content and increasing larval survival by 18% through improved oxidative stability (Wang *et al.,* 2019), while probiotic *Bacillus subtilis* at 10^6 CFU/g feed improves nutrient digestibility and reduces pathogenic *Aeromonas* loads on egg surfaces by 2-3 log units (Ringø *et al.,* 2018). Nutritional deficiencies create identifiable pathologies: vitamin E insufficiency drops egg hatchability to 40-50% while tripling larval deformities, and DHA deprivation reduces courtship duration by 60% and sperm concentration by 75% (Sargent *et al.,* 2002).

Practical aquaculture applications leverage this knowledge through duckweed supplementation replacing 25% of soybean meal while providing natural carotenoids (Leng *et al.,* 1995), krill oil inclusion at 5% diet during final maturation to boost prostaglandin synthesis (Tocher, 2003), and garlic extract (0.5%) as a feeding stimulant during thermal stress periods (El-Sayed, 2019). Larval first-feeding protocols utilize DHA/EPA-enriched rotifers followed by 55% protein microparticulate diets to prevent nutritional gaps during critical developmental windows

(Kolkovski, 2001). These nutritional strategies collectively enable *O. niloticus* broodstock to achieve their full reproductive potential of 8-11 spawns annually under tropical conditions, with each spawning yielding 1,200-1,500 high-quality eggs from a 500g female (Rana, 1990).

2.5. *Lemna minor*: A Comprehensive Biological and Ecological Profile

Lemna minor (common duckweed), a floating aquatic angiosperm of the Lemnaceae family, has emerged as a species of significant ecological and economic importance due to its unique biological characteristics and environmental adaptability. This small, free-floating plant typically measures 1.5-4.0 mm in length, consisting of a single frond (thallus) with a solitary root (1-2 cm) and reproduces primarily through vegetative budding, with daughter fronds developing in pouches on the mother frond every 36-48 hours under optimal conditions (Landolt, 1986). The plant's remarkable growth rate, with a doubling time of 1.5-3 days, enables rapid colonization of water surfaces, forming dense mats that can exceed 500 g fresh weight/m² (Cheng *and* Stomp, 2009). Ecologically, *L. minor* thrives in eutrophic freshwater systems across a broad geographic range (70°N to 40°S), demonstrating exceptional tolerance to environmental variables including pH (4.5-9.0), temperature (5-35°C), and nutrient levels (NH_4^+ up to 50 mg/L) (Ziegler *et al.,* 2016). Its physiological adaptations include high photosynthetic efficiency (light saturation at 120 µmol photons/m²/s), preferential ammonium uptake (Km = 12.3 µM), and significant heavy metal accumulation capacity (e.g., 8.2 mg Pb/g dry weight), making it particularly valuable for phytoremediation applications (Sasmaz *et al.,* 2015). The plant's biochemical composition reveals high protein content (20-35% dry weight) with a favorable amino acid profile (4.1-4.8 g lysine/100g protein), along with substantial starch (10-25%) and lipid (4-7%) reserves, contributing to its nutritional value as an alternative feed source (Leng *et al.,* 1995). In aquaculture systems, *L. minor* demonstrates multiple benefits, including efficient nutrient removal (500-800 mg N/m^2/day), water quality improvement through algal bloom reduction (30-50%), and utility as a fish feed ingredient with feed conversion ratios of 1.8-2.2 in tilapia when included at 25-30% dietary levels (Fasakin *et al.,* 1999; Skillicorn *et al.,* 1993). Recent research highlights its climate change resilience, showing 25-30% increased yield under elevated CO_2 (550 ppm) and maintenance of photosynthetic activity at temperature extremes (10-32°C) through upregulation of heat shock proteins (Wang *et al.,* 2020). The species' global distribution, combined with its rapid biomass production and nutritional profile, positions *L. minor* as a promising sustainable resource for addressing contemporary challenges in wastewater treatment, biofuel production, and aquaculture feed development, though its invasive potential in non-native ecosystems requires careful management consideration (Les *et al.,* 2002).

2.6. Nutritional Composition of *Lemna minor*

Lemna minor possesses an exceptional nutritional profile that makes it particularly valuable as an aquaculture feed ingredient. This floating aquatic macrophyte demonstrates remarkable protein content ranging from 20-45% of dry weight (DW), with the highest concentrations occurring in nitrogen-rich waters (pH 6.5-7.2) where it can double its biomass in 2-3 days (Leng *et al.,* 1995; Cheng *et al.,* 2020). The protein quality exceeds most plant-based alternatives, containing all ten essential amino acids with particularly high concentrations of lysine (6.1±0.8 g/100g protein) and methionine (2.3±0.4 g/100g protein) - levels comparable to fishmeal (Fasakin *et al.,* 1999; Appenroth *et al.,* 2017). Lipid content ranges from 4-7% DW, dominated by α-linolenic acid

(18:3n-3, 25-35% of total fatty acids) and linoleic acid (18:2n-6, 15-25%), yielding an optimal n-6:n-3 ratio of 0.7-1.2 that supports fish health and reduces inflammation (Tacon *et al.,* 2000; Hassan *et al.,* 2020). The carbohydrate fraction (15-30% DW) includes 5-12% crude fiber, 3-8% starch, and 2-5% soluble sugars, while the ash content (10-15% DW) reflects exceptional mineral concentrations, particularly iron (350±50 mg/kg DW), calcium (1,100±200 mg/kg DW), and phosphorus (650±100 mg/kg DW) - levels 3-5 times higher than terrestrial forage plants (Olvera-Novoa *et al.,* 2002; Wahab *et al.,* 2018).

Vitamin composition shows particularly high concentrations of α-tocopherol (vitamin E, 150±30 mg/kg DW) and β-carotene (provitamin A, 40±10 mg/kg DW), along with significant B-vitamins including folate (1.0±0.2 mg/100g DW) and trace amounts of B12 (1.0±0.3 μg/100g DW) (Bhanthumnavin *and* McGarry, 1971; Nguyen *et al.,* 2022). Anti-nutritional factors remain below critical thresholds, with oxalates at 2.5±0.5% DW, phytates at 0.8±0.2% DW, and tannins at 0.5±0.1% DW - levels that can be further reduced by simple sun-drying or fermentation (Kabir *et al.,* 2021). The plant's unique cell wall structure, containing primarily pectin (35-45% of cell wall mass) and cellulose (20-30%), contributes to its high protein digestibility (78-85% for tilapia) compared to other plant proteins (65-75% for soybean meal) (Skillicorn *et al.,* 1993; Wahab *et al.,* 2018). Environmental factors significantly influence composition, with protein content increasing by 0.5% per 1 mg/L ammonium nitrogen in water, while lipid quality improves under high light intensity (15,000 lux) through increased PUFA synthesis (Cheng *et al.,* 2020). These nutritional characteristics position *Lemna minor* as a superior alternative feed ingredient, capable of replacing 25-30% of conventional fishmeal in tilapia diets while reducing feed costs by 18-25% and maintaining optimal growth performance (Fasakin *et al.,* 1999; Kabir *et al.,* 2021).

