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Behavioural response, welfare, and performance of Nile tilapia (*Oreochromis niloticus*) under different water temperatures

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Abstract
This work was carried out to detect behavioural response, performance and welfare of Nile tilapia fingerlings under different water temperatures. Fingerlings were reared in eight 90-L aquaria in duplicate for 60 days with 15 fish per aquarium at different temperatures (20 °C (group1), 24 °C (group 2), 28 °C (group 3), and 32 °C (group 4). The results showed that group 4 was higher in surfacing, surface swimming and aggressive behaviour than other groups. While, group 3 was higher in middle swimming behaviour and crossing test than other groups, but bottom swimming behaviour was higher in group 1 than other groups. Also final body weight of group 3 was markedly greater than other groups, finally, it is concluded that a temperature 28 °C is the optimum for achievement of Nile tilapia welfare due to high body weight, lowering in surfacing, scratching and aggressive behaviour, increasing the ability of Nile tilapia for practicing swimming behaviour.

Keywords: Nile tilapia, water temperature, behaviour, welfare, crossing test

1. Introduction
Aquaculture is an essential source of fish and fish products which provides a good source of protein and important micronutrients for a well-balanced nutrition and good health [1]. There is an increasing demand for fish meat over the world as it contains a healthy and high-quality protein, so fish culture showed a remarkable distribution in the last years [2]. Tilapia is the prime fish species for culturing the wide-world. It is the species of choice because of its rapid growth rate, easily farming and having the ability to bear to environmental cues, and resistance to disease [3]. Nile tilapia is in constant interaction with its environment through the gills and skin, so water quality (temperature, dissolved oxygen, PH, ammonia, nitrite, and salinity) is crucial for its welfare [4]. Water temperature is one of the most fundamental factors affecting the performance of Nile tilapia, chiefly in subtropical and temperate areas, which are characterized by seasonal disturbance in temperature [5]. Water temperature influences the growth parameters, welfare, and behaviour of aquatic organisms [6]. Water temperature has a major impact in fish farming because of its ability to make a powerful impact on fish biochemical reactions and performance [7, 8]. An increase of water temperature impacts greatly on the growth of fish by elevating their feed intake [9]**.** Principally, tilapia stop their feed intake when water temperature decreases below 15 °C and are unable to reproduce below 20 °C [10]. High temperatures can increase anger and hostility in aquatic animals and increase their desire to fight [11]. Temperature is an essential environmental factor in aquatic animals that can significantly impact the aggressive behaviour of fish [12]. During severe winter weather (low water temperature) fish move into deeper into ponds, form large aggregations, reduce feeding consumption and aggression decreased [13]. Low temperature results in sluggishness by slowing down the digestion speeding of fish [14]. High water temperature could result in decreasing feed consumption and growth parameters that have been observed in Nile tilapia. Also found that the survival rate of Nile tilapia, Oreochromis niloticus was notably lower when it was raised at the lower and upper levels of its optimum water temperature [15]. Culturing of Nile tilapia at high water temperature caused mortality due to Long term exposure of low dissolved oxygen level that resulted in the complete respiratory arrests in fish, so temperature and oxygen are the most essential determinants regulating the survival and

physiology of all aquatic living organisms. Despite the ability of Nile tilapia to resist a wide range of temperatures, the over and suboptimal temperature causes a decline in the growth rates [16]. Tilapia grows well in a high water temperature ranging from 25 to 28 °C owing to their ability to practice all activities efficiently [17]. For this reason, the aim of this work is to show the influence of water temperature on behaviour, welfare, and performance of Nile Tilapia.

2. Materials and methods

The present work was carried out at Fish management and Behaviour Research Unit, Department of Veterinary Public Health of Faculty of Veterinary Medicine, Zagazig University. The present study was started from the mid of February to mid of April of (2019) to investigate the effect of water temperature of aquaria on the behaviour and performance of Nile Tilapia.

2.1. Fish management and water hygiene

By the arrival of fish, it was acclimated to aquarium water temperature to avoid stress. For handling transportation, a hand net made from nylon was used separately for each aquarium to avoid transmission of infection. Fish were kept at the following aquarium parameters according to (Ndwiga, 2015) [18] in the table (1):

Table 1: Aquarium parameters of the experiment.

Parameters	Level
Dissolved Oxygen	6.5 ± 0.5 mg/L
Total Ammonia	< 0.02 mg/L
Nitrite	< 0.05 mg/L
PH	8 ± 0.5
Salinity	0.5 ± 0.1 ‰
Water Hardness ($CaCO_3$)	190 ± 10 mg/L

A total number of 150 apparently healthy Nile Tilapia fingerlings with average body weight 34g were obtained from a private fish farm at Ismailia Governorate. Fish were transported to the fish Research Unit of Faculty of Veterinary Medicine, Zagazig University, where fish were acclimated for two weeks in a cement pond. Then fish were transported to Fish Management and Behaviour Research Unit, Department of Veterinary Public Health, and divided into four duplicated aquaria that were subjected to four water temperatures (20, 24, 28, 32 ºC). Tilapia fingerlings were acclimated at their aquaria by increasing water temperature by 2 ºC per day in all treatments for a week according to (Xie *et al*., 2011) [19].When each treatment reached the required water temperature, the fish were acclimated for a week before the start of the experiment. Fifteen fish were randomly allocated in each aquarium, at the beginning of the experiment; the fish were starved for 2 days before recording their average initial weight, at the termination of the experiment, all the fish in each aquarium were netted, weighed individually and their average final weight was recorded. A fully prepared 8 glass aquaria measured (100 x 30 x 40) was used for four water temperatures (20, 24, 28, 32 ºC) that were tested, and one duplicate aquarium was allocated to each temperature. Each aquarium was supplied with continuous aeration, and the water temperature was maintained at the required degrees by a thermostatically controlled heater and thermometer and it was controlled by a temperature-monitor. Aquaria were cleaned by removal third of aquarium water five times weekly then replaced by dechlorinated water from the water storage tank. Each aquarium contained electrical aerator, and filter to remove the organic waste matter in each aquarium and source of dissolved oxygen, heater (Thermostat) and aquarium net for fish handling and transporting. The basal diet was produced by the Cairo Poultry Processing Company (CPC). It was formulated in the form of dry floated pellets, to meet the nutrient requirements of Nile Tilapia fingerling.

The ingredients and proximate composition of the experimental diet are presented in this table (2) according to (NRC, National Research Council), 1993) [20]:

Table 2: Chemical composition of the diets used in the experiment.

