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Estimating some physiological parameters in the blood of *Tilapia zillii* fingerlings during adaptation to different salinities

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Abstract - Two experiments were carried out on fingerlings of *Tilapia zillii* to estimate the effect of direct transfer from the control salinity of 15 psu to the different salinities of 1.5, 7.5, 15 and 30 psu during the periods of 6, 24, 48 and 96 hrs for short term effect experiments, and after 56 days for long term effect experiment in the salinities of 1.5, 7.5, 15 and 30 psu. Some physiological parameters (P.C.V.; osmolality; water content; Na+, K+ and Cl-, ions; glucose; total protein) were tested in the two experiments. Results of PCV showed a direct proportion with salinity increase, the time (96 hrs) was enough to reach the stable state of PCV. Fishes reach a new stable range of PCV in the salinities of 1.5, 7.5 and 15 psu after 56 days from transfer. Fishes seem hyperosmotic in the salinities of 1.5 and 7.5 psu and hyposmotic in the salinities of 15 and 30 psu. Plasma osmolality of the blood was significantly greater at 30 psu than the control salinity (15 psu), while it was significantly lower in the salinities of 1.5 and 7.5 psu at the time 96 hrs. Plasma osmolality decreased to values close to those of the three salinities 1.5, 7.5 and 15 psu at the time 56 days, indicating a strong osmoregulatory capacity of *T. zillii* fish. Water content in the muscles of *T. zillii* had inverse relationship with salinity increase in short term effect experiment. It was decreased significantly ($P \leq 0.05$) at the time 56 days from other times in the short term effect experiment at salinities 1.5, 7.5 and 15 psu. Sodium and chloride ions concentrations had a direct proportion with salinity increase in the short term effect experiment. After 56 days from transfer, sodium and chloride ions concentrations reached a stable state and had close values at the three salinities (1.5, 7.5 and 15 psu), Potassium concentration values had a direct proportion with salinity increase and the values decreased with time at all salinities. However, the potassium concentration had a lower value in contrast to sodium and chloride ions. Glucose concentrations in the plasma of *T. zillii* had a direct proportion with salinity increase after 96 hrs transfer to different salinities (1.5, 7.5, 15 and 30 psu). The glucose of plasma decreased significantly ($P \leq 0.05$) at all salinities (1.5, 7.5 and 15 psu) at the time 56 days compared to its levels at times of short term effect experiment. Protein concentration in the plasma decreased significantly ($P \leq 0.05$) in the three salinities (1.5, 7.5 and 30 psu) compared with the control salinity (15 psu) at the time 96 hrs of short term effect experiment. After 56 days from transfer, plasma protein values in the three salinities (1.5, 7.5 and 15 psu) had a significant increase from other times in the short term effect experiment at all salinities. The results indicated that the salinity increase caused an increase in PCV, osmotic pressure, plasma ions, glucose, total protein; of *T. zillii* plasma and decrease in water content of muscles and fish seemed hyperosmotic in the salinities of 1.5, 7.5 psu and hyposmotic in the salinities of 15, 30 psu, also the time of 96 hrs was not enough for fish to reach the stable osmotic pressure at all salinities.

Keywords: Physiological parameters, blood, *Tilapia zillii*, fingerlings.

Introduction

In teleost fishes, alternation of environment (water pH, Salinity or heavy metal pollution) cause physiological response such as secretion of hormones (Growth hormone, Prolactin or Cortisol), fluctuation of plasma ion, osmolality and glucose, changes in water balance and oxygen consumption rate which are the main parameters of osmoregulation (Potts *et al.*, 1987; McCormick *et al.*, 1989; McCormick, 1996; Lin *et al.*, 2000; Weng *et al.*, 2002).

Maintenance of blood osmolality and ion concentrations at levels different from those in the external medium is called Osmoregulation (McCormick, 1995). Salinity is one of the main environmental factors exerting a selective pressure on aquatic organisms. Salinity adaptation depends on the ability to osmoregulate, based on the maintenance of ion and water balance accomplished through different mechanisms at different sites in the teguments, gills, gut and intestine (Evans and Claiborne, 2008).

Tilapia zillii (Gervais, 1848) is an African and Middle Eastern native tilapiine fish (Chakrabarty, 2004). *T. zillii* is highly euryhaline i.e. can tolerate a wide range of salinity (Bayoumi, 1969; El-Zarka *et al.*, 1970; Fryer and Iles, 1972; Chervenski and Horing, 1973; Meyer, 2002), therefore, it is able to extend its geographic distribution into habitats of a wide salinity range. El-Sayed (2006) mentioned that *Tilapia zillii; Oreochromis mosambicus* and *O. aureus* are the most salinity-tolerant tilapia species. *T. zilli* is one of the most valued fish in North Africa. It constitutes an important part of Egypt and Morocco fish production especially in the brackish lagoons of Morocco, Senegal River, Egypt and Libya (Mahomoud *et al.,* 2011).

Among tilapia species *T. zillii* is a suitable model for studies on osmoregulatory mechanisms, because this euryhaline tilapia is adapted to a wide range of salinity from freshwater to seawater, furthermore, it possesses excellent salinity tolerance for surviving even in concentrated seawater (Dang, 1985; Suresh and Lin, 1992; Kultz *et al.*, 1995; Nakano *et al.*, 1997).

T. zillii is found in highly saline water (36-45psu) in many tropical and subtropical regions (Balarin and Hatton, 1979; El-Sayed, 2006). it also reproduce at 29-30 psu and can tolerate 45 psu in gradual transfer (El-Sayed, 2006).

The present study throw some lights on *Tilapia zillii* physiological response to salinity transfer in short term effect (6 , 24 , 48 and 96 hrs.) and long term effect (56 day) of different salinities (1.5 , 7.5 , 15 and 30 psu) in estimating some physiological factors (packed cell volume PCV, osmolainty, sodium (Na^+), potassium (K^+) and chloride (Cl^-) ions concentrations, water content of muscles, glucose level and total protein in blood plasma.

Materials and Methods

Experimental Fishes:

Tilapia zillii fishes were collected from Shatt Al-Basrah Canal which has a salinity mean of 17.35 ± 0.77 psu, by two types of nets:

1- Seine Nets (30 m length, 4 m highest, 2x2 cm mesh size).

2- Cast Nets (10 m diameter, 2x2 cm mesh size).

A total of 1000 fishes were collected from April 2011 to December 2012. Fishes were classified according to Beckman (1962). Fishes were held in cooling boxes and transfer to the Marine Science Center (MSC) aquaculture station. Fiberglass tanks were used for fish acclimation. The salinity in these tanks is 15 psu which is close to the salinity of the fish natural environment. Fishes were left for one week to be acclimated in the tanks.

