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Effect of Salinity on the Growth, Survival, Body Composition and Fatty Acid Profile of Juvenile Nile Tilapia, *Oreochromis niloticus*

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Abstract. In attempts to improve aquaculture performances, the examination of environmental factors affecting the healthy growth and nutritional profile of fish have been of great interest over the years. This study evaluated the effects of salinity on the growth, survival, proximate and fatty acid compositions of juvenile Nile tilapia (*Oreochromis niloticus*, 5.93 ± 0.51 g). Using commercial diets, salinity treatments of 9, 18 and 27 ppt (Experiment I) and 5, 10 and 15 ppt (Experiment II) were applied, with 0 ppt (freshwater) serving as the control for both experiments. In both of two rearing experiments, the highest weight gain was obtained in the control group (0 ppt) followed by 5 ppt group in Experiment II, while the higher salinity groups exhibited lower weight gains. Regarding final survival, a mass mortality was observed in the highest salinity group (27 ppt), whereas other groups achieved high survival rates of 83.0-93.3% in Experiment I and 97.0-100.0% in Experient II. The nutritional profile results from both trials strongly indicate an inverse relationship between salinity and the protein and lipid contents of the fish body. Both experiments demonstrated similar trends, showing that the fish reared in 0 ppt exhibited higher contents of highly unsaturated fatty acids, which are crucial dietary components for both of finfish and humans. Overall, results suggest that a salinity range of 0 ppt to 5 ppt is optimum for achieving good growth, survival, high nutritional quality, and fair palatability in juvenile Nile tilapia.

Key words: Nile tilapia, *Oreochromis niloticus*, Juvenile, Salinity, body composition

Introduction

Nile tilapia *Oreochromis niloticus* (Linnaeus, 1758) is widely cultured commercially in freshwater environments around the world. Despite its good adaptation capacity to relatively wide range of salinity, it is considered less tolerant than the other tilapia species such as *O. mossambicus*[1]. Many countries experience freshwater shortages due to sea level rise and desertification from climate change, as well as competition with agriculture activities, and other industries, prompting the development of tilapia aquaculture in brackish and seawater zones[2]. Salinity is one of the most extensively studied environmental parameters in aquaculture, as it significantly influences the development, growth, survival, self-defence responses, metabolic and osmoregulation processes of aquatic animals[1]. Assessing the correlation between salinity and the body composition of fish is essential in

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providing information needed by fish feed scientists and nutritionists in dietary information[1, 3].

Evaluating fish quality is crucial in aquaculture activities because it greatly influences market value and determines the carcass quality of fish. According to Nakagawa et al. (2007), the criteria used to evaluate cultured fish include meat quality, durability of freshness, colour, metabolism, disease resistance, stress response and growth. However, factors that dominate carcass quality include body composition, such as lipid content and fatty acid composition, which are known to significantly influence the taste and texture of fish flesh[3]. Fats enhance the taste and acceptability of foods, while lipid components generally determine texture, flavour, and aroma of foods[4, 5]. Additionally, determining the protein composition of fish is crucial in evaluating the physical attributes of the carcass such as the hardness and texture of meat and its maintenance of freshness[3, 5].

The effects of salinity on the growth and production of Nile tilapia have been well studied over the years. However, understanding the effects of salinity on the body composition and production of Nile tilapia when they are in young stage have not been well studied to date. Therefore, the purpose of the present study was to document the effects of salinity on the fish growth, survival, proximate composition and fatty acid profiles of juvenile Nile tilapia.