Composition	
Fish meal, 66%	20
Soybean meal, 44%	20
DDGS, 28%	10
Yellow corn	15
Corn gluten, 62%	4.55
Rice bran	26.45
Vegetable oil	3.50
L-Lysine HCL 98%	-
D L- Methionine	-
Calcium carbonate	-
Vitamin. mineral premix*	0.50
Total %	**100**
Calculated composition	
DM, %	86.47
CP, %	32.01
EE, %	11.47
CF, %	4.27
Ash, %	7.60
NFE, %	34.06
Ca, %	0.89
P, %	1.19
Lysine, %	1.85
Methionine, %	0.71
DE, Kcal/ kg**	3007.46

Vitamin and mineral mixture (kg/ diet) (Vit. A 6000 I.U, D3 2.000 I.U, E 500mg, k3 12.0 mg, C. 1.000mg. B1 10mg, B2 15.0mg, B6 7.5mg, B12 0.1mg, Biotin 0.2mg, Folic acid 0.4mg, choline Hcl 1.0g inosit. 3000.0mg, pantothenic acid 50.0mg, Nicotinic acid 100mg, P-Aminobenzonic acid 50 mg, iron 80mg, copper 5g, zinc 40g, Sodium selenite 100 mg, and potassium iodide 300mg, and cobalt sulfate 100 mg). digestible energy calculation based on values of protein 3.5 kcal/gm, fat 8.1 kcal/gm, NFE 2.5 kcal/gm according to (Essam *et al*., 2020) [21]. Feeding was three times daily at (9:00 AM), at (1:00 PM), and at (4:00 PM), and Fish was fed 6 days a week. Feed only as much as they can eat within **(**5 min) according to (Scheurmann, 2000) [22]. The daily amount of food was kept constant at 3% of the total biomass of fish, throughout the experimental time according to (Chowdhury, 2011) [23]**.** It was adjusted almost every 2 weeks, when the entire population of each aquarium was weighed. Nile Tilapia was identified by short plastic strips applied in the dorsal fin of seven fish to facilitate observation for the fish during the experimental period according to (Khalil *et al*. 2016) [24].

2.2. Medication

Potassium permanganate (2mg / 1L), Oxytetracycline (50mg / 1Kg b.wt.) Both were used to treat from columnaris disease that affects most freshwater fish during stress, NaCl (1gm/1L) used as a protection against fish disease (2) times per week after the water change.

2.3 Observation and Data collection

Behaviour was recorded in the period between (09:00 Am) till (04:00 Pm) for (8) weeks by using focal sample technique, Visually by using a notebook for recording behaviour, a stopwatch, multipurpose counter, and video camera according to (Dawkins, 2007) [25]. The behavioural observations were performed as the following: each treatment group was observed two times daily 15 minutes/time (7.5 minutes/aquarium) (65sec/fish) for 4 days/week at the circularly predetermined time. Intervals through (8) hours weekly for 4 groups according to (Soltan *et al*., 2008) [26]. The observed behavioural patterns were recorded as the following:

Table 3: The observed behaviours**.**

Behavioural	Description
Swimming Behaviour	Mean frequency and time (sec) / 8 hours of the Swimming of fish that means rapidly or slowly movement without any behaviour activity in different aquarium areas (surface, middle and bottom), according to (Chen *et al*., 2001) [27].
Scratching (chafing) behaviour	Mean frequency and time (sec) / 8 hours of scratching behaviour that means rubbing any part of the body against any object, according to (Fall, 2005) [28] and Neto *et al*., (2020) [29].
Aggressive behaviour	Mean frequency and time (sec) /8 hour's observation of the fighting and means the act of initiating an attack according to (Fall, 2005) [28], Barreto *et al*., (2011) [30] and Brandão *et al*., (2018) [31].
Number of midline crossing	The aquarium was divided by a midline externally, and the number of midline crossings from fish through 5 minutes was detected for each aquarium according to the protocol and calculations of (Scott *et al*. 2003) [32].

2.4. Live fish performance

To calculate average body weight, every 15 days all fish in each group were weighted then divided the total weight of fish by the number of fish in each group according to (Khalil *et al*. 2016) [24].

2.5. Statistical analysis

All experiments data were collected, arranged, summarized and then analyzed using SPSS version 21 Statistical Analysis System package [33]. Results expressed as Mean ±SD.

1. The mixed model ANOVA test was used to test behavioural parameters for different groups during consecutive weeks of the experiment. The interaction plot was used to compare between means of each behavioural parameters in different groups at weeks of the experiment.
2. One- way analysis of variance (ANOVA) test was applied to test differences at a bodyweight of fish at different groups. Tukey's honesty significant test was applied after significant results.
   P.value < 0.05 was considered statistically significance.

Results

Table 4: Mean ± SD of Surface Swimming behaviour in the four groups during eight weeks of experiment.

	G1	G2	G3	G4	G1	G2	G3	G4
	Surface Swimming Frequency				Surface Swimming Duration			
W1	0.34 ± 0.22	1.22 ± 0.4	0.68 ± 0.31	1.09 ± 0.43	3.45 ± 2.42	12.95 ± 6.2	6.61 ± 4.15	16.34 ± 5.12
W2	0.41 ± 0.3	1.66 ± 0.47	1 ± 0.27	1.91 ± 0.48	6.48 ± 5.18	18.95 ± 6.03	10.93 ± 5.51	20.16 ± 8.44
W3	0.27 ± 0.15	1.29 ± 0.19	0.82 ± 0.19	1.32 ± 0.6	4.84 ± 3.53	14.18 ± 3.76	9.58 ± 2.85	15.59 ± 9.87
W4	0.75 ± 0.32	1.09 ± 0.29	0.88 ± 0.38	1.98 ± 0.55	11.24 ± 5.06	12.59 ± 6.18	9.04 ± 4.28	21.7 ± 10.1
W5	0.81 ± 0.46	1.21 ± 0.55	1.2 ± 0.48	2.2 ± 0.76	11.23 ± 7.08	13.38 ± 8.1	11.07 ± 3.23	21 ± 8.85
W6	0.91 ± 0.25	1.31 ± 0.52	0.84 ± 0.41	2.41 ± 0.73	11.84 ± 5.66	13.2 ± 4.82	6.98 ± 3.18	17.25 ± 5.95
W7	0.75 ± 0.23	0.99 ± 0.49	0.32 ± 0.27	2.02 ± 0.78	14.97 ± 5.69	12.65 ± 7.94	2.93 ± 2.73	14.36 ± 6.16
W8	1.15 ± 0.47	1.27 ± 0.51	0.63 ± 0.18	2.45 ± 0.65	15.84 ± 8.65	13.25 ± 7.4	7.22 ± 3.21	18.9 ± 4.55

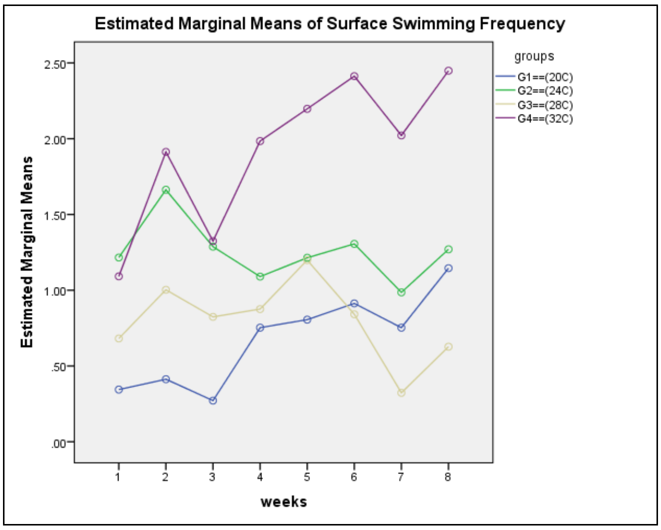

Fig 1: Interaction plot of Surface Swimming frequency for four groups during eight weeks of experiment.

