

Tolerance of fry of three tilapia varieties exposed to low temperatures

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Abstract

Tilapia are known for their tolerance to wide ranges of water quality, diets and farming systems. However, their performance is affected in countries with harsh winters, with almost zero growth, high mortality and consequently low economic efficiency during this period. In view of the above, this study was developed with the aim of evaluating the cold tolerance of fry from three contemporary groups of three varieties of tilapia, two commercial varieties and one local variety (MGTUP), developed by the Federal University of Lavras. A total of 3,240 animals were used, distributed according to age in three tanks. The experimental design was a completely randomized factorial arrangement, consisting of nine treatments (3 contemporary groups × 3 genetic groups) with three repetitions per treatment. During the experimental period, daily evaluations were carried out to count mortality, measure water temperature and dissolved oxygen levels. The fish survival time data (in days) was submitted to multivariate Cox regression to evaluate the effects of genetic groups and contemporaries, and their interactions on survival time. Older fry are more resistant to cold stress. Notably, the MGTUP variety is cold tolerant, with survival rates close to 90 %, and is superior in all age groups. These findings suggest that the use of tilapia varieties adapted to the climate of the region where they will be grown may significantly mitigate losses in aquaculture, especially in regions prone to cold.

Keywords: Cold Resistance. Fingerlings. Genetic Groups. *Oreochromis niloticus*.

Introduction

In recent years, Brazilian aquaculture has grown substantially in response to the growing demand for healthy food and the depletion of natural fish stocks, prompting the use of effective methods for selecting fillet yields and management to increase the survival rates of farmed animals (REZENDE *et al.*, 2023; BEZERRA *et al.*, 2008). Among various species, tilapia (*Oreochromis niloticus*) has emerged as the most cultivated, with production exceeding 4.4 million tons and prospects for further expansion (FAO, 2022; PEIXE BR, 2023). Recognized for its adaptability, tilapia achieves optimal production under specific conditions including proper nutrition, effective production systems, health management, and crucially, the physicochemical properties of the water (VERAS

et al., 2013). The success of tilapia farming significantly hinges on water quality and feed management (HASHIM, 2005).

Temperature is a key abiotic factor influencing fish metabolism, affecting vital life processes such as growth, swimming ability, reproductive performance, and overall welfare (JIAN *et al.*, 2003; MOURA *et al.*, 2007). As ectothermic animals, fish are highly susceptible to thermal fluctuations, which can impact their physiological and metabolic functions (WALBERG, 2011; PANG *et al.*, 2013). For tilapia production, the optimal temperature range is 18-32°C, with peak performance near 27°C. Temperatures nearing the upper limit can promote pathogen proliferation, whereas lower temperatures suppress the immune system,

increasing susceptibility to infections and production losses (KUBITZA, 2000).

Cold temperatures adversely affect metabolic rates, physiological conditions, and hematological profiles in fish, leading to stress-induced immune system compromises and high mortality rates (SIGNOR, 2007). This phenomenon has sparked numerous studies exploring temperature effects on growth and survival in various fish species (OLIVEIRA *et al.*, 2013; HE *et al.*, 2014; NYTRØ *et al.*, 2014; FERNANDES *et al.*, 2018). Specifically, tilapia fry and juveniles exhibit decreased survival rates during colder periods due to reduced metabolism and low cold tolerance (CHARO-KARISA *et al.*, 2004). In light of these considerations and the critical role of temperature in fish farming, this study was developed with the objective of evaluating the cold tolerance of three tilapia varieties in their early production phase.

Material and methods

His study was carried out at the Fish Culture Station of the Department of Animal Science at the Federal University of Lavras. Located in Lavras-MG, the area has an average annual temperature of 19.7°C. The research spanned 110 days, from May 1, 2017, to August 19, 2017. A total of 3240 fish, initially allocated for performance testing, were included in the study. The winter period offered an opportunity to collect additional data such as mortality rates. All experimental procedures complied with ethical standards, adhering to protocol CEUA/UFLA 016/13 by the university's ethics committee on animal use.

There were used 1080 fish of the chitralada strain from the Geneforte Farm, named "Commercial Variety 1" (CV1), 1080 fish derived from the GIFT program carried out in the Philippines and named "Commercial Variety 2" (CV2) and 1080 fish of the adapted

variety developed in the fish farming sector of the Federal University of Lavras (MGTUP). The animals used had an average weight of 9.27 ± 6.56 g. The fish were categorized into three contemporary groups (CG) based on age: Group one (G1), born between December 1 and 15, 2016; Group two (G2), born between December 16 and 31, 2016; and Group three (G3), born between January 1 and 15, 2017.

