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Behavioral Responses of Rainbow Trout (*Oncorhynchus mykiss*) in the Face of Some Environmental Factors (Temperature and Dissolved Ammonia)

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Abstract

The aim of this study was assessed behavioral responses of rainbow trout (*Oncorhynchus mykiss*) exposed to some of the environmental factors (temperature and dissolved ammonia). For this purpose, 16 fish in a group control, treating ammonia (treatment 1) and temperature (treatment 2) with four replications was placed in tanks cylindrical volume of 5 l (height 10 cm, diameter section 29 cm) that each tank has a water inlet and outlet, respectively. For all treatments tested to two periods of time (Went and Back) 42 min and a 10-min period (Fixed) were divided. Pattern of fish swimming movements simultaneously from the digital camera (Canon, SX230 Hs, 5.0-70 mm) was recorded. Swimming pattern includes six characteristic average swimming speed, total distance traveled, acceleration level (leap), percentage movements, mean shift displacement and the average distance from the center. Went step includes increasing temperature values of 17°C to 32°C for temperature treatment and ammonia increasing from 0.05 mg/L to 10 mg/L for treatment of ammonia. In contrast, the back step includes reducing values listed to initial amount was. Between swimming patterns of treatment compared with control there was a significant difference (p<0.05). Between swimming patterns of treatment there was significant difference in comparison with each other (p<0.05). The results of this study suggest that behavioral responses that can be used as early signs of environmental changes.

***Keywords:** Behavioral responses; Swimming pattern; Rainbow trout (Oncorhynchus mykiss); Environmental changes (temperature and ammonia)*

Introduction

Fish like other organisms to ensure its survival and spread of generation, must adapt to its environment [1]. Aquaculture, in addition to being a growing industry in recent years is a reliable way to secure supplies of natural ecosystems as well [2]. Intensive fish culture, spontaneously obstacles such as transportation, density, feeding, water quality and disease risk [3]; these problems led to the reduction of water quality which this event reduced profitability associated [4]. Salinity, temperature, ammonia, dissolved oxygen reduced and diseases are the limiting factors of affecting the quality of culture [5].

Fish are cold blooded animals (poikilothermic). Body temperature is equal to the ambient temperature water. Metabolism of rainbow trout at a lower temperature than 20°C higher performance and at a temperature of 15°C best performances [6]. Many fishes in the natural environment can tolerate a wide range of changes in temperature; however, the temperature increase should not occur suddenly or in other words the thermal shock [7,8]. Ammonia in the aquatic environment in two form ammonium ions (NH_4^+) and molecular form ammonium (NH_3) in aqueous and biological fluids found [9], that molecular shape it is dangerous to aquatic organisms. Ammonia with impact on neuronal function affects brain activity [7]. Ammonia poisoning has symptoms such as anxiety,

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increased breathing and irregular moving operculum, swim near the surface, increased mucus and skin pallor, intense swimming and loss of balance; in severe infections we see fish jumping out of water, swimming unbalanced and mortality with an open mouth [9].

To this day there have been several studies on the fish behavior. For example, Smith and Bailey [10] examined the behavior of fish in relation to environmental changes [11]. Effect of sub-lethal levels of pesticides on the swimming behavior of fish examined; Also, Vogel et al., [12], studied on identification of specific behaviors sub-lethal concentrations of toxins in fish studied. Previous studies indicate the fact that the manipulation and physiological changes, can lead to changes in fish behavior [13]; so, familiarity with certain behaviors (natural and unnatural) to manage stress is critical [4,14]. Behavioral changes in living beings Faced with environmental stresses and different concentrations of toxins, new information and innovative compared to traditional methods of toxicology (e.g., short-term and long exposure in fish exposed to deadly toxins and poisons as well as case fatality rate) in provide which gives access to this data of the previous track is not possible [11,15,16]; However, studies of behavior is growing slowly, because changes in behavior may be related to environmental factors such as hunting, Hunting bait, stress resistance, longevity and reproduction [17].

The study, the answer to the question of whether fish faced with environmental changes incidence of the same behavioral pattern or depending on the type of change, will develop a different behavioral pattern. Knowing this, it is vital for water resource management. Better and wider understanding of the behavioral characteristics of the physiological characteristics aquaculture and understanding of the relationship between the two leads to the efficient and effective management.

Material and Methods

Two hundred rainbow trout with an average weight of 5 g of hatchery and breeding (Dashte Sabz 2km, Firuzkuh, Tehran, Iran) was prepared. The fish were transferred to the Aquaculture salon of Faculty of Fisheries, University of Agricultural Sciences and Natural Resources, Gorgan, Iran. The fish in a circular fiberglass tanks 270 l in similar circumstances to adapt to the laboratory conditions were maintained for two weeks. Dewatering volume of 250 l for each tank, the number of fish in each tank was 50. During this time the fish to the diet Biomar (FFT1) for two meals a day and about 3% by weight fish were feeding.

Ammonia used in these tests, product laboratory doctor Mojalali (No. 15, alley Shahrokh, near the Abbas Abad, Street Suhrawardi north, Tehran, Iran) with a purity of 25 percent. Was used for heating water of six water heaters (electric element waterproof), 200 volts Manufacturing Co. Atman.

We used to prepare the test environment of 16 cylindrical tank with a volume of 5 liter (height of 10 cm and a diameter of 29 cm). Each of the tanks has a water inlet and outlet ducts, which the two channels from each other, were at the farthest distance possible. Water inlet and outlet ducts, respectively, on the walls and bottom of tank were created. Quickly enter and exit the water in all reservoirs was 2 ml/sec. Video data at each stage of the tests by four digital cameras (Canon, SX230 Hs, 5.0-70 mm) were recorded (FIG. 1).

After completing adaptation time, twelve fish (control, treatment temperature, the ammonia treatment) were randomly selected from primary storage tanks and two five-liter containers (test environment) were transferred; each five-liter tank contains only one fish. For greater compatibility, this transfer was done 24 hrs before the start of the experiment. Also at this time the fish were not feeding. Physicochemical conditions of water during the period of adaptation was fixed (temperature 16.4°C, ammonium 0.025 mg/Lit, 80% oxygen saturation and pH = 7.6), and was similar in all tanks.

Experiment was divided into three stages. Firstly, the increase in ammonia for ammonia treatment from 0.05 mg/Lit to 10 mg/Lit, and increasing the temperature for the treatment temperature from 17°C to 32°C, respectively. Time the stage was set for 42 min. The second stage was 10 min and continues to be tested first stage. At this stage the fish are exposed to each treatment for 10 min at 32°C and a concentration of 10 mg/Lit of ammonia was used. The Third stage test, contrary first stage the test. This stage includes reducing the amount of ammonia and temperature in the order of 10 mg and 32°C to its initial value at the beginning of first stage the test (FIG. 2).