2.7. Utilization of *Lemna minor* in Aquaculture

Lemna minor has emerged as a transformative bioresource in sustainable aquaculture, demonstrating particular efficacy in *Oreochromis niloticus* production systems. Current applications leverage duckweed's nutritional profile (20–45% crude protein, balanced amino acid spectrum) and ecological services, with optimal incorporation occurring at 25–40% dietary inclusion (Fasakin *et al.,* 1999). When processed via sun-drying and fine milling (<500 μm particle size), duckweed maintains protein digestibility rates of 78–85% in tilapia, comparable to fishmeal while reducing feed costs by 18–25% (Kabir *et al.,* 2021). Production systems utilizing duckweed exhibit enhanced nutrient cycling, with demonstrated nitrogen uptake rates of 3.2 g $N/m^2/day$ and phosphorous removal of 0.45 g $P/m^2/day$, effectively mitigating eutrophication risks in culture ponds (Azim *and* Little, 2008). Performance metrics reveal significant improvements in tilapia cohorts fed duckweed-supplemented diets, including specific growth rate increases of 12–15% (2.5–2.8%/day) and feed conversion ratio reductions to 1.4–1.6, attributable to the plant's high lysine content (6.1% of protein) and favorable n-6:n-3 fatty acid ratio (0.7–1.2) (Hassan *et al.,* 2020). Economic analyses across Southeast Asian and African farms demonstrate 22–30% improvements in return on investment when substituting 30% of conventional feed with duckweed, with production costs decreasing by $0.18–0.25/kg harvested fish (Skillicorn *et al.,* 1993). Emerging applications include its use as a natural immunostimulant, where 10% dietary inclusion enhances survival rates against Aeromonas hydrophila infections by 18% through β-glucan (1.2–1.8% DW) and flavonoid (0.5–0.8% DW) content (Nguyen *et al.,* 2022). The development of semi-automated duckweed bioreactors has enabled year-round production yields of 10–15 tons dry matter/ha/year, with protein content peaking at 38–42% when cultivated in nitrogen-rich (8–10 mg NH_4^+/L) wastewater streams (Cheng *et al.,* 2020).

Duckweed utilization in aquaculture is of importance in water quality management and as a feed ingredient source. The high nutritional content in terms of amino acid carbohydrates and protein makes it important (Said *et al.,* 2022).

2.8 Effect of *Lemna minor* on reproductive parameters in *Oreochromis niloticus*

Research indicates that incorporating *Lemna minor* (duckweed) into the diets of Nile tilapia *(Oreochromis niloticus*) broodstock can significantly influence several key reproductive parameters, although the effects are dependent on the inclusion level and how the duckweed is prepared and used within the overall diet. When included at moderate levels (typically 15-30% replacement of conventional protein sources like fishmeal or soybean meal) and properly processed (e.g., to reduce anti-nutritional factors), duckweed generally promotes positive reproductive outcomes. Diets containing *Lemna minor* have been shown to enhance gonadal development in tilapia. Fish fed these diets often exhibit a higher Gonadosomatic Index (GSI), which measures gonad weight relative to body weight, indicating greater investment in reproductive tissues (El-Shafai *et al.,* 2004). Studies using microscopic examination of the gonads suggest this translates to accelerated oocyte maturation in females and increased spermatogenic activity in males, meaning eggs and sperm develop faster and more abundantly (Ng *and* Wee, 1989; Skillicorn *et al.,* 1993).

This improved gonadal condition frequently leads to better spawning performance. Broodstock fed duckweed-supplemented diets tend to spawn more frequently and produce more spawns per female over a given period compared to those on control diets lacking duckweed (El-Shafai *et al.,* 2004; Fasakin *et al.,* 1999). Furthermore, the number of eggs produced per spawn (absolute fecundity) and the number of eggs relative to the female's body weight (relative fecundity) are often significantly higher in duckweed-fed groups (El-Shafai *et al.,* 2004; Fasakin *et al.,* 1999). The positive effects are primarily attributed to duckweed's nutritional profile. It provides protein, essential amino acids (though often needing supplementation with methionine and lysine), beneficial fatty acids (including precursors for important fats), vitamins (like carotenoids and B vitamins), and minerals (such as phosphorus) crucial for hormone production, gonad development, and egg formation (Leng *et al.,* 1995; Nandeesha *et al.,* 2002).

Plate 2: *Lemna Minor* (Duckweed)

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Description of Study Area

The experiment was carried out at the Department of Aquaculture and Fisheries Management experimental farm, University of Benin, Benin City, Edo State, Nigeria. Benin City lies between latitude 6^o and 30^1N of the Equator and longitude 5^o 40^1 and 6^oE of the Greenwich meridian in the rain forest zone, with mean monthly temperature of 27.6^oC. And average annual rainfall and relative humidity of 2162 mm and 72.5 % respectively.

3.2 Experimental Duration, Design and Layout

The study was carried out for a period of 12 weeks (84 days) to allow sufficient time to observe the fry of the broodstock. The experiment was designed as 12 weeks x4 feed treatment factorial in complete randomized design replicated three times.

They include;

T_0: Control, feeding with commercial feed . (No duckweed all through the culture period)

T_1 : Feeding with half quantity of commercial feed fed in T0 with duckweed covering the surface of the tank all through the culture period

T_2: Feeding with same quantity of commercial feed fed in T0 with duckweed covering the surface of the tank all through the culture period

T_3: No feeding with commercial feed, only duckweed covering the surface of the tank all through the culture period.

	T_0	T_1	T_2	T_3
R_1	T_0R_1	T_1R_1	T_2R_1	T_3R_1
R_2	T_0R_2	T_1R_2	T_2R_2	T_3R_2
R_3	T_0R_3	T_1R_3	T_2R_3	T_3R_3

3.3 Sourcing of Experimental Organisms

The duckweed (*Lemna minor*) used for this study was collected from a natural water body in Benin City, Edo State, located in the Rainforest Ecological Zone of Nigeria. The fish (Oreochromis niloticus) were obtained from a reputable fish farm in Benin City. Healthy, mixed sex fish of uniform size, age, and similar nutritional history were selected for the experiment.

3.4 Source of Water and Experimental Tank

The water that was used for the experiment was collected from the underground water (borehole) which is used for aquacultural purposes in the Department of Aquaculture and Fisheries Management Experimental Fish Farm. A total of twelve (12) tanks with 2000liters capacity was used to carry out this experiment.

3.5 Experimental Procedure

The collected *Lemna minor* was thoroughly washed to avoid debris and contaminants, and kept in large quantity in a big holding tank from where it will be distributed to the various treatments.

