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Physiological Responses of Angolan Tilapia (Oreochromis Angolensis) in Different Salinity Gradients

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Abstract

Several species of tilapia are considered ideal for aquaculture in different production systems due to their rusticity. Oreocrhomis Angolensis, originally found in fresh and brackish waters, is a little-known species and little explored by aquaculture. It can be a candidate for generating strong hybrids that can be used for industrial production. This study aimed to evaluate the performance of Oreochromis angolensis in different salinities. For this purpose, 108 juveniles of 1.37 ± 0.23g and 3.57 ± 0.25cm, acclimated for 8 days and cultured for 30 days in the following salinities: 5; 10; 15; and 20g, three replicates each, in 9-liter tanks with aeration and thermostated bath. Biometrics and analysis of water quality parameters were performed. Weight gain was lowest in the 5g treatment and highest in the 20g treatment, with an average of 0.60g. TCE was not limited by salinity. Feed conversion was not good in any of the treatments. Survival was 90% in the 20g treatment and 100% in the others, during the 30 days of culture. Therefore, Angolan tilapia is a species with a wide euryhaline capacity and these results will help future studies regarding the appropriate salt concentrations for the growth of this species in different salinities.

Keywords: Angolan Tilapia, Salinity, Physiological Responses, Performance, Resistance

Introduction

Aquaculture basically consists of removing organisms from their natural environment to artificial systems or captivity. Success depends on the use of rustic species capable of adapting to environmental variations. In any environment where there are environmental variations, adaptations emerge as an alternative to ensure the survival, reproduction, development, geographic distribution and diversification of species [1].

Aquaculture is currently the most important alternative to reverse the problems caused by the reduction of fishery resources and meet the demand for animal protein. Fish farming as an economic activity has grown significantly in recent years. The amplification of this activity must be done through knowledge about the species to be exploited. Therefore, basic and applied research must be encouraged so that the activity has greater profitability.

The different species of tilapia are adapted to a wide diversity of habitats, reflecting very variable environmental conditions, which include physical, chemical and biological parameters. They are considered ideal for aquaculture and can be used in different systems and production scales due to their hardiness [2]. The species Oreochromis niloticus, O. aureus, O. urolepis hornorum and O. mossambicus are the most prominent in aquaculture production. However, there are more than 70 species of tilapia identified, including O. Angolensis [3].

The species Oreochromis angolensis originally described as Sarotherodon is a native lineage of Angola abundant in the Bengo River, lower Cuanza River up to the Cambambe waterfalls. Its maximum length is 20.4 cm, adult males have white spots on the pelvic and dorsal fins, and both sexes have them on the anal fins and at the base of the caudal fin [4]. It may be a strong candidate for industrial production, however there is a large gap in information about its zootechnical performance in different environments. The production of native species at an industrial level requires concrete information about their sensitivity to environmental parameters to ensure safe management and, at the same time, profit.

Knowledge of the sensitivity of fish to salt, based on a diversity of osmoregulatory interactions, is one of the basic principles for choosing a species to be used in environments susceptible to saline changes Many freshwater species can suffer severe stress and threat due to their physiological inability to cope with extreme osmotic stress [5].

Stress resulting from fish handling, capture, sorting and transportation can lead to significant losses in production systems. To reduce the effects of stress in these procedures, common salt has been recommended, especially after handling and during fish transportation. However, tolerance to salt concentrations varies among fish species.

Research problem What are the physiological responses of Angolan tilapia (Oreochromis angolensis) in different salinity gradients?

Hypotheses Null hypothesis: Performance of Angolan tilapia is not limited by chronic exposure to different salinities.

Alternative hypothesis: Performance of Angolan tilapia is limited by chronic exposure to different salinities.

Methodology

The type of study used was experimental with a qualitative-quantitative approach, naturally aided by bibliographic analysis. Stratified sampling, 108 juvenile Angolan tilapia (Oreochromis angolensis) of the two sons-in-law, size 1.37 ± 0.23g and average length 3.57 ± 0.25cm were used.

General objective The main objective of this work is to evaluate the performance of Angolan tilapia (Oreochromis angolensis) in different salinities.

Specific objectives

- To verify the effects of salinity on the growth and weight gain of Angolan tilapia.
- Identify the salt concentrations that least harm the performance of Angolan tilapia.
- To evaluate the influence of salinity on the survival of Oreochromis angolensis.
- Provide the necessary support to help reduce economic losses caused by changes in salinity.

Theoretical Basis

Angolan Tilapia

According to Froese and Pauly, (2018) “the species Oreochromis angolensis (a tilapia, with no common name) is found in the Bengo River, associated lakes and lagoons, lower Cuanza River to the Cambambe Falls”.

Figure 1: Angolan tilapia Oreochromis angolensis, Source- Ernst Swartz. Licensed under Creative Commons BY 4.0 Available: https://www.gbif.org/occurrence/1230403069. (July 20, 2018).

Oreochromis angolensis is the name for this species, it was originally described as Sarotherodon [6]. It has the following taxonomic classification:

- **Kingdom:** Animalia
- **Sub-Kingdom:** Bilateria
- **Infra-Kingdom Deuterostomia Phylum:** Chordata
- **Subphylum:** Vertebrata Infraphylum: Gnathostomata
- **Superclass:** Actinopterygii
- **Class:** Teleostei
- **Superorder:** Acanthopterygii Order: Perciformes
- **Suborder:** Labroidei Family: Cichlidae
- **Genus:** Oreochromis
- **Species:** Oreochromis angolensis [7].

Asexual males reach their peak of growth at 20.4 cm. They have between 15–17 dorsal spines; 11–12 soft dorsal rays; 3 anal spines; 8–10 soft anal rays, 29 vertebrae, external maxillary teeth in females and bicuspid in young males, slightly lateral unicuspid in mature males with many unicuspids, up to about 20 cm, almost all external teeth are unicuspid and the posterior ones are enlarged; thin and dense pharyngeal teeth, with the lower pharyngeal bone blade longer than the toothed area, 21-26 gill rakers on the lower part of the first arch, 29 vertebrae, 15-16 rarely 17 dorsal spines, 27-28 dorsal rays, non-scaly caudal fin, simple genital papilla for both sexes, elongated, bifid and with tassels in the male; white spots on the pelvic fins of mature males and on the dorsal, anal and base of the caudal fins in both sexes [4].

It is abundant in cold water environments, tropical climate, brackish, pelagic benthos, found in the Bengo River, associated lakes and lagoons, in the lower Cuanza River and at the Cambambe waterfalls [6].

Hybrid species of the Chilidae family, of the Oreochromis, Sarotherodon and Tilapia genera, are called tilapia. They originate from Africa, and are found mainly in the basins of the Nile and Chad rivers and in the lakes of central-west Africa [8]. They are among the most suitable fish for farming in tropical regions. There are 77 species of tilapia, of which 22 are used commercially, and the most cultivated in the world is the Nile Tilapia. These fish reproduce naturally throughout the year, as long as there are suitable conditions, thus presenting the need to analyse basic environmental factors (physical, chemical and biological factors) and the study of their interactions. They inhabit lentic environments, influenced by several hydrometeorological factors such as evapotranspiration, rainfall, winds, atmospheric pressure and solar radiation, presenting an unstable pH and low concentration of dissolved oxygen [3]. 1.2. Fish sensitivity to salt Knowledge of fish sensitivity to salt, based on a diversity of osmoregulatory interactions, is one of the basic principles for choosing a species to be used in environments susceptible to saline changes [5]. Many freshwater species can suffer severe stress and threat due to their physiological inability to cope with extreme osmotic stress. Salt has been used for the transportation and management of freshwater fish as a stress mitigator [9]. For juvenile freshwater fish, salinity can affect nitrogen excretion, influence the activity of digestive enzymes due to the presence of salt in the intestinal tract content and, consequently, digestibility [10].