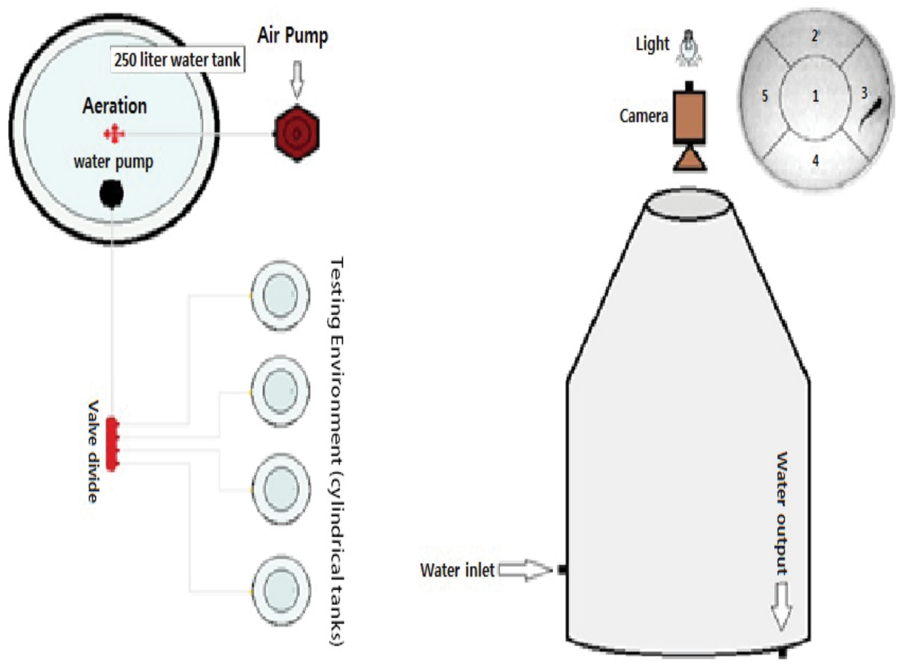

FIG. 1. **Schematic diagram represents test environment with its components and systems for recording and collecting information. 250-liter water storage tank, aeration with an air pump, water pump to deliver water to the test environment, tap and fixed in each division to have a steady stream of tanks, environment test has a water input and output, digital video cameras with detachable memory for recording images and the light source 60 amperes for better recording.**

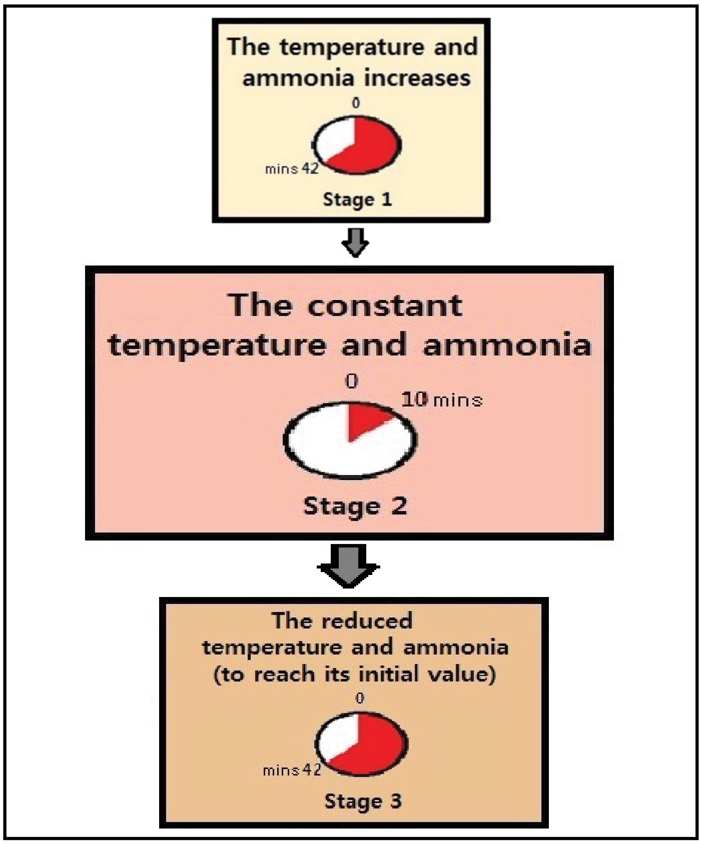

FIG. 2. **Schematic diagram is showing the time each test interval with the testing process.**

During the three stages of testing, except the desired parameters (temperature and ammonia) other water physicochemical parameters were fixed and unchanged.

The images produced by the software to analyze images and pictures Adobe after effects (AAE CS6) were analyzed. Fish movement patterns according to the TABLE 1 were evaluated in three periods. Testing was divided into 10 equal time intervals (eleven minutes). Five elementary interval (go) is related to the increase in temperature, and ammonia and five period end (back) of the decrease in temperature and ammonia (FIG. 3).

Data obtained using one way ANOVA (ANOVA) was evaluated using SPSS software. Significant differences between the mean levels of 0.05 via LSD test were performed.

Results and Discussion

Between swimming patterns of treatments (treatment temperature and ammonia treatment) compared with the control group there was a significant difference ($p<0.05$). Also, the swimming patterns of treatments compared with each other, there are also significant differences ($p<0.05$); The results of the observations are shown in TABLE 2. Analysis of the data by using Spearman correlation indicators of the average swimming speed, total distance traveled (TABLE 3), the accelerating and changing the direction of movement indicated in the treatment ammonia (TABLE 4); While in the treatment temperature, only between the average swimming speed and total movements correlation was detected (TABLE 5). Comparison patterns of fish swimming in the same concentration, represents a significant difference between the pattern of fish swimming in step one compared with step third ($p<0.05$).

The fishes at high temperatures and high concentrations of ammonia in an overall assessment have the highest average swimming speed, highest average distance from the center, highest average change the angle movement and highest movement. Has the highest of the fish were high concentrations of ammonia, the index continues the test (Stage 3) have declined sharply. Other word fish in this stage often remained unmoved near the water inlet; Due to stop and stay resident Fishes in the third stage, the bulk of the movement fishes at this stage as accelerating interpreted because the average speed of fishes in the third stage due to a long stop Fishes, very small.

