COMPARATIVE STUDY BETWEEN JUVENILE AND ADULTS OF YELLOWFIN SEABREAM *Acanthopagrus latus* IN PLASMA BIOCHEMICAL INDICATORS INDUCED BY DIFFERENT SALINITIES

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ABSTRACT

Laboratorial experiments were carried out on juvenile and adults of *A. latus* to estimate and compare the effect of direct transfer from the control salinity of 1.5 psu to the different salinities of 1.5, 7.5, 15, 30 and 45 psu during the periods of 6, 24, 48 and 96hrs for short term effect experiments. Some physiological parameters (PCV, Osmotic pressure, muscles water content, total protein, glucose, sodium Na^+, potassium K^+ and chloride Cl^-) were tested in plasma of the two age stages. Results for all parameters showed a clear ability of adults to Osmoregulate and adapt a wide range of salinities ranged from 1.5 psu to 45 psu and explain why juvenile die at the salinity 45 psu after 48 hrs of transport by showing the insufficiency of juvenile to Osmoregulate and the high exhaustion of body activities in this salinity.

The results indicated that the salinity increase caused an increase in plasma PCV, osmotic pressure, (sodium, potassium and chloride ions), total protein and glucose. Also, a decrease in water content of muscles of juvenile and adults. The time of 96 hrs was not enough for juvenile to reach the stable condition at high salinities, while adults showed more constancy at the end time of experiment in all salinities.

INTRODUCTION

Salinity adaptation in euryhaline teleosts is a complex process involving a set of physiological responses to differing ion regulatory requirements (1). The organs involved in osmoregulation in teleosts include the opercular membrane, gill, gut, kidney, and urinary bladder (2). The gill is the primary organ that responds to the critical problem of salinity changes in teleost fish (2). Gill, as the most important osmoregulatory organ in fish (3; 2), absorbs Na^+ and Cl^- in low salinity environments and rapidly secretes these ions in relatively high salinity environments (4; 2). The transport of Na^+ and Cl^- across the teleost gill epithelium is facilitated by the chloride cells which absorb ion in freshwater while secrete ions in seawater (5; 2; 6) achieving that with the driving force provides from the universal membrane-bound enzyme Na^+K^+ATPase (NKA) (2).

The biochemical parameters consider as bio-indicators in a biological system which can be related to exposure to, or effects of, a contaminant compound or environmental stress. They are measures of the rates of chemical reactions or the amounts of biochemical products in cellular or sub cellular systems (7).

The hematocrit or paced cell volume (PCV) is one of the primary indices (directly measured) for the physiological state of the fish (8). Osmotic pressure in blood of teleost fish is about 280–360 mosmol.kg^{-1}, and is tightly regulated in a species-dependent range of salinities (9). Marine teleosts have a blood concentration lower than that of seawater (10), such as the Atlantic cod which are hypo-osmotic regulators, maintaining plasma osmolality at about one-third of that of full-strength seawater (11).

The high differences in osmotic pressure between the blood and external environment in fishes transferred to high saline mediums induces the loss of water from fish body surface to the external hyper saline medium which caused a decrease in water content of muscles (12).

Glucose level in fish blood is known to be very useful as a criterion for diagnosis of liver and muscle tissue functions (13). Salinity is one of the environmental factors affecting glucose concentration in fish blood (14; 15), by packing glucose to blood from its storage organs to satisfy the increasing demand for energy in osmoregulatory organs during salinity acclimation (16).

Environmental alterations caused increase synthesis or breakdown of proteins which are involved in major physiological events therefore the assessment of the protein content can be considered as a diagnostic tool to determine the physiological phases of organism (17). Total plasma protein concentration is a measure of all of the different proteins in plasma (18).

Fishes of *A. latus* are widely distributed in shallow coastal waters, lagoons and estuaries of the Indo–West Pacific included Arabian Gulf to Australia (19; 20). Adults spawn in coastal waters and the larvae move to estuaries (21). They are economically important for fishery and aquaculture, they considered as one of the leading fish species with high mariculture potential (22).