Mixed model ANOVA test results showed that the effect of weeks of the experiment significantly produced a significant difference in the reading of surface swimming frequency among groups P- value (0.001) By looking to the interaction plot Figure (1) and Table (4) both clearly showed the differences among groups during the weeks of the experiment, in the first week the highest behaviour recorded was in the group2 (1.22 ± 0.4), the behaviour in group 3,4 was lower in average than group2, and the lowest behaviour was recorded in group 1. By going on weeks of the experiment, the behaviour showed a decrease in week 4 in group 2 and showed more increase in group 3 than the other groups. In the last week group 4 showed more increase than groups 1, 2.while group 3 showed a decrease in the behaviour. The general look to the interaction plot showed that group 1 (0.27 ± 0.15) showed the minimum values of surface swimming behaviour compared to the other three groups, and group 4 (2.45± 0.65) nearly showed the highest values after the first week of the experiment.

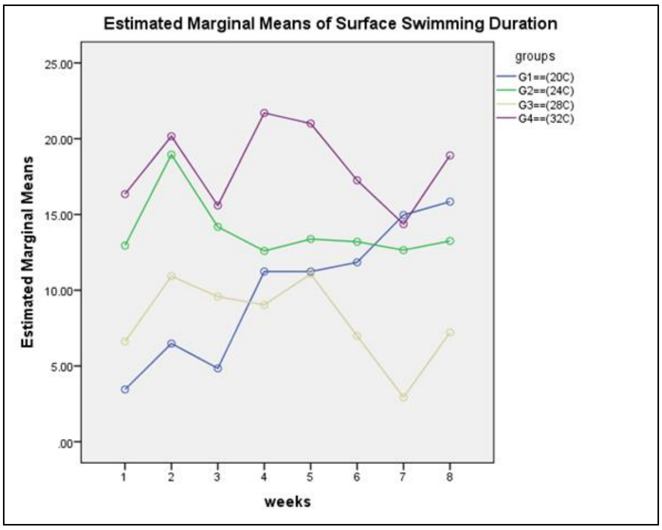

Fig 2: Interaction plot of Surface Swimming duration for four groups during eight weeks of experiment.

Mixed model ANOVA test results showed that the effect of weeks of the experiment significantly produced a significant difference in the reading of surface swimming duration among groups P- value (0.001) By looking to the interaction plot Figure (2) and Table (4) both clearly showed the differences among groups during the weeks of the experiment, in the first week the highest behaviour recorded was in the group 4 (16.34 ± 5.12), the behaviour in group 2, 3 was lower in average than group 4, and the lowest behaviour was recorded in group 1. By going on weeks of the experiment, the behaviour showed a decrease in week 4 in group 2 and showed more increase in group 3 than the other groups. In the last week the highest behaviour recorded was in group4 (13.82±7.57). The general look to the interaction plot showed that group 3 (2.93 ± 2.73) showed the minimum values of surface swimming behaviour compared to the other three groups, and group 4 (21.7 ± 10.1) nearly showed the highest values after the first week of the experiment.

Table 5: Mean ± SD of Middle Swimming behaviour in the four groups during eight weeks of experiment.

	G1	G2	G3	G4	G1	G2	G3	G4
	Middle Swimming Frequency				Middle Swimming Duration			
W1	1.09 ± 0.14	1.86 ± 0.73	1.63 ± 0.25	1.88 ± 0.44	12.48 ± 2.79	16.56 ± 8.46	24.09 ± 5.86	23.72 ± 5.5
W2	1.2 ± 0.45	1.93 ± 0.33	1.61 ± 0.34	2.09 ± 0.49	18.39 ± 6.56	18.68 ± 5.78	20.07 ± 5.81	14.93 ± 5.68
W3	1.23 ± 0.32	1.61 ± 0.31	1.52 ± 0.39	2.18 ± 0.43	17.81 ± 4.96	18.34 ± 6.67	21.93 ± 9.37	23.11 ± 7.95
W4	1.4 ± 0.36	1.48 ± 0.44	1.36 ± 0.58	1.88 ± 0.69	21.18 ± 7.95	19.88 ± 7.69	17.98 ± 10.42	13.82 ± 4.86
W5	1.41 ± 0.44	1.48 ± 0.41	1.66 ± 0.44	2.09 ± 0.42	19.06 ± 8.56	17.73 ± 5.78	22.14 ± 7.67	15.64 ± 6.08
W6	1.45 ± 0.37	1.47 ± 0.47	1.68 ± 0.65	2.34 ± 0.52	16.02 ± 5.04	16.57 ± 6	23.45 ± 9.17	15.88 ± 5.22
W7	1.2 ± 0.41	1.2 ± 0.36	1.72 ± 0.66	2 ± 0.52	15.77 ± 9.47	13.88 ± 5.04	24.38 ± 10.45	14.25 ± 6.06
W8	1.34 ± 0.6	1.43 ± 0.5	1.54 ± 0.22	1.7 ± 0.67	17.06 ± 8.44	17.61 ± 10.03	24.61 ± 4.07	10.2 ± 5.15

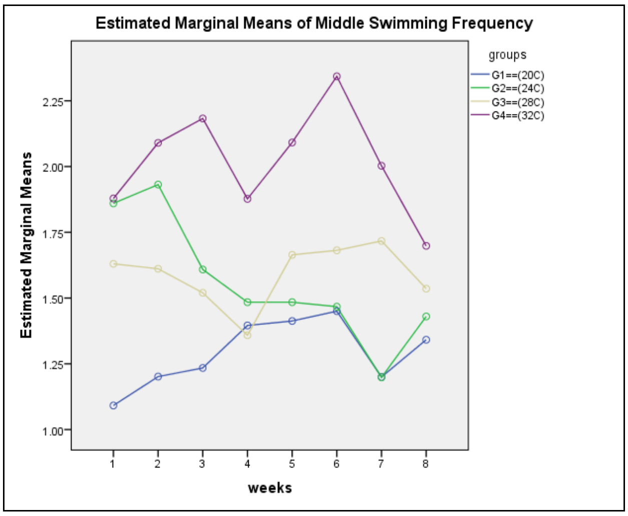

Fig 3: Interaction plot of Middle Swimming frequency for four groups during eight weeks of experiment.