Average speed of fish swimming in the ammonia treatment proportional to the increase in ammonia concentration increased. Total distance traveled, and average change in the direction of the displacement was increased. Increasing the concentration of the ammonia was also led to an increase in the total movement, percent move and average change the angle movement. Fishes, at this concentration have signed, including switch sequentially direction, a disorder in the movement of gill cover, dark skin, restlessness and finally had to jump in the water. Increased ammonia concentration (40 mg), ultimately swimming unbalanced, spin to your round and death of the fish with an open mouth.

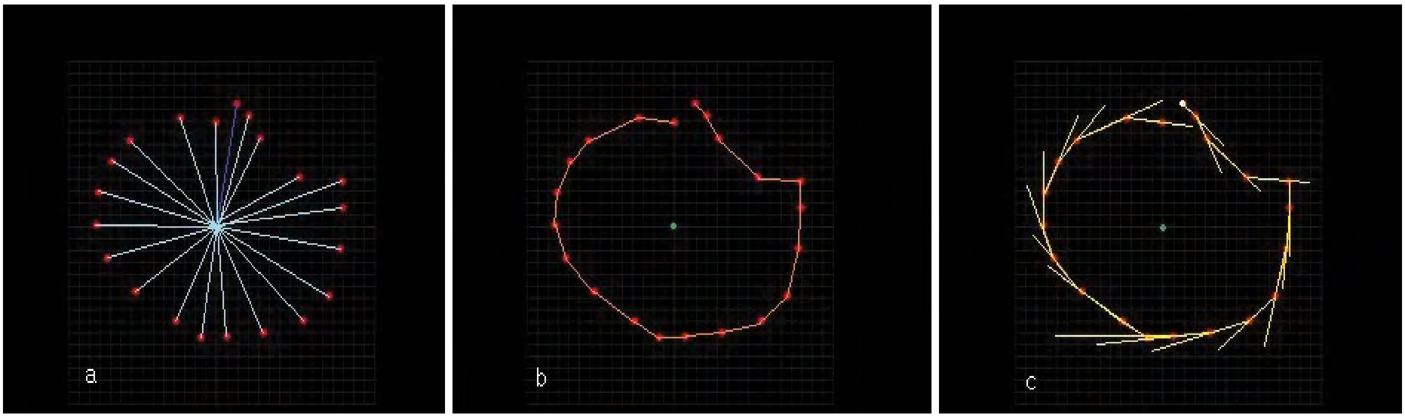

FIG. 3. **Trace the trajectory of fish in the first twenty seconds of motion (images using Adobe After Effects software to analyze images and photos obtained). a) The average distance from the center; b) The total movement; c) The average angle of movement.**

TABLE 1**. Characteristics measured in relation to the swimming pattern, items listed in the table, from table Kane et al. [17] have adapted and optimized.**

Behavioral parameters	Definition
Average speed swimming (Aver. Speed)	Average speed of the fish at the time 20 seconds, when the fish move to x cm.
Total movement (Tot. Move.)	Total movement of fish in 20 seconds.
Acceleration (Relative burst)	The total distance that the fish in the time interval X (the equivalent a second), his speed is more than double the average pace in twenty seconds.
Percent move (Per. Move)	When the fish the equivalent two-thirds of their body length to be replaced, has moved. The number of moving parts in Total elapsed time, multiplied by the percentage represents the percentage of motion.
Average change the angle movement (A.C.A.M)	The angle differences point X2 to X1 when the point X0 is beginning to move.
Average distance from center (A.D.C)	Average Distance from the center of the fish tank in total elapsed time.

TABLE 2. **Shows swimming pattern changes during the stages of the experiment in the treatments and control group. a) Average of swimming speed (cm/sec.), b) Total movement (cm), c) Acceleration (cm), d) Percent move (%), e) Average change the angle movement (θ˚), f) Average distance from center (cm).**

Stage 1					Stage 2		Stage 3			
	0-11 min	11-22 min	22-32 min	32-42 min	10 min		0-11 min	11-22 min	22-32 min	32-42 min
					5 min	5 min				
Control	2.50 ± 0.32[b]	3.31 ± 0.20[a]	2.94 ± 0.23[b]	3.75 ± 0.52[b]	3.31 ± 0.54[c]	3.40 ± 0.16[b]	3.80 ± 0.25[b]	2.28 ± 0.20[b]	4.80 ± 0.16[a]	3.11 ± 0.28[b]
Treatment ammonia	3.52 ± 0.10[a]	3.14 ± 0.92[a]	3.59 ± 0.65[b]	2.56 ± 0.06[c]	4.04 ± 0.54[b]	7.26 ± 1.89[a]	2.61 ± 0.46[c]	0.91 ± 0.22[c]	0.75 ± 0.30[b]	0.31 ± 0.08[c]
Treatment temperature	3.9 ± 0.32[a]	4.04 ± 0.21[a]	4.95 ± 1.27[a]	5.83 ± 0.40[a]	5.23 ± 0.21[a]	7.30 ± 0.27[a]	5.17 ± 0.23[a]	4.66 ± 0.41[a]	4.46 ± 0.31[a]	1.7 ± 0.14[a]

2a) Average speed swimming (cm/Sec.); Values are expressed as mean ± SD. Same alphabets indicate no significant difference between the values of the same column.

Stage 1					Stage 2		Stage 3			
	0-11 min	11-22 min	22-32 min	32-42 min	10 min		0-11 min	11-22 min	22-32 min	32-42 min
					5 min	5 min				
Control	50.06 ± 6.48[b]	66.28 ± 4.08[a]	58.95 ± 4.62[b]	74.99 ± 10.33[b]	66.34 ± 1.11[c]	68.08 ± 3.26[b]	76.18 ± 4.96[b]	45.54 ± 4.06[b]	96.08 ± 3.25[a]	62.23 ± 5.60[b]
Treatment ammonia	70.40 ± 1.98[a]	62.91 ± 18.22[a]	71.90 ± 13.10[b]	51.20 ± 1.21[c]	80.80 ± 10.77[b]	145.91 ± 37.25[a]	52.17 ± 9.16[c]	18.32 ± 4.18[c]	14.96 ± 3.89[b]	6.28 ± 1.64[c]
Treatment temperature	78.00 ± 6.32[a]	80.76 ± 4.34[a]	99.00 ± 25.43[a]	116.75 ± 8.06[a]	104.60 ± 4.31[a]	146.10 ± 5.49[a]	103.40 ± 4.41[a]	93.20 ± 8.32[a]	89.20 ± 6.32[a]	74.00 ± 2.80[a]
Values are expressed as mean ± SD. Same Alphabets indicate no significant difference between the values of the same column.										

2b) Total movements (cm).