Short term effect of salinity on fishes:

Four different salinity concentrations (1.5, 7.5, 15 and 30 psu) were tested. Eight containers (200 L) were used in two replicates for each salinity. The containers were provided with aerators, they were covered with nets to prevent fish from jumping out. They were filled with tap water free from chlorine. The salinities of containers were corrected according to the designed experiment using marine salt from Aquamedic company (Bissendrof, Germany) with major elements Na^{+}, Mg^{+2}, Ca^{+2}, K^{+}, Cl^{-}, $So_4^{=}$, HCO_3^{-}, Sr^{+} (11000, 1200, 420, 350, 19700, 2200, 180, 16 mg/L respectively). Thirty fishes were transferred directly from the control salinity 15 psu to the containers of different salinities. After direct transfer to the different salinities, fishes were sampled after 6, 24, 48 and 96 hrs for measuring some physiological parameters. Six fishes were killed from each salinity at the times 6, 24, 48 and 96 hrs after being anesthetized by clove oil by putting fish in container with water in the same salinity containing clove oil (Durvill and Collet, 2001). The total length (cm) and total weight (g) of the fish were recorded. Blood samples were collected from the caudal veins for PCV and osmolality measurements by using heparinized capillary tubes with a length of 75 mm and diameter 1.1 mm from the inside and 1.5 mm from the outside. Samples of muscles were also taken for estimating their water content.

Long term effect of salinity on fishes:

Eight 200 L containers were used for maintaining of *T. zillii* using different salinities (1.5, 7.5, 15, and 30 psu) with a duplicate for each salinity. *T. zillii* were transferred from the acclimation tanks to the laboratory and distributed to containers with 30 fish each. Fish were acclimated at laboratory conditions for one week with feeding on artificial diet. After the acclimation period, the fish were fed the same artificial diet in acclimation period with protein contents of 38.25 %, the feeding process prolonged for 56 days.

After growth experiment of 56 days fish were then used for determining the effect of salinity on osmoregulation (PCV; osmotic pressure; Na^{+}, Cl^{-}, K^{+} concentrations; water content of muscles; glucose level and protein content in plasma) as a long term effect of salinity on these parameters.

Effect of salinity on fish osmoregulation:

Packed Cell Volume (PCV):

After fish were anesthetized with 0.05 ml/l Clove Oil, blood samples from the short term and the long term effect experiments were collected after cutting the caudal peduncle and drafting blood by a heparinized capillary tube with 75 mm length and 1.1 mm diameter from the inside and 1.5 mm from the outside.

The capillary tubes were then put in a microcentrifuge (haematokrit 210) with a speed of 3500 rpm for 5 minutes to separate plasma from the blood. Packed Cell Volume (PCV) was measured by estimating Haematocrit ratio (%) using Micro-Capillary Reader type DAMON/IEC.

Osmotic pressure:

Blood plasma was collected from the capillary tubes after finishing the PCV measurements by a Micro Syringe with a volume of 100 µm, 50 µm of plasma was taken to ependroff tubes with a volume of 0.5 ml. Osmolality (mosmol.Kg^{-1}) was measured by the freezing point depression measurement method using the cryoscopic osmometer (OSMOMAT 030). Since the freezing point depression is directly proportional to the dissolved parts, the OSMOMAT 030 measures the osmolality directly (Saoud *et al.*, 2007).

Sodium (Na^+) and Potassium (K^+) concentrations in the plasma:

The plasma was diluted 100 times in distilled water and kept in plastic plen tubes with a volume of 12 ml and kept frozen at -20°c until measurements.

Sodium (Na^+) and potassium (K^+) ions estimated by a Flame-Photometer (PFP7) after calibrating it with standard solutions of sodium chloride (NaCl) (5, 10, 15, 20, 25, 50, 100 ppm), and potassium chloride (KCl) (5, 10, 15, 20 ppm) and with Deionize Water (DIW) as blank. After getting the ion concentration from the calibration curve of the standards, the results were converted to mmol/L from the relationship between mmol/L and ppm:

1 mmol/L sodium(Na^+) = 23 ppm, 1 mmol/L potassium (K^+) = 39 ppm

Chloride (Cl^-) concentrations in the plasma:

The test:

The chloride (Cl^-) ion concentration in the plasma was estimated using commercial kit (BIOLABO SA, 02160 maizy, France) in a colorimetric method according to Florence and Farrar (1971) for estimating chloride ion. All reagents that provided by manufacturer were ready for use which are: (Thiocyanate reagent R1 and Standard R2).

Assay procedure:

All specimens and reagents were transported to room temperature (18-25 °C) before use.

- 1000 µl of thiocyanate reagent R1 was added to the blank, standard and assay tubes.
- 10 µl of DIW was added to the blank tube.
- 10 µl of standard R2 was added to the standard tube.
- 10 µl of specimen was added to the assay tube.
- Tubes were mixed well and left for 5 minutes at room temperature.
- The absorbance of standard and assay were recorded at 500 nm against reagent blank using a spectrophotometer model (Humalyzer Primus).
- Chloride concentration (mmol/L) was calculated from the following equation.

$$\text{Cl}^- \text{ concentration (mmol/L)} = \frac{\text{(Assay) Abs}}{\text{(Standard) Abs}} \times \text{standard concentration (100 mmol/L)}$$

Water content of the muscles:

After removing scales and skin from the region under the dorsal fin, a piece of muscle tissue was taken and washed in distilled water to remove external salts. Moisture of the muscles was conducted by drying samples in an oven at 105 °C. Water content of the muscles was calculated from the equation:

$$\text{Water Content (\%)} = \frac{\text{Wet weight - Dry weight}}{\text{Wet weight}} \times 100$$

Glucose level in the plasma:

The test:

The plasma was kept frozen at -20°c in ependorf tubes until measurements. Glucose concentration (mmol/L) in the plasma was estimated by using a commercial kit (RANDOX laboratories/UK) in a colorimetric method (Tietz, 1990).

All reagents that provided by manufacturer were ready for use which are: (Buffer R1a, GOD-PAP Reagent R1b and standard CAL).

Reagent preparation:

The working reagent was prepared by reconstitute the contents of GOD-PAP Reagent R1b with a portion of Buffer R1a and then the entire contents of R1a was transferred to R1b, mix well and stored at 2-8 °C.

Assay Procedure:

All specimens and reagents were kept at room temperature (18-25 °C) before use.

- 10 µl of specimen (plasma of fish) was added to the sampling tube.
- 1000 µl of standard CAL was added to the standard tube.
- 1000 µl of working reagent was added to the sample, standard and reagent blank tubes.
- The tubes was mixed well and incubated for 10 minutes at 37 °C.
- The absorbance of the sample and standard was read at 500 nm against the reagent blank using a spectrophotometer (Humalyzer Primus).
- Glucose concentration (mmol/L) was calculated from the following equation:

$$\underset{\text{(mmol/L)}}{\text{Glucose concentration}} = \frac{\text{Sample (Abs)}}{\text{Standard (Abs)}} \times \underset{\text{(5.49 mmol/L)}}{\text{standard concentratiom}}$$

Total Protein in the Plasma:

The test:

The Biuret method was used for estimating the total protein (g/100ml) in the plasma by using a commercial kit (BIOLABO SA, 02160 maizy, France) in a colorimetric method (Henry *et al.,* 1974).