Salinity variations can also induce increases in oxygen consumption and alter locomotor activity and food intake, thus affecting the animal's growth [9, 11]. Therefore, for salt to be used to improve handling and transportation practices, it is necessary to know its acute and chronic effect on different species and development stages.

In the larviculture of neotropical fish, the use of salt has enabled survival and growth results similar to and/or superior to those recorded in larvae cultivated in freshwater, with tolerance to different salinity gradients depending on the species [12].

Laboratory studies on the tolerance of different fish species to water salinity are carried out using dilutions of non-iodized common salt with local non-chlorinated water to assess the acute effects of salinity in 96-hour tests [13]. Through these acute tests, the lethal salinity limits can vary to lower levels, since the fish are not previously acclimatized. Although salinity variations can occur quickly in the natural environment, they are never instantaneous as in osmotic shock tests performed in the laboratory [14]. Demonstrating the average lethal concentrations for native and commercial fish species, with various saline gradients, under identical conditions regarding dilution water quality, allows for an unequivocal comparison of their sensitivity [15].

Sodium chloride in fish has effects such as: stimulating mucus secretion, both in the skin and gills, reducing ammonia levels in the blood and constricting gill filaments [16]. It is considered effective, when used at non-harmful levels, for a prolonged period of time, for the control of Cichlidogyrus sclerosus, Gyrodactylus sp, Trichodina sp and Ichthyophthirius sp. [17]. However, tolerance to water salinity varies between species, with one of the main factors being the time of exposure of the fish. Therefore, assessing the tolerance to water salinity for fish is essential to allow the safe use of common salt [18].

Some fish species have the ability to permanently adapt to varying salinity conditions in the farming environment, as they can regulate their osmotic pressure [19]. However, this constant control of the body's salts through osmoregulation represents an energy expenditure for the animal [20]. It is estimated that energy expenditure during osmoregulation can represent a loss of approximately 10 to 50% in fish metabolism [21]. Even if there is control of this energy expenditure, the animal's metabolism will direct its reserves to other main metabolic functions, such as growth. According to Basti (2011) and Freitas (2010), the study of the effects of salinity, for a prolonged period in the culture environment, serves as an important tool for the production of fish fry, where understanding these effects on the environment is directly related to maximizing growth and survival, in addition to the management and profitability of production.

Factors that Promote Salt Loss in Fish: Salts and minerals also exist in rivers, lakes, and ponds. These mineral salts, even in small quantities, constantly influence the osmotic balance of fish [22].

Fish blood contains approximately 0.9% salt, and in fish, sodium ions ($Na+$) represent approximately 75% to 80% of these salts. Freshwater fish tend to lose salts through osmosis, since water that contains few salts is in direct contact with the blood capillaries of their gills [15].

Freshwater fish have strategies to maintain osmotic balance. An important strategy for fish to maintain this balance is associated with the neuroendocrine system. This system induces the production of harmones that act on the tissues responsible for osmoregulation [23]. Among these harmonies, prolactin can be cited as one of the most important. Prolactin is one of the main hormones in osmoregulation, acting on the entry of ions into tissues, causing changes in the metabolism of animals. Prolactin is responsible for reducing the permeability of the gills and increases mucus production. It reduces the permeability of the epithelium, reducing the absorption of NA+, CL and water. Increases plasma CA2+, minimizing the loss of salts from the blood to water. It also influences the functioning of the urinary bladder and kidneys, to reabsorb large amounts of urine salts and discard large amounts of water, which is continuously absorbed by osmosis [5].

Main Effects of Salinity Variations: on teleosts Salinity is composed of the concentration of ions dissolved in water [9]. Marine fish have a blood osmotic concentration (about 300-350 mOsm/kg) approximately 1/3 the osmotic concentration of seawater (about 1,000 mOsm/kg). In this way, they are hyposmotic in relation to the environment in which they live. Thus, they present two general osmoregulatory problems: entry of salts by diffusion and loss of water by osmosis [24]. The development and growth of teleost fish are generally influenced by environmental salinity [25].

According to Silva (2019), water salinity can influence the nutritional status of euryhaline teleosts by changing the metabolic cost of osmoregulation and reorganization of energy metabolism. In addition to reducing the energy required for osmoregulation, salinity can directly affect fish growth by affecting food consumption rates or the ability to digest food efficiently [18]. Tolerance to salinity variation in fish depends on their own physiological, biochemical and morphological adjustment to a given salinity [13]. Salinity can affect osmoregulatory processes, due to changes in the number of chloride cells and/or modulation of Na+ +, K+ -ATPase (NKA) activity. Therefore, some adverse factors may occur when marine teleosts are raised in low salinities, which may lead to an increase in food consumption and a reduction in their growth rate [10]. Marine teleosts when exposed to low salinity in the environment tend to passively lose ions such as Na+, Cl-, K+, Mg2+ and Ca2+. This loss must be compensated by the active uptake of these ions through water and/or food [16]. The demands and functioning mechanisms of ionoregulatory pathways change depending on environmental salinity, diet, activity, development stage and a variety of stressors [24]. Therefore, acclimatization to salinity change requires structural and metabolic reorganization to meet the increased energy demand associated with exposure to the new environment.

Teleost fish are osmoregulators, that is, they maintain the osmotic concentration of the blood different from the environment in which they live, and all teleost fish are considered osmoregulators [26]. There are some species of teleost fish with the ability to survive large salinity ranges, these being called euryhaline. Osmoregulation is an energy-demanding process, and fish growth can be maximized through selected salinities that would reduce energy expenditure to maintain homeostasis [13]. Some studies have shown that energy expenditure for carrying out osmoregulation can reach 50%. However, the metabolic cost of osmoregulation is proportional to the osmotic gradient to which the fish is exposed [3]. Thus, the reduction in metabolic costs for osmotic regulation may reflect the reallocation of this energy to other physiological processes, such as increased growth [18].

Physiological Adaptations of Fish to Stress

Stress can be defined as a condition in which the dynamic physiological balance, or homeostasis, of a given organism is disturbed or influenced by an intrinsic or extrinsic stimulus, called a stressor [1].

The main mechanisms of neuroendocrine control of stress responses in fish are comparable to those existing in mammals and other terrestrial animals, although many reactions are typical of the group [10]. Like higher animals, fish have a natural ability to respond physiologically to stressors, to control the imposed disturbances. However, when response mechanisms are forced beyond their normal limits, the body's response can be detrimental to the animal's health [16].

Exposure to stressors can be acute or chronic. Acute usually occurs during handling, in conditions such as capture, biometry and transport. Chronic conditions occur in conditions that expose fish, for long periods, to stressful situations, such as farming in an environment with poor water quality or frequent fluctuations in physical-chemical parameters and overcrowding, which can lead to a decrease in growth rate, reproduction and greater vulnerability to diseases [1].

The stress response, triggered by stressors, comprises a series of physiological changes that are classified into primary, secondary and tertiary responses. Among the primary responses, the increase in the secretion of catecholamines, adrenaline and noradrenaline and cortisol in the plasma stands out, while among the secondary responses are the metabolic responses, such as changes in blood glucose, lactic acid, hepatic and muscular glycogen; hematological responses, such as changes in hematocrit and lymphocyte number, and also responses that affect hydromineral balance, such as changes in the concentrations of chloride, sodium, potassium, proteins and plasma osmolarity. The main tertiary responses are changes that lead to a drop in productive performance and a decrease in resistance to diseases [18].