The experiment utilized 27 ponds, each with a capacity of 1.73 m³ and housing 120 fish, distributed across masonry tanks with a total volume of 60 m³. Each contemporary and genetic group was equally represented across all tanks. Feeding was conducted twice daily (at 8:00 and 14:00) with commercial feed (36 % protein), administered until apparent satiety. Water temperature, dissolved oxygen, and pH were measured twice daily (at 9:00 and 15:00) using a YSI Pro 20 portable pHmeter and oximeter. A 15-day acclimatization period was followed by daily mortality recordings.

The experimental design was a completely randomized factorial arrangement, consisting of nine treatments (3 contemporary groups × 3 genetic groups) with three replicates per treatment. Survival time data of the fish (in days) underwent multivariate Cox regression analysis (COX, 1972) to evaluate the effects of genetic and contemporary groups, and their interaction, on survival time. This was analyzed using the "survival" library in R statistical software version 3.4.2. Significant factors from the Cox regression analysis were further examined with Kaplan-Meier survival curves (EFRON, 1988). Lifespan estimates derived from these curves were compared using the log-rank test, fitted to a chi-squared distribution. Due to multiple comparisons, the α value was adjusted using the Bonferroni method. The physico-chemical parameters of the water were tested using the Tukey test ($p < 0.05$).

Results

The water quality parameters, including pH and dissolved oxygen levels, remained consistent across all tanks, with no significant variations observed. Similarly, average water temperatures during the experimental period were stable (Figure 1). However, the region experienced notable rapid temperature drops during the winter, making temperature fluctuations wider. The temperature range between the tanks, as well as the temperature collected within each tank, were not statistically significant.

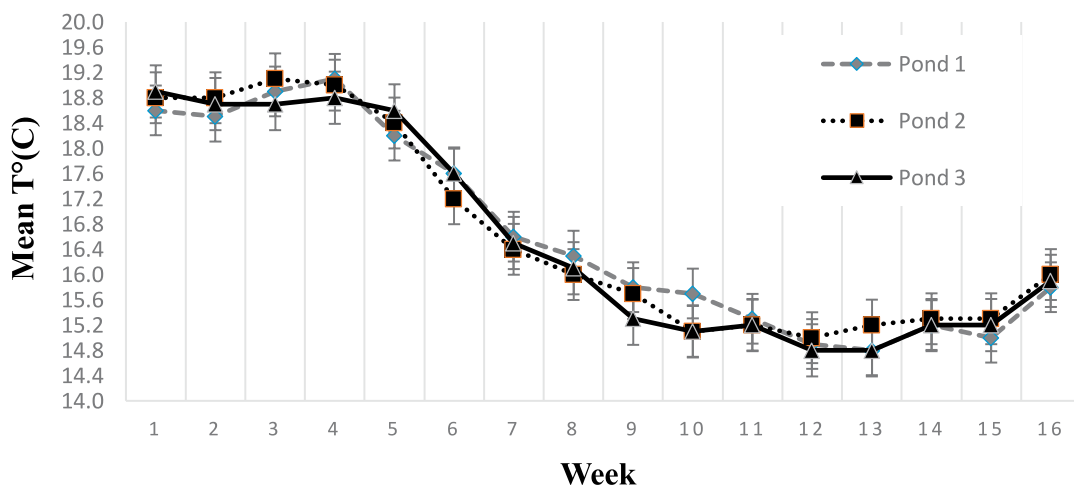
A significant interaction was found between tilapia variety and contemporary group in the multivariate Cox regression analysis. This indicates a clear association between mortality rates and both genetic and age groups. The MGTUP variety displayed the lowest mortality rates across all age groups compared to the other commercial varieties, with a p-value less than 0.016. Notably, older fish within this variety demonstrated greater cold tolerance, with a significant difference ($p < 0.008$) noted in their survival rates (Table 1).

The older group of tilapia was more tolerant to cold, with a significant drop in survival rates observed only after 90 days of exposure to

high temperature ranges, coinciding with water temperatures close to 14°C. Commercial Variety 1 (CV1) showed a late start to the decline in survival, but once started, the survival rate fell sharply, especially in the first two generations, reaching 25 % (Figure 2. B). In contrast, Commercial Variety 2 (CV2) showed a drop in survival rate shortly after the start of the experiment, but the survival rate remained above 85 % (Figure 2. C). The first contemporary group of the MGTUP variety showed remarkably high survival rates, maintaining a population survival of close to 90 %. However, it was clear that the youngest fish of this variety had a lower tolerance to cold (Figure 2.A). Despite the rapid drop in the survival rate, CV2 performed better than CV1, since the latter showed a sharper reduction in the survival rate and when deaths began, the survival rate reached close to 20 % in the youngest groups.