Materials and Methods

Fish Sampling and Experimental Design

For the rearing experiments, juveniles of Nile tilapia *Oreochromis niloticus* were sampled from the wild stock in a freshwater stream tributary to the Nikko River, Aichi Prefecture, Japan and then transferred to the wet laboratory of Mie University in Mie Prefecture where a week's acclimation of fish was conducted. This study involved two separate feeding trials with different salinity treatments. The first experiment (Experiment I) was undertaken for 8 weeks with 120 healthy test fish (average body weight 6.45 ± 0.27 g) without any external malformation or damages. The fish were randomly stocked into 12 rearing tanks (45 L capacity) at an initial stocking density of 10 fish per tank. Salinity treatments for rearing water included 0, 9, 18 and 27 ppt, with three replicates per treatment. The second experiment (Experiment II) narrowed the salinity treatments to 0, 5, 10 and 15 ppt, with three replicates per treatment, similar to Experiment I. The stocking density in Experiment II was 12 fish per tank, with 144 fish (average body weight of 5.42 ± 0.34 g). In both experiments, the 0 ppt (freshwater) group served as the control. Due to time constraints and to avoid the influence of seasonal temperature declines, Experiment II lasted for 6 weeks.

Experimental Setup

Both rearing trials involved similar experimental setups. Salinity (ppt: parts per thousand, ‰) of each rearing water was monitored using a salinity refractometer (Atago, Tanaka Sanjiro, Japan), and the fish were gradually acclimated by increasing salinity by 3 ppt every two days until the final salinity was reached. Test fish were fed commercial formula diets (Otohime EP-1 and 2, 1.3-2.0 mm in diameter, approx. 48% dietary crude protein; Nisshin Marubeni Feed, Japan) ad libitum at 3% of body weight (dry basis) twice a day. The fish were subjected to a photoperiod of 12 h light and 12 h darkness using fluorescent lights. Growth measurements were conducted fortnightly after anesthetising the fish with 100 ppm MS222 (Tricaine Methanesulfonate, Sigma-Aldrich, Japan). Survival was monitored daily every morning throughout the experimental period. Half of the rearing water was replaced every three days, and uneaten food and faeces were manually siphoned out daily to maintain good water quality.

Water quality parameters were monitored weekly. Dissolved oxygen was measured using a DO-55X9 DO meter (Lutron Electronics, Taiwan), and pH was measured with a Docu-pH meter (Sartorius, Germany). Other parameters such as the contents of Ammonia (NH_3), Nitrate (NO_3), Nitrite (NO_2) and Phosphate (PO_4) were measured using the DR/850 Colorimeter (Hach, Canada) with their respective reagents. Water quality parameters were maintained throughout the experimental period, with ambient room temperatures of 28 ± 2°C and 22 ± 3°C for Experiments I and II, respectively.

Body Composition Analysis

Final body weights were measured individually at the end of the experiment. After measurement, due to their insufficient body size, test fish from triplicated tanks were pooled into a single sample for each treatment group, sacrificed using MS222 and eviscerated. The sampled flesh was ground using a kitchen blender and analysed for body composition according to the standard proximate analysis procedure[6,7]. Fatty acid composition was determined in the laboratory using gas chromatography[7,8].

Statistical Analysis

One-way analysis of variance (ANOVA) was used to determine differences between treatment means, which

were deemed significant at $P < 0.05$. The analyses and interpretations of data were examined through the statistical package of SPSS 16.0.

Results and Discussion

Maintaining optimal water quality is crucial in aquaculture as it supports the healthy growth and normal development of aquatic species. The water quality data obtained from the two rearing experiments, excluding the highest salinity group of 27 ppt in Experiment I (where mass mortalities exceeding 60-90% of the test fish in each tank occurred), were as follows: dissolved oxygen: 5.15–5.62 ppm, water temperature: 20–30ºC, ammonia: 0.05–0.26 mg L^{-1}, nitrite: 0.36–0.38 mg L^{-1}, nitrate: 13.01–18.78 mg L^{-1} and pH: 8.15–8.58. These values were maintained within the optimal or acceptable ranges for rearing Nile tilapia as previously reported[9], indicating that water quality parameters were not limiting factors for the growth of the test fish during the experiment.