Mixed model ANOVA test results showed that the effect of weeks of the experiment produced non-significant difference in the reading of middle swimming frequency among groups P- value (0.2) By looking to the interaction plot Figure (3) and Table (5) both clearly showed the differences among groups during the weeks of the experiment, in the first week, the highest behaviour recorded was in the group 4 (1.88 ± 0.44), the behaviour in group 2, 3 was lower in average than group 4, and the lowest behaviour was recorded in group 1. By going on weeks of the experiment, the behaviour showed a decrease in week 4 in groups 2, 3 while showed a marked increase in group 1, 4. In the last week group 3 was the only group showed an increase but group 4 was still the highest in the behaviour while the groups 1, 2 showed a decrease. The general look to the interaction plot showed that group 1 (1.09 ± 0.14) showed the minimum values of middle swimming behaviour compared to the other three groups, and group 4 (2.34 ± 0.52) nearly showed the highest values after the first week of the experiment.

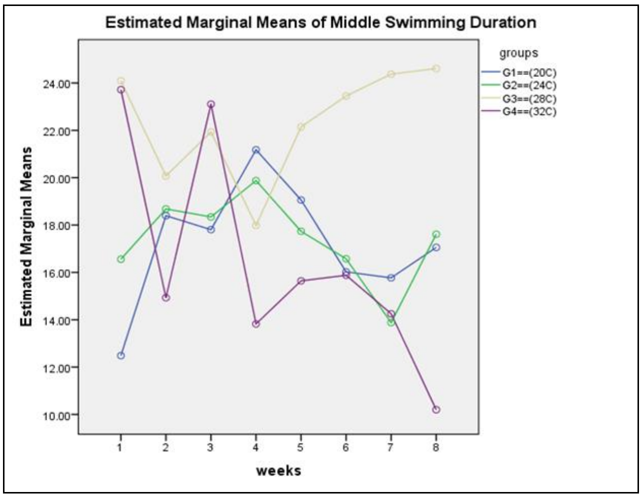

Fig 4: Interaction plot of Middle Swimming duration for four groups during eight weeks of experiment.

Mixed model ANOVA test results showed that the effect of weeks of the experiment significantly produced a significant difference in the reading of middle swimming duration among groups P- value (0.01) By looking to the interaction plot Figure (4) and Table (5) both clearly showed the differences among groups during the weeks of the experiment, in the first week the highest behaviour recorded was in the group 3 (24.09 ± 5.86), the behaviour in group 2, 4 was lower in average than group3, and the lowest behaviour was recorded in group 1. By going on weeks of the experiment, the behaviour showed a decrease in week 4 in groups 3, 4 and showed more increase in group 1 than group 2. In the last week group 3 was the only group showed a marked increase in the behaviour while the other groups showed a decrease. The general look to the interaction plot showed that group 4 (10.2 ± 5.15) showed the minimum values of middle swimming behaviour compared to the other three groups, and group 3 (24.61 ± 4.07) nearly showed the highest values after the first week of the experiment.

Table 6: Mean ± SD of Bottom Swimming behaviour in the four groups during eight weeks of experiments.

	G1	G2	G3	G4	G1	G2	G3	G4
	Bottom Swimming Frequency				Bottom Swimming Duration			
W1	1.66 ± 0.26	1.68 ± 0.49	1.23 ± 0.34	1.09 ± 0.2	41.24 ± 6.33	17.32 ± 7.79	11.57 ± 2.43	10.77 ± 4.48
W2	1.38 ± 0.18	1.09 ± 0.32	0.91 ± 0.33	1.39 ± 0.25	32.83 ± 7.57	9.06 ± 6.29	7.66 ± 4.89	9.66 ± 3.26
W3	1.72 ± 0.41	1 ± 0.36	1 ± 0.3	1.24 ± 0.37	30.88 ± 4.54	10.66 ± 4.49	8.98 ± 2.59	8.82 ± 1.12
W4	1.31 ± 0.48	1.09 ± 0.32	1 ± 0.5	1.56 ± 0.66	19.52 ± 6.87	9.95 ± 4.8	5.84 ± 2.92	9.95 ± 4.72
W5	1.11 ± 0.4	1.24 ± 0.56	0.71 ± 0.37	1.31 ± 0.55	18.09 ± 10.39	13.04 ± 5.89	6.75 ± 4.18	7.81 ± 5.63
W6	1.45 ± 0.47	1.04 ± 0.47	0.86 ± 0.47	1.15 ± 0.57	20.4 ± 4.06	9.38 ± 4.72	5.15 ± 1.83	5.82 ± 2.78
W7	1.27 ± 0.29	1.34 ± 0.29	1.06 ± 0.31	1.36 ± 0.64	16.15 ± 8.17	10.54 ± 3.42	7.04 ± 3.2	7.15 ± 2.65
W8	1.04 ± 0.45	0.93 ± 0.39	0.81 ± 0.36	1.11 ± 0.6	9.72 ± 4.96	4.45 ± 1.29	3.88 ± 2.53	6.07 ± 2.54

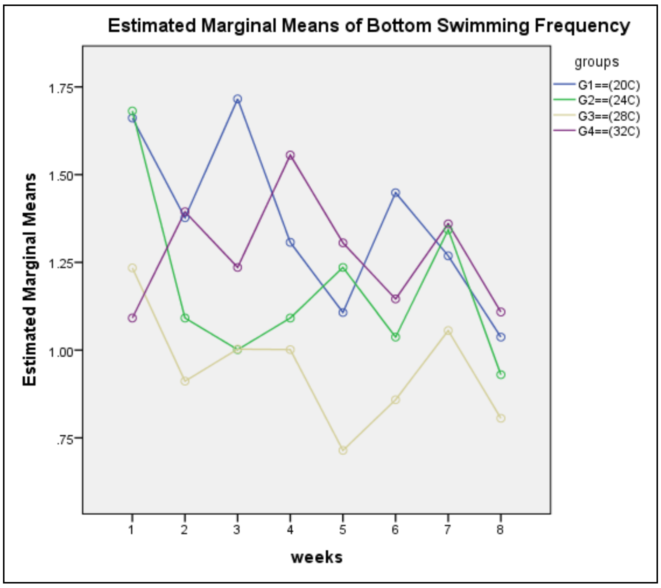

Fig 5: Interaction plot of Bottom Swimming frequency for four groups during eight weeks of experiment

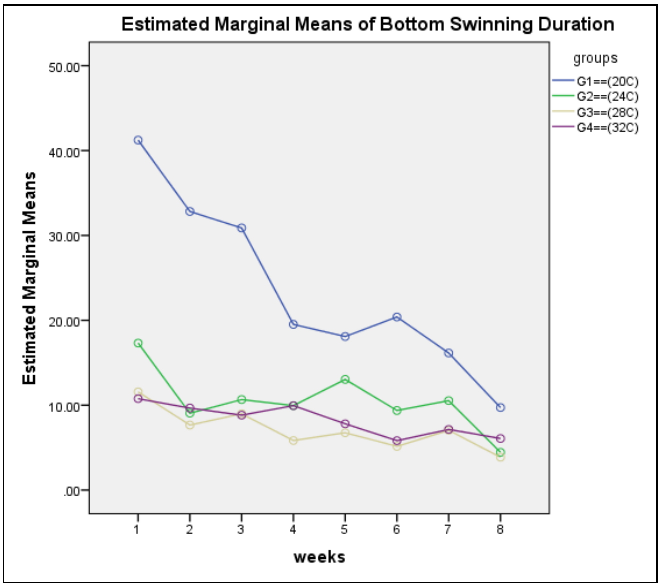

Fig 6: Interaction plot of Bottom Swimming duration for four groups during eight weeks of experiment.