All reagents that provided by manufacturer were ready for use which are: (biuret reagent R1 and standared R2).

Assay Procedure:

All specimens and reagents were kept at room temperature (18-25 °C) before use.

- 1000 µl of biuret reagent R1 was added to the reagent blank, standard and assay tubes.
- 20 µl of standard R2 was added to the standard tube.
- 20 µl of specimen was added to the assay tube.
- 20 µl of DIW was added to the reagent blank tube.
- Tubes were mixed well and left for 10 minutes at room temperature.
- The absorbance of standard and assay was measured at 550 nm against the reagent blank using a spectrophotometer (Humalyzer Primus).
- Total protein in the plasma (g/100ml) was calculated from the following equation:

$$\text{Total Protein (g/100ml)} = \frac{\text{Abs (Assay)}}{\text{Abs (standard)}} \times \text{standard concentration (6g/100ml)}$$

Statistical analyses:

Values were compared using a one-way and two-way analysis of variance (ANOVA) and Revised Least Significant Difference (RLSD) to compare the variances between salinities and time. $P < 0.05$ was set as the significance level using SPSS program. Values were expressed as mean ± S.E.M. (the standard error of the mean) (Stell and Torrie, 1960).

Results

Packed Cell Volume (PCV %):

Figure (1) shows the PCVs percentage (%) of *T. zillii* blood in the direct transfer experiment from 15 psu as a control to different salinities (1.5, 7.5, 15 and 30) psu at different times (6, 24, 48 and 96) hrs (short term effect) and after 56 days (long term effect). There was a decrease in PCV values in the salinities 1.5 psu and 7.5 psu at all times, except at 6 hrs for the salinity 7.5 psu; whereas the PCV increased in the salinity 30 psu at all times. PCV showed a direct proportion with the salinity increase. After 56 days, there were close values of PCV at the three salinities (1.5, 7.5 and 15 psu) which were 30.42 %, 31.33 % and 31.34 %, respectively. Statistical analysis showed that there were significant differences ($P \leq 0.05$) in PCV values between the control and the salinities 1.5, 7.5 psu and there was no significant differences ($P > 0.05$) in PCV values between the control and salinity 30 psu and between the salinities 1.5 and 7.5 psu, there were no significant differences ($P > 0.05$) in PCV % during times of experiments except between 24 hrs and 56 days.

Osmotic Pressure:

Figure (2) shows the osmotic pressure ($mOsmol.Kg^{-1}$) of *T. zillii* plasma in the direct transfer experiment from 15 psu to different salinities (1.5, 7.5, 15 and 30 psu) after 6, 24, 48 and 96 hrs and after 56 days. There was a decrease in the osmotic pressure values at salinities 1.5 and 7.5 psu compared with the control at all times, whereas at salinity 30 psu there was an increase in the osmotic pressure values from the control at all time. Osmotic pressure values decreased with time and reached close values after 56 days (227.4, 261.2 and 263.8 $mOsmol.Kg^{-1}$ for the salinities 1.5, 7.5 and 15 psu, respectively). Statistical analysis showed that there were significant differences ($P \leq 0.05$) in the osmotic pressure values between all salinities,

also there were significant difference ($P \leq 0.05$) of the osmotic pressure values between times except at 6, 24 hrs and 48, 96 hrs. The time 56 days had significant difference ($P \leq 0.05$) from the other times (6, 24, 48 and 96 hrs). The data from the two experiments were pooled and plotted against the osmotic pressure at different salinities (Fig. 3). The slope and intercept of the graphs of water and blood osmolality to *T. zillii* were used to calculate the state of the fish. It is apparent that the fish was hyperosmotic at salinities 1.5, 7.5 psu and hyposmotic at salinities of 15, 30 psu.

Water content of the muscles:

Figure (4) shows that the water content (%) of *T. zillii* muscles increased at salinities 1.5 and 7.5 psu at all times of the experiments; whereas the salinity 30 psu decreased in water content values from the control salinity 15 psu at all times, whereas at salinity 30 psu there was a decrease in water content at all times. The water content after 56 day of transfer showed significant increase ($P \leq 0.05$) at salinities 1.5, 7.5 psu which were 79.95 %, 78.40 % and 73.47 %, respectively. Statistical analysis showed that there were significant differences ($P \leq 0.05$) in the muscle water contents between all salinities but there were no significant differences ($P > 0.05$) between times except at time (56) days.

Sodium (Na^+) Concentration:

Figure (5) shows Sodium (Na^+) concentration ($Mmol.L^{-1}$) of *T. zillii* plasma at different salinities. There was decrease in (Na^+) concentration at salinities 1.5 and 7.5 psu at all times, whereas at salinity 30 psu an increase in (Na^+) concentration after 6 hrs had occurred. The values were 91.43 $Mmol.L^{-1}$, 97.02 $Mmol.L^{-1}$ and 104.23 $Mmol.L^{-1}$, at the three salinities 1.5, 7.5 and 15 psu, respectively. Statistical analysis showed that there were significant differences ($P \leq 0.05$) in (Na^+) concentration values between the control salinity 15 psu and the salinities 1.5, 7.5 psu and there were no significant differences ($P > 0.05$) between the control salinity 15 psu and the salinity 30 psu. However, there were significant differences ($P \leq 0.05$) in (Na^+) concentration at 56 days and the rest of times, while there were no significant differences ($P > 0.05$) between times 6 and 48 hrs and 24 and 96 hrs.

Potassium (K^+) Concentration:

Figure (6) indicates a decrease in the K^+ concentration of *T. zillii* at salinities 1.5 and 7.5 psu from the control salinity 15 psu at all times, while the salinity 30 psu showed an increase in the K^+ concentration. These values were 2.66 $Mmol.L^{-1}$, 4.73 $Mmol.L^{-1}$ and 5.3 $Mmol.L^{-1}$ at salinities 1.5, 7.5 and 15 psu, respectively after 56 days of transfer. Statistical analysis showed that there were significant differences ($P \leq 0.05$) in the K^+ concentration values between all salinities. and there were significant differences ($P \leq 0.05$) in the K^+ concentration values during all times of the experiments.

Chloride (Cl^-) concentration:

Direct transfer of *T. zillii* fishes from the control salinity 15 psu to different salinities 1.5, 7.5, 15 and 30 psu cause a significant decrease ($P \leq 0.05$) in the chloride ion (Cl^-) concentration values at salinities (1.5, 7.5) psu and significant increase ($P \leq 0.05$) in the chloride ions at salinity 30 psu during all times.