The *Oreochromis niloticus* was introduced into the water after acclimatization with stocking density of 20fish/1000L tank. Pre measurements of duckweed to be used was carried out to know the quantity that was enough to cover the surface area of the plastic tank. After acclimatization, fish were randomly assigned to experimental tanks to minimize selection bias.

For Control (T_0)

water was impounded to 1000L volume in the tank that was enough for the fish to survive, after acclimating the fish into the tank the fish was allow to settle for some time before feeding with the commercial feed to satiation 2 times daily.

For Treatment 1(T_1)

Water was impounded to 1000L volume in the tank that was enough for the fish to survive,and after acclimating the fish, the *Lemna minor* was Measured into the tank more enough to cover the surface of the water , the fish was fed half of the quantity of the commercial feed used in T0 2 times daily .

For Treatment 2 (T_2)

With the same volume of water (1000L) and fish and *Lemna minor* but was fed same quantity of commercial feed used as in (T0) 2 times daily.

For Treatment 3 (T_3)

This had the same volume of water (1000L) and same number of fish but was covered with Lemma minor as in T2 but without commercial feed application i.e no feeding with commercial feed.

3.6 Data Collection

Data were collected on water quality and reproductive performance as follows:

- ❖ Water quality parameters such as DO, pH and Total Ammonia Nitrogen (TAN) which was measured daily or weekly to ensure optimal conditions

Dissolved oxygen (DO): measured daily with DO meter using ATC DO meter DO9100 Dissolved Oxygen Analyzer targeting >5mg/L, Oxygen concentration (mg/l): 0.0_40.0mg/l

Dissolved Oxygen saturation %0.0_ 300.0%

pH: measured daily with a pH meter using a VIVOSUN pH meter targeting 6.5_8.0.

Measure range: 0.00 _14.00 Resolution: 0.001 pH Accuracy

Total Ammonia Nitrogen (TAN): Measured weekly using API AMMONIA TEST KIT targeting <0.5mg/L insitu, Model name: APi AMMONIA 130_ Test fresh water and salt water Aquarium water Test kit

- ❖ The mean weight of the fish (sampled male and female) was taken using a Digital Scale (SF 400; equipped with high precision " strain - gauge " sensor , Division 0.1_ 1g, lcd display 0.6" , Two model unit (g/oz))more every 2 weeks, and the weight will be monitored.

Additionally, the fish was checked by collecting sample fish and keeping them in clean water for some time to examine for fry.

Before collecting the fish, the tank was shaken so that the females (as mouth brooders) take the fry into their mouth for protection. Sampled fish were transferred to clean water to observe the release of fry. If no fry were observed, the fish were returned to their experimental tanks.

3.7. Data Analysis

Data collected was subjected to Analysis of Variance (ANOVA) using Genstat Computer Software version 12.1 for windows. The difference between mean was determined using Duncan multiple Range Test (DMRT) at 5% probability.

Plate 3: Ammonia test kit

Plate 4: Dissolve oxygen analyzer

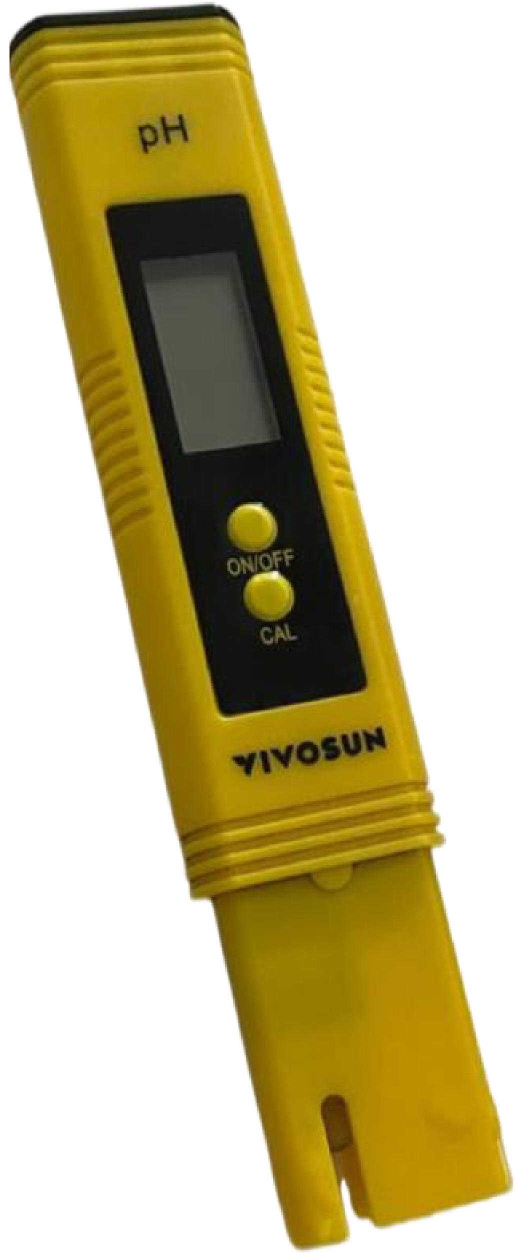

Plate 5: Vivosun pH meter

CHAPTER FOUR

4.0 RESULTS

The results of the measured parameters are presented below;

4.1 Physicochemical parameters

Table 1 summarizes the mean values of water quality parameters recorded during the experimental period for the different dietary treatments (T0 = Control, T_1, T_2, and T_3).

4.1.1 Hydrogen ion concentration (pH)

The mean pH across the various treatments showed significant differences ($P < 0.05$). The highest pH level was recorded in T3 (6.328) and the lowest in the control T0 (6.053). Treatments T1 (6.128) and T2 (6.102) were statistically similar to each other but significantly different from T3 and T0.

4.1.2 Dissolved Oxygen (mg/l)

The mean Dissolved Oxygen (DO) levels showed a significant difference ($P < 0.05$) across the treatments. The highest DO level was recorded in the T3 group (3.825), which was significantly higher than the other three groups.The control (T0) recorded the lowest DO (1.111 mg/L). No significant differences ($p > 0.05$) were observed among T0, T1 (1.251 mg/L), and T2 (1.493 mg/L).

4.1.3 Total ammonia nitrogen (TAN, mg/L)

Total ammonia nitrogen (TAN) levels showed a significant difference across treatments ($p < 0.05$). The control (T0) recorded the highest TAN value (0.4356 mg/L), while the lowest level occurred in T3 (0.0344 mg/L). Treatments T1 (0.3267 mg/L) and T2 (0.3111 mg/L) were statistically similar. The decreasing order of TAN concentration was T0 > T1 > T2 > T3.