Mixed model ANOVA test results showed that the effect of weeks of the experiment produced non-significant difference in the reading of bottom swimming frequency among groups P- value (0.1) By looking to the interaction plot Figure (5) and Table (6) both clearly showed the differences among groups during the weeks of the experiment, in the first week the highest behaviour recorded was in the group 2 (1.68 ± 0.49), the behaviour in group 1, 4 was lower in average than group2, and the lowest behaviour was recorded in group 4. By going on weeks of the experiment, the behaviour showed a decrease in groups 1, 2 and 3 while showed an increase in group 4. In the last week all groups showed a decrease, but group 4 was the highest in the behaviour. The general look to the interaction plot showed that group 3 (0.71 ± 0.37) showed the minimum values of bottom swimming behaviour compared to the other three groups, and group 1 (1.72 ± 0.41) nearly showed the highest values after the first week of the experiment.

Mixed model ANOVA test results showed that the effect of weeks of the experiment significantly produced a significant difference in the reading of bottom swimming duration among groups P- value (0.001) By looking to the interaction plot Figure (6) and Table (6) both clearly showed the differences among groups during the weeks of the experiment, in the first week the highest behaviour recorded was in the group1(41.24 ± 6.33), the behaviour in group 2,3 was lower in average than group1 and the lowest behaviour was recorded in group 4. By going on weeks of the experiment, the behaviour showed a decrease in all groups, but group 1 was the highest. In the last week the behaviour showed a decrease in all groups, but group 1 was still the highest in the behaviour. The general look to the interaction plot showed that group 4 (3.88 ± 2.53) showed the minimum values of bottom swimming behaviour compared to the other three groups, and group 1 (32.83 ± 7.57) nearly showed the highest values after the first week of the experiment.

Table 7: Mean ± SD of Scratching behaviour in the four groups during eight weeks of experiment

	G1	G2	G3	G4	G1	G2	G3	G4
	Scratching Frequency				Scratching Duration			
W1	0 ± 0	0.07 ± 0.1	0.04 ± 0.09	0.13 ± 0.18	0 ± 0	0.23 ± 0.3	0.18 ± 0.47	0.59 ± 0.89
W2	0.04 ± 0.06	0.07 ± 0.19	0.11 ± 0.11	0.05 ± 0.14	0.14 ± 0.26	0.18 ± 0.47	0.27 ± 0.29	0.18 ± 0.47
W3	0 ± 0	0.16 ± 0.17	0.04 ± 0.09	0 ± 0	0 ± 0	0.59 ± 0.69	0.09 ± 0.24	0 ± 0
W4	0.02 ± 0.05	0 ± 0	0.04 ± 0.09	0.04 ± 0.09	0.05 ± 0.14	0 ± 0	0.07 ± 0.19	0.09 ± 0.24
W5	0.14 ± 0.24	0.04 ± 0.09	0.11 ± 0.13	0.14 ± 0.38	0.64 ± 1.11	0.18 ± 0.47	0.23 ± 0.29	0.43 ± 1.13
W6	0.07 ± 0.19	0.04 ± 0.09	0.06 ± 0.07	0.16 ± 0.12	0.43 ± 1.13	0.09 ± 0.24	0.13 ± 0.16	0.47 ± 0.37
W7	0.05 ± 0.14	0.18 ± 0.14	0.04 ± 0.06	0.13 ± 0.24	0.18 ± 0.47	0.59 ± 0.53	0.07 ± 0.12	0.36 ± 0.71
W8	0 ± 0	0.11 ± 0.13	0.02 ± 0.05	0.04 ± 0.09	0 ± 0	0.27 ± 0.33	0.04 ± 0.09	0.18 ± 0.47

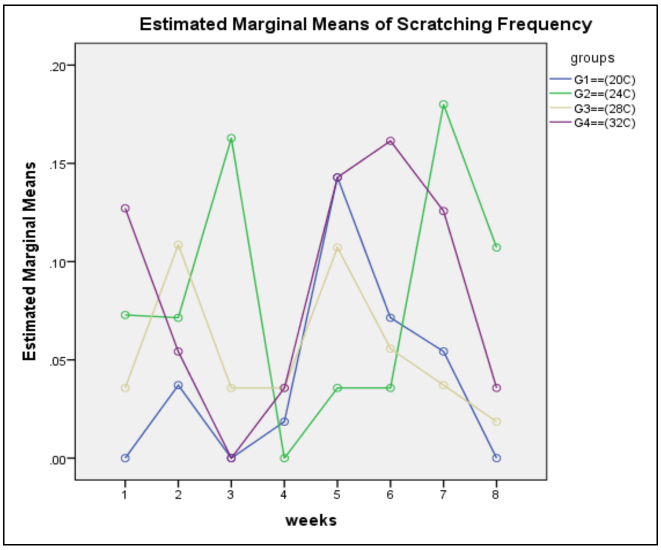

Fig 7: Interaction plot of scratching frequency for four groups during eight weeks of experiment.

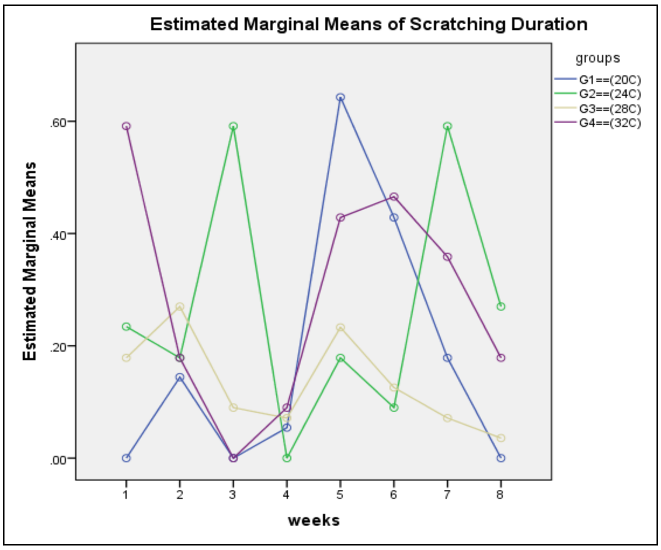

Fig 8: Interaction plot of scratching duration for four groups during eight weeks of experiment.