In the current study some plasma biochemical parameters was measured in order to investigate the patterns of responses and changes caused by the different salinities in different age stages, so the aim of current study was to determine the *in vitro* effects of salinity on biochemical characteristics of juvenile and adults of yellowfin sea bream *A. latus* and make a comparison between the two life stages of this species.

MATERIALS AND METHODS

Experimental groups

A. latus juvenile and adults were collected from two stations of Shatt Al-Arab River (juvenile from OmAlresas and adults from Bradeia) which has a salinity mean of (1.46 ± 0.20) psu, by two types of nets, Seine Nets (30m length, 4m highest, 2x2 cm mish size) and Cast Nets (10 m diameter, 2x2 cm mish size). a total of 600 fishes of *A. latus* juvenile and adults were collected from July to October 2011. Fishes were held in cooling boxes and transfered to the laboratory and were divided randomly into five groups (24 fish in each group and two replicates per treatment). Two groups were held in five different salinities at concentrations of 1.5, 7.5, 15, 30 and 45 psu. The first group was considered as control. Ten 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. 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).

Fish sampling and Blood collection

After direct transfer to the different salinities, six fishes were killed from each salinity at the times (6, 24, 48 and 96) hrs after being anesthetized by clove oil by putting it in a container with water in the same salinity they taken from and adding clove oil to the water (23). There total length (cm) and total weight (g) were recorded. Blood samples were collected from the caudal veins 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 for PCV measurements. Plasma was collected from capillary tubes by a Micro syringe with a volume of 100µm, transferred to ependorf tubes with a volume of 0.5 ml and kept in freeze at -20 c° for glucose, protein and chloride (Cl^-) measurements. Plasma from other capillary tubes was diluted 100 times in distilled water and kept in plastic plen tubes with a volume of 12 ml and kept frozen in -20c° for sodium Na^+ and potassium K^+ measurements.

Scales and skin samples were removed from the region under the dorsal fin and a piece of muscle tissue was taken and washed in distilled water to remove external salts and kept frozen at -20 °c in plastic Petri dish for water content measurements.

Packed Cell Volume (PCV) and Osmotic pressure measurements

Capillary tubes were put in a micro centrifuge (model haematokrit 210) at (3500) rpm for 5 minutes to separate plasma from blood. PCV was measured by estimating Haematocrit ratio (%) using Micro-Capillary Reader type DAMON/IEC.

After PCV measurements, osmolality ($mosmol.Kg^{-1}$) was measured directly in the plasma ependorf tubes with a volume of 0.5 ml by the freezing point depression measurement method using the cryoscopic osmometer (model OSMOMAT 030 manufactured by GONOTEC, Germany) (24).

Water content in muscles

Analysis of water content of muscles was conducted by drying samples in an oven at (105) c°. and calculated from the equation:

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

Glucose Analysis

Glucose concentration (mmol/L) in plasma was estimated by using a commercial kit (RANDOX laboratories/UK, 1775 GL) in a colorimetric method according to (25) using a spectrophotometer (model Humalyzer Primus) at a wavelength of 500 nm.

Protein Analysis

The Biuret method was used for estimating the total protein (g/100ml) in plasma by using a commercial kit (BIOLABO SA, 02160 maizy, France, LP87016) in a colorimetric method using a spectrophotometer model (Humalyzer Primus) at a wavelength of 550 nm.. The reaction principles were described by (26).

Ion Analysis

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

1 mmol/L sodium(Na^+) = 23 ppm

1 mmol/L potassium (K^+) = 39 ppm

Chloride (Cl^-) ion in plasma was estimated by a commercial kit manufactured by (BIOLABO SA, 02160 maizy, France) in a colorimetric method using a spectrophotometer model (Humalyzer Primus) at a wavelength of 500 nm. depending on the principles of (27) for estimating chloride ion.

Environmental and Survival rate Analysis

Four environmental factors (tempreture, D.O, salinity, pH) were measured daily using the YSI instrument model (**556 MPS**). Survival rate was estimated from the following equation:

Survival Rate (%) = (N2/N1)×100 (28)

N1 = number of fishes at beginning of experiment.

N2 = number of fishes at end of experiment.