In the aquaculture industry, producing healthy, well-grown fish with high survival rates is crucial for success. Table 1 illustrates the growth and survival of fish during the test periods, while Fig. 1 shows the final body weights (g) of test fish in each salinity group from two rearing experiments. In Experiment I, juvenile fish with average initial weights of 6.07-6.69 g grew to average weights of 19.63-28.04 g after 8 weeks of feeding. The average weight gain (%) was highest in the control group at 336.2%, while the other groups showed significantly lower rates of 222.3-228.6% compared to the 0 ppt control group. In Experiment II, the fish grew from average initial weights of 5.49-5.59 g to 11.22-16.99 g after 6 weeks of feeding. The growth rates in the higher salinity groups of 10 ppt (103.1%) and 15 ppt (122.0%) were significantly lower (P<0.05) compared to those in the control (204.1%) and 5 ppt (197.1%), with no statistically significant difference between the control and 5 ppt groups, as shown in Fig. 1.

Regarding survival rates, in Experiment I, continuous mortality was observed in each of the triplicated treatments during the rearing period. Consequently, the highest salinity group of 27 ppt exhibited the lowest average survival rate of 23.0% by the end of the 8-week rearing period. However, other groups in Experiment I achieved higher average survival rates of 83.0-93.3%. In Experiment II, due to a slight lower rearing temperature

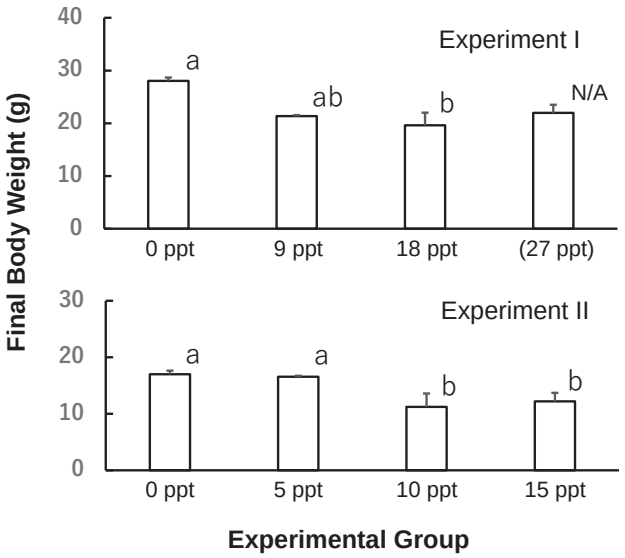

Fig. 1. Average final body weights (g) of juvenile Nile tilapia reared in different salinity treatments in Experiment I and II. Columns with different letters are significantly different ($P < 0.05$). Bars denote mean ± SD. The data for the 27 ppt group in Experiment I was obtained from a few surviving fish following a mass mortality event. Therefore, the final body weight for the 27 ppt group was considered not available (N/A) for the comparison with other experimental groups.

Table 1. Growth and survival data of juvenile Nile tilapia in different salinity treatments

Experiment I	**Control (0 ppt)**	**9 ppt**	**18 ppt**	**27 ppt**
Initial body weight (g)	6.43 ± 0.13[a]	6.63 ± 0.46[a]	6.07 ± 0.95[a]	6.69 ± 0.15[a]
Final body weight (g)	28.04 ± 0.63[a]	21.37 ± 0.17[ab]	19.63 ± 2.38[b]	21.98 ± 1.52[b]
Average weight gain (%)	336.2	222.3	223.9	228.6
Final survival rate (%)	83.3 ± 2.1[a]	93.3 ± 0.6[a]	83.0 ± 1.2[a]	23.0 ± 1.5[b]
Experiment II	**Control (0 ppt)**	**5 ppt**	**10 ppt**	**15 ppt**
Initial body weight (g)	5.59 ± 0.09[a]	5.57 ± 0.07[a]	5.53 ± 0.04[a]	5.49 ± 0.03[a]
Final body weight (g)	16.99 ± 1.91[a]	16.56 ± 3.41[a]	11.22 ± 0.60[b]	12.19 ± 2.73[b]
Average weight gain (%)	204.1	197.1	103.1	122.0
Final survival rate (%)	100.0 ± 0.0[a]	100.0 ± 0.0[a]	100.0 ± 0.0[a]	97.0 ± 0.0[a]

Values are represented as mean obtained from duplicate analyses data or mean ± SD of triplicate groups.
Different superscript letters in a row indicate significantly different at P<0.05.
The average weight gains for each salinity group were calculated based on the combined data from all replicated treatments.