Mixed model ANOVA test results showed that the effect of weeks of the experiment produced non-significant difference in the reading of scratching frequency among groups P- value (0.5) By looking to the interaction plot Figure (7) and Table (7) both clearly showed the differences among groups during the weeks of the experiment, in the first week the highest behaviour recorded was in the group 4 (0.13 ± 0.18) the behaviour in group 2, 3 was lower in average than group 4 and the lowest behaviour was recorded in group 1. By going on weeks of the experiment in week 4, the behaviour showed a decrease in group 2, 4 and showed an increase in group1 while group 3 was stable. In the last week the behaviour showed an increase in group 2 and showed a decrease in group 1, 3 but group 4 increased then became stable. The general look to the interaction plot showed that group 1 (0.02 ± 0.05) showed the minimum values of scratching behaviour compared to the other three groups, and group 2 (0.18 ± 0.14) and 4 (0.16 ± 0.12) nearly showed the highest values after the first week of the experiment.

Mixed model ANOVA test results showed that the effect of weeks of the experiment significantly produced non-significant difference in the reading of scratching duration among groups P- value (0. 4) By looking to the interaction plot Figure (8) and Table (7) both clearly showed the differences among groups during the weeks of experiment, in the first week the highest behaviour recorded was in the group 4 (0.59 ± 0.89), the behaviour in group 2,3 was lower in average than group 4 and the lowest behaviour was recorded in group 1. By going on weeks of the experiment in week4, the behaviour showed a decrease in groups 2, 3 and 4. While showed an increase in group1. In the last week group 2 showed more increase in the behaviour than group 4 while group 1, 3 showed a decrease. The general look to the interaction plot showed that group 1 (0.05 ± 0.14) showed the minimum values of scratching behaviour compared to the other three groups, and group 2 (0.59 ± 0.53) and 4 (0.59 ± 0.89) nearly showed the highest values after the first week of the experiment.

Table 8: Mean ± SD of Aggressive behaviour in the four groups during eight weeks of experiment.

	G1	G2	G3	G4	G1	G2	G3	G4
	Aggressive Frequency				Aggressive Duration			
W1	1.22 ± 0.64	1.52 ± 0.52	0.81 ± 0.59	0.73 ± 0.87	3.25 ± 2.66	3.13 ± 1.11	1.68 ± 1.13	1.38 ± 1.52
W2	0.59 ± 0.45	0.59 ± 0.47	0.9 ± 0.3	0.9 ± 0.91	1.38 ± 1.11	1.54 ± 1.32	1.77 ± 0.58	2.04 ± 2.36
W3	1.24 ± 0.58	1.13 ± 0.89	0.61 ± 0.54	1.48 ± 0.58	2.98 ± 0.97	2.72 ± 2.39	1 ± 0.8	2.47 ± 0.88
W4	1.22 ± 0.59	0.52 ± 0.36	0.4 ± 0.24	2.57 ± 0.54	2.32 ± 1.31	1.18 ± 0.86	0.48 ± 0.28	5.02 ± 1.07
W5	1.2 ± 0.61	0.84 ± 0.6	0.5 ± 0.24	2.38 ± 1.2	2.72 ± 1.6	1.7 ± 1.19	0.86 ± 0.39	4.93 ± 2.73
W6	1.22 ± 0.69	0.89 ± 0.44	0.36 ± 0.42	3.02 ± 2.09	3.06 ± 2.12	2.11 ± 1.05	0.57 ± 0.64	6.38 ± 5.67
W7	1.15 ± 0.87	1.4 ± 0.81	0.31 ± 0.34	4.52 ± 2.05	2.27 ± 1.65	2.47 ± 1.53	0.41 ± 0.41	10.27 ± 4.43
W8	0.97 ± 0.34	1.45 ± 0.63	0.25 ± 0.16	3.06 ± 1.81	2.84 ± 1.4	2.61 ± 1.08	0.34 ± 0.23	6.79 ± 4.4

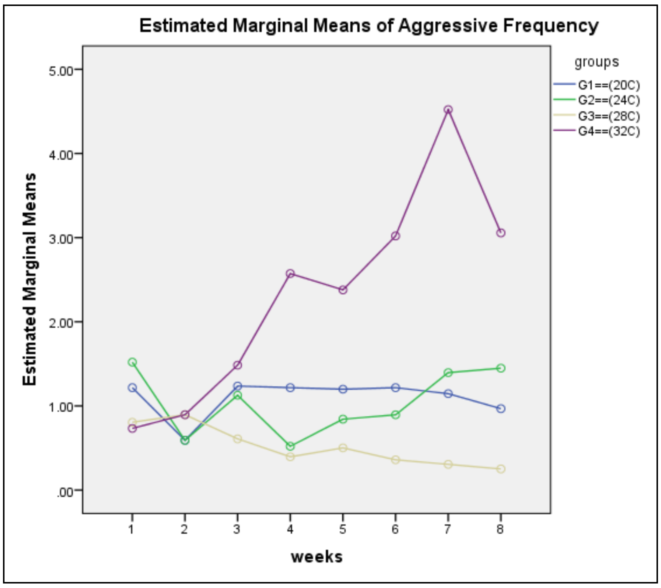

Fig 9: Interaction plot of Aggressive frequency for four groups during eight weeks of experiment.

Mixed model ANOVA test results showed that the effect of weeks of the experiment significantly produced a significant difference in the reading of aggressive frequency among groups P- value (0.001) By looking to the interaction plot Figure (9) and Table (8) both clearly showed the differences among groups during the weeks of the experiment, in the first week the highest aggressive behaviour recorded was in the group 2 (1.52 ± 0.52), the behaviour in group 1, 3 was lower in average than group2, and the lowest behaviour was recorded in group 4. By going on weeks of the experiment, the behaviour showed increase in week 4 in group 4 and showed a decrease in other groups. In the last week groups 1, 3 showed a decrease in the behaviour while group 4 showed more increase than group 2. The general look to the interaction plot showed that group 3 (0.25 ± 0.16) showed the minimum values of aggressive behaviour compared to the other three groups, and group 4 (4.52 ± 2.05) nearly showed the highest values after the first week of the experiment.

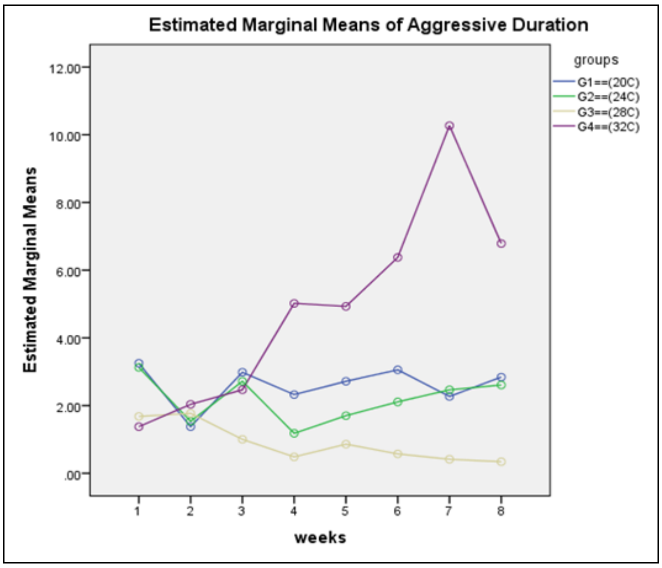

Fig 10: Interaction plot of Aggressive duration for four groups during eight weeks of experiment.