Statistical Analysis

Values were compared using a one-way analysis of variance (ANOVA) and Revised Least Significant Difference (RLSD) to compare the variances between salinities in different times. ($P < 0.05$) was set as the significance level using SPSS program. Values were expressed as the (mean ± S.E.M.) (the standard error of the mean) (29).

RESULTS

Experimental groups

Total length and weight for juvenile of *A. latus* were (10.41 ± 0.12)cm and (22.97 ± 0.89) gm respectively and for adults of *A. latus* were (20.22 ± 0.34)cm and (166.25 ± 1.68) gm respectively. Environmental factors of water containers of juvenile for temperature (30.18 ± 0.47) c^o, Dissolved Oxygen (4.70 ± 0.2) mg/l and pH (8.10 ± 0.1). While environmental factors in containers of adults were (22.82 ± 0.05) ^{o}c for temperature, (5.48 ± 0.1) mg/l for D.O. and (7.9 ± 0.12) for pH.

A. latus adults were survived (100)% in all salinities (from 1.5 to 45) psu at all times of the experiment. While *A. latus* juvenile were survived (100)% in the salinities (1.5, 7.5, 15, 30) psu, and all fishes were die in the salinity 45 psu after 48 hrs of direct transfer.

Paced Cell Volume (PCV)

Tables (1) and figure (1) explain the PCVs measurement (%) for *A. latus* juvenile and adults blood in the short term effect experiment of different salinities (1.5, 7.5, 15, 30 and 45 psu) at different times (6. 24, 48 and 96 hrs). PCV values in juvenile and adults of *A. latus* correlated directly with the salinity increase in all times of experiments, and the peak one (60.65 ± 2.4)% obtained in adults in the salinity 45 hrs at 48 hrs.

Generally we can see from figure (1) that juvenile of *A. latus* had the highest PCV values at the time 24 hrs in all salinities, then it start to decrease at times (48, 96 hrs) but still higher than the first time 6 hrs. this reflect the unstable condition at these fishes. While in adults of *A. latus* PCV values decrease gradually with time and the time 96 hrs had the lowest values in all salinities, which mean adults had more constancy status from juvenile. Statistical analysis for juvenile showed there were significant differences ($P < 0.05$) in PCV values between the control salinity 1.5 psu and the other salinities (7.5, 15, 30) psu and there were no significant differences ($P > 0.05$) in PCV values between the salinities (7.5, 15, 30) at the end time (96) hrs of experiment. While adults showed there were no significant differences ($P > 0.05$) in PCV values between the control salinity 1.5psu and the salinities (7.5, 15, 30) psu and there were significant differences ($P < 0.05$) between the salinity 45 psu and the salinities (1.5, 7.5) psu at the end time (96) hrs of experiment.

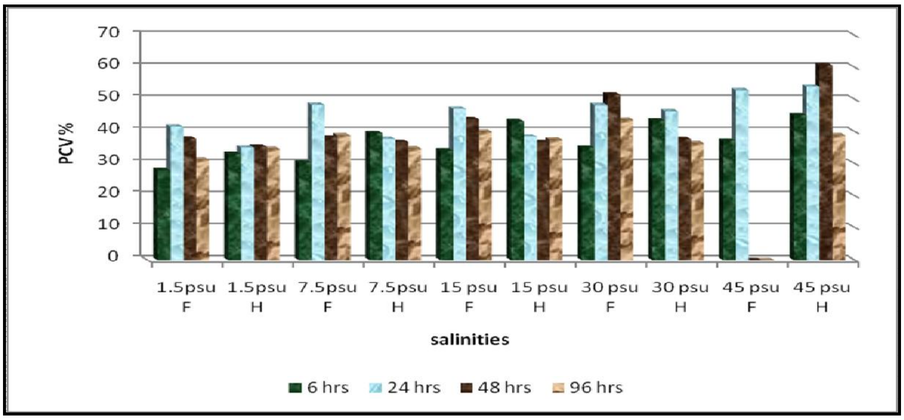

Figure (1): PCV (%) for *A. latus* juvenile and adults blood in the short term effect experiment of different salinities

Table (1): PCV (%) for *A. latus* juvenile and adults blood in the short term effect experiment of different salinities (Mean ± S.E.).