	Stage 1				**Stage 2**		**Stage 3**			
	0-11 min	11-22 min	22-32 min	32-42 min	10 min		0-11 min	11-22 min	22-32 min	32-42 min
					5 min	5 min				
Control	15.56 ± 3.7^{a}	0.00 ± 0.00^{b}	0.00 ± 0.00^{b}	0.00 ± 0.00^{b}	6.72 ± 1.59^{c}	0.00 ± 0.00^{c}	0.00 ± 0.00^{c}	0.00 ± 0.00^{c}	9.18 ± 1.34^{c}	0.00 ± 0.00^{b}
Treatment ammonia	0.00 ± 0.00^{c}	0.00 ± 0.00^{b}	5.70 ± 0.38^{b}	14.46 ± 5.62^{a}	23.06 ± 5.62^{a}	0.00 ± 0.00^{a}	22.62 ± 2.39^{a}	11.17 ± 2.10^{b}	13.17 ± 1.97^{b}	0.00 ± 0.00^{b}
Treatment temperature	7.15 ± 1.42^{b}	18.32 ± 0.62^{a}	60.87 ± 1.83^{a}	11.16 ± 1.05^{a}	10.47 ± 1.05^{b}	0.07 ± 0.01^{a}	16.72 ± 0.41^{b}	53.21 ± 1.23^{a}	44.58 ± 0.49^{a}	1.33 ± 0.66^{a}
1.34 Values are expressed as mean ± SD. Same Alphabets indicate no significant difference between the values of the same column.										

2c) Acceleration (cm).

	Stage 1				**Stage 2**		**Stage 3**			
	0-11 min	11-22 min	22-32 min	32-42 min	10 min		0-11 min	11-22 min	22-32 min	32-42 min
					5 min	5 min				
Control	80.00 ± 9.13^{b}	99.25 ± 0.50^{a}	98.00 ± 0.00^{a}	85.00 ± 4.08^{a}	95.00 ± 0.00^{b}	95.00 ± 5.77^{ab}	98.00 ± 5.77^{a}	90.00 ± 5.77^{a}	98.25 ± 0.50^{a}	95.00 ± 0.00^{b}
Treatment ammonia	98.00 ± 0.00^{a}	98.00 ± 0.82^{b}	75.00 ± 5.77^{b}	75.00 ± 7.07^{b}	98.00 ± 0.00^{a}	98.00 ± 0.00^{a}	60.00 ± 4.08^{b}	40.00 ± 7.07^{c}	40.00 ± 0.00^{c}	5.00 ± 0.00^{c}
Treatment temperature	98.00 ± 0.00^{a}	80.00 ± 0.00^{c}	80.00 ± 4.08^{b}	85.00 ± 5.77^{a}	90.00 ± 0.00^{c}	91.25 ± 4.79^{b}	95.00 ± 0.00^{a}	81.25 ± 7.50^{b}	75.00 ± 5.77^{b}	98.0 ± 0.00^{a}
	Values are expressed as mean ± SD. Same Alphabets indicate no significant difference between the values of the same column.									

2d) Percent move (%).

	Stage 1				**Stage 2**		**Stage 3**			
	0-11 min	11-22 min	22-32 min	32-42 min	10 min		0-11 min	11-22 min	22-32 min	32-42 min
					5 min	5 min				
Control	27.68 ± 2.17^{c}	34.11 ± 1.76^{b}	29.68 ± 1.57^{b}	31.16 ± 1.44^{b}	38.95 ± 1.01^{c}	43.31 ± 1.60^{b}	38.53 ± 4.90^{a}	29.94 ± 1.98^{b}	46.65 ± 4.11^{a}	39.5 ± 3.82^{a}
Treatment ammonia	32.78 ± 3.17^{a}	26.22 ± 1.38^{c}	27.2 ± 1.61^{b}	26.43 ± 2.48^{c}	67.83 ± 6.53^{a}	102.00 ± 12.35^{a}	24.75 ± 6.60^{b}	27.25 ± 2.63^{b}	21.32 ± 1.76^{b}	2.14 ± 1.44^{c}
Treatment temperature	32.1 ± 0.62^{a}	43.16 ± 0.63^{a}	47.9 ± 9.43^{a}	54.73 ± 0.31^{a}	48.37 ± 0.52^{b}	41.05 ± 1.05^{b}	42.32 ± 0.94^{a}	39.27 ± 0.32^{a}	43.64 ± 0.87^{a}	1.60 ± 0.70^{b}
1.61 Values expressed as mean ± SD. Same Alphabets indicate no significant difference between the values of the same column										

2e) Average changes the angle movement (ϴ°).

Stage 1					**Stage 2**		**Stage 3**			
	0-11 min	11-22 min	22-32 min	32-42 min	10 min		0-11 min	11-22 min	22-32 min	32-42 min
					5 min	5 min				
Control	9.4 ± 0.60a	9.09 ± 0.66b	8.79 ± 0.75b	9.15 ± 0.44b	8.22 ± 0.36b	7.23 ± 0.85b	7.43 ± 0.65b	4.94 ± 0.36b	7.40 ± 0.85c	8.20 ± 0.55c
Treatment ammonia	8.02 ± 0.42b	10.50 ± 1.31a	12.20 ± 0.72a	11.78 ± 1.15a	12.80 ± 1.36a	10.69 ± 1.13b	11.52 ± 0.41a	11.50 ± 0.54a	10.44 ± 0.61a	11.81 ± 0.95a
Treatment temperature	9.37 ± 0.69a	10.32 ± 0.60a	11.20 ± 0.72a	9.45 ± 1.03b	11.42 ± 0.34b	11.42 ± 0.41a	10.54 ± 0.41a	10.44 ± 1.32a	9/35 ± 0.91b	1.24 ± 0.09b
Values are expressed as mean ± SD. Same Alphabets indicate no significant difference between the values of the same column										

2f) Average distance from center (cm).

TABLE 3. **Shows the correlation between parameters investigated in conjunction with the pattern of fish swimming in the ammonia treatment.** **

	Aver. Speed	Tot. Move.	Acceleration	Per. Move	A.C.A.M	A.D.C
Aver. Speed	-	1.00**	-0.44	0.92**	0.82**	0.11
Tot. Move.	-	-	-0.44	0.92**	0.82**	0.11
Acceleration	-	-	-	-0.16	-0.25	0.45
Per. Move	-	-	-	-	0.73*	-0.06
A.C.A.M	-	-	-	-	-	0.02
A.D.C	-	-	-	-	-	-
Correlation at a significance level of 0.01. * Correlation at a significance level of 0.05.						