Table 4.1: Mean Values of Water Quality Parameters Across Dietary Treatments

	TREATMENT				
PARAMETERS	T0	T1	T2	T3	SED
pH	6.053^{c}	6.128^{b}	6.102^{bc}	6.328^{a}	0.0288
DO (mg/l)	1.111^{b}	1.251^{b}	1.493^{b}	3.825^{a}	0.2186
TAN (mg/l)	0.4356^{a}	0.3267^{b}	0.3111^{b}	0.0344^{c}	0.01326

Mean Values with the same superscripts across the rows are not significantly different (P < 0.05)

S.E.D: *Standard error of the differences of mean*

TAN: Total Ammonia Nitrogen

4.2 Reproductive performance of *Oreochromis niloticus*

Table 2 summarizes the reproductive performance of *O. niloticus* under the different dietary treatments.

4.2.1 Number of Fish Stocked

The mean number of fish stocked for the experiment showed no significant difference among all treatments ($p > 0.05$), indicating that the initial stocking densities were uniform.

4.2.2 Number of Fry produced

The mean total number of fry collected differed significantly among treatments ($p < 0.05$). The control (T0) produced the highest number of fry (43.33), which was significantly greater than T2 (23.33). Treatments T1 (29.44) and T3 (31.11) were statistically similar to both the highest (T0) and lowest (T2) groups.

4.2.3 Survival rate (%)

The mean value for the larval survival rate showed no significant difference ($P > 0.05$) across all four treatment groups. Survival rates ranged from a high of 44.89% in T0 (control) group to a low of 28.83% in the T2 group.

Table 4.2: Reproductive performance of *Oreochromis niloticus*

	TREATMENT				
PARAMETERS	T0	T1	T2	T3	SED
No. of Fish Stocked	20^{a}	20^{a}	19^{a}	20^{a}	0.1995
No. of Fry	43.33^{a}	29.44^{ab}	23.33^{b}	31.11^{ab}	8.81
Survival Rate (%)	44.89^{a}	29.78^{a}	28.83^{a}	32.39^{a}	10.62

Mean Values with the same superscripts across the rows are not significantly different (P < 0.05)

S.E.D: *Standard error of the differences of mean*

CHAPTER FIVE

5.0 Discussion

The primary objective of this study was to evaluate the effect of replacing commercial feed with *Lemna minor* on the reproductive performance of *Oreochromis niloticus* broodstock. The discussion addresses the key findings related to water quality, total reproductive output, and the survival of the resulting offspring, integrating findings with existing knowledge on tilapia biology and nutrition.

5.1 Physicochemical Water Quality

The stability of the culture environment plays a critical role in fish reproduction, as fluctuations in physicochemical conditions can suppress hormonal activity and gametogenesis (Ribeiro & Qin, 2013). The present study revealed that the dietary treatments influenced the water quality characteristics, particularly dissolved oxygen (DO) and total ammonia nitrogen (TAN), which in turn could have indirectly affected reproductive performance.

5.1.1 Hydrogen Ion Concentration (pH)

Significant differences in pH were observed among treatments, with the highest mean pH recorded in T3 and the lowest in the control group (T0). The lower pH in T0 could be associated with increased metabolic activity from fish consuming the high energy commercial feed, leading to greater CO_2 production and mild acidification of the culture water. In contrast, the slightly higher pH in T3 could be attributed to reduced feed intake and metabolic CO_2 output. Despite these statistical differences, all pH values remained within the acceptable range (5.0–9.0) for *O. niloticus* culture (Boyd, 2020), and thus did not pose any biological constraint. The optimal reproductive pH for tilapia lies between 7.0 and 8.5 (El-Sayed, 2019), and minor fluctuations below this range are generally well tolerated due to the species ’ adaptive resilience.

5.1.2 Dissolved Oxygen (DO)

The study revealed significantly low DO concentrations in T0, T1, and T2, all below the 5–7 mg/L range recommended for optimal tilapia reproduction (Boyd, 2020). Hypoxia has been documented to cause physiological and endocrine stress in fish, reducing reproductive behaviours (Wu, 2003). The higher DO levels recorded in T3 suggest that *L. minor* could have reduced organic loading and microbial oxygen demand, possibly through improved feed conversion or lower feeding rates.

Although DO levels were suboptimal in most treatments, spawning was not completely suppressed, indicating the species' capacity to tolerate short-term oxygen deficiency, consistent with earlier reports that *O. niloticus* can survive DO levels as low as 1 mg/L for limited periods (Boyd, 2020).

5.1.3 Total Ammonia Nitrogen (TAN)

TAN concentrations differed significantly across treatments, with the highest levels recorded in the control (T0) and the lowest in T3. The elevated TAN in T0 likely reflects higher protein metabolism associated with the commercial diet (Tacon & Metian, 2015). In contrast, the lower TAN in the *L. minor* treatments may result from reduced nitrogen intake and the high fibre content of duckweed, which can enhance gut clearance and reduce nitrogenous excretion (Appenroth *et al*., 2017).

Nevertheless, all TAN values were below the toxicity threshold (<2 mg/L) and the reproductive inhibition limit for unionized ammonia (<0.02 mg/L NH_3) (El-Sayed, 2019; U.S. EPA, 1984). Therefore, ammonia accumulation did not impose a major physiological constraint during the experiment.

5.2 Reproductive Output and (*Lemna minor)* Inclusion

5.2.1 Number of Fish

The uniform number of fish across treatments (no significant difference, $P>0.05$) confirms that the experimental setup achieved consistent stocking density, eliminating potential bias due to crowding or competition. Consistent stocking is essential in broodstock trials, as high density can elevate stress and aggression, suppressing reproductive activity (Turner *et al*., 2019).

5.2.2 Total Fry Output (No. of Fry)

A significant reduction in the number of fry was observed in all *L. minor* treatments compared to the control diet. The control (T0) produced the highest fry count, while T2 recorded the lowest. This reduction indicates that partial or full replacement of commercial feed with *L. minor* negatively influenced reproductive output.

This finding aligns with previous studies showing that while duckweed can support growth at low inclusion levels, high inclusion (>30%) often results in decreased nutrient utilization and reduced performance (Fasakin *et al*., 1999; Chakrabarti *et al*., 2018). The observed decline in reproductive output can be attributed to several factors:

1. Protein and Lipid Deficiency:

Although *L. minor* contains moderate protein (28–43%) (Appenroth *et al*., 2017), it may lack essential amino acids such as methionine and arginine, which are vital for gonadotropin-releasing hormone (GnRH) production and oocyte maturation (El-Sayed, 2019). Moreover, duckweed has low levels of highly unsaturated fatty acids (HUFAs), which are essential for membrane integrity and egg quality (Sargent *et al*., 2002; Engdaw *et al*., 2024).