Time	1.5 psu		7.5 psu		15 psu		30 psu		45 psu	
	F	H	F	H	F	H	F	H	F	H
6hrs	27.92 ±2.50 b	33.05 ±0.73 C	30.34 ±1.5 ab	39.51 ±1.13 B	34.10 ±2.50 a	43.17 ±1.61 A	34.93 ± 3.08 a	43.36 ±0.45 A	37.06 ±1.85 a	45.20 ±0.63 A
24hrs	41.53 ±1.28 b	35.00 ±0.38 D	48.30 ±1.27 a	37.67 ±0.52 C	47.16 ±2.99 ab	38.47 ±0.72 C	48.22 ±4.16 ab	46.36 ±1.28 B	52.89 ±2.36 a	53.94 ±1.21 A
48hrs	37.73 ±4.39 b	35.28 ±0.79 B	38.14 ± 1.48 b	36.7 ±0.56 B	43.84 ±2.86 ab	36.67 ±0.53 B	51.46 ±2.79 a	37.61 ±0.99 B	——	60.65 ±2.40 A
96hrs	31.28 ±2.26 b	34.60 ±1.05 B	38.86 ±2.66 a	34.72 ± 0.77 B	40.00 ±1.92 a	37.45 ± 1.43 AB	43.67 ±2.09 a	36.31 ± 1.24 AB	——	38.90 ±0.86 A

*The capital letters (A,B,C) means a significant differences among the groups of *A. latue* adults.

**The small letters (a,b,c) means a significant differences among the groups of *A. latue* juveniles.

Osmotic Pressure

Tables (2) and figure (2) explain the Osmotic Pressure (mOsmol.Kg^{-1}) for *A. latus* juvenile and adults plasma in the short term effect experiment of different salinities (1.5, 7.5, 15, 30 and 45 psu) at different times (6. 24, 48 and 96 hrs). Results showed that there were an increase in osmotic pressure values from the control salinity 1.5 psu to all other salinities (7.5, 15, 30, 45) psu at all times of the experiments in juvenile and adults of *A. latus*. The osmotic pressure correlated directly with salinity increase in both juvenile and adults of *A. latus*.

Figure (2) showed that adults of *A. latus* had highest values of osmotic pressure from juvenile at all salinities and times, and the values of osmotic pressure in adults still arise with time until the end time 96 hrs of experiment in all salinities. This reflect the high efficiency for osmoregulation at these fishes and the time 96 hrs is not enough to reach the stable state in osmotic pressure at these fishes. While in juvenile osmotic pressure values were close in the same salinity at different times (6, 24, 48, 96) hrs and correlated directly with salinity increase. The highest value of osmotic pressure obtained in juvenile was (370.67 ± 0.67) mOsmol.Kg^{-1} in the salinity 45 psu at the time 24 hrs, this value is so lower than the highest one in adults which was (514.29 ± 2.45) mOsmol.Kg^{-1} in the salinity 45 at the end time 96 hrs of experiment, this explain the insufficient of juvenile to osmoregulate and the fish die at this salinity.

Statistical analysis for juvenile and adults showed there were significant differences ($P < 0.05$) in osmotic pressure values between all salinities at the end time (96) hrs of experiments, except between the control salinity 1.5psu and the salinity 7.5 psu in adults were no significant differences ($P > 0.05$).

Table (2): 0smotic pressure (mOsmol.Kg-1) for *A. latus* juvenile and adults plasma in the short term effect experiment of different salinities (Mean ± S.E.).