Mixed model ANOVA test results showed that the effect of weeks of the experiment significantly produced a significant difference in the reading of aggressive duration among groups P- value (0.001) By looking to the interaction plot Figure (10) and Table (8) both clearly showed the differences among groups during the weeks of experiment, in the first week the highest aggressive behaviour recorded was in the group1 (3.25 ± 2.66), the behaviour in group 2,3 was lower in average than group 1 and the lowest behaviour was recorded in group 4. By going on weeks of the experiment, the behaviour showed an increase in week 4 in group 4 and showed a decrease in other groups. In the last week group 4 showed more increase than groups 1, 2 in the behaviour, while group 3 showed a decrease in the behaviour. The general look to the interaction plot showed that group 3 (0.34 ± 0.23) showed the minimum values of aggressive behaviour compared to the other three groups, and group 4 (10.27 ± 4.43) nearly showed the highest values after the first week of the experiment.

Table 9: Mean ± SD of crossing test in the four groups during eight weeks of experiment.

	G1	G2	G3	G4
W1	0.85 ± 0.46	1.75 ± 0.31	2.75 ± 0.41	2.71 ± 0.67
W2	0.77 ± 0.27	2.74 ± 0.66	2.65 ± 0.33	2.85 ± 0.37
W3	1.25 ± 0.44	3.11 ± 0.34	2.81 ± 0.41	2.74 ± 0.6
W4	1.17 ± 0.27	2.58 ± 0.2	2.67 ± 0.24	2.65 ± 0.54
W5	1.23 ± 0.29	2.21 ± 0.22	2.84 ± 0.25	2.47 ± 0.5
W6	1.5 ± 0.4	2.6 ± 0.29	2.65 ± 0.32	2.59 ± 0.83
W7	0.99 ± 0.29	2.45 ± 0.35	2.65 ± 0.47	2.52 ± 0.25
W8	1.44 ± 0.47	2.55 ± 0.35	2.49 ± 0.29	2.62 ± 0.49

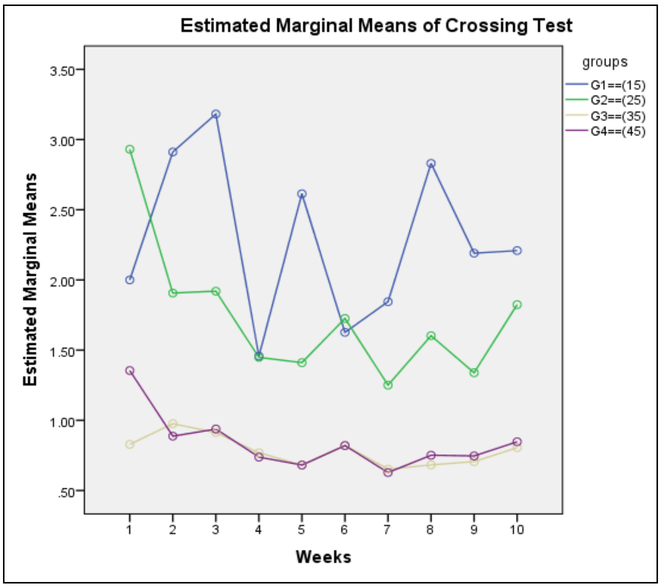

Fig 11: Interaction plot of crossing test for four groups during eight weeks of experiment.

Mixed model ANOVA test results showed that the effect of weeks of the experiment significantly produced a significant differences in the reading of crossing test among groups P-value (0.001). By looking to the interaction plot Figure (11) and Table (9) both clearly showed the differences among groups during the weeks of the experiment, in the first week the highest crossing test value recorded was in the group2 (2.93±1.26), the crossing test value in group1,4 was lower in average than group2 and the lowest behaviour was recorded in group 3. By going on weeks of the experiment, the crossing test showed an increase in group1, while showed a decrease in other groups. In the last week, group 1 showed a decrease but was still the highest in the crossing test while the other 3 groups showed an increase. The general look to the interaction plot showed that group 3 (0.65 ± 0.23) showed the minimum values of crossing test compared to the other three groups, and group 1 (3.18 ± 0.40) nearly showed the highest values after the first week of the experiment.

Table 10: Mean ± SD of Effect of water temperature of aquaria on average body weight per gram.

	G1	G2	G3	G4	Sig.
Initial W	34.35 ± 0.71	34.33 ± 0.675	34.40 ± 0.68	34.34 ± 0.674	0.999
W2	35.65 ± 1.94^{b}	38.56 ± 0.92^{ab}	40.88 ± 0.96^{a}	38.80 ± 0.89^{ab}	0.038
W4	36.37 ± 1.99^{b}	40.47 ± 0.99^{a}	42.61 ± 1.07^{a}	41. 25 ± 1.08^{a}	0.001
W6	35.88 ± 1.93^{b}	41.83 ± 1.215^{a}	43.61 ± 1.21^{a}	42.62 ± 1.299^{a}	0.001
W8	37.87 ± 2^{b}	43.85 ± 1.12^{a}	45.61 ± 1.24^{a}	44.77 ± 1.294^{a}	0.001

^{abc} Means in the same rows with different superscripts are significantly different at (P<0.05).
G1 = Temperature (20 ºC)
G2 = Temperature (24 ºC)
G3 = Temperature (28 ºC)
G4 = Temperature (32 ºC)
W = Week

4. Discussion

Despite of the ability of Nile tilapia to resist a wide range of water temperatures, the over and suboptimal water temperature results in a reduction in the growth parameters [34] and alteration in Ingestive behaviour leading to low feeding rate [35]. The optimum temperature for feeding and growth is between 26 -30°C [7]. The water temperature of about 20 – 25 ^{0}C causes a decrease in growth [18]. The data showed in the Table (3) and Figures (1, 2) revealed that water temperature of aquaria markedly affects surface swimming behaviour frequency and duration among groups, where group (4) (2.45± 0.65 bout) (7.75 ± 8. 43 sec) showed the highest means of surface swimming frequency and duration respectively, while group 1 (0.27 ± 0.15 bout) showed the lowest means of surface swimming frequency but the lowest means of surface swimming duration was recorded in group 3 (2.45± 0.65 sec). These results may attribute to low dissolved oxygen in the aquarium. These findings agreed with the result obtained by Islam *et al*., (2020) [16] who found that the decrease of water quality because of anthropogenic nutrient and sediment load associated with increased water temperature reduced the dissolved oxygen level in the aquatic system and this lowered dissolved oxygen with high water temperature due to less capacity of water to hold oxygen at higher water temperature as mentioned by Conte (2004) [36] impacts the metabolic demand for oxygen and swimming behaviour of fish and this affects badly on the welfare of Nile tilapia.