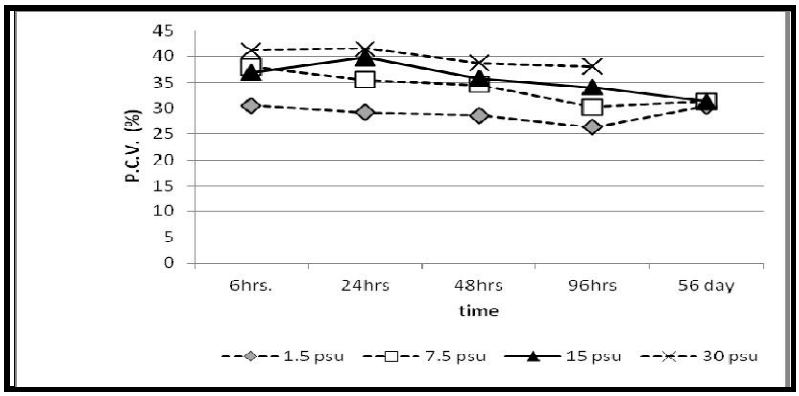

Figure 1. P.C.V. measurement (%) of *T. zillii* blood in the short and long term effects experiments at different salinities.

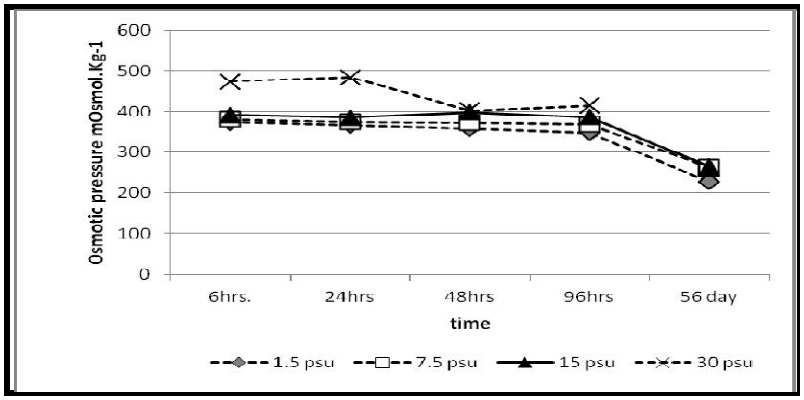

Figure 2. Osmotic Pressure (mOsmol.Kg^{-1}) of *T. zillii* plasma at different salinities.

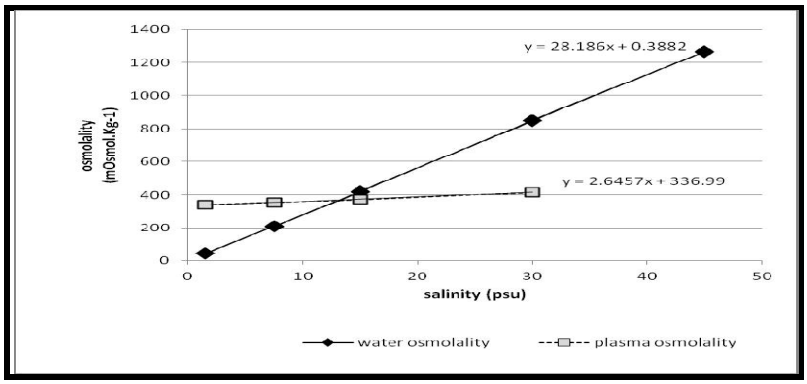

Figure 3. Saline medium and plasma osmolality of the *T. zillii* fingerlings maintained at salinities from 1.5 psu to 45 psu (values are pooled from the two experiments).

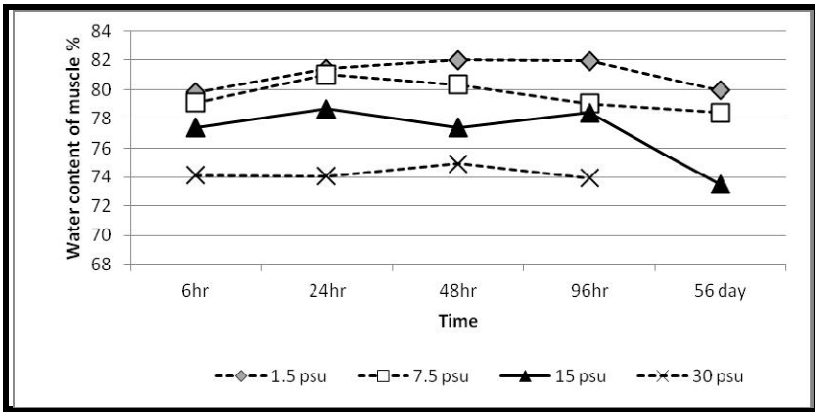

Figure 4. Water content of *T. zillii* muscles at different salinities.

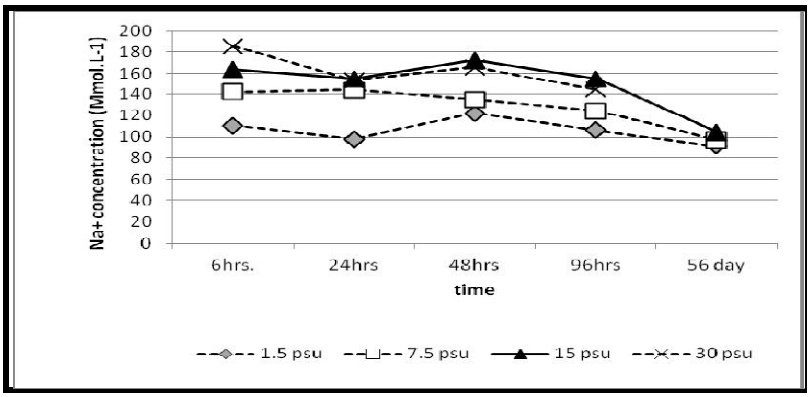

Figure 5. Sodium (Na+) concentration (Mmol.L^{-1}) of *T. zillii* plasma at different salinities.

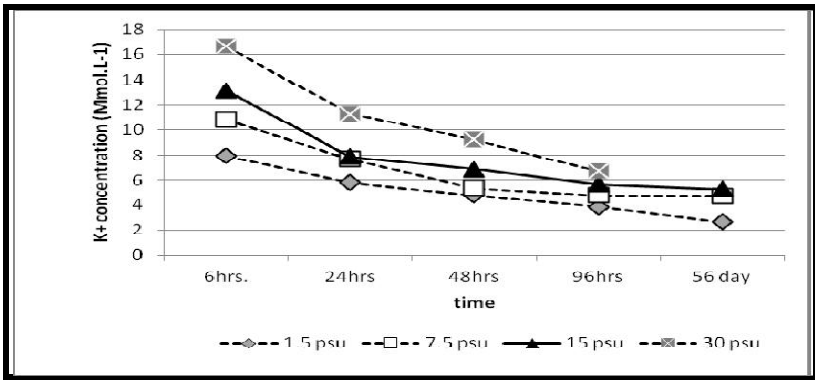

Figure 6. Potassium K^{+} concentration (Mmol.L^{-1}) of *T. zillii* at different salinities.