Time	1.5 psu		7.5 psu		15 psu		30 psu		45 psu	
	F	H	F	H	F	H	F	H	F	H
6hrs	305.33 ±0.88 b	345.55 ± 2.69 E	315.00 ±0.00 b	359.22 ±2.19 D	334.00 ±0.58 a	394.82 ±2.18 C	345.00 ± 1.00 a	414.00 ±2.17 B	348.00 ±12.00 a	483.10 ±1.91 A
24hrs	306.33 ±0.88 e	366.50 ±1.74 E	317.00 ±1.15 d	383.93 ±1.23 D	337 ±2.52 c	411.00 ±8.31 C	345 ±1.00 b	421.25 ±2.27 B	370.67 ±0.67 a	502.77 ±2.72 A
48hrs	308.67 ±0.33 b	377.91 ±1.63 C	316.33 ± 0.88 b	382.12 ±1.39 C	344.67 ±4.91 a	386.40 ±1.54 C	357.67 ±7.84 a	413.67 ±3.11 B	—	503.80 ±8.25 A
96hrs	307.67 ±0.88 d	386.08 ±6.67 D	314.67 ±0.33 c	387.77 ± 1.06 D	336.67 ±3.18 b	399.46 ± 1.62 C	352.33 ±0.88 a	433.42 ± 1.85 B	—	514.29 ±2.45 A

*The capital letters (A,B,C) means a significant differences among the groups of *A. latue* adults.
**The small letters (a,b,c) means a significant differences among the groups of *A. latue* juveniles.

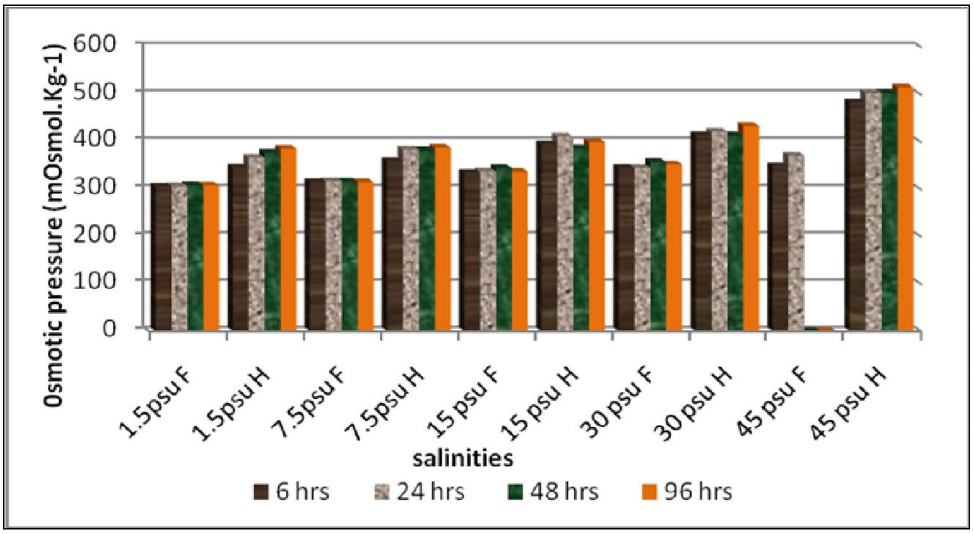

Figure (2): 0smotic pressure (mOsmol.Kg-1) for *A. latus* juvenile and adults blood in the short term effect experiment of different salinities.

Water content

Table (3) and figure (3) explain the water content (%) for *A. latus* juvenile and adults muscles in the short term effect experiment of different salinities (1.5, 7.5, 15, 30 and 45 psu) at different times (6. 24, 48 and 96 hrs). Results showed that there were a decrease in water content values with salinity increase at all times of the experiments in juvenile and adults of *A. latus*. The water content correlated reversely with salinity increase in both juvenile and adults of *A. latus*.

Statistical analysis for juvenile showed there were significant differences ($P < 0.05$) in water content values between all salinities at the end time (96) hrs of experiment. While adults showed there were no significant differences ($P > 0.05$) in water content values between the salinities [(1.5 and 7.5), (7.5 and 15), (15 and 30) and (30 and 45)]psu at the end time (96) hrs of experiment.

Table (3): Water content (%) for *A. latus* juvenile and adults muscles in the short term effect experiment of different salinities (Mean ± S.E.).