In general, the MGTUP variety outperformed the others during the experimental period, maintaining more than 60 % survival ($p < 0.008$). Its mortality curve was comparatively lower than that of the commercial varieties. The varieties available on the market, selected for daily weight gain, showed low tolerance to low temperatures, with survival rates of less than 50 % in genetic group two and 30 % in genetic group one.

Figure 1. Average water temperature (T°) in degrees Celsius (C) of the ponds during the experimental period.



Source: Elaborated by the authors.

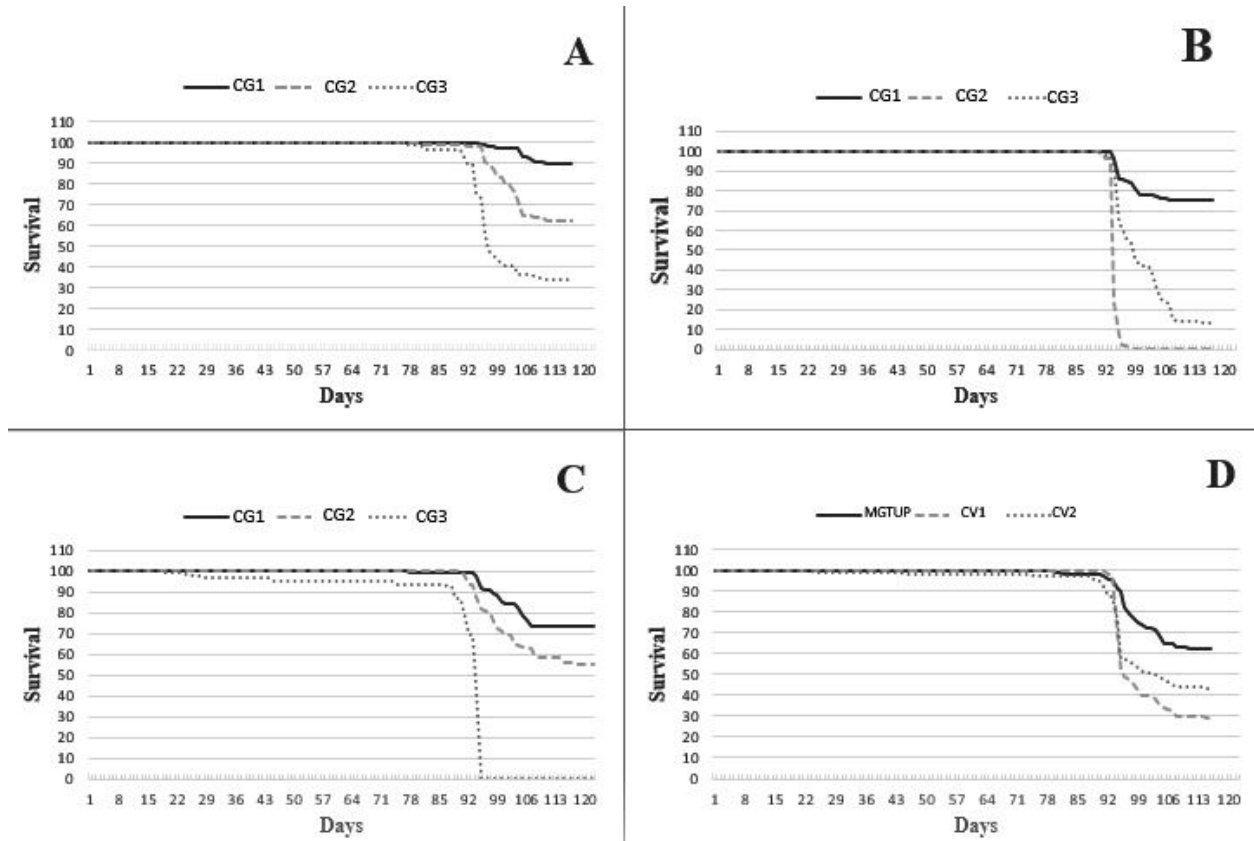
Table 1. Estimate (confidence interval) of life expectancy (days) of fry from three contemporary groups of three varieties of tilapia reared at low temperatures (Figure 1).