The results presented in the Table (4) and Figures (3, 4) revealed that there was a significant difference among groups in middle swimming behaviour frequency and duration. Where the lowest means of middle swimming behaviour was shown in both group 1 (1.09 ± 0.14 bout) in frequency, but group 4 (10.2 ± 5.15 sec) in duration. While the highest means of middle swimming frequency in group 4 (2.34 ± 0.52 bout) but group 3 (24.61 ± 4.07 sec) in duration, these results agreed with that recorded by Donaldson *et al*., (2008) [37] who found that change in water temperature resulted in compromising locomotion of fish and alteration in swimming behaviour.

As well as data presented in the Table (5) and Figures (5, 6) showed that bottom swimming behaviour frequency insignificantly affected by water temperature of aquaria among groups, but bottom swimming behaviour duration significantly differed among groups where group 1 (1.72 ± 0.41 bout) (32.83 ± 7.57 sec) showed the highest means of bottom swimming frequency and duration respectively, while group 3 (0.71 ± 0.37 bout) and group 4 (3.88 ± 2.53 sec) showed the lowest means of bottom swimming behaviour frequency and duration respectively. These results agreed with the result obtained by Wingfield (2003) [13] who mentioned that during winter weather (low water temperature) fish move into deeper into the bottom of ponds. This data also agreed with Brandão *et al*., (2018) [31] who observed that a 6° C decline in water temperature than the optimum water temperature decreases swimming activity. These results are also similar to Green and fisher (2004) [38] who found that changing water temperature impacts critical swimming speed and spontaneous swimming activity. These findings disagreed with Claireaux *et al.*, (2006) [39] who found that both swimming behaviour and activity increased by elevating the water temperature. This contrast may be attributed to the difference in species. All these findings indicate that the lower and higher water temperature of aquaria impacts badly on the welfare of Nile tilapia.

Regarding, the results presented in the Table (6) and Figures (7, 8) showed that scratching behaviour (frequency and duration) insignificantly varied among groups, group 4 (0.16 ± 0.12 bout) (0.59 ± 0.89 sec) showed the highest means of scratching behaviour frequency and duration respectively, while group 1 (0.02 ± 0.05 bout) (0.05 ± 0.14 sec) showed the lowest means of scratching behaviour frequency and duration respectively. These results seem to be roughly parallel with the results recorded by Sriyasak *et al*., (2015) [40] who mentioned that increasing water temperature caused an acceleration of organic matter decomposition leading to increasing the level of un-ionized ammonia that becomes more toxic to fish by elevating PH and water temperature causing irritating to fish. As well as these results agreed with Grier and Burk (1992) [41] who mentioned that fish practice body care behaviour especially scratching activity for removal of surface irritation by rubbing body surface against any object in the aquarium and this contributes to decreasing Nile tilapia welfare. These findings also correlated with Neto *et al*., (2020) [29] who showed that repeated scratching behaviour is considered as a stereotypical behaviour owing to repeatedly rubbing the body against any object, fish can remove the protective mucus layer, become injured and more prone to fungal and bacterial diseases, causing high mortality and poor welfare.

Concerning the aggressive behaviour (frequency and duration) as shown in the Table (7) and Figures (9, 10) the results revealed that water temperature of the aquaria had a significant effect on aggressive behaviour, where it was the highest in group 4 (4.52 ± 2.05 bout) (10.27 ± 4.43 sec) frequency and duration respectively, while group 3 (0.25 ± 0.16 bout) (0.34 ± 0.23 sec) showed the lowest means of aggressive behaviour frequency and duration respectively. These results agreed with that recorded by Campos *et al*., (2017) [42] who observed that the elevation of water temperature caused an increase in aggressive interactions. These results also agreed with that observed by Su *et al*., (2020) [43] who mentioned that temperature is an essential environmental factor in aquatic animals because it significantly influences the desire of fight and alter aggression during the fighting and found that the frequency of aggressive behaviour was significantly affected by the temperature due to increasing agonistic behaviour with the increase of temperature to 32°C. The obtained results were supported with that obtained by Brandão *et al*., (2018) [31] who demonstrated that the aggressive patterns of fish species may be influenced by an alteration in water temperature since a reduction in water temperature resulted in a decrease in the all patterns of aggressive behaviour, On the other hand, Gherardi *et al*., (2013) [11] mentioned that the difference in a water temperature did not influence on the aggressive behaviour of the fish. This disagreement may be owing to the change in species. So optimum water temperature improves Nile tilapia welfare.

Data in the Table (8) and Figure (11) revealed that midline crossing test (frequency) significantly affected by water temperature of aquaria, where group 3 (2.84 ± 0.25 bout) showed the highest mean frequency of crossing test and the lowest in group 1 (0.77 ± 0.27 bout). This finding attributed to temperature plays an essential role in fish activity and the increase of water temperature increases the respiratory mechanisms in fish leading to reduction of dissolved oxygen concentrations as recorded by Sorensen *et al*., (2014) [44]. This result also agreed with Johansen *et al*., (2006) [45] who mentioned that both dissolved oxygen concentrations and dissolved oxygen demand vary with water temperature. This result goes hand by hand with the data cited by Islam *et al*., (2020) [16] who reported that water quality parameters affected on swimming behaviour. For example, decreased dissolved oxygen levels (hypoxia) resulting from high water temperature of aquaria as in group 4 can decrease the swimming speed and activity.

The data presented in Table (9) revealed that average body weight significantly affected by water temperature of aquaria throughout weeks of experiment, where final body weight was the greatest in group 3 (45.61 ± 1.24^a g) and the lowest in group 1 (37.87 ± 2^b g). These results agreed with those obtained by Donaldson *et al*., (2008) [37] who found that variation in water temperature influence growth by decreasing feed intake. So the low water temperature of aquaria causes reductions in food intake and a decrease in growth. As well as the data agreed with Ndwiga, (2015) [18] who confirmed that the optimum water temperature range that showed the best growth for Nile tilapia is between 25 ^{0}C – 30 ^{0}C.

5. Conclusion

In the present work, the behavioural response of Nile tilapia fingerlings to low and high water temperature indicates that fish feel discomfort, pain and sufferance due to reduction of dissolved oxygen, loss of appetite and inability to express natural behaviour. All that in turn lead to poor welfare. While rearing of Nile tilapia fingerlings at optimum temperature (28°C) provides good welfare conditions owing to the optimum growth performance and the ability to express their natural behaviour.

6. Recommendation

It is recommended that water temperature must be taken into consideration due to its great impact on Nile tilapia growth and welfare for achieving maximum productivity and profitability.

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