Time	1.5 psu		7.5 psu		15 psu		30 psu		45 psu	
	F	H	F	H	F	H	F	H	F	H
6hrs	77.84 ±0.20 a	77.67 ±0.95 A	77.18 ± 0.16 a	77.22 ±0.001 AB	76.52 ±0.31 ab	75.63 ±0.002 BC	75.62 ± 0.23 b	74.09 ±0.007 CD	74.86 ±0.75 c	73.85 ±0.43 D
24hrs	78.49 ±0.57 a	77.99 ±0.85 A	77.03 ±0.21 ab	76.47 ±0.005 AB	75.56 ±0.007 b	75.55 ±0.009 B	75.23 ±0.97 b	73.68 ±0.20 C	72.16 ±0.006 c	72.47 ±0.35 C
48hrs	81.33 ±0.71 a	77.33 ±0.32 A	77.68 ± 0.12 b	75.75 ±0.29 B	75.99 ±0.005 c	75.37 ±0.004 C	75.11 ±1.1 d	73.41 ±0.005 D	—	71.44 ±0.20 E
96hrs	80.65 ±0.001 a	77.12 ±0.10 A	78.18 ±0.006 b	75.62 ± 0.20 B	75.81 ±0.15 c	75.17 ± 0.005 C	74.64 ±0.42 d	73.32 ± 0.11 D	—	70.02 ±0.61 E

*The capital letters (A,B,C) means a significant differences among the groups of *A. latue* adults.

**The small letters (a,b,c) means a significant differences among the groups of *A. latue* juveniles.

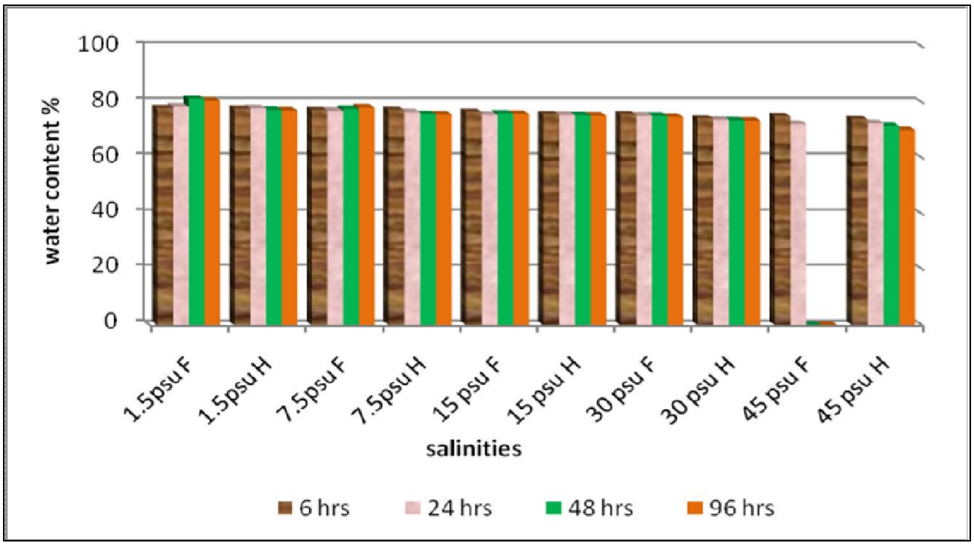

Figure 3: Water content (%) for *A. latus* juvenile and adults blood in the short term effect experiment of different salinities

Total protein

Table (4) and figure (4) explain the total protein (gm/100 ml) for *A. latus* juvenile and adults plasma in the short term effect experiment of different salinities (1.5, 7.5, 15, 30 and 45 psu) at different times (6. 24, 48 and 96 hrs). Total protein values correlated directly with salinity increase and it decrease with time at each salinity in both juvenile and adults of *A. latus*.

In a comparison between juvenile with adults of *A. latus* we can see from figure (4) that juvenile had the highest values of total protein in the high salinities (30, 45) psu at the first time 6 hrs of experiment. It decrease with time in the salinity 30 psu and reached to 6.63 gm/100 ml at the end time 96 hrs, but in the salinity 45 psu total protein values reached to the highest one (9.41) gm/100 ml and decreased to 7.18 gm/100 ml at the time 24 hrs. This reflect the high exhausted of body activities in juvenile to adapt the high salinities and the inability of them to adequate and fish die at the salinity 45 psu. While adults of *A. latus* had a gentle arise of total protein values with salinity increase and the values decrease with time at each salinity even in the salinity 45 psu which decreased from 5.53 mg/100 ml at the time 6 hrs to 3.30 mg/100 ml at the end time 96 hrs of experiment. This explain the high efficiency of adults to adapt the very high salinities and its return to the stable state in total protein values even in the highest salinity 45 psu. Statistical analysis for juvenile showed there were significant differences ($P < 0.05$) in total protein values between all salinities at the end time (96) hrs of experiment. While adults showed there were no significant differences ($P > 0.05$) in total protein values between all salinities at the end time (96) hrs of experiment.