Chloride ion concentration values begin to decrease after 6 hrs to the next 24, 48, 96 hrs, and reached 108.45, 123.62, 134.02 and 142.72 $Mmol.L^{-1}$ after 96 hrs at salinities 1.5, 7.5, 15 and 30 psu, respectively. After 56 day the values reached 126.36 $Mmol.L^{-1}$, 132.76 $Mmol.L^{-1}$ and 142.49 $Mmol.L^{-1}$, respectively at salinities 1.5, 7.5 and 15 psu (Fig. 7). Statistical analysis showed there were significant differences ($P \leq 0.05$) of chloride ion values between all salinities and there were no significant differences ($P > 0.05$) between the times 6 and 24) hrs, 24 and 48 hrs and 48 and 96 hrs. The time 56 days had no significant differences ($P > 0.05$) with other times, except with 96 hrs.

Glucose level in the plasma:

Figure (8) shows the Glucose concentration ($Mmol.L^{-1}$) in *T. zillii* plasma at different salinities and various times. The results showed that there was a significant decrease ($P \leq 0.05$) of plasma glucose concentration values at salinities 1.5 and 7.5 psu and a significant increase ($P \leq 0.05$) in plasma glucose concentration values at salinity 30 psu compared with the control salinity 15 psu. The glucose concentration values began to decrease after 6 hrs till the end of the experiment reaching 4.37, 5.33, 5.98 and 7.9 $Mmol.L^{-1}$ after 96 hrs at the salinities 1.5, 7.5, 15 and 30 psu, respectively. There were significant differences ($P \leq 0.05$) between times at all salinities. The results for the long term effect of different salinities after 56 days showed a decrease in the glucose concentration at all salinities after 56 day of transfer in contrast to the values obtained from the short term effect experiment. The glucose concentration values in the three salinities 1.5, 7.5 and 15 psu were 0.48 $Mmol.L^{-1}$, 2.36 $Mmol.L^{-1}$ and 3.43 $Mmol.L^{-1}$, respectively. Statistical analysis showed significant differences ($P < 0.05$) in the glucose concentration values in *T. zillii* plasma between the time 56 days and other times at all salinities.

Total protein in the plasma:

The total plasma protein (gm/100 ml) of *T. zillii* plasma at different salinities and various times are show in figure (9). At the time 6 hrs there was a significant increase ($P \leq 0.05$) in the plasma protein at salinities 1.5 and 7.5 psu from the control salinity 15 psu; and a significant decrease ($P \leq 0.05$) at salinity 30 psu. Then plasma protein decreased at salinities 1.5 and 7.5 psu and reached values of 2.104 and 2.610 gm/100 ml, respectively after 96 hrs. In contrast to salinities 15 and 30 psu when the values increased to 4.083, 3.675 gm/100ml, respectively at the time 96 hrs. After 56 day of transfer the plasma protein values in the three salinities 1.5, 7.5, 15 psu were higher than those in the short term experiment, 2.818, 5.692 and 8.425 gm/100ml, respectively.

The results of the long term effect of different salinities after 56 days have a direct proportion with salinity increase and significantly increased ($P \leq 0.05$) in relation to other times at each salinity. Statistical analysis showed that there were significant differences ($P < 0.05$) in the plasma protein between the salinities 1.5, 7.5, 30 psu compared with the control, and there were no significant differences ($P > 0.05$) between the salinities 7.5 and 30 psu. Also there were no significant differences ($P > 0.05$) between all times of the short term effect experiment.

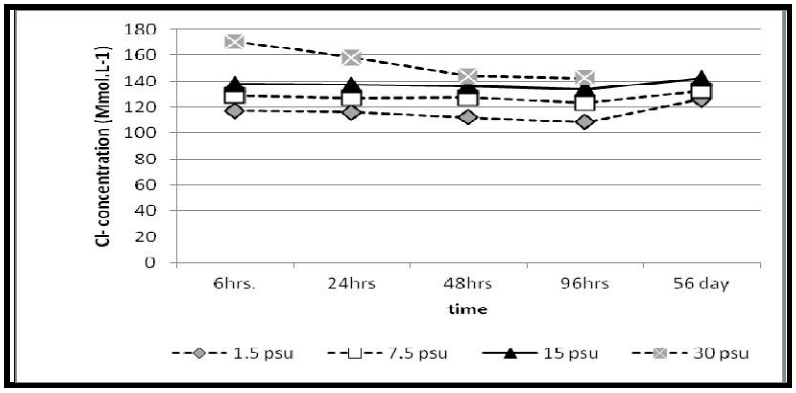

Figure 7. Chloride (Cl^-) concentration (Mmol.L-1) of *T. zillii* plasma at different salinities.

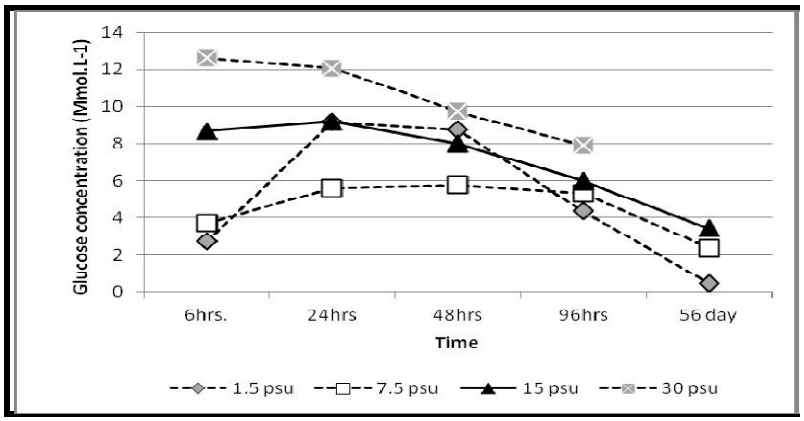

Figure 8. Glucose concentration ($Mmol.L^{-1}$) of *T. zillii* plasma at different salinities.

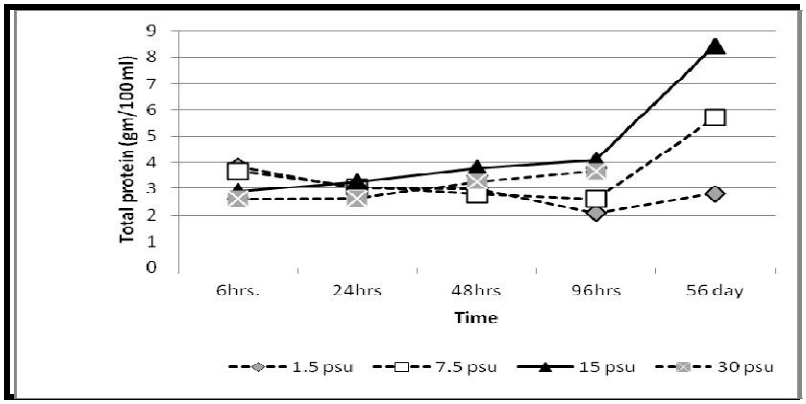

Figure 9. Total plasma protein (gm/100 ml) of *T. zillii* plasma at different salinities.