TABLE 4. **Evaluate the pattern of fish swimming in the same concentrations ammonia in the ammonia treatment.**

Parameters	Ammonia concentrations (ml/lit)	Stage 1	Stage 3	*p*
Average Speed swimming (cm/sec.)	0.09	3.52 ± 0.99	0.31 ± 0.82	0.00**
	2.68	3.14 ± 0.92	0.75 ± 0.19	0.02*
	5.23	3.59 ± 0.65	0.91 ± 0.21	0.00**
	7.96	2.56 ± 0.60	2.61 ± 0.46	0.84
	10	4.04 ± 0.54	7.26 ± 1.89	0.03*
Total movement (cm)	0.09	70.40 ± 1.99	6.28 ± 0.82	0.00**
	2.68	62.91 ± 18.22	14.96 ± 3.89	0.02*
	5.23	71.90 ± 13.1	18.32 ± 4.18	0.00**
	7.96	51.2 ± 1.21	52.17 ± 9.16	0.84
	10	80.80 ± 10.77	145.90 ± 1.89	0.03*
Acceleration (cm)	0.09	0.00 ± 0.00	0.00 ± 0.00	1
	2.68	0.00 ± 0.00	13.17 ± 1.97	0.00**
	5.23	5.7 ± 0.38	11.17 ± 2.10	0.02*
	7.96	14.2 ± 1.21	22.62 ± 2.39	0.03*
	10	23.06 ± 2.23	0.00 ± 0.00	0.00**
Percent move (%)	0.09	98.00 ± 0.00	5.00 ± 0.00	0.00**
	2.68	98.00 ± 0.82	40.00 ± 0.00	0.00**
	5.23	75.00 ± 5.77	40.00 ± 7.07	0.00**
	7.96	75.00 ± 7.07	60.00 ± 4.08	0.01**
	10	98.00 ± 00	98.00 ± 0.00	1
Average change the angle movement (θ˚)	0.09	32.78 ± 3.17	2.14 ± 0.95	0.00**
	2.68	26.22 ± 1.38	21.32 ± 1.76	0.05*
	5.23	27.20 ± 1.61	27.25 ± 2.63	0.97
	7.96	26.43 ± 2.48	24.75 ± 6.60	0.65
	10	67.83 ± 6.53	102.00 ± 12.35	0.3*
Average Distance from center (cm)	0.09	8.02 ± 0.42	11.80 ± 0.95	0.00**
	2.68	10.50 ± 1.30	10.44 ± 0.61	0.93
	5.23	12.20 ± 0.72	11.50 ± 0.54	0.17
	7.96	11.78 ± 1.15	11.52 ± 0.41	0.68
	10	12.80 ± 2.23	10.69 ± 1.13	0.05
Values are expressed as mean ± SD. ** There is a significant difference 0.01. * There is a significant difference 0.05.				

TABLE 5. **Shows the correlation between parameters investigated in conjunction with the pattern of fish swimming in the temperature treatment.**

	Aver. Speed	Tot. Move.	Acceleration	Per. Move	A.C.A.M	A.D.C
Aver. Speed	-	1.00**	-0.24	0.11	0.43	0.55
Tot. Move.		-	-0.24	0.11	0.43	0.55
Acceleration			-	-0.76*	0.16	0.26
Per. Move				-	-0.48	-0.11
A.C.A.M					-	0.05
A.D.C						-
**Correlation at a significance level of 0.01. * Correlation at a significance level of 0.05.						

TABLE 6**. Evaluate the pattern of fish swimming in the same temperature in the temperature treatment.**

	Temperature (°C)	Stage 1	Stage 3	*p*
Average Speed swimming (cm/sec.)	17	3.90 ± 0.32	3.70 ± 0.14	0.29
	21	4.04 ± 0.31	4.46 ± 0.31	0.07
	25	4.95 ± 1.27	4.66 ± 0.41	0.68
	28	5.83 ± 0.40	5.17 ± 0.23	0.03*
	32	5.23 ± 0.21	7.30 ± 0.27	0.00**
Total movement (cm)	17	78.00 ± 6.32	74.00 ± 2.8	0.29
	21	80.76 ± 4.33	89.20 ± 6.32	0.07
	25	99.00 ± 25.43	93.20 ± 8.31	0.68
	28	103.40 ± 4.61	116.75 ± 8.06	0.03*
	32	104.60 ± 4.31	146.07 ± 5.49	0.00**
Acceleration (cm)	17	7.15 ± 1.42	10.33 ± 0.66	0.07
	21	18.23 ± 0.62	44.58 ± 0.49	0.00**
	25	60.87 ± 1.83	53.21 ± 1.23	0.00**
	28	11.16 ± 1.05	16.72 ± 0.41	0.00**
	32	10.47 ± 0.38	0.07 ± 0.01	0.00**
Percent move (%)	17	98.00 ± 0.00	98.00 ± 0.00	1
	21	80.00 ± 0.00	75.00 ± 5.77	0.13*
	25	80.08 ± 2.04	81.25 ± 7.50	0.78
	28	95.00 ± 0.00	85.00 ± 5.77	0.01**
	32	90.00 ± 0.00	91.25 ± 4.79	0.64
Average change the angle movement (θ˚)	17	32.10 ± 0.62	35.60 ± 0.70	0.00**
	21	43.16 ± 0.63	43.64 ± 0.87	0.41
	25	43.16 ± 0.63	39.27 ± 0.32	0.00**
	28	54.73 ± 0.31	39.27 ± 0.32	0.00**
	32	48.37 ± 0.52	41.05 ± 1.05	0.00**
Average Distance from center (cm)	17	9.37 ± 0.69	9.24 ± 0.09	0.72*
	21	10.32 ± 0.59	9.35 ± 0.09	0.01**
	25	11.20 ± 0.72	10.44 ± 1.44	0.35
	28	9.45 ± 1.03	10.54 ± 0.42	0.09
	32	9.32 ± 1.15	11.42 ± 0.34	0.01**
Values are expressed as mean ± SD. ** There is a significant difference 0.01. * There is a significant difference 0.05.				

Average speed of fish swimming in the temperature treatment in the first stage of the experiment with increasing the temperature is increased. The average swimming speed, total movement and percent move against of ammonia treatment, in the treatment temperature during the third stage test compared with the first stage of testing in most similar temperatures not significantly different (p>0.05). Fishes at elevated temperatures (28°C to 32°C) behavioral symptoms (like ammonia treatment) such as swimming fast, dark color, shifting the direction of swimming, jumping out of the water and were restless.

Interpretation of swimming pattern changes. In the first stage to the third stage is very difficult. For example, if we want decide about total average swimming speed in the first stage compared to the the third stage in the ammonia treatment, shall observe that the average swimming speed In the first stage (3.37 cm/sec) compared to the third stage (2.14 cm/sec), there is no Significant difference ($p>0.05$). While compared to the same point to point of ammonia concentration in the ammonia treatment is very evident this difference ($p<0.05$). Therefore, the information contained in the diagrams are important.