The term salt encompasses a very large group of chemical compounds. According to basic chemistry, the definition of a salt is very simple: salt is a compound that when mixed in water ionizes into a cation other than H+ and an anion other than OH. Among the most common salts used in lakes and captivity for various purposes are sodium bicarbonate (NaHCO3), calcium chloride (CaCl2), magnesium chloride (MgCl2) and potassium permanganate (KMnO4).

Salinity is simply the ratio of the mass of salt to the mass of water and is usually measured in g/kg (grams per kilogram) or g/l (grams per liter). We have two ways of measuring the salinity of water in freshwater ponds and lakes: using a refractometer and using a conductivity meter (TDS), [27].

Fish blood has a salinity of about 9 g/L (0.9% salt) and a pH of 7.4. Approximately 77% of the salt in the blood is sodium and chloride. The remainder is composed mainly of bicarbonate, potassium and calcium. Therefore, sodium chloride or common salt is one of the most used chemical substances in the transportation of freshwater fish [28].

Handling and transportation are unavoidable procedures throughout the production process in any fish farm. In general, both procedures expose the fish to a series of stressful stimuli that can trigger physiological adaptation responses in the fish. The blood of freshwater fish contains concentrations of salts, in the form of ions, which must be kept in balance with the environment. This osmotic balance is maintained by mechanisms that occur in the gills (Figure 1), by active flow of salts in the gills of these fish, maintaining the ionic balance [25].

Freshwater fish lose salts (ions) to the external environment by diffusion through the gills, through the body surface and through excretion in feces and urine (Fig. 1).

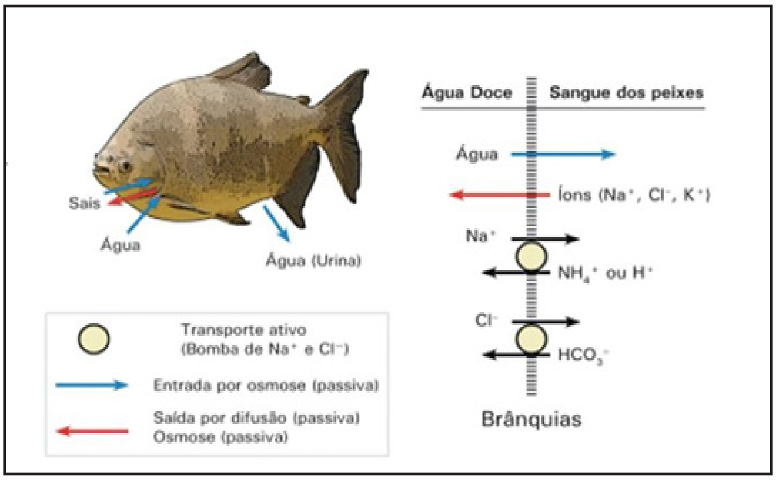

Figure 2: Mechanism of osmoregulation in freshwater fish. Source- Fábio Sian Martins, 2015.

Stress is defined as a non-specific response of the organism to something that is demanded of it, which can affect the osmoregulatory balance of fish. The physiological reactions caused by stressors involve adaptive mechanisms that allow fish to maintain homeostasis in the presence of any adverse stimulus, due to the mobilization of energy reserves, such as blood glucose and liver glycogen [25]. Because when fish are handled or transported, they are forced to consume extra energy to maintain osmoregulation (water balance).

Cortisol increases the permeability of cell membranes, accentuating the loss of salts from the blood into water, as well as the entry of water (hydration) into the body [29]. This can result in significant fish mortality during and, more commonly, one to two weeks after transport. Even if they do not die as a direct consequence of the osmoregulatory imbalance, surviving fish may succumb to disease due to the suppression of their immune system caused by the stress to which they have been subjected. This immunosuppression condition can occur in fish of all ages, but is more pronounced in fry and juveniles [30].

For each fish species, the maximum tolerance to salt can be defined by the acute toxicity test in the laboratory, generally characterized by the average lethal concentration (LC50) in a period of 24 to 96 hours, to define the concentration that leads to the death of 50% of the fish. However, the fish farmer can and should previously test the concentration to be used on a small batch of fish, before using it on all the fish [31].

The intensity of physiological changes depends on the species of fish, its nutritional status, its health, water conditions, among other factors. The use of salts, particularly sodium chloride, can reduce the severity of this stress and increase fish survival after routine fish farming practices [32].

During operations preceding transport (fishing, handling, classification by size, debugging and loading), fish suffer some damage to their bodies (loss of scales, lesions caused by parasites or handling, others) and lose part of the protection provided by mucus and scales. In this sense, bathing with salt stimulates the production of mucus [16].

It is necessary to know in advance the average lethal concentration (LC50%) for each species of fish. However, before applying salt baths to any fish, it is recommended to test in advance on a batch of 10 fish and observe the reaction, the time in which half of them lose balance during the bath (exposure time to the bath), as safe concentrations depend on the size of the fish (age) and their health status [33]. Short baths (lasting minutes) use high concentrations of salt, while long baths use low concentrations. Therefore, this prior assessment of the tolerance to water salinity, for each species of fish, is essential to allow the use of salt safely before using it in a batch, and it is also important to define the bath time.

Salt must be added before starting to load the fish. A 1,000 L box must have a water level between 600 L and 700 L if it is to receive a 300 kg load of fish. In practice, there will be a displacement of 1 L of water for each kilo of fish. In general, 4 kg to 5 kg of salt can be placed in the transport box, with 600 L to 700 L of water, for most fish species [34]. Preferably, this quantity of salt should be dissolved in a bucket or container, stirring vigorously until it is completely dissolved and then it will be ready to be added to the transport box, providing constant aeration.

Use of salt in the prophylaxis and control of fish ectoparasites

Parasitic and bacterial infections are among the biggest causes of economic losses in fish farming. Several signs of abnormal behavior caused by these agents can be observed in sick fish, such as lethargy (slow movement), anorexia (lack of appetite), loss of balance (fish swimming in a spiral or vertical motion), grouping at the surface or in the water, agitated breathing (increased opercular beat), excessive production of mucus causing an opaque appearance, erosion of the skin and/or fins, inflamed or pale gills, inflamed abdomen and, sometimes, presenting bloody fluid, swollen and stiff anus, and xophthalmia (eye prominence), apathy, fish isolated from the school and death [33].

Several chemotherapeutic products are used in the prophylaxis and treatment of diseases, but these can compromise the environment, being toxic to fish and also to humans. Many of these products have usage limitations, depending on the levels of dissolved oxygen in the water, ammonia levels, pH, temperature, fish population density, water volume and organic matter content in the tanks/culture pond [35, 33]. This does not apply to common salt, when used in adequate concentrations and periods, it can be effective in controlling some external parasites (ectoparasites), and has therefore been recommended for the prophylaxis and treatment of various ectoparasitoses in freshwater fish [36].

Baths in water containing salt not only dehydrate the parasites, leading to their death, but also allow the replacement of salts (sodium and chloride) in the fish's blood, facilitating the reestablishment of osmoregulatory balance and improving the fish's health [23]. In fish farming, therapeutic interventions with salt can be done in different ways, but long and short-term baths are the most frequently used [33].