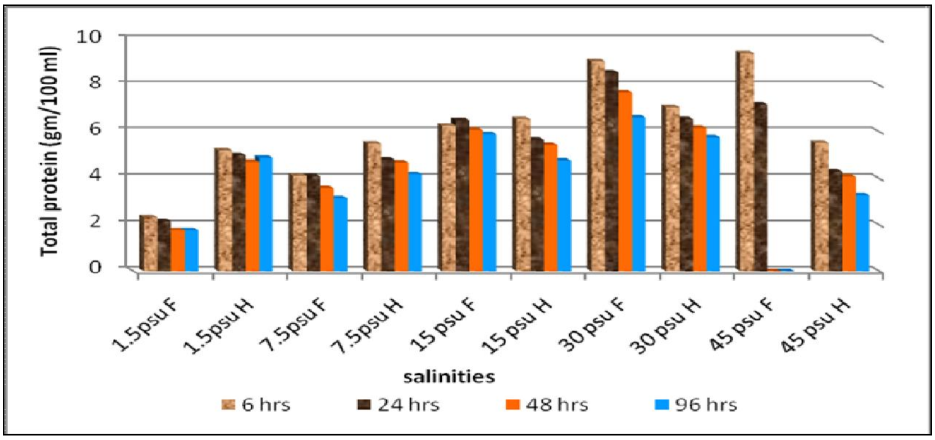

Figure (4): Total protein (gm/100 ml) for *A. latus* juvenile and adults plasma in the short term effect experiment of different salinities

Table (4): Total protein (gm/100 ml) for *A. latus* juvenile and adults plasma in the short term effect experiment of different salinities (Mean ± S.E.).

Time	1.5 psu		7.5 psu		15 psu		30 psu		45 psu	
	F	H	F	F	F	H	F	H	F	H
6hrs	2.34 ±0.001 d	5.22 ±0.44 B	4.12 ± 0.004 c	5.50 ±0.57 B	6.27 ±0.006 b	6.56 ±0.44 AB	9.07 ± 0.18 a	7.07 ±0.48 A	9.41 ±0.54 a	5.53 ±0.00 A
24hrs	2.17 ±0.007 e	5.01 ±0.31 A	4.10 ±0.002 d	4.82 ±0.99 A	6.51 ±0.008 c	5.66 ±0.33 A	8.57 ±0.13 a	6.56 ±1.17 A	7.18 ±0.26 b	4.30 ±0.20 A
48hrs	1.77 ±0.009 d	4.72 ±0.52 A	3.60 ± 0.004 c	4.69 ±1.00 A	6.08 ±0.006 b	5.44 ±0.23 A	7.70 ±0.29 a	6.17 ±1.12 A	—	4.08 ±0.47 A
96hrs	1.77 ±0.005 d	4.90 ±0.68 A	3.17 ±0.005 c	4.18 ± 0.65 A	5.90 ±0.001 b	4.78 ± 0.40 A	6.63 ±0.11 a	5.76 ± 1.28 A	—	3.30 ±0.24 A

*The capital letters (A,B,C) means a significant differences among the groups of *A. latue* adults.

**The small letters (a,b,c) means a significant differences among the groups of *A. latue* juveniles.

Glucose

Table (5) and figure (5) showed the glucose concentration ($Mmol.L^{-1}$) for *A. latus* juvenile and adults plasma in the short term effect experiment of different salinities (1.5, 7.5, 15, 30 and 45 psu) at different times (6. 24, 48 and 96 hrs). Glucose concentration values correlated directly with salinity increase in both juvenile and adults.