Diagram patterns of fish swimming in the ammonia treatment (DIAGRAM 1), shows the main parameters investigated follow the pattern polynomial graph with a gradient increase or decrease.Average speed of movement in the face of rising levels of ammonia rainbow trout follows a polynomial Diagram with a gradient increase. The average speed of fish in the face of decreasing levels ammonia follows a quadratic Diagram with a gradient decline. In the first stage test with a gradual increase ammonia, increasing the average speed of swimming fish pattern does not follow a consistent trend; In other words, up to concentration of 8 mg/Lit average swimming speed slowly increases. Through the ammonia concentration of this point, immediately increasing average swimming speed. Interpretation of diagram of total movement, the accelerating and average change the angle movement similar graph of average swimming speed of fish.

The average distance from the center of the diagram indicates that the these indicators to ammonia treatment in both the testing phase (Stage 1 and Stage 3) was higher than the control ($p<0.05$). There was also the conditions on the treatment temperature. The fish tend to swim near the walls of the tank and hit the wall can be interpreted as a the desire of to leave fish and trying to move away from the source of contamination.

DIAGRAM 2, related to the changes of swimming pattern in the temperature treatment. Unlike ammonia treatment, the comparison between the indicators obtained at the same temperature shows between the average swimming speed, total movement and move percent in the first stage test compared to the last stage of testing for the most part, there is no significant difference (TABLE 6). Although the average swimming speed diagram in the first phase of testing is is a logarithmic diagram with the slope of the diagram while the third stage is is a polynomial diagram with a steep downward; But it is important to note that unlike ammonia treatment, the average swimming speed, followed by the total movement in the temperature treatments (three phase test) is more than the control group.

Accelerating (Relative burst), indicating the distribution of the data. Whatever it is low Presence of uniform motion at a steady rate per unit of time. Unlike the control group, the the treatment ammonia and temperature the treatment often we witness the fragmentation and lack of uniformity. Diagram of rate accelerating in the first stage and the third stage is in the form of a parabola with the difference that the return diagram has more height. The value of this index in both stage tests higher than the control group (DIAGRAM 2c). By looking at the the diagram (DIAGRAM 2d), we find that the percen move with increasing temperature is reduced. The temperature treatment unlike ammonia treatment percent move at the beginning of the first stage of testing is reduced and fish spends the greater part of the time constant.However, from the middle of the track to the next percent move further and at the end of the first part is the amount of control group.

Look at a pattern of fish swimming in the control group was a constant and uniform pattern. in most situations the fish are swimming. Fish more often than in the control group were hovering in the middle of the container and the water inlet. Dispersion of movement or rather the accelerating rate was zero in many times and the fish were moving quite smoothly.During the test there was no alternate route change, color change, jumping out of water, lack of balance the body and fish mortality in the control group.

Pattern of fish swimming in the ammonia treatment was in direct relation with ammonia. Increasing the amount of ammonia leads to increased the average of swimming speed. Fish by moving towards the wall and outlet of water trying to find a way out of the environment. With the continuous increase of ammonia in the tank the amount accelerating will rise and mucus secretion caused by the darker color of the fish. Following the first stage of test increase of ammonia leads to increased continuously accelerating, scattering move, continuously changing the direction of swimming, restlessness, darkening of the skin caused by mucus secretion and jumping out of water. The third stage of test in ammonia treatment was different from the other group; the Fishes were mainly

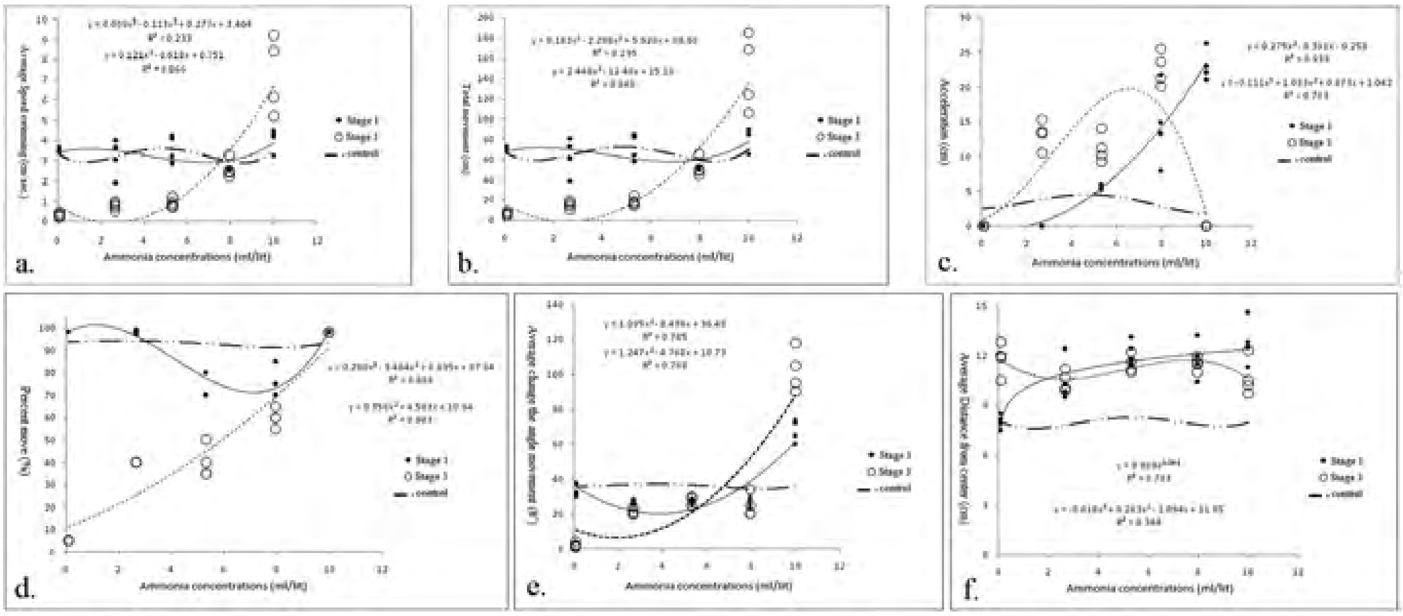

DIAGRAM 1. **Shows changes in the pattern fishes swimming towards the concentration of ammonia in the ammonia treatment. a) Average speed swimming (Aver. Speed). b) Total movement (Tot. Move.). c) Acceleration (Relative burst). d) Percent move (Per. Move). e) Average change the angle movement (A.C.A.M). f) Average distance from center (A.D.C)**