Carrying out treatments in large-scale nurseries is generally unfeasible from a practical and economic point of view. It is generally necessary to do three treatments every three days for greater effectiveness. However, each case must be carefully analyzed, as sometimes highly parasitized and weakened fish do not tolerate repeated handling and treatment. In these cases, a single prolonged treatment may be the most appropriate [23].

Salt should also be used during quarantine. Salt is the second most used product in the treatment of ichthyophthriasis and, depending on the species of fish, treatments with 1 g to 5 g/L for a period of 7 to 32 days reduce the number of trophonts, in addition to helping to recover the osmotic balance caused to the fish by the infection. On the other hand, high concentrations (15 g to 20 g/L) in short baths, lasting 20 to 60 minutes, are not effective against ichthyophthriasis [38]. In Pacus with monogeniosis, a disease caused by Monogenea helminths, baths with 228 mg/L were efficient in treating the disease, while doses from 550 mg/L, for 10 minutes, proved to be lethal for the fish, as the animals presented gill hemorrhage, mucous membrane detachment and corneal opacity [39]. However, for tambaquis, concentrations of up to 8 g/L of salt were not efficient in eliminating the monogenetics Anacanthorus spathulatus, Notozothecium janauachensis and Mymarotheciun boegeri and also caused stress in the fish [25].

Salt has little effect in the treatment of adult monogeneans, but can more efficiently eliminate oncomiracidia [33]. A salt concentration of 9 g/L in the water impaired the survival of the monogenean Aphanoblastella chewatus and the viability of its eggs in jundiás after 10 days [37].

Infections by parasitic crustaceans are difficult to prevent or treat, and the first indication of infestation in fish farms usually occurs after they are observed on the bodies of fish [33]. Salt is effective in eliminating crustacean ectoparasites in the fish's body, but it can help in the recovery of injuries caused by them. Infestation with the crustacean Perulernaea gamitanae causes serious lesions in the gills and mouth of tambaquis, so after eliminating the parasites with a suitable product (e.g. diflubenzuron), three consecutive treatments with 4/L to 6 g/L (4 kg/m3 to 6 kg/m3) of salt can be used in the pond [35]. This same procedure with salt was used to recover lesions on the skin of Tambaqui, caused by the isopod Braga patagonica, improving the appearance of the fish for commercialization. However, this product accumulates in the muscles of the fish, which cannot be consumed before [30, 27].

Table 1: Guidance for the correct use of salt in different circumstances of fish farming.

Objective	Kg/1,000 liters	Time
Continuous use – normal conditions	3 to 5	Indefinite time
Parasite control (protozoa)	50	Baths of 20 seconds to 2 minutes
Fungal control	20	Baths of 5 to 20 minutes
Prevention of environmental disease in the gills	10	Baths of 2 to 4 hours with weekly intervals
Nitrite toxicity	$(g/m^3)=[6x(NO\text{-}mg/L)\text{-}(Cl\ mg/L)]/0.6$	Time – until levels decrease
Parasite and disease prevention	3	Indefinite time

Baths with high salt concentrations should not exceed 20 minutes. excessive amounts of salt, which can harm the general biology of the system (killing bacterial colonies and thousands of microorganisms). Fish are at their limit when they begin to lose their balance in salt water. If this happens, immediately remove the fish from the salt bath and place it back in the pond water. Animal resistance changes according to the species [37].

Salt does not treat diseases, it acts exclusively by increasing the density of the water, causing the fish to produce more mucus and become more resistant to diseases. Direct application to the pond water requires some care to avoid causing serious problems [38].

Calculation of Salt Dosage in Therapeutic or Transport Baths: First, you must know the volume of the container that will be used for the bath and calculate the desired amount of salt [30]. For example, when you want a 2% concentration (equal to 20 g for 1 L of water) of salt for 10 L of water, simply follow a rule of three:
20 g for 1 liter
X= 200 g of salt
X ------10 liters

Therefore, 200 g of salt should be used for 10 L of water, at this concentration of 2%.

Calculation of Salt Dosage in a Cultivation Pond: First, the size of the pond, in m3, must be known, by making the following calculation [30]:
Nursery with 30 m long, 20 m wide and 1.5 m deep at 10 x 15 x 1.2
= 180 m3 (= 180,000 L of water)

Then, the amount of salt to be used in the desired procedure must be calculated.

For example: Use of 2% salt, which is equal to 20 g/L, in a 180 m3 pond (equal to 180,000 L of water).

20 g 1 Litro
X= 3600 kg
X 180.000 L

Therefore, 3600 kg of salt should be used for this 180 m3 pond of water, at this 2% salt concentration.

Methodology

Characterization of the Study Area

The experiment was carried out in the Parasitology Laboratory of the Faculty of Natural Sciences of the University of Namibe, for 30 days, in 12 reservoirs with a capacity of 9 L and 9 juveniles each, made of cooking oil containers cut in half (Fig. 3). Water from the laboratory tap was used. To reduce changes in the results due to external factors, all water parameters such as reservoir volume and water column height were kept as similar as possible. The physical-chemical parameters of the water, temperature, salinity and pH were measured daily in the morning. The fish were removed from the cement tank existing at the institution and then transferred to the laboratory, where they were kept in the reservoirs with supplemental aeration by means of a portable air compressor. After 1 day of rest and fasting, they were acclimated for eight days to accept conventional food and the presence of incandescent lamps 24/7.

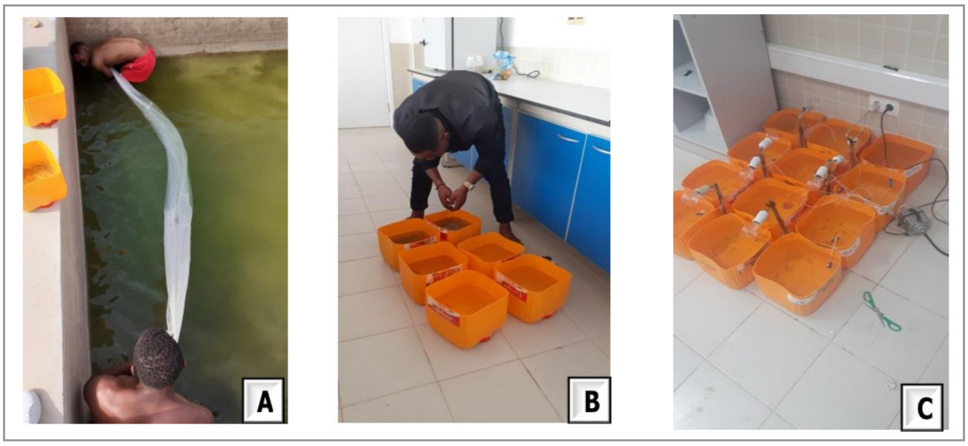

Figure 3: Fishing process (A); juvenile storage in the laboratory (B) and start of acclimatization (C). Source - personal archive.

Experimental Design

After the acclimatization period, 108 juveniles with an average weight of 1.37 ± 0.23g and length of 3.57 ± 0.25cm were subjected to 4 treatments: 5; 10; 15; and 20g of salt/L, with three replicates each, in a completely randomized design. The reservoirs were kept in a thermostatic bath system with 100w incandescent lamps as a heat source, which maintained the average water temperature at 28.4°C. To prevent fish from overflowing due to attraction to the light, the reservoirs were covered with mosquito netting.

Figure 4: Treatments 5; 10; 15; and 20g and their respective replicates, coupled to a portable air compressor and 100w incandescent bulbs. Source - Personal archive.