Discussion

Packed cell volume (PCV):

The present results showed that the PCV of *T. zillii* increased at salinities 1.5 and 7.5 psu, but not so at salinity 30 psu. But there was a decrease in the values after 6 hrs. However, a stable state was reached after 96 hrs. The present result of PCV is in agreement with the findings of Zeitoun *et al.* (1974), who reported significant increase of hematocrit values ($P \leq 0.05$) with an increase in salinity from 10 psu to 20 psu in the rainbow trout *Salmo gairdineri*. Dheer *et al.* (1986) reported that blood parameters of *Channa punctata* were affected by changes in salinity. In contrast Verdegem *et al.* (1997) reported that salinity had no significant ($P > 0.05$) influence on hematocrit in hybrid red tilapia (*Oreochromis niloticus* (Linnaeus) × *O.mossambicus* (Peters). Walker *et al.* (1989) found that haematocrit (Hct) was significantly lower ($P \leq 0.005$) in saline-acclimated *Catostomus commersoni* than in the control animals. The same results for PCV values were noted in many studies on the grass carp (Maceina *et al.* 1980), spotted gar (Smatresk and Cameron, 1982) and carp (Hegab & Hanke, 1982). Indeed, the decreases in haematocrit and plasma protein and hemoglobin concentrations in the study of Wilkes and McMahon (1985) after 24 hrs transporting the freshwater fish *Catostomus commersoni* to hyper saline medium [0.94% sodium chloride /300 mosmol/L] suggest that the plasma volume was expanded during saline acclimation.

Osmotic Pressure:

Fish plasma osmolality in the present study ranged between 227.6 $mOsmol.kg^{-1}$ and 483.67 $mOsmol.kg^{-1}$, in fish transferred to salinities from 1.5 psu to 30 psu, respectively. After 56 days of transfer, plasma osmolality decreased to close values at the three salinities 1.5, 7.5 and 15 psu indicating of a strong osmoregulatory capacity of the *T. zillii* fish. Results of Saoud *et al.* (2007) are in agreement with the present study. They showed that the *Siganus rivulatus* had an increase in osmotic pressure values with a salinity increase ranging from 10 psu to 50 psu and that they can maintain a relatively stable blood osmolality (between 398 and 435 $mOsmol.kg^{-1}$). They found that the blood osmolality of the fish reared at 40 psu (408.3 $mOsmol.kg^{-1}$) was significantly greater than that of fish reared at 25 and 30 psu (383.6 and 387.0 $mOsmol.kg^{-1}$, respectively). Also, blood osmolality of fish reared at 20 and 25 psu (416.8 and 419.8 $mOsmol.kg^{-1}$, respectively) was significantly greater than the blood osmolality of fish reared at 10 and 15 psu (378.6 and 373.4 $mOsmol.kg^{-1}$, respectively). In contrast Lin *et al.* (2003) found no effect of salinities ranging from tap water psu to 35 psu on blood osmolality of milkfish *Chanous chanous*; and Hwang *et al.* (1989) found no differences in the blood osmolality of *Oreochromis mossambicus* reared in fresh water or in salt water. Laiz-Carrión *et al.* (2005) found a small but significant difference in the blood osmolality of gilthead sea bream reared at 12 psu and 6 psu but no difference was found between the blood osmolalities of fish reared at 12 psu and 38 psu. The slope of the curve in Figure (3) depicting blood plasma osmolality increased with salinity is much smaller than that depicting the increase in water osmolality and that in support of the argument that the teleostan fish *T. zillii* tend to be strong

osmoregulators. Similar results are reported by Sampaio and Bianchini (2002) working with the flounder *Paralichthys orbignyanus*, they found that when salinity only slightly affects plasma osmolality, a species is probably adapted to face the salinities it lives in. Therefore, the present results suggest that *T. zillii* is adapted to survive in low salinity rivers in Iraq (Tigres, Euphrates and Shatt Al-Arab) as well as in the moderate salinity environments of Shatt Al-Basrah canal. Imsland *et al.* (2003) reported a significant effect of temperature on osmoregulation of turbot *Scophthalmus maximus*. They found that when fishes reared at 10 °C, 14 °C and 22 °C, salinity had a significant effect on osmoregulation, but at 18 °C salinity did not affect blood osmolality. Küçük *et al.* (2013) pointed out that the plasma osmolality were not significantly affected until the salinity reached to as much as 16 psu in blue tilapia *Oreochromis aureus*. Walker *et al.* (1989) mentioned that the increase in plasma osmolality was primarily due to an increase in sodium and chloride concentrations. Because the plasma concentrations of potassium, calcium and magnesium remained constant, it is reasonable to assume that the increases in sodium and chloride concentration were due to a net influx during the acclimation process rather than an osmotic loss of water.

Water content of muscles:

The present study showed that water content in *T. zillii* muscles was decreased significantly with salinity increase from the control, and increased significantly with salinity decrease. Water content of the muscles remained in close values at all times to each salinity. After 56 days of transfer, the water content of the muscles decreased significantly ($P \leq 0.05$) from other times, but still had a significant increase in the salinities (1.5, 7.5) psu from the control. Many studies were coincided with the present results of the water content in the fish muscles. Water content of the catfish (*Mystus vittatus*) was found to decrease with an increase in salinity (Arunachalam and Reddy, 1979). Küçük *et al.* (2013) found that high salinity caused alterations in the water content of the muscles which were significantly decreased in 150% SW and 200% SW. Muscle water content reduced in the grass carp (Maceina and Shireman, 1979), in Mozambique tilapia when transfer from 10 psu to 20 psu (Lee *et al.*, 2005) and in goldfish (Luz *et al.*, 2008) at 10 psu of salinity. However, muscle water content (around 79 to 82%) did not change at tap water to 10 psu of salinity (Overton *et al.*, 2008). Many studies showed a decrease in muscles water content in fish transfer from fresh water to sea water, in *Salmo trotta* (Madsen and Bern, 1992), *Salmo gairdinery* (Madsen, 1990a), *Salvelinus alpines* (Finstad *et al.*, 1989), *Salmo trutta trutta* (Madsen, 1990b), Gilthed seabream (Sangiao-Alvarellos *et al.*, 2003). Woo and Chung (1995) found that the water content of *Pomacanthus imperator* muscles increased in the low saline media.

Sodium, potassium and chloride concentrations:

The present results of sodium and chloride ion in *T. zillii* are in agreement with many studies indicating that the two ions increased with salinity increase. Morgan *et al.* (1997) reported that sodium and chloride ions showed similar patterns in *Tilapia mozambique* cultured in fresh water

when transferred to higher salinities; sodium was about 136 mMol.L^{-1} in the FW and raised to about 155 mMol.L^{-1} and 170 mMol.L^{-1} in 12 psu and 25 psu, respectively; chloride ion increased around 165 mMol.L^{-1} in the 25 psu, while it was about 140 mMol.L^{-1} in FW and 12 psu. Overton *et al.* (2008) also indicated that sodium and chloride concentrations increased at higher salinities than 8 psu.