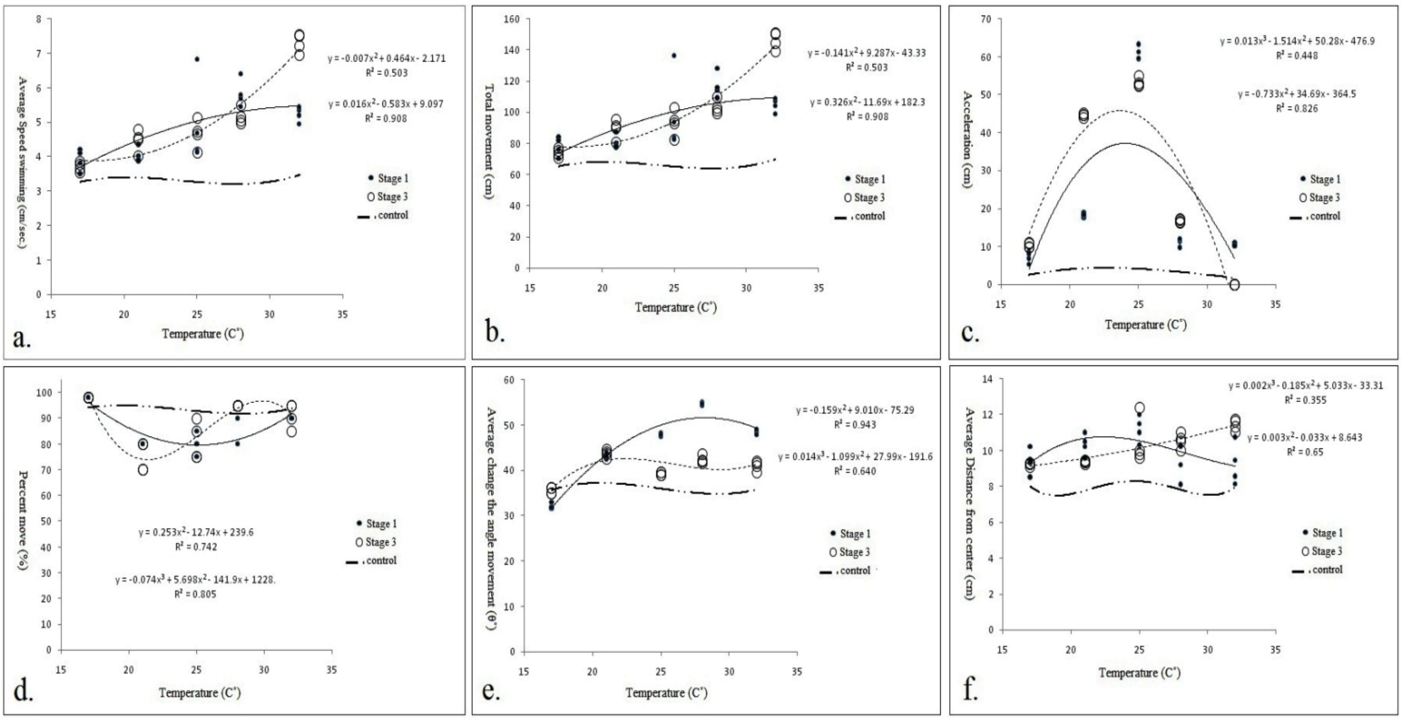

DIAGRAM 2. **Fishes swimming the pattern changes with respect to temperature changes in the temperature treatment. a) Average speed swimming (Aver. Speed). b) Total movement (Tot. Move.). c) Acceleration (Relative burst). d) Percent move (Per. Move). e) Average changes the angle movement (A.C.A.M). f. Average distance from center (A.D.C).**

fixed and immobile near the wall or water inlet.

Although the trend of average swimming speed, total movement, the amount of accelerating, average change the angle movement and distance from the center in the first stage of testing in temperature treatment were almost like the ammonia treatment, but the pattern fish swimming in the temperature treatment has a unique feature. Percent move of fish in the temperature treatment during the first stage of the test fell to lower levels of the control group. Although at the end of the first stagepercent move reachesto the control group, but did not exceed the average of the control group. The interesting point is that despite reduction of percent move, due to higher average speed swimming of fish temperature treatment than the control group total movement in this stage more than the control group. Most of the difference between treatments swimming pattern is related to the third stage of testing. At this stage the pattern of fish swimming in temperature treatment for the most part similar was with the first stage (unlike ammonia treatment).

General symptoms such as darkening of the skin, lack of stability, restlessness, switch sequentially the direction of swimming, jumping out of the water and swim in the water surface were recorded in both treatments (at the end of the first stage and the second stage of test).

Previous studies indicate the fact that the manipulation and physiological changes can be force a change in the fish behaviors [13]. In this study were examined the effect of temperature and ammonia on the fish swimming patterns. Swimming patterns studied include the Average speed swimming, the total movement, the accelerating (Relative burst), the Percent move, Average change the angle movement and the distance from the center. The results showed that changes of temperature and ammonia influence on the swimming patterns. The results clearly show there are significant differences between the patterns of fish swimming in different stages of testing; also treatments compared with each other and compared with the control, there was a significant difference ($p<0.05$). The average speed rainbow trout swimming was increased in the face of increasing amounts of ammonia and temperature. The total movement and distance from the center as an indicator to describe the trend of fish to escape from the environment commensurate with increasing amount of the ammonia and temperature, increased ($p<0.05$).

Kane et al. [17], studied a video-based movement analysis system to quantify behavioral stress responses of fish. The results of their study showed that fishes that are exposed to environmental stress (different amounts of poison MS222) swimming patterns have different in terms of average swimming speed, total movement, Average changes the angle movement and the complexity of the path compared with the control group ($p<0.05$). The results of their studies were consistent with the results of our study. Rainbow trout exposed to different concentrations of dissolved ammonia and different temperatures compared with the control group have different swimming patterns in terms of average swimming speed, total movement, accelerating, percent move, Average changes the angle movement and distance from the center ($p<0.05$).

Thurston et al. [9] stated, poisoning the ammonia has a variety of symptoms such as anxiety, increased breathing and irregular operculum moves, swimming close to the surface, increased mucus and skin discoloration (color change), swimming a fast and loose the balance that this was in accordance with the present investigation; The fish were exposed to high levels of the ammonia solution, showed a collection of symptoms including jumping out of water and rising motion the branchial cover. Also the results of the ammonia toxicity preliminary tests showed that fish exposed to hyper acute the ammonia concentrations (67 ml/lit) have a set of symptoms such as jumping out of water, unbalanced swimming, quick movements, unusual operculum moves and mortality with open mouth that this set of symptoms with Thurston et al. [9] studies were well correlated.