The salt was added gradually, with 5g of salt/L being distributed every 8 hours, 5g at a time, in this way the desired salinities were reached at the end of 24 hours. Non-iodized table salt was used. Measurements were made using a precision scale.

Figure 5: Precision scale used to measure salt. Source - Personal archive.

The fish were fed twice a day with extruded 35% PB feed, purchased from the Missombo larviculture center. It was previously ground in a homemade mortar (pestle) to adapt it to the mouth size and palatability of the juveniles.

The water used was taken from the laboratory tap, and was changed weekly. The fish from each treatment were placed in a box with the respective salt level, while the reservoirs were cleaned and later refilled with the same amount of water and salt.

At the end of the experimental period, biometry was performed to assess the growth and weight gain of the fish. Using a precision scale, Petri dish and ichthyometer (Fig. 6).

Figure 6: Biometrics of juveniles at the end of the experiment, with ichthyometer (1), precision scale (2) and Petri dish (3). Source- Personal archive.

Water Quality Analysis
Salinity, pH and temperature were measured once a day using a benchtop multiparameter probe and digital thermometer (Fig. 7), and expressed as weekly averages.

Figure 7: Benchtop multiparameter probe (A) and digital thermometer (B). Source- Personal archive.

Performance

1. With the data obtained, the following were calculated:
2. Survival: S = (nf / ni) × 100; where nf is the number of fish at the end of the experiment and ni is the number of fish at the beginning of the experiment.
3. Weight gain: GP = pf - pi; where pf is the final average weight and pi is the initial average weight.
4. Daily specific growth rate: TCE = [(ln pf – ln pi) / t] × 100; where pf and pi are the weights of the juveniles (g) at the end and beginning of the experiment, respectively, and t is the time of the experiment (days).
5. Apparent feed conversion: CAA = ac / gp; where ac is the total amount of food offered during the experimental period and gp is the weight gain of the fish.
6. Fulton condition factor: K = (pf / cf3) × 100; where pf and cf are the weight (mg) and length (mm) of the juveniles at the end of the experiment.

Statistical Analyses

The results were evaluated using Microsoft Office Excel 2010. All data are expressed as mean ± standard deviation.

Presentation and Discussion of Results

Results

Water Quality Parameters: No significant differences were observed in temperature, salinity and pH values between the different salinities during the evaluation periods. The temperature varied according to the position of the tanks in relation to the lamps and proximity to the wall, with the highest temperatures recorded in the 20g treatment.

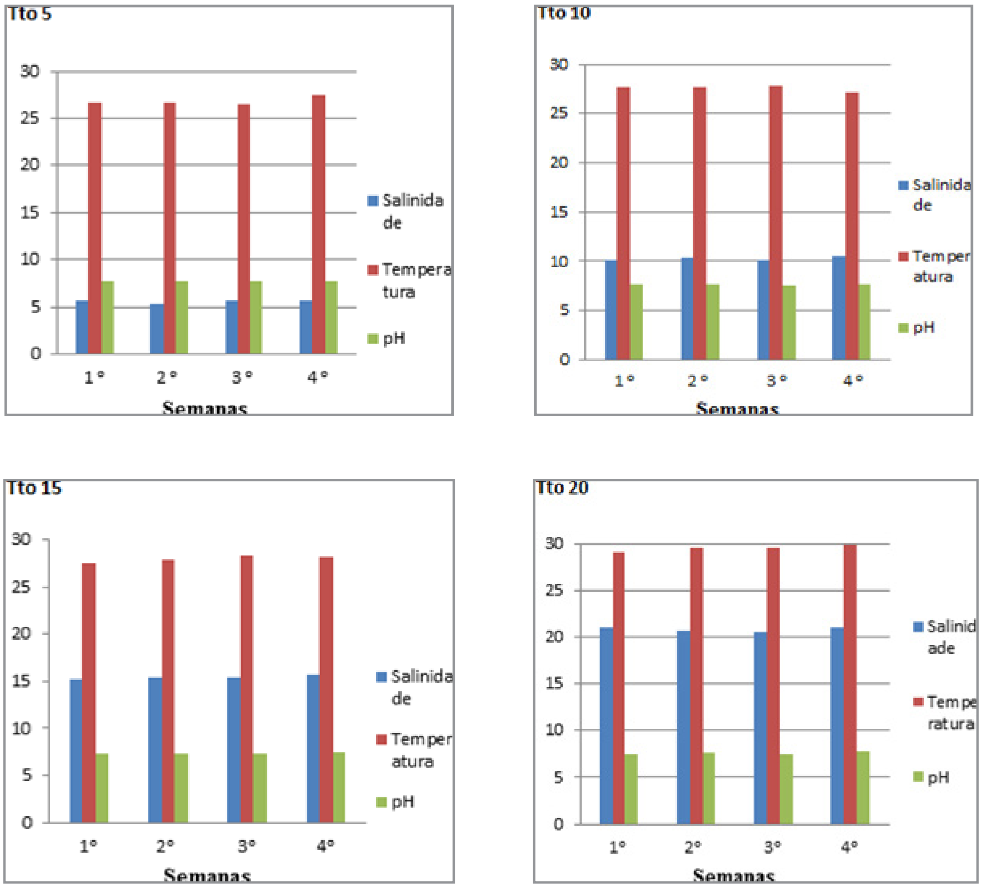

Figure 8: Variation of water physical quality parameters at different experimental salinities of juvenile Oreochromis angolenses during the experimental period. Source: Personal archive.

Performance: The data obtained after biometry at the end of the experimental period demon-strated that the performance of Oreochromis angolensis at different salinities for 30 days showed better performance in the 20g treatment (Table 2).

Table 2. Performance (Mean ± SD) of juvenile Oreochromis angolensis kept at salinities of 5; 10; 15 and 20g; for 30 days.

Salinity	5	10	15	20
Initial length (cm)	3,53±0,15	3,50±0,2	3,43±0,41	3,80±0,1
Final length (cm)	4,30±0,17	4,67±0,28	4,13±0,15	4,83±0,28
Initial weight (g)	1,23±0,15	1,50±0,20	1,15±0,13	1,60±0,07
Final weight (g)	1,37±0,19	2,03±0,24	1,42±0,13	2,27±0,40

Weight Gain: The results showed that the 20g salinity provided the greatest weight gain and that the 5g and 15g salinities were the ones that least favored weight gain (Fig. 9). However, none of the treatments led to weight loss.

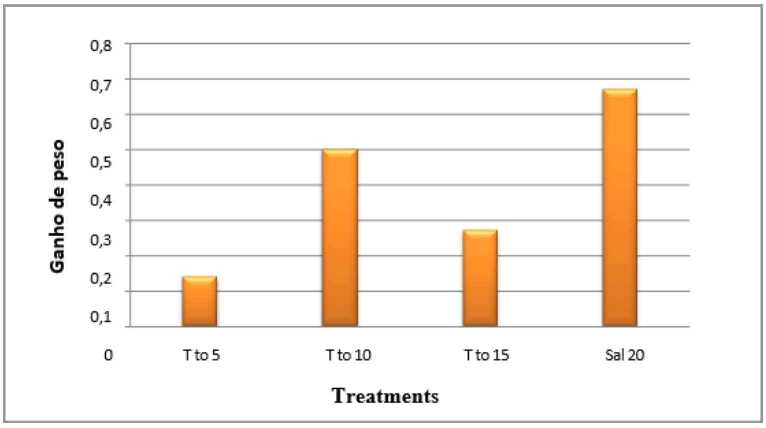

Figure 9: Variation in weight gain (WG) for salinities 5g, 10g, 15g and 20g after 30 days of cultivation.