Sodium and chloride concentrations instantly increased in 16 psu and continued to increase in the upper salinities (Küçük *et al.,* 2013). A study by Walker *et al.* (1989) showed significant increases above the control values ($P \leq 0.001$) in the concentration of sodium and chloride after 10 days of exposure to an environmental salinity of 300 mOsmol.kg^{-1} NaCl in the fresh water stenohaline teleost *Castostomus commersoni.* Four days of exposure to maximum sub-lethal salinity (0.94%, 300 mOsmol.L^{-1}) of the freshwater stenohaline teleost *Catostomus commersoni* resulted in an increase in the plasma concentrations of both sodium and chloride ions but a decrease in the Na^+/Cl^- ratio (Wilkes and McMahon, 1985).

In contrast no change in plasma Na^+ level has been reported in the rainbow trout upon exposure to salinity for five days (Shepherd *et al.*, 2005), the unaltered plasma Na^+ during the initial phases of salinity exposure in this study indicates the capacity of the fishes to regulate the whole body mineral status during salinity exposure. Woo and Chung (1995) pointed out that sodium ion concentration in *Pomacanthus imperator* plasma decreased from 163 mMom.L^{-1} to 92 mMom.L^{-1} in fish transfer from 33 psu to 7 psu. Marshall *et al.* (1999) reported that sodium ion in the killifish plasma rises in the first 24 hrs of transfer to seawater and return to the new levels after full acclimation to seawater.

The present results reported that potassium values had a direct proportion with the salinity increase and the values decreased with time at all salinities. However, the potassium concentrations in the *T. zillii* plasma have lower values in contrast with sodium and chloride ions. There were significant different ($P \leq 0.05$) at all salinities and all times.

The present study is in agreement with Madsen and Bern (1992) who found an increase in potassium ion in *Salmo trutta* fishes transferred from fresh water to sea water after 48 hrs of transfer. Potassium ion increased in the tilapia fishes plasma after two weeks of transfer to sea water (Vijayan *et al.*, 1996). A study by Ahmed (2005) recorded an increase in potassium ion in *Liza abu* plasma transferred from fresh water to higher water salinities; it reached 10 and 14 mMom.L^{-1} in salinities of 7 and 15 psu, respectively.

The present study in is contrast with that of Walker *et al.* (1989) who found no significant changes in the concentrations of potassium, calcium and magnesium after 10 days of exposure to an environmental salinity of 300 mosmol.kg^{-1} NaCl in the fresh water stenohaline teleost *Castostomus commersoni.* Potassium did not change significantly in mozambique tilapia, *O. mossambicus* (Morgan *et al.*, 1997) in FW, 12 psu and 25 psu; in perch, *Perca fluviatilis* at 0 to 10 psu (Overton *et al.*, 2008) and in shi drum, *Umbrina cirrosa* at 4, 10, 40 psu (Mylonas *et al.*, 2009). Potassium was not altered significantly in blue tilapia (*Oreochromis aureus*) transferred to five different saltwater treatments: 8 psu, 12 psu, 16 psu, 20 psu and 24 psu (Küçük *et al.*, 2013). McCormick and Naiman (1984) found no direct

increase of potassium ion in *Salvlinus fentinalis* when transferred to high salinities in contrast with other ions, and remained constant until the third day of transfer to the salinity 32 psu.

Although there was a high gradient in concentration between the blood and the external medium, potassium ion was found in large amounts inside the cell and it is regulated in many directions of seawater acclimation; therefore, there was no confusion of potassium ion regulation in contrast to sodium and chloride ions (McDonald and Milligan, 1992). Analysis of plasma potassium, calcium, magnesium and sodium concentration revealed no significant differences ($P > 0.05$) in the various treatments of different salinity levels effect (0, 2, 4, 7, 10 psu) on Kutum fishes *Rutilus frisiim* the endemic fish to the Caspian sea (Gholampoor *et al*., 2011).

Glucose level in the plasma:

The present results showed that the glucose concentrations in the *T. zillii* plasma had a direct proportion with salinity increase. There was a significant decrease in the plasma glucose at the salinities 1.5 and 7.5 psu from the control, while there was a significant increase in plasma glucose at the salinity 30 psu from the control. These results are in agreement with many studies which had an increase in plasma glucose in response to the short term effect of salinity transfer. In a study by Jeanette *et al.* (2007) the plasma glucose levels increased significantly with the increase in the environmental salinity and temperature. After 56 days of transfer to different salinities plasma glucose decreased significantly at all salinities (1.5, 7.5 and 15 psu). A similar result was found by Küçük *et al.* (2013) for the blue tilapia (*Oreochromis aureus*), who concluded that the return of glucose to the basal level in fish kept for a relatively longer period of salinity exposure indicates that this osmotic challenge does not pose any serious stress to this fish. In a study by Urbinati and Carneiro (2006) the blood glucose levels in Matrinxã *Brycon amazonicus* was elevated after transport to saline media at 0.0, 1.0, 3.0 psu NaCl as a stress response characteristic; while the blood glucose levels after transporting fish to 6.0 psu of salt suggests no stress response and concluded that the unchangeable levels of blood glucose in fish exposed to 6.0 psu of salt indicate that it help matrinxã fish to maintain homeostasis after transporting stress. In contrast with other studies like Morgan *et al.* (1997), Mylonas *et al.* (2009) and Arjona *et al.* (2009) they found that glucose did not change during the salinity exposure. Küçük *et al.* (2013) found that there were no significant differences in the mean of plasma glucose concentration (around 5 to 7 mM) in the tilapia fish *Oreochromis aureus* reared at different salinities (8, 12, 16, 20, and 24 psu). They attributed this result to the acclimatization of the fish to SW before the experiment started. As detected in the study by Gholampoor *et al.* (2011), changes in plasma glucose and cholesterol of kutum fingerlings *Rutilus frisii* exposed to different salinities were not significant ($P > 0.05$). Sumpter (1997) suggested that most of the teleost fishes showed stress responses characteristic to a short term exposure, e.g. glucose elevation. Liver glycogen decrease and blood glucose increase were also registered in tilapia *Oreochromis mossambicus* subjected to confinement stress (Vijayan *et al*., 1997).

Total protein in the plasma:

The present study showed that there was a significant decrease in plasma protein concentration in relation to salinity increase. After 56 days of transfer, plasma protein values had a significant increase from other times in at all salinities and the two salinities 1.5 and 7.5 psu had a significant decrease from the control at the time 56 days. The present results for the plasma protein concentration at different salinities are in agreement with the results of Kelly and Woo (1999) who found a decrease in plasma protein with a salinity increase in the sea bream. In a study by Martinez-Alvarez *et al.* (2002) they found a decrease in the total plasma protein of *Acipenser naccarii* fish when salinity increased from 15 to 29 psu. In contrast, Woo and Chung (1995) found that the total plasma protein levels in *Pomacanthus imperator* was high in fishes transferred to salinities 15 and 22 psu, while it decreased in sea water. A study by Gholampoor *et al.* (2011) showed no significant difference (P>0.05) among the plasma protein concentrations of kutum fingerlings *Rutilus frisii* at different salinities (0, 2, 4, 7 and 10 psu). The study of Arnason *et al.* (2013) showed that different salinities (ranged from 6 to 32 psu) and an abrupt increase in salinity had limited or no effects on plasma protein concentration in the Atlantic cod *Gadus morhua*.