(28 ± 2°C and 22 ± 3°C for Experiments I and II, respectively) and a two-week shorter rearing period, all groups showed high survival rates with 100% survival in the 0, 5 and 10 ppt groups and 97.0% in the 15 ppt group.

The study also investigated the body compositions of moisture, crude protein, crude lipid and ash content of juvenile Nile tilapia. Determining these essential components is important as they affect sensory attributes of a fish, such as freshness, taste and appearance[3]. Table 2 shows the proximate compositions of test fish in both experiments. The results from Experiment I reveal an inverse relationship between salinity and the protein and lipid contents in the test fish, indicating that an increase in salinity cause a decrease in those components. Although the protein contents of the fish carcass ranged from 14.4% to 16.1% in the saline water groups and 16.5% in the control group (0 ppt), the protein contents obtained in Experiment II (salinity 0 to 15 ppt) ranged from 17.5% to 18.4%, which was a smaller difference compared to the results in Experiment I. Those findings are consistent with a previous study with different cultured fish sizes, which reported protein contents ranging from 17.9% in brackish water to 22.2% in the freshwater[10].

Regarding crude lipid contents, some studies have reported that freshwater fish tend to have lower lipids in their muscles compared to fish species in brackish or marine water[11, 13]. However, the present study on juvenile Nile tilapia contradicts these findings. As shown in Table 2, the percentage of crude lipid contents decrease with increasing salinity from 4.0% to 3.0-3.8% in Experiment I, which aligns with the research findings reported by Olopade et al. (2016)[10]. Conversely, in Experiment II, lipid and protein contents showed slight variations, yielded higher percentages in the 5 ppt and 15 ppt salinity treatments. The results for the second experiment show a slightly greater range of protein and crude lipid contents, fluctuating from 17.5% to 18.4% and 3.7% to 4.5% respectively, compared to the results obtained in Experiment 1.

Based on the results from both experiments, juvenile Nile tilapia cultured in the 0 ppt to 5 ppt groups exhibited higher lipid content compared to those in higher salinities. The high lipid content in the fish muscle is responsible for good palatability or good taste[13], implying that the tilapia raised between 0 ppt and 5 ppt may have better taste than those cultured in higher salinities.

Moreover, the observed moisture content, ranging from 73% to 76%, indicates that the moisture percentage in the fish muscles across all salinity treatments was within acceptable levels according to previous studies[3, 10, 12, 13]. It has been reported that the moisture content remains relatively constant throughout the same developmental stages of fish. Meanwhile, the levels of crude protein and lipids, which were important nutritional elements, increased, whereas the moisture and ash content decreased[6, 13]. In the present experiments at salinity levels 0 ppt (Experiment I) and 0-5 ppt (Experiment II) the highest growth and lowest moisture content of 73.3% and 74.5-74.6% were recorded, respectively. In terms of ash content, the levels were similar across all salinity treatments in both experiments, ranging between 2.3% to 2.7%. It is clear that salinity did not significantly affect the ash contents in fish. These combined results indicate that juvenile Nile tilapia cultured in rearing waters of 0 ppt to 5 ppt will be better sources of protein and lipid and may exhibit good palatability with a firm and cohesive flesh.