Specific Growth: The growth rate (GRR) was quite different among the treatments. The best GRR was in the 20g treatment, followed by 10g. However, none of the salinities had a negative effect (Fig. 4).

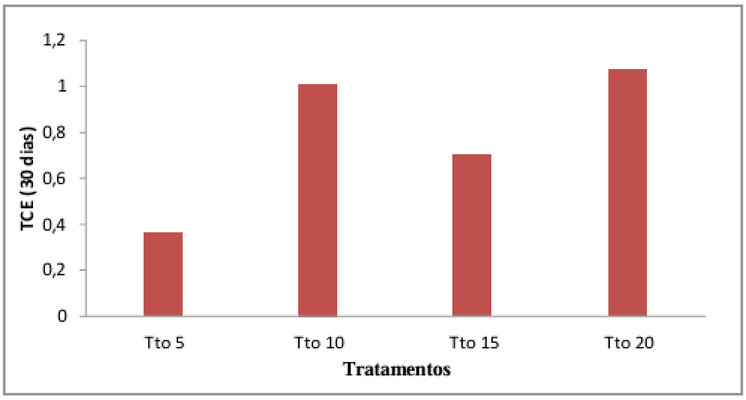

Figure 10: Specific growth rate curve (TCE) of Angolan tilapia juveniles after 30 days of exposure to salinities of 5; 10; 15 and 20g 3.1.4. Feed conversion (FCR)

The feed conversion of Oreocromis angolensis was not good in any of the treatments. However, the feed was better utilized in the 20g treatment, followed by the 10g treatment (Fig. 11).

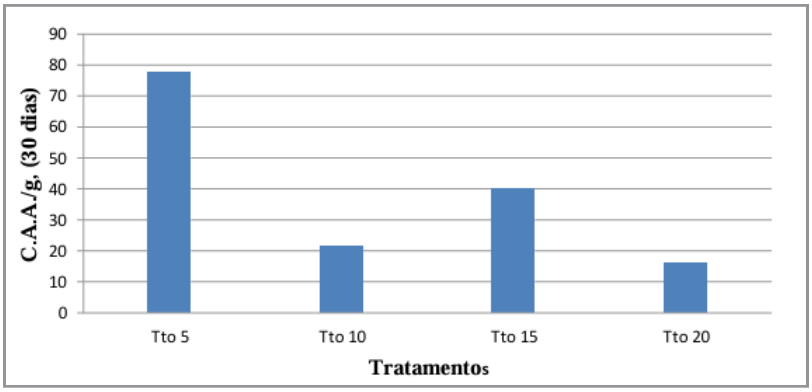

Figure 11: Average apparent feed conversion (AFC).

Condition factor (k) The condition factor values did not show many differences between the treatments. Only the salinity 5g remained with a value very different from the others.

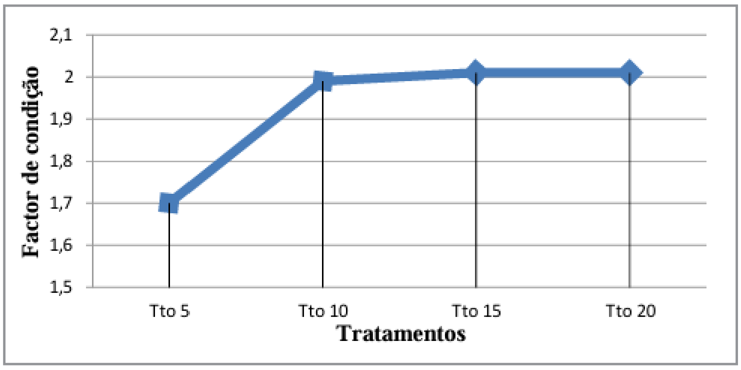

Figure 12: values of the condition factor of different salinities

Survival: Angolan tilapia proved to be completely resistant to gradual exposure to high salt concentrations, with 100% survival being recorded at salinities of 5g; 10; 15; and 90% at 20g, during the 30 days of exposure. It is worth noting that the drop to 90% at 20g salinity only occurred after the 25th day of the experimental period and from then on no cases of mortality were recorded.

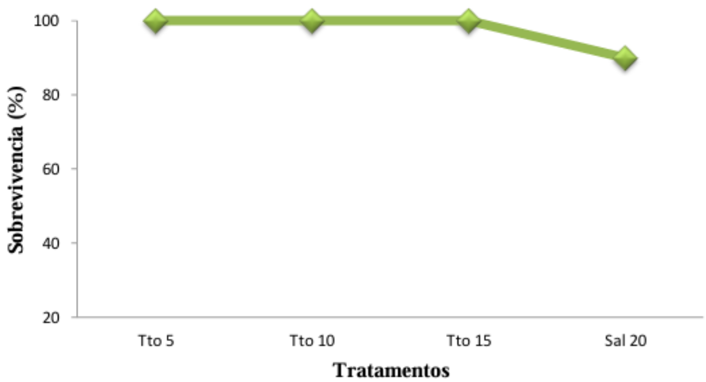

Figure 13: Survival rate (S) for salinities 5g, 10g, 15g and 20g after 30 days of cultivation.

Discussion

Physical Parameters of the Water: Small differences in temperature values were observed between treatments, however, the values of all treatments remained within ideal values for tilapia, demonstrating the potential of 100-watt incandescent lamps as an alternative source of heat, which maintained the average water temperature at 28.4°C [40]. The differences recorded in temperature were probably related to their position. It is known that the optimum temperature for tilapia cultivation is in the range of 28 - 30ºC, with no growth below 20ºC [43].

The pH values were the most homogeneous among the treatments. Water pH values can influence fish osmoregulation, but it is known that tilapia should be kept at a pH of 7.0–8.0, ideal [2643].

The salinity described in the literature for adequate growth of Nile tilapia is <10 ppt and 25 ppt for Oreochromis mossambicus (Mozambican tilapia), and the fish do not show any difference in survival at this salinity [2].

Weight Gain: Oreochromis angolensis obtained a significantly small weight gain at the different salinities, however it showed some tendency to increase weight at the salinity of 20g (Fig. 1). This does not corroborate, who found good tolerance of Nile tilapia to salinities of up to 15 g L, however, with a reduction in its growth, with the ideal salinity for growth being up to 08 g L. Better performance at a salinity of 20g may have resulted from the balance in the maintenance of osmoregulation, which justifies this being the optimal salinity, corroborating for red tilapia [3, 2]. Salinity can limit fish growth, as maintaining osmoregulation leads to greater energy expenditure and negative consequences on the biomass gain of the cultivated species [2]. The energy expenditure required for homeostasis at different salinities can vary from 20 to 50% of the energy expenditure consumed by the fish [41]. This justifies the low weight gain in the present study. A study conducted by Carraro, et al., Júnior, (2007) with tambaqui, which is also a ducicola species, demonstrated that its growth when fed with feed with high proportions of salt in its composition was superior, given the lower energy expenditure with osmoregulation, which increased the energy available for growth, which did not happen in the present study, since the weight gain in both treatments was low.

The increase in the salinity of the environment results in a higher concentration of chloride ions (Cl-) in the gill epithelium [29]. The internal salt concentration of duchicola fish changes when grown in salinized water, and, consequently, there is an increase in Na+, K+ -ATPase activity in the gill epithelium, which is marked in growth [42].