	Genetics groups		
	MGTUP	CV1	CV2
CG1	89,00 Aa (85,74-92,26)*	83,03 Ab (80,86-85,20)	85,23 Ab (83,92-86,54)
CG2	86,82 Aa (84,91-88,73)	79,99 Bb (79,55-80,43)	81,96 Bc (81,04-82,88)
CG3	80,49 Ba (79,18-81,80)	83,95 Aa (83,03-84,87)	77,83 Bb (75,38-80,28)

Legend: CG1: Contemporary group 1, CG2: contemporary group 2 e CG3: contemporary group 3. Estimates followed by capital letters in the same column are significantly different according to the log-rank test ($p < 0.008$). Estimates followed by lowercase letters in the same row are significantly different according to the log-rank test ($p < 0.016$). *(lower bound - upper bound).

Source: Elaborated by the authors.

Figure 2. Survival rate of different tilapia varieties during winter. A - survival as a function of time for three contemporary groups (CG) of the MGTUP variety. B - survival as a function of time for three contemporary groups of the commercial variety one. C - survival as a function of time for three contemporary groups of commercial variety two. D - survival as a function of time for the genetic groups evaluated.



Source: Elaborated by the authors.

Discussion

The tank water temperature significantly impacts the physiological and behavioral condition of fish. To maintain homeostasis, aquatic animals must regulate their body temperature in accordance with their external environment (LEMBI, 2001). During the adaptation to temperature changes, three phases are observed: physiological and behavioral adjustments, alterations in the corticotropin-interrenal axis and osmoregulation, and finally, a phase characterized by reduced resistance to fungi and bacteria (MAZEAUD *et al.*, 1977; SILVA *et al.*, 2021). Physiologically unstable animals often exhibit impaired swimming abilities, fungal growth on fins and body, and increased mortality (SARDELLA *et al.*, 2007; FERNANDES *et al.*, 2018). These symptoms, including erratic swimming and fungal infections, were also observed in our study, often preceding rapid mortality.

Notably, when temperatures approached 14° C, there was a significant increase in mortality, suggesting a critical threshold for tilapia survival. The minimum lethal temperature for Nile tilapia is reported to be around 7.4° C (BEHRENDIS *et al.*, 1990). However, different studies have reported varying lethal temperature ranges for tilapia, likely due to differences in the genetic makeup of the strains studied (SIFA *et al.*, 2002; ATWOOD *et al.*, 2003; CHAROKARISA *et al.*, 2005; SILVA *et al.*, 2021).

This study also highlighted the maternal effect on cold tolerance. Offspring from females adapted to colder environments demonstrated higher survival rates (NITZAN *et al.*, 2016). Although our experiment did not specifically estimate maternal effects or perform complete diallel crossing, the lower mortality observed in the MGTUP variety, bred in the same environmental conditions as the experiment, supports this observation. Behrends *et al.* (1996) suggest that the adaptive capacity of each genetic and contemporary group is crucial,

with older animals typically showing greater resilience to climatic changes. Consistently, older fish in all varieties in our study demonstrated better tolerance to temperature drops.

Breeding programs take into account maternal effects and the age or stage of development of the animals, since the survival rate is directly related to the age of the fish (BEZERRA *et al.*, 2008). These factors are crucial as the amount and composition of calf and mitochondrial DNA can directly influence the progeny's production characteristics (BROWN *et al.*, 2006; FERNANDES *et al.*, 2018). Common behavior among tropical fish in winter includes reduced food intake and appetite, leading to physiological stress and increased susceptibility to diseases. However, pre-winter feeding with high lipid content can provide necessary reserves for the winter period, even in the absence of feeding (LEMLY, 1996; BENDIKSEN, 2003; BIRO *et al.*, 2004; ECKMANN, 2004; IBARZ *et al.*, 2005). In this study, food consumption and feed conversion were not taken into account, since priority was given to the cold tolerance of the varieties, with the aim of leaving the winter with as many animals as possible, making it possible to obtain a greater biomass of fish at the end of the cycle. Further studies taking into account not only survival, but also weight gain and apparent feed conversion could corroborate the perception of the need to use fish adapted to the climate in order to reach the end of the cycle without major losses caused by climatic events.

Conclusion

Several factors influence the cold tolerance of tilapias. The MGTUP variety, adapted to the local environmental conditions of the study, showed greater tolerance to temperatures between 14 and 19° C. Additionally, animals older than six months exhibited higher survival rates, underscoring the importance of genetic adaptation and age in cold tolerance.

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