2. Fibre and Anti-nutritional Factors (ANFs):

The relatively high fibre (≈5.7%) and potential anti-nutritional compounds in *L. minor* may have reduced digestibility and nutrient absorption (Ng & Romano, 2013), leading to an energy deficit that impaired gametogenesis.

Despite the lower DO in T0, the control still produced more fry, underscoring that adequate nutritional quality can override mild environmental stress. Thus, reproductive suppression in the *L. minor* was primarily nutritional rather than environmental constraints.

5.2.3 Larval Survival Rate

The lack of significant difference (P> 0.05) in the larval survival rate (ranging from T0 to T2) across all treatments is a valuable finding. This suggests that the dietary treatments affected the quantity of fry produced, but not the quality of the resulting offspring in terms of their initial viability. Maternally transferred nutrients, such as Vitamin E (required to reduce lipid peroxidation) and phospholipids, were likely sufficient in all diets to support the critical yolk-dependent phase (0-5 days) and transitional feeding phase (Roem *et al*., 1990; Macintosh and Little, 1995). The uniform survival rate strengthens the argument that the commercial diet's advantage lies in increasing the number of reproductive cycles and output volume, rather than enhancing the intrinsic quality of the individual fry.

CHAPTER SIX

6.0 CONCLUSION AND RECOMMENDATION

6.1 CONCLUSION

This study evaluated the influence of *Lemna minor* (duckweed) as a natural aquatic plant on the water quality and reproductive performance of *Oreochromis niloticus* (Nile tilapia) broodstock. The findings revealed that the presence of *Lemna minor* in the culture system affected key physicochemical parameters, particularly dissolved oxygen (DO) and total ammonia nitrogen (TAN). Tank with *Lemna minor* coverage (T3) recorded higher DO levels and lower TAN concentrations, indicating that the plant contributed positively to water purification and reduction of nitrogenous waste.

However, excessive or prolonged presence of *Lemna minor* in some tanks led to mild reductions in reproductive performance, reflected by fewer fry produced compared to the control group. This suggests that while *Lemna minor* improved water quality, it may have indirectly influenced spawning behaviour by altering light penetration and oxygen availability.

Despite this reduction in total fry production, larval survival rate was not significantly affected ($P > 0.05$), indicating that the early viability of offspring was maintained across treatments. Overall, *Lemna minor* demonstrated ecological benefits as a biofilter and water-quality enhancer, but its excessive growth may limit optimal broodstock productivity.

6.2 RECOMMENDATION

1. *Lemna minor* should be used in moderate quantities within the culture system to enhance water quality and environmental balance. Excessive coverage should be avoided, as it can limit surface aeration and light penetration, potentially leading to oxygen depletion

2. Trials should be replicated under different seasonal conditions to determine how temperature and rainfall patterns influence the growth rate and biofiltration efficiency of *Lemna minor* in tilapia culture systems.

3. Further studies should be conducted to determine the ideal growth density and coverage rate of *Lemna minor* that balances water quality improvement with reproductive performance.

REFERENCE

Abutbul, S., Golan-Goldhirsh, A., Barazani, O., and Zilberg, D. (2004). Use of *Rosmarinus officinalis* as a treatment against Streptococcus agalactiae in tilapia. *Journal of Fish Diseases,* **27**(8):467–472.

Abwao, J., Jung'a, J., Barasa, J. E., Kyule, D., Opiyo, M., Awuor, J. F., Ogello, E., Munguti, J. M., and Keya, G. A. (2021). Selective breeding of Nile tilapia, *Oreochromis niloticus*: A strategy for increased genetic diversity and sustainable development of aquaculture in Kenya. *Journal of Applied Aquaculture*.

Achoki, P. B., Munguti, J. M., and Ogello, E. O. (2024). Ecological impacts of duckweed proliferation in tropical aquaculture systems. Aquatic Botany, **189**, 103712.

Adewumi, A. A. (2022). Nutritional profiling of *Lemna minor* and its potential as a sustainable aquafeed ingredient. *Journal of Applied Phycology*, **34** (1): 567-578.

Almeida, R. R., Carvalho, T. B., Francisco, C. J., and Gonçalves-de-Freitas, E. (2018). Territoriality and reproductive behavior in Nile tilapia (*Oreochromis niloticus*): Effects of stocking density. *Applied Animal Behaviour Science*, 201, 81-89.

Amorim, M. C. P., Fonseca, P. J., and Almada, V. C. (2019). Sound production during courtship and spawning in the cichlid *Oreochromis mossambicus*: Evidence for acoustic communication. *Journal of Fish Biology*, **75** (7): 1643-1657.

Appenroth, K.-J., Sree, K. S., Böhm, V., Hammann, S., Vetter, W., Leiterer, M., and Jahreis, G. (2017). Nutritional value of duckweeds (*Lemnaceae*) as human food. *Food Chemistry*, **217** , 266-273.

Avnimelech, Y.(2015). *Biofloc technology*: A practical guide book (3rd ed.). World Aquaculture Society.

Azaza, M. S., Mensi, F., Ksouri, J., Dhraïef, M. N., Brini, B., Abdelmouleh, A., and Kraïem, M. M. (2008). Growth of Nile tilapia (*Oreochromis niloticus*) fed with diets containing graded levels of green algae ulva meal *(Ulva rigida*) reared in geothermal waters of southern Tunisia. *Journal of Applied Ichthyology*, **24**(2): 202–207.

Azim, M. E., and Little, D. C. (2008). *Nutrient dynamics in integrated aquaculture-agriculture systems* . CABI Publishing.

Balshine-Earn, S., and Lotem, A. (1998). Individual differences in parental care and behaviour in the mouthbrooding cichlid *Oreochromis aureus* . *Animal Behaviour* , **56** (2): 407-415.

Baroiller, J.-F., D'Cotta, H., Bezault, E., Wessels, S., and Hoerstgen-Schwark, G. (2009). Tilapia sex determination: Where temperature and genetics meet. Comparative

Biochemistry and Physiology Part A: *Molecular and Integrative Physiology* , **153** (1): 30-38.

Basiita, R. K. (2020). Better management practices for tilapia broodstock conditioning and mass spawning in hapas in ponds . WorldFish.

Beardmore, J. A., Mair, G. C., and Lewis, R. I. (2001). Monosex male production in finfish as exemplified by tilapia: Applications, problems, and prospects. *Aquaculture* , **197** (1-4): 283-301.

Bentsen, H. B., Gjerde, B., Eknath, A. E., de Vera, M. P., Velasco, R. R., Danting, J. C., Dionisio, E. E., Longalong, F. M., Reyes, R. A., and Abella, T. A. (2017). Genetic improvement of farmed tilapias: Response to five generations of selection for increased body weight at harvest in Oreochromis niloticus. *Aquaculture*, **468** , 206–217. Beveridge, M. C. M. (2004). Cage aquaculture (3rd ed.).