Henry and Athinison [14] examined the effects of three copper concentrations on motion behavior and swimming patterns of Bluegills (*Lepomis macrochirus*). The results of their study showed that fish were exposed to different concentrations of copper showed symptoms such as anxiety and aggression. They also stated that percent move and total movement in fishes exposed to different concentrations of copper were significantly different to the control group ($p<0.05$). The results of percent study were consistent with the results of studies [14]. According to the results of the present study and previous studies, it can be concluded increase swimming speed and movements are fish public response in dealing with all kinds of contaminants. These responses due to fishes tend to escape the pollution source.

Little et al. [18], studied the effects of sub-lethal levels of six agricultural pesticides on the rainbow trout swimming patterns. They in their study examined the four behavior factors, including the move or swimming, percent move, feeding behavior and the ability to escape from predators. At last little and colleagues, said that the fishes for 96 hr were exposed to these toxins has different behavioral responses to the control group. They also in another part of their study, stating that the behavioral responses the six different treatments, although have common points but there are significant differences. According to data obtained from the ammonia treatment and temperature treatment in all three-testing stage we can see the results of this experiment are consistent with the results of the Little and colleagues experiments [18]. Pattern fishes swimming at all stages of the experiment, different than the control group. Nevertheless, in the first stage of experiment, the average swimming speed and total movement in both treatments were similar process (the upward trend), but there are differences. In the first stage tests, the percent move of temperature treatment unlike ammonia treatment not only did not exceed the level of the control group, but also fell below the surface of control group and only at the end of the first stage to reach their level. The third stage of tests showed that the biggest difference between treatments and control groups ($p<0.05$).

Little and Finger [11], studied the effect of sub lethal levels of toxins on fish swimming behavior that results of their study were similar to our results. Their studies show that the fish swimming behaviors is impaired in the face with varying amounts of pollutants; this difference was observed in all treatments (ammonia treatment and temperature treatment).

Israeli and Kimmel [13], examined goldfish behaviors (*Carassius auratus*) in face of hypoxia-stressed using computer vision monitoring. The results of their study were three approaches; the first approach was to investigate the changes in the fish swimming speed along with distance from the center. In addition to these factors, the complexity of the path and jump movements (Acceleration) also was studied. The results of their study showed that fish swimming speed reduced exposed to low-oxygen conditions (hypoxia-stressed). Based on the results of the study of Israel and Kimmel [13], Distance from the center increased in the fish that were exposed to low-oxygen conditions; these findings were consistent with the results of the stress test temperature and ammonia in the present study. To verify the results of tests Israeli and Kimmel [13] and present study, it can be concluded that fish exposed to environmental stresses have the desire to centrifugal from the source of contamination. Escape, most fish response to environmental stresses and this can be inferred from the data collected in the field characteristic distance of the center.

Brewer et al., [19] studied the behavioral and physiological rainbow trout in response with a chemical inhibitor of cholinesterase (Cholinesterase-inhibiting). The results of their study showed speed and movement in a linear ways by the treatments had a sharp decrease compared to the control group. They also stated, fishes that are a long time (96 hr) was exposed to chemical inhibitors compared to treatment (24 hr) have lower swimming speed and the passed linear ways. These effects did not improve even after the fish have been placed in fresh water for 48 hr. The average change the angle movement is character to say, the fish move in a straight line ways. Whatever rate of average change the angle movement higher, rate a linear movement of fish reduced and they has a more curved and complexity in their ways. However, Brewer and colleagues studied on cholinesterase-inhibiting, but the results of this study were consistent with the results of their study. Since Ammonia effect on nerve cell activity Svobodova et al., [7], may be ammonia as well as inhibitors of cholinesterase with effect on neurotransmitters, leading to changes in nervous system function of fish.

Ortega et al., [20], stated sub-lethal amounts of ammonia leads to an increase in plasma Cortisol levels and behavioral changes such as excessive mobility and suppress the appetite of the fish. The results of the ammonia treatments are corresponding to Comments Ortega and colleagues; mobility offish increased in the face of sub-lethal amounts of ammonia. However, this increased mobility is not permanent and then drops sharply.

Xu Jian-yu et al. [21], studied behavioral response of tilapia (*Oreochromis niloticus*) to ammonia stress monitored by computer vision. They put groups of young fish exposed to concentrations of low (0.13 ml/lit), medium (0.79 ml/lit) and high (2.65 ml/lit) ammonia. Well, as increased ammonia, swimming speed and distance from the center increased. Although our study was limited to individual study on rainbow trout, but the results of the ammonia treatment were consistent with the results of the experiments Xu Jian-yu and colleagues [21].

Kang et al. [22], studied the effect of chemical KCN and NaCN on small freshwater fish, Medaka (*Oryziaslatipes*). The results of the studies showed, these chemicals affect fish swimming speed and movement pattern; other words, with increasing concentrations of these chemicals in the environment fish swimming speed is also increasing. These results were consistent with the results obtained in the present study; Average Speed of swim in the treatment temperature, increased in the first stage of tests (temperature rise) and more than the average of the control group.

Yi Hang et al., [23], studied the effect of different concentrations of Deltamethrin in zebra fish (*Danio rerio*), the results of their testing showed that pollution has an effect on fish swimming patterns. Results of their studies were consistent with the results of our study, the swimming patterns of temperature and ammonia treatments compared to the control group, a significant difference ($p<0.05$). Yi hang et al., [23], in the other part of the results of their studies have stated that by changing the concentration of Deltamethrin, there is no significant difference between swimming speed in different concentrations that it did not match our study. With increasing temperature, the ammonia and swimming Speed were different in the different ranges ($p<0.05$).

We did not an article on the effects of sub-lethal levels of environmental pollution on fish behavior, for this reason the possibility of comparing the results of temperature and the ammonia treatments in the third stage tests did not exist with other studies. More resources in the field of behavioral responses of fishes in the face of environmental changes have attunement look. More reviews and studies are limited to a time period in which the fish is faced with a constant amount of toxins or environmental pollutants. Therefore, we have designed a system that during the time of testing levels of temperature and the ammonia to form a uniform increase so that we can study fish behavior from the starting point of pollution up to its maximum point. Thus, our research approach than previous studies was a new approach This study points out the importance of further study in the field of fish behavior in the face of pollutants. The results showed that fish exposed to environmental changes (temperature, the ammonia), show specific behavioral responses. The better identification of these patterns leads to better manage the aquaculture farms. The present study as a basis for understanding the road ahead and the journey requires more studies and more comprehensive.

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