The performance of the biomass in culture with high salinity may be related to the genetic inheritance of the strain used, since fish from the cross with Oreochromis mossabicus withstand higher salinity [43].

Several species of fish can adapt to temporary unfavorable environmental conditions, as is the case of hybrid red tilapia (Silva, 2019). However, changes in salinity beyond the body concentration of different species, especially freshwater species, can cause them to lose or gain water or salts irregularly. To stabilize this adaptation mechanism and maintain the amount of salts in the organism at a certain gradient, fish expend energy [40]. This energy expenditure can lead to economic losses, as it is directly linked to the growth and weight gain of the animal, which is not desired in the production chain.
3.2.3. Specific growth

The specific growth of Oreocromis angolensis was not limited by the different salinities during the experiment. The lowest value recorded at salinity 5g may be related to the low temperature compared to the other treatments, caused by its position in relation to the lamps. These growth performance results of the present study, which did not interfere with salinities of up to 20g, confirm the rusticity characteristic of the species. A similar result was observed by Silva and Ruhan (2013) when evaluating the growth responses of juvenile tilapia of the species O. mossambicus raised at different salinity levels [44]. These results can be attributed to the ability of different species to adapt, giving them the possibility of production in brackish or marine environments.

There are also reports showing that some species of Tilapia have better growth performance in saline environments than in freshwater [22]. The ability of different species of tilapia to survive and grow in saline waters is quite varied and several factors can have an influence, including: genetic load, age, sex, temperature, climate and pH. Figueiredo et al (2021) also demonstrated that there were no significant differences in the growth of Oreochromis niloticus grown at different salinities in biofloc systems, salinities 02 and 08g, presenting growth of (242.21 ± 16.53 and 217.10 ± 16.27) respectively, in relation to treatments 15 (149.74 ± 22.91) and 22 g/L (122.46 ± 16.58). 3.2.4. Feed conversion (FCR)

Throughout the experimental period, feed conversion was not good for any of the salinities. However, the 20g salinity appears to be the one that allowed the best use of the feed, followed by the 10g salinity. However, this poor use cannot be attributed solely to the salt concentration; we believe that it was also influenced, among other factors, by the conditions of the tank where the fish came from (it is a cement tank without oxygenation and water renewal, it only has natural food from primary production); the low affinity of the fish with the commercial feed and the reduction in the buoyancy of the feed after it was ground.

The greater use of the feed in the 20 and 10g treatments demonstrates that energy expenditure was lower, allowing more energy to be available for growth [2]. The values found in this study were much higher than those found by other authors obtained an average of 0.66 CAA for red tilapia at the same salinities in 60 days found CAA of 1.23 and 2.02 [2, 45]. It is known that feed conversion for Nile tilapia should be between 1.4-1.8 and 1, [46].

Condition Factor (k): The values of the condition coefficient (b) for fish can assume values between 2.5 and 4.0 or 2.0 and 3.5, [47]. Generally, the values of b are around 3.0 (isometric growth). The relationship between weight and body length allows the calculation of a parameter that determines the degree of well-being of the fish, and is what is called the condition factor. Thus, nutrition, diseases and contaminants are highly interrelated with fish farming [48]. Insufficient nutritional intake can alter the condition factor. The condition factor allows comparisons between fish populations that are subjected to different conditions of climate, temperature, feeding, density, etc. [45]. The results of this study are within the expected parameters, demonstrating that growth remained proportional to weight.

Survival: The results of this study demonstrated a broad tolerance of the species Oreochromis angolensis to chronic exposure to different salinities, since only after 25 days of experimentation was a 10% drop in survival observed in treatment 20. From then on, no cases of mortality were recorded. Tolerance of freshwater species to high salinities may be related to the presence of the NKCC protein, as it promotes salt secretion after transfer to saline waters [43]. This result corroborates Silva (2019) in which the beginning of the decline in the survival of red tilapia was related to the smaller size of the organisms and higher salinities, caused by the imbalance in the hydromineral balance at salinities above 18 PSU, caused by the increase in the concentrations of Sodium (Na+) and Potassium (K+) in relation to the concentrations of ions in freshwater.

This hardiness to high salinity makes Angolan tilapia an excellent candidate for producers in regions with high susceptibility to saline variations. They can also become excellent candidates for integrated aquaculture systems with marine shrimp, since they explore different niches in the aquatic environment [26]. Tilapia is a filter-feeding species, so polyculture with other species may demonstrate some efficiency in controlling and reducing diseases. Tilapia tend to reduce viral load and contribute to maintaining water quality [49]. Tilapia also promote the movement and removal of a considerable part of organic matter from the system, control the growth of unwanted macroalgae, positively affect the survival rate of shrimp and prevent the multiplication of Vibrio spp [50]. Which produces pathogenic toxin in shrimp farms [51].

Conclusion

The results of this study demonstrated that the Angolan tilapia juveniles tested exhibited great euryhaline plasticity. Their growth, weight gain and feed conversion were enhanced in the 20g treatment, although it was at this salinity that they demonstrated a slight tendency towards mortality. The salt concentrations that least affected their performance were 20g and 10g. However, the results of the 5g and 15g treatments were not significantly different, leading us to consider that this tilapia is a candidate for cultivation in environments with salinities equal to or lower than 20g for a prolonged period. This confirms the hypothesis that the performance of Angolan tilapia is not limited by chronic exposure to different salinities.

Suggestions

As a continuation of the studies on the physiology of Angolan tilapia in stressful situations, we present the following suggestions:

- Evaluate the hematological profiles of Angolan tilapia in different salinities.
- Identify the isoosmotic point of Angolan Tilapia.
- Evaluate the performance of Angolan Tilapia at different temperatures; pH and O2.
- Identify the acute tolerance of Angolan Tilapia to different salinities.

Acknowledgements

Final course work prepared for the degree of Bachelor in Aquaculture in the Aquaculture Department.

I dedicate this work to my shields.

“My father and my mother” Acknowledgments First of all, to God who orchestrated this entire process, not only during these years as a university student, but at all times. He is the greatest Master there is.

To my parents my shields “Manuel Tchiloya and Ana Tchimuma”; to my brothers “Adriano; Lucia; Germana; Quim; Jayne; Titus; Nelson; Cleide; and Emanuel” who are always present, never let me get discouraged, thank you very much. Only you give me courage and strength to continue fighting and trying to win in life.

Professor Eunice Cassoma, for being tireless in providing guidance and technical-scientific support.

To Professor Edson Mangueira, as co-supervisor for the guidance, hard and tiring work throughout the entire experience period.

I thank my girlfriend “Blandina” for having spent such difficult days with me in the last year, she helped a lot with my emotional health; To my colleagues, Abdalay Tuma, Adelino Leitão, Fernando Garcias; Manuel Ponte and Rafael, who transcended into brotherhood, always gave the necessary strength.

I thank you all from the bottom of my heart.