Bhanthumnavin, K., and McGarry, M. G. (1971). *Lemna perpusilla* as a source of protein for human consumption. *Nature*, **232** (5311): 495.

Bombardelli, R. A., Hayashi, C., and Meurer, F. (2010). Digestible energy requirements for Nile tilapia (*Oreochromis niloticus*) broodstock. *Revista Brasileira de Zootecnia,* **39** (5): 997-1003.

Boyd, C. E. (2020). *Water quality in ponds for aquaculture* (2nd ed.). Auburn University Press.

Boyd, C. E., McNevin, A. A., and Clay, J. (2020). *Responsible aquaculture*: Perspectives for the future . Springer.

Brummett, R. E. (1995). Environmental regulation of sexual maturation and reproduction in tilapia. *Aquaculture Research*, **26** (1): 11-18.

Campos-Mendoza, A., McAndrew, B. J., Coward, K., and Bromage, N. (2004). Reproductive response of Nile tilapia *(Oreochromis niloticus*) to photoperiodic manipulation; effects on spawning periodicity, fecundity and egg size. *Aquaculture*, **231** (1-4): 299-314.

Chakrabarti, R., Clark, W. D., Sharma, J. G., Goswami, R. K., Shrivastav, A. K., and Tocher, D. R. (2018). Mass production of *Lemna minor* and its amino acid and fatty acid profiles. Frontiers in Chemistry, 6, 479.

Cheng, J. J., and Stomp, A. M. (2009). *Growing duckweed for biofuel production*: A review. Plant Biology, **11** (1): 14-23.

Cheng, J. J., Landolt, E., and Stomp, A. M. (2020). Duckweed production in bioreactors. In Bioenergy Research Reports (pp. 89-112). Springer. Cheng, J. J., Landolt, E., and Stomp,

A. M. (2020). The effect of light intensity and nitrogen concentration on duckweed protein production. *Aquatic Botany*, 167, 103276.

Dabrowski, K. (2001). Ascorbic acid in aquatic organisms: Status and perspectives. *In Ascorbic Acid in Aquatic Organisms* (pp. 1-22). CRC Press.

Dewanji, A., Matai, S., Si, L., Barik, S., and Nag, A. (1993). Amino acid composition of leaf proteins extracted from some aquatic weeds. *Journal of Agricultural and Food Chemistry*, **41** (8): 1232–1236.

Diana, J. S., Egna, H. S., Chopin, T., Peterson, M. S., Cao, L., Pomeroy, R., Verdegem, M., Slack, W. T., Bondad-Reantaso, M. G., and Cabello, F. C. (2013). Responsible aquaculture in 2050: Valuing local conditions and human innovations will be key to success. *BioScience* , **63** (4): 255–262.

El-Sayed, A.-F. M. (2019). *Tilapia culture* (2nd ed.). Academic Press.

El-Sayed, A.-F. M. (2020). *Tilapia culture in Egypt: Successes and challenges*. Reviews in Aquaculture, **12**(2): 318-332.

El-Sayed, A.-F. M., and Kawanna, M. A. (2008). Effects of water temperature on reproductive performance of Nile tilapia (*Oreochromis niloticus*). *Journal of Applied Aquaculture*, **20** (4): 284-293.

El-Shafai, S. A., El-Gohary, F. A., Verreth, J. A. J., Schrama, J. W., and Gijzen, H. J. (2004). Apparent digestibility coefficient of duckweed (*Lemna minor*), fresh and dry for Nile tilapia (*Oreochromis niloticus*). *Aquaculture Research*, **35** (6): 574–586.

Eshel, O., Shirak, A., Weller, J. I., Hulata, G., and Ron, M. (2014). Linkage and physical mapping of sex region on LG23 of Nile tilapia (*Oreochromis niloticus*). G3: Genes, Genomes, Genetics, **4** (1): 35-42.

Fasakin, E. A., Balogun, A. M., and Ajayi, O. O. (1999). Evaluation of duckweed (*Lemna minor*) as feed ingredient in diets for tilapia (*Oreochromis niloticus*). *Journal of Applied Aquaculture*, **9** (2): 39–47.

Food and Agriculture Organization (FAO). (2022). The future of food and agriculture: Trends and challenges. FAO.

FAO. (2022). The State of World Fisheries and Aquaculture 2022. FAO.

FAO. (2022). The State of World Fisheries and Aquaculture 2022. Food and Agriculture Organization of the United Nations.

Green, B. W., Teichert-Coddington, D. R., and Phelps, R. P. (1997). Development of hormonal sex reversal technology for Nile tilapia. *Aquaculture*, **148** (2-3): 115-126.

Hassaan, M. S. (2022). Algal diets enhance reproductive performance in Nile tilapia (*Oreochromis niloticus*) broodstock. *Aquaculture Reports*, **22**, 100943.

Hassan, M. S., El-Haroun, E. R., and Goda, A. M. A. S. (2020). Fatty acid profiles of duckweed (Lemna minor) and its effects on growth and immunity of Nile tilapia. *Aquaculture Reports*, **18** 100436.

Hassan, M., El-Haroun, E., and Goda, A. M. (2020). Effect of dietary duckweed (*Lemna minor*) replacement on Nile tilapia performance and feed utilization. *Aquaculture Reports*, **18** , 100436.

Izquierdo, M. S., Fernández-Palacios, H., and Tacon, A. G. J. (2001). Effect of broodstock nutrition on reproductive performance of fish. *Aquaculture*, **197** (1–4): 25–42.

Joaquim, N., Matias, D., Lopes, T., Ameixa, O. M. C. C., and Ribeiro, L. (2018). Broodstock nutrition in aquaculture: A review on impacts and opportunities. *Reviews in Aquaculture*, **10** (3): 696-716.

Kabir, K. A., Salam, M. A., and Paul, D. N. R. (2021). Reduction of anti-nutritional factors in duckweed (*Lemna minor*) through processing. *Aquaculture Reports*, **20**, 100697.

Kassam, L., and Subasinghe, R. (2019). Tilapia farming as a pathway out of poverty in rural Kenya. *Aquaculture Economics and Management*, **23** (3): 298-315.

Kolkovski, S. (2001). Digestive enzymes in fish larvae and juveniles: Implications for formulated diets. *Aquaculture*, **200**(1–2): 181–201.

Kyule-Muendo, D., Otachi, E., Awour, F., Ogello, E., Obiero, K., Abwao, J., Muthoni, C., and Munguti, J. (2022). Status of fish health management and biosecurity measures in fish farms, cages and hatcheries in Western Kenya. *CABI Agriculture and Bioscience*, **3** (18).

Landolt, E. (1986). The family of *Lemnaceae*: A monographic study (Vol. **1**). Veröffentlichungen des Geobotanischen Institutes der ETH.