Fatty acids are structural components of lipids that

Table 2. Whole body proximate composition (%) of juvenile Nile tilapia in different salinity treatments

Proximate composition	Moisture	Crude Protein	Crude Lipid	Crude Ash
Experiment I				
Commercial Feed	3.1	41.2	8.8	13.5
Initial	81.9	14.1	0.9	2.5
Control (0 ppt)	73.7	16.5	4.0	2.7
9 ppt	74.7	16.1	3.8	2.5
18 ppt	75.3	14.4	3.0	2.7
27 ppt	N/A	N/A	N/A	N/A
Experiment II				
Commercial Feed	6.0	50.8	13.9	12.8
Initial	77.6	16.3	2.0	2.9
Control (0 ppt)	74.5	17.5	4.4	2.5
5 ppt	74.6	18.4	4.5	2.3
10 ppt	76.1	17.5	3.7	2.5
15 ppt	74.2	18.3	4.5	2.5

In the table, ppt: parts per thousand; N/A: no available data because of mass mortality.

play an important role in both fish physiology and human diets[14]. As illustrated in Tables 3 and 4, important ten fatty acids were evaluated in the test fish which includes some important essential fatty acids (EFAs); C20:5n-3 (EPA), C22:6n-3 (DHA), C18:3n-3 and C18:2n-6. These are categorized as polyunsaturated fatty acids (PUFAs) and offer numerous health benefits for both of fish and humans[15]. EPA and DHA are important members of n-3 highly unsaturated fatty acids (n-3HUFAs) and are essential for proper early development, healthy aging, and are considered beneficial in the treatment of several diseases[16].

In terms of fatty acids, the results from the present study (Tables 3 and 4) showed that juvenile Nile tilapia reared in freshwater (0 ppt) by feeding commercial pellets exhibited similar or slightly higher levels of PUFAs such as C18:2n-6, C18:3n-3, and C20:5n-3, which are important EFA components of the human diet[17]. However, the contents of C22:6n-3 (DHA), one of the most important n-3 HUFAs[3,4], demonstrated a clear positive correlation with salinity in Experiment I with higher rearing temperature (28 ± 2°C). It also showed much higher contents (11.7-14.0%) than those from other PUFAs at lower rearing temperature treatment (Experiment II). These findings are consistent with Suloma et al. (2008), who documented higher n-3 HUFAs content in marine and brackish water fishes compared to fishes from freshwater including Nile tilapia. The differences in the duration of both experiments and the rearing water temperatures (28 ± 2°C and 22 ± 3°C for Experiments I and II, respectively) could be a contributing factor to the variations in fatty acid

Table 3. Fatty acid composition (%) of juvenile Nile tilapia in different salinity treatments in Experiment I

Fatty acid	Control (0 ppt)	9 ppt	18 ppt	27 ppt
C14:0	2.6	3.0	3.1	N/A
C15:0	0.4	0.4	0.0	N/A
C16:0	13.0	14.7	13.6	N/A
C18:0	3.8	4.0	4.4	N/A
C18:1n-9	15.0	16.1	16.4	N/A
C18:2n-6	5.9	5.1	6.6	N/A
C18:3n-3	1.5	1.1	1.8	N/A
C20:4n-6	ND	ND	ND	N/A
C20:5n-3	6.6	4.8	5.5	N/A
C22:6n-3	6.6	7.8	8.4	N/A
ΣPUFA	20.6	18.8	22.3	N/A
Σn-3HUFA	13.2	12.6	13.9	N/A

In the table, ND: not detected; N/A: no available data because of mass mortality.
ΣPUFA: Total % of poly unsaturated fatty acids (PUFAs) contents in the table.
Σn-3HUFA: Total % of n-3 highly unsaturated fatty acids (HUFAs) contents in the table.