Stress or infection is known to influence the plasma protein activity in the Atlantic cod *Gadus morhua* (Magnadóttir *et al*., 2001, 2010, 2011). Plasma protein levels were positively correlated with fish size (Arnason *et al*., 2013). Levels of plasma protein in cod have been found to be influenced by season (Magnadóttir *et al*., 2001) and thus the difference in plasma protein levels between the two experiments in the present study may be attributed to the larger time span of the long term effect experiment. The addition of salt to the water in the study of Urbinati and Carneiro (2006) has been used to mitigate stress and improve survival in fishes. This study investigated the effects of sodium chloride (0.0, 1.0, 3.0 and 6.0 psu) on the levels of plasma total protein in adult matrinxã (*Brycon amazonicum*) after 4 hrs transport, and during a 96-h recovery period, the total plasma protein did not change during the experiment.

Conclusion

This study confirms that the salinity increase caused an increase in PCV, osmotic pressure, plasma ions, glucose, total protein; and decrease in water content of muscles of *T. zillii* plasma and fishes seemed hyperosmotic in the salinities 1.5, 7.5 psu and hyposmotic in the salinities 15, 30 psu, also the time 96 hrs was not enough for fishes to reach the stable osmotic pressure in all salinities.

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تقدير بعض العوامل الفسلجية في دم اصبعيات اسماك التيلابيا *Tilapia zillii* خلال الأقلمة على ملوحات مختلفة

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المستخلص - تم إجراء تجربتين على أصبعيات اسماك *Tilapia zillii* التجربة الأولى هي تجربة التأثير القصير الأمد لتقدير تأثير النقل المفاجئ من ملوحة السيطرة 15 جزء بالألف إلى الملوحات (1.5 و 7.5 و 15 و 30) جزء بالألف خلال الأوقات (6 و 24 و 48 و 96) ساعة. والتجربة الثانية هي تجربة التأثير الطويل الأمد لتقدير التأثير الطويل الأمد للملوحات (1.5 و 7.5 و 15 و 30) جزء بالألف بعد 56 يوما من النقل. تم قياس بعض العوامل الفسلجية في التجربتين مثل مكداس الدم والضغط الازموزي ورطوبة العضلات وايونات الصوديوم والبوتاسيوم والكلورايد والكلوكوز والبروتين الكلي. أظهرت نتائج مكداس الدم وجود تناسب طردي مع زيادة الملوحة والوقت 96 ساعة هو كافي للوصول إلى حالة الاستقرار في قيم مكداس الدم، بعد 56 يوما من النقل وصلت الأسماك إلى مستوى مكداس دم ثابت في الملوحات (1.5 و 7.5 و 15) جزء بالألف. تبدو الأسماك أعلى ازموزيا في الملوحات (1.5 و 7.5) جزء بالألف وأوطئ أزموزيا عند الملوحات (15 و 30) جزء بالألف. أظهرت النتائج وجود زيادة معنوية في الضغط الازموزي عند الملوحة 30 جزء بالالف ونقصان معنوي عند الملوحات (1.5 و 7.5) جزء بالألف عن ملوحة السيطرة عند الزمن 96 ساعة من تجربة التأثير القصير الأمد للملوحات المختلفة .بعد 56 يوماً من النقل انخفض الضغط الازموزي لقيم متقاربة في الملوحات الثلاث (1.5 و 7.5 و 15) جزء بالألف ما يدل على قدرة التنظيم الأزموزي العالية لاسماك *T. zillii*. امتلكت قيم المحتوى المائي في عضلات أسماك *T. zillii* تناسبا عكسيا مع زيادة الملوحة في تجربة التأثير القصير الأمد، انخفضت قيم المحتوى المائي في العضلات انخفاضا معنويا ($P \leq 0.05$) في الزمن 56 يوما عن بقية الأزمان الأخرى في تجربة التأثير القصير الأمد للملوحات المختلفة (1.5 و 7.5 و 15) جزء بالألف. امتلكت قيم تراكيز ايوني الصوديوم والكلورايد في بلازما الدم تناسبا طرديا مع زيادة الملوحة في تجربة التأثير القصير الأمد، وبعد 56 يوم وصلت قيم تراكيز ايونات الصوديوم والكلورايد إلى حالة الاستقرار وكانت قيم متقاربة في الملوحات الثلاث (1.5 و 7.5 و 15) جزء بالألف .يمتلك تركيز ايون البوتاسيوم تناسبا طرديا مع زيادة الملوحة وقد انخفضت قيمه بمرور الوقت في كل الملوحات. مع هذا فان تركيز ايون البوتاسيوم في بلازما دم اسماك *T. zillii* يمتلك قيما منخفضة بالمقارنة مع تراكيز ايوني الصوديوم والكلورايد. تمتلك تراكيز الكلوكوز في بلازما دم اسماك *T. zillii* تناسبا طرديا مع زيادة الملوحة بعد مرور 96 ساعة من نقل الأسماك إلى الملوحات (1.5 و 7.5 و 15 و 30) جزء بالألف. بعد مرور 56 يوما من النقل إلى الملوحات (1.5 و 7.5 و 15) جزء بالألف انخفضت قيم تراكيز الكلوكوز معنويا ($P \leq 0.05$) في الملوحات الثلاث عن مستوياتها في تجربة التأثير القصير الأمد للملوحات المختلفة. انخفضت قيم البروتين الكلي في بلازما دم اسماك *T. zillii* بصورة معنوية ($P \leq 0.05$) في الملوحات الثلاث (1.5 و 7.5 و 30) جزء بالألف عن ملوحة السيطرة 15 جزء بالألف بعد 96 ساعة من بدء تجربة التأثير القصير الأمد للملوحات المختلفة, وبعد مرور 56 يوما ازدادت قيم البروتين في الملوحات الثلاث (1.5 و 7.5 و 15) جزء بالألف عن مدياتها في بقية الأزمان في تجربة التأثير القصير الأمد للملوحات المختلفة. أشارت النتائج أن زيادة الملوحة أدت إلى زيادة في قيم مكداس الدم والضغط الازموزي وايونات البلازما والكلوكوز والبروتين الكلي في بلازما دم اسماك *T. zillii* ونقصان في محتوى الماء في العضلات. تبدو الأسماك أعلى ازموزيا في الملوحات (1.5 و 7.5) جزء بالألف وأوطئ ازموزيا عند الملوحات (15 و 30) جزء بالألف، أيضا فان الوقت 96 ساعة هو غير كافي للأسماك للوصول إلى حالة الاستقرار في الضغط الازموزي في كل الملوحات.