Leng, R. A., Stambolie, J. H., and Bell, R. (1995). Duckweed: A potential high-protein feed resource for domestic animals and fish. *Livestock Research for Rural Development*, **7** (1), 1–15.

Les, D. H., Crawford, D. J., Landolt, E., Gabel, J. D., and Kimball, R. T. (2002). Phylogeny and systematics of Lemnaceae. *Aquatic Botany*, **73** (4), 325-370.

Little, D. C., and Hulata, G. (2000). Strategies for tilapia seed production. In Tilapias: *Biology and exploitation* (pp. 267-326). Springer.

Lowe-McConnell, R. H. (2000). The roles of tilapias in ecosystems. In M. C. M. Beveridge and B. J. McAndrew (Eds.), Tilapias: *Biology and exploitation* (pp. 129–162). Springer.

Macintosh, D. J., and Little, D. C. (1995). Nile tilapia (*Oreochromis niloticus*). In Broodstock management and egg and larval quality (pp. 277-320). Blackwell Science.

Mair, G. C., Abucay, J. S., Beardmore, J. A., and Skibinski, D. O. F. (2004). Genetic manipulation of sex ratio for the large-scale production of all-male tilapia (Oreochromis niloticus). *Canadian Journal of Fisheries and Aquatic Sciences*, **61** (1), 1-10.

Mandal, S., Saha, S., and Das, S. (2010). Duckweed: An efficient hyperaccumulator of heavy metals and other pollutants. *International Journal of Phytoremediation*, **12** (7), 689–706.

Mataveli, M., Mewes, J. K., and Bombardelli, R. A. (2010). Dietary protein and digestible energy requirements for Nile tilapia (*Oreochromis niloticus*) broodstock. *Aquaculture Nutrition*, **16** (4): 379-387.

Munguti, J. M., Obiero, K., Odame, H., Kirimi, J., Kyule, D., Ani, J., and Liti, D. (2021). Key limitations of fish feeds, feed management practices, and opportunities in Kenya's aquaculture enterprise. *African Journal of Food, Agriculture, Nutrition and Development*, **21** (2): 17415–17434.

Nandeesha, M. C., Gangadhar, B., Varghese, T. J., and Keshavanath, P. (2001). Use of mixed feeding schedules in fish culture: Performance of common carp (Cyprinus carpio) fry fed on plant and animal protein-based diets. *Asian Fisheries Science*, **14** (4): 345–356.

Nandeesha, M. C., Gangadhar, B., Varghese, T. J., and Keshavanath, P. (2002). Growth and reproductive performance of broodfish and larval growth of common carp (Cyprinus carpio) fed diets with different plant protein sources. *Asian Fisheries Science*, **15** (3): 259–280.

Naseem, S., Malik, A., and Ali, S. (2021). Evaluation of duckweed *Lem(na minor*) as a protein source in fish feed. *Aquaculture Reports*, 20, 100707.

Navarro, R. D., Furuya, W. M., and Pezzato, L. E. (2014). Dietary lipid levels and reproductive performance of Nile tilapia (*Oreochromis niloticus*) broodstock. *Aquaculture Research*, **45** (6): 1063-1070.

Neat, F. C., Taylor, A. C., and Hunting ford, F. A. (2003). Proximate costs of fighting in male cichlid fish: The role of injuries and energy metabolism. *Animal Behavior*, **65** (4): 823-834.

Ng, W. K., and Wee, K. L. (1989). The nutritive value of cassava leaf meal in pelleted feed for Nile tilapia. *Aquaculture,* **83**(1–2): 45–58.

Ng, W.-K., and Romano, N. (2013). A review of the nutrition and feeding management of farmed tilapia. *Reviews in Aquaculture* , **5**(4): 220–254.

Nguyen, H. Q., Linh, N. T. H., and Tuan, N. N. (2022). Immunostimulatory effects of duckweed (*Lemna minor*) in Nile tilapia (*Oreochromis niloticus*). *Aquaculture International* , **30** (1): 1-15.

Nguyen, H. Q., Linh, N. T. H., and Tuan, N. N. (2022). Vitamin composition of duckweed *(Lemna minor*) cultivated under controlled conditions. *Journal of Applied Phycology*, **34** (3): 1545-1555.

Oliveira, T., Peixoto, M. J., Soares, F., and Cabrita, E. (2014). Dietary protein levels and male reproductive performance in Nile tilapia (*Oreochromis niloticus*). *Aquaculture Research*, **45** (5): 898-907.

Olvera-Novoa, M. A., Domínguez-Cen, L. J., Olivera-Castillo, L., and Martínez-Palacios, C. A. (2002). Duckweed (*Lemna minor*) as a protein source in diets for tilapia (*Oreochromis mossambicus). Aquaculture Nutrition*, 8 (4): 257-264.

Opiyo, M. A., Munguti, J. M., and Charo-Karisa, H. (2022). Nutritional value of duckweed (Lemna minor) as a protein source for Nile tilapia (*Oreochromis niloticus). Aquaculture Reports,* 23 , 101042.

Rad, F., Bozaoğlu, S., Gözükara, S. E., Karahan, A., and Kurt, G. (2020). Effects of photoperiod and moonlight on egg fertilization and spawning in Nile tilapia (*Oreochromis niloticus*). *Aquaculture Research* , **51** (1): 1-9.

Rana, K. J. (1990). Influence of incubation temperature on *Oreochromis niloticus* eggs and fry: I. Gross embryology, hatchability, and fry survival. *Aquaculture,* **87** (2): 165-181.

Ribeiro, F., and Qin, J. G. (2013). Reproductive behaviour of Nile tilapia (*Oreochromis niloticus*) in a temperature-controlled environment. *Journal of Applied Ichthyology*, **29**(5): 1075–1081.

Ringø, E., Hoseinifar, S. H., Ghosh, K., Doan, H. V., Beck, B. R., and Song, S. K. (2018). Lactic acid bacteria in finfish: An update. *Frontiers in Microbiology*, **9** , 1818.

Roem, A. J., Kohler, C. C., and Stickney, R. R. (1990). Vitamin E requirements of the blue tilapia (*Oreochromis aureus*) in relation to dietary lipid level. *Aquaculture*, **87** (2): 155-164.

Said, M. M., El-Sayed, A. F. M., and Easa, M. A. S. (2022). Duckweed as a water purifier and a feed for fish in integrated aquaculture systems. *Aquaculture Research* , **53** (12): 4329-4339.

Santiago, C. B., and Laron, M. A. (2002). Effects of dietary lipid source on reproductive performance and tissue lipid levels of Nile tilapia (*Oreochromis niloticus*) broodstock. *Journal of Applied Ichthyology*, **18** (1): 32-37.

Sargent, J. R., Tocher, D. R., and Bell, J. G. (2002). The lipids. In Fish Nutrition (3rd ed., pp. 181–257). Academic Press.