Table 4. Fatty acid composition (%) of juvenile Nile tilapia in different salinity treatments in Experiment II

Fatty acid	Control (0 ppt)	5 ppt	10 ppt	15 ppt
C14:0	0.7	3.9	3.6	3.9
C15:0	0.3	0.3	0.3	0.3
C16:0	17.9	18.8	16.5	17.9
C18:0	5.5	5.3	5.1	5.0
C18:1n-9	21.0	21.5	18.8	21.6
C18:2n-6	2.1	1.8	2.1	1.9
C18:3n-3	0.6	0.5	0.5	0.5
C20:4n-6	1.1	1.0	1.6	1.3
C20:5n-3	3.7	2.7	3.4	3.0
C22:6n-3	13.8	11.7	14.0	12.5
ΣPUFA	21.3	17.7	21.6	19.2
Σn-3HUFA	17.5	14.4	17.4	15.5

ΣPUFA: Total % of poly unsaturated fatty acids (PUFAs) contents in the table.
Σn-3HUFA: Total % of n-3 highly unsaturated fatty acids (HUFAs) contents in the table.

composition. Additionally, C20:4n-6 was undetectable in Experiment I at higher water temperatures (28 ± 2°C) for unknown reasons, while Experiment II displayed results for different salinity treatments. Nevertheless, there have been no studies evaluating the effects of different salinities and rearing temperatures on the body fatty acid compositions of Nile tilapia through long-term rearing experiments when they were juvenile. Therefore, it is necessary to conduct such research to elucidate the relationship between rearing conditions and the nutritional profiles of fish bodies, in order to produce better aquaculture products in the future. The results of both experiments demonstrated variations in fatty acid composition across different salinity treatments; however, it is evident that juvenile Nile tilapia reared in low salinity rearing water with commercial feeds will be better sources of various EFAs for human consumption.

In conclusion, juvenile Nile tilapia cultured in freshwater exhibited more favorable results compared to those in higher salinities. Although it is feasible to raise juvenile Nile tilapia in higher salinity conditions than freshwater, their growth performance and carcass quality are likely to be inferior to those cultured at lower salinities. Overall, this study supports the idea that a salinity range of 0 ppt to 5 ppt (rearing water systems with freshwater to very low saline water conditions) is optimal for achieving higher nutritional quality, fair palatability, and better survival of juvenile Nile tilapia, *Oreochromis niloticus.*

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幼期のナイルティラピア *Oreochromis niloticus* の成長、生残、体成分と脂肪酸組成に与える飼育水塩分の影響

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要旨

環境要因が養成魚の成長や生残あるいは栄養品質に与える影響を調べる試みが、養殖成績の向上の見地から長年にわたり大きな関心を集めている。本研究では、幼期の成長段階にあるナイルティラピア（*Oreochromis niloticus*, 5.93 ± 0.51 g）を用いて、市販の配合飼料を用いた長期の給餌試験後の成長、生残、魚体の一般組成と脂肪酸組成に与える飼育水塩分濃度の影響を検討した。飼育試験は 2 回実施し、0 ppt（淡水）を対照区として 9、18、27 ppt（実験 I）および 5、10、15 ppt（実験 II）の 4 実験区をそれぞれ設け評価した。両実験共に、体重の平均増重率は 0 ppt 区で最も高く、他区はそれより低い値を示した。生存率に関しては、実験 I の最も高い塩分区（27 ppt）で継続的な大量の斃死が観察されたが、他区については実験 I では 83.0-93.3%、実験 II では 97.0-100% の高い生存率が得られた。また、飼育水塩分が魚体のタンパク質および脂質含量に逆相関の影響を与えることが強く示唆され、0 ppt 区でそれらの含量が最も高くなった。さらに 0 ppt 区で飼育された魚は重要な栄養成分である高度不飽和脂肪酸の魚体中の含量が高塩分区と同じかやや高くなる傾向を示した。今回の一連の飼育試験の結果は、幼期のナイルティラピアにおいて良好な飼育成績および高い栄養的品質を得るためには、0 から 5 ppt の淡水からごく低い塩分の範囲の飼育水が好適であることを示している。

キーワード：ナイルティラピア，*Oreochromis niloticus*，稚魚，塩分，体組成