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Appendices

Appendix I (Experimental Protocol)

Experimental Protocol

- Reservoirs: Yellow 20L cooking oil containers, cut in half
- Label the reservoirs
- Organize them in 3 columns in the laboratory
- Oxygenation system: Portable air diffuser coupled to hospital serum systems
- Collect the sample: fish the Angolan Tilapia juveniles in the institution's fishery with a mosquito net. Biometry: Using ichthyometer and scale After 2 days of accommodating the sample in the laboratory
- Acclimatization: Rest the fish in the system for 5 days, giving oxygen feed and heating the water.
- Addition of salt: 5g/L; 10g/L; 15g/L; 20g/L; where 5g x 9L=25g/Rev. At intervals of 6 hours
- Feed: extruded 35PB will be given twice a day, coming from the Missombo Larviculture Center.
- Water renewal: Every 7 days (the new water will be previously salinized with 50g; 100g; 150g; 200g; respectively)
- Analysis of water quality parameters: will be monitored once a day, namely, Salinity; Temperature; Oxygen; PH; (with multiparameter probe)
- Performance
- It will be monitored according to the specificity of each index, being Survival (every 24h); CR (Daily); GP (at the end); TCE (at the end);

Experimental Design

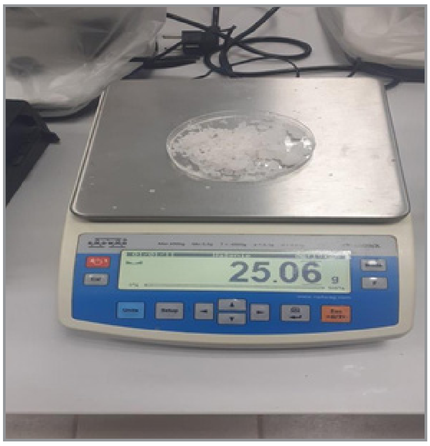
- The experiment will be carried out at the UNIBE Fisheries Faculty; It will be a 30-day trial;
- The fish will be removed from the reservoir that the institution has and will be distributed in 12 reservoirs with a capacity of 9L each, with supplementary aeration by means of an air compressor.

Acclimatization will take place one week before the start of the experiment. The following treatments will be submitted: 0.0; 5; 10; 15 and 20.0 g of salt/L. The salt will be added gradually, distributed every 6 hours, so that the desired salinity levels are reached at the end of 24 hours. The water will be heated with 100w incandescent lamps.

Appendix II (Process of Fishing Juveniles in the Fisheries Sciences College Fisheries Nursing Facility)

Appendix III (Time When Juveniles Arrive at the Laboratory)

Appendix IV (Preparation of Salt for Gradual Increase in Reservoirs)



Annex I (Letter of Request for Authorization to use Some of the Institution's Resources)

UNINBE
FACULDADE DE CIÊNCIAS DAS PESCAS

To
His Excellency, Coordinator of the Management Committee of the Faculty of Fisheries Sciences

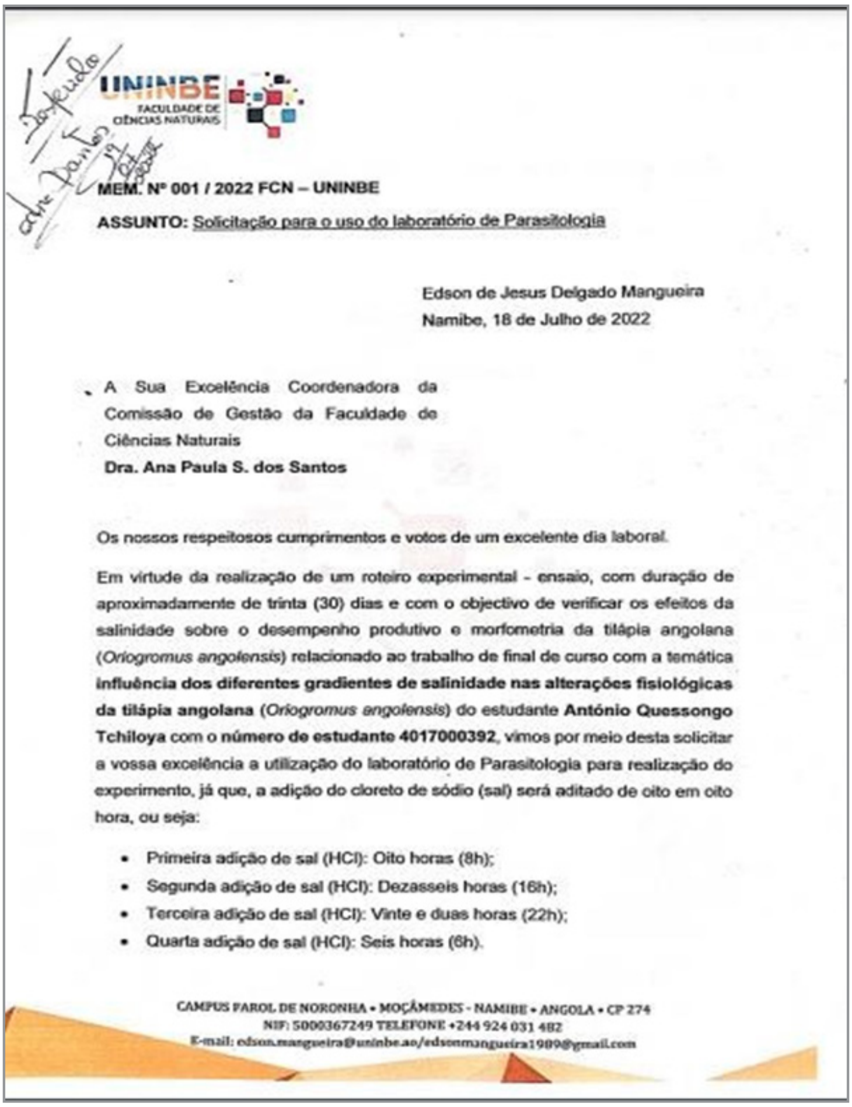
Subject: Request for authorization to use some of the institution's resources

I, António Quessongo Chiloia, a student at the Faculty of Natural Sciences, enrolled in the 5th year of the degree course in Aquaculture, aspiring to the degree of Bachelor. I need to apply the experimental component of the final course work.

I hereby request the Coordinator of the Management Committee of the Faculty of Fisheries Sciences to kindly authorize the use of some of the institution's resources, such as space (laboratory); precision scales; 4 oxygen pumps; multiparameter probe; water and electricity.

Hoping that my request will be duly addressed, I reiterate my best wishes and consideration.

Annex II (Positive Approval of the Application for Use of the Pasitology Laboratory)



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MEM. Nº 001 / 2022 FCN – UNINBE

ASSUNTO: Solicitação para o uso do laboratório de Parasitologia

Edson de Jesus Delgado Mangueira
Namibe, 18 de Julho de 2022

A Sua Excelência Coordenadora da Comissão de Gestão da Faculdade de Ciências Naturais
Dra. Ana Paula S. dos Santos

Os nossos respeitosos cumprimentos e votos de um excelente dia laboral.

Em virtude da realização de um roteiro experimental - ensaio, com duração de aproximadamente de trinta (30) dias e com o objectivo de verificar os efeitos da salinidade sobre o desempenho produtivo e morfometria da tilápia angolana (*Oriogromus angolensis*) relacionado ao trabalho de final de curso com a temática **influência dos diferentes gradientes de salinidade nas alterações fisiológicas da tilápia angolana** (*Oriogromus angolensis*) do estudante **António Quessongo Tchiloya** com o **número de estudante 4017000392**, vimos por meio desta solicitar a vossa excelência a utilização do laboratório de Parasitologia para realização do experimento, já que, a adição do cloreto de sódio (sal) será aditado de oito em oito hora, ou seja:

- Primeira adição de sal (HCl): Oito horas (8h);
- Segunda adição de sal (HCl): Dezasseis horas (16h);
- Terceira adição de sal (HCl): Vinte e duas horas (22h);
- Quarta adição de sal (HCl): Seis horas (6h).

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