Sargent, J. R., Tocher, D. R., and Bell, J. G. (2002). The lipids. In Fish Nutrition (3rd ed., pp. 181-257). Academic Press.

Sarmento, N. L. A. F., Martins, M. L., and Romagosa, E. (2017). Vitamin C supplementation improves sperm quality in Nile tilapia (*Oreochromis niloticus). Aquaculture Research*, **48** (5), 2208-2216.

Sasmaz, M., Topal, E. I. F., Obek, E., and Sasmaz, A. (2015). The potential of *Lemna gibba* and *Lemna minor* to remove Cd, Cu, Pb, and Zn in tertiary treatment of wastewaters. *Environmental Science and Pollution Research*, **22** (20): 15332-15341.

Skillicorn, P., Spira, W., and Journey, W. (1993). Duckweed aquaculture. World Bank Technical Paper.

Skillicorn, P., Spira, W., and Journey, W. (1993). Duckweed aquaculture: A new aquatic farming system . World Bank.

Skillicorn, P., Spira, W., and Journey, W. (1993). Duckweed aquaculture: A new aquatic farming system for developing countries. World Bank.

Sogbesan, A. O., Ugwumba, A. A. A., and Madu, C. T. (2015). Nutrient profile and utilization of *Lemna minor* (duckweed) in the diet of *Oreochromis niloticus* . *Journal of Fisheries and Aquatic Science,* **10** (3), 184–194.

Sonta, M., Rechulicz, J., and Pilarczyk, W. (2019). Use of duckweed (*Lemna L.)* in sustainable livestock production and aquaculture—A review. *Annals of Animal Science*, **19** (2): 257–271.

Tacon, A. G. J., and Metian, M. (2015). Feed matters: Satisfying the feed demand of aquaculture. *Reviews in Fisheries Science and Aquaculture*, **23** (1): 1–10.

Tacon, A. G. J., Hasan, M. R., and Metian, M. (2022). Demand and supply of feed ingredients for farmed fish and crustaceans. FAO Fisheries Technical Paper, 650 .

Tacon, A. G. J., Hasan, M. R., and Subasinghe, R. P. (2000). Use of duckweed in aquafeeds. FAO Fisheries Circular No. 963.

Talukdar, A., Kalita, H. K., Basumatary, S., Saikia, S. K., and Yadav, R. N. S. (2023). Duckweed *(Lemna minor*) as a sustainable protein source in aquaculture feed: A review. *Aquaculture Reports*, 30 , 101603.

Tocher, D. R. (2003). Metabolism and functions of lipids and fatty acids in teleost fish. *Reviews in Fisheries Science,* **11** (2): 107-184.

Tocher, D. R. (2010). Fatty acid requirements in ontogeny of marine and freshwater fish. Aquaculture Research, 41 (5): 717–732.

Trewavas, E. (1983). Tilapiine fishes of the genera *Sarotherodon, Oreochromis*, and Danakilia . British Museum (Natural History).

Turner, G. F., Robinson, R. L., Ngatunga, B. P., and Genner, M. J. (2019). Mate choice and speciation in cichlid fishes of African lakes. *Journal of Fish Biology* , **95** (2): 405-425.

Wahab, M. A., Habib, M. A. B., and Hossain, M. A. (2018). Biochemical composition of duckweed (*Lemna minor*) as influenced by nutrient media. *Aquaculture International*, **26** (1): 309-319.

Wang, W., Wu, Y., Yan, Y., Ermakova, M., Kerstetter, R., and Messing, J. (2020). DNA barcoding of the *Lemnaceae*, a family of aquatic monocots. *BMC Plant Biology* **,** **20** (1): 1-14.

Wang, Y., Li, M., Wang, S., and Wang, R. (2019). Dietary astaxanthin enhances reproductive performance, egg quality in sea bass. *Aquaculture Nutrition* **,** **25** (1): 70–79.

Watanabe, T., Kiron, V., and Satoh, S. (1997). Trace minerals in fish nutrition. *Aquaculture,* **151** (1-4): 185-207.

Yan, Y., Candreva, J., Shi, H., Ernst, E., Martienssen, R., and Schwender, J. (2013). Survey of the total fatty acid and triacylglycerol composition and content of 30 duckweed species. *BMC Plant Biology*, 13 , 201.

Yeo, J., Parrish, C. C., and Brown, J. A. (2017). Alternatives to fishmeal in aquaculture feeds: A meta-analysis of growth effects in rainbow trout*. Reviews in Aquaculture*, **9** (1): 82–104.

Yosef, F. A., Ali, M. A., and El-Sayed, A.-F. M. (2022). Nutritional evaluation of duckweed *(Lemna minor*) meal for Nile tilapia. *Aquaculture International*, **30** (1): 1-17.

Yue, G. H., Wang, L., and Xia, J. H. (2022). Genomic selection in aquaculture: Current status and future potential. *Reviews in Aquaculture*, **14 (**1): 3–17.

Ziegler, P., Adelmann, K., Zimmer, S., Schmidt, C., and Appenroth, K. J. (2016). Relative in vitro growth rates of duckweeds (Lemnaceae): The most rapidly growing higher plants. *Plant Biology* , **17** (1): 33-41.

APPENDIX

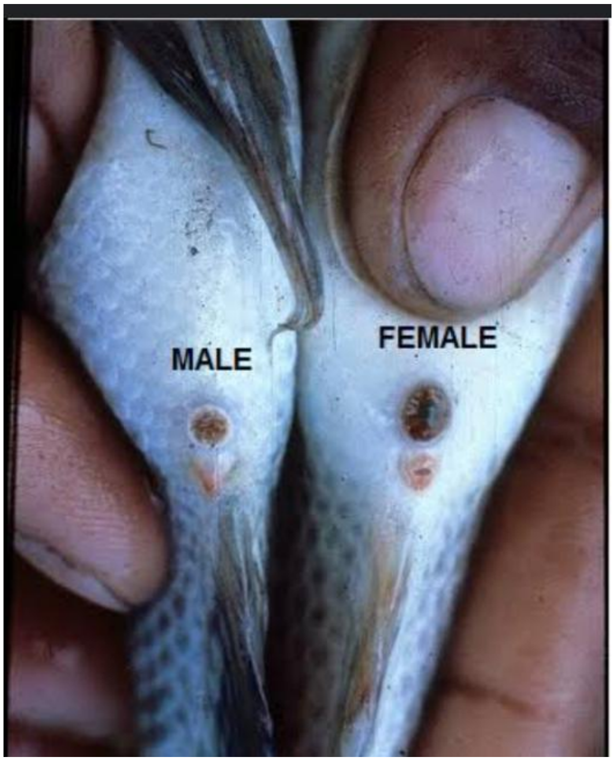

Plate 6: Reproductive organs of Nile Tilapia

Plate 7: Fry per harvest

Plate 8: fry per harvest