Generally we can see from figure (5) that adults of *A. latus* had highest values of glucose from juvenile at all salinities and times. The highest value of glucose concentration obtained in adults in the salinity 45 psu at the time 24 hrs which was (24.31 ± 1.33) ($Mmol.L^{-1}$), it decreased to (14.50) $Mmol.L^{-1}$ at the end time 96 hrs of experiment. This reflect the high demand to glucose in adults for energy supplement needed for high salinity adaptation. While in juvenile of *A. latus* glucose concentration values were close in the same salinity at different times (6, 24, 48, 96) hrs and correlated directly with salinity increase. The highest value of glucose concentration obtained in juvenile was (10.98 ± 0.84) $Mmol.L^{-1}$ in the salinity 45 psu at the time 6 hrs, this value is so lower than the highest one in adults in the same salinity. This explain the insufficient of juvenile to supply glucose for different metabolic activities that complain with salinity adaptation and the fish die at this salinity. Statistical analysis for juvenile showed there were significant differences ($P < 0.05$) in glucose concentration values between all salinities at the end time (96) hrs of experiment. While adults showed there were no significant differences ($P > 0.05$) in glucose concentration values between the salinities (7.5, 15, 30) psu at the end time (96) hrs of experiment.

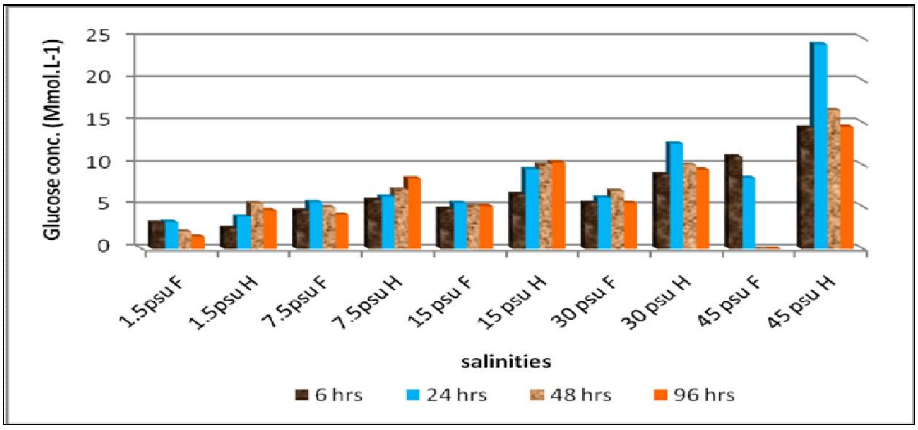

figure (5): Glucose concentration (Mmol.L-1) for *A. latus* juvenile and adults plasma in the short term effect experiment of different salinities.

Table (5): Glucose concentration (Mmol.L-1) for *A. latus* juvenile and adults plasma in the short term effect experiment of different salinities (Mean ± S.E.).

Time	1.5 psu		7.5 psu		15 psu		30 psu		45 psu	
	F	H	F	H	F	H	F	H	F	H
6hrs	3.04 ±0.34 c	2.39 ±0.33 D	4.53 ± 0. 4 bc	5.81 ±0.17 C	4.73 ±0.21 bc	6.50 ±0.63 C	5.45 ± 0.31 b	8.91 ±0.008 B	10.98 ±0.84 a	14.40 ±0.18 A
24hrs	3.13 ±0.26 c	3.76 ±0.005 D	5.56 ±0.29 b	6.19 ±0.30 D	5.45 ±0.10 b	9.49 ±0.48 C	6.06 ±0.71 b	12.51 ±0.91 B	8.44 ±0.001 a	24.31 ±1.33 A
48hrs	2.03 ±0.51 c	5.41 ±0.47 D	4.92 ± 0.001 b	7.04 ±0.30 C	5.11 ±0.003 b	10.08 ±0.007 B	6.89 ±0.52 a	10.03 ±0.18 B	—	16.47 ±0.24 A
96hrs	1.44 ±0.009 c	4.56 ±0.43 C	4.06 ±0.68 b	8.38 ± 1.60 B	5.08 ±0.001 b	10.32 ± 0.22 B	5.45 ±0.1 a	9.44 ± 0.001 B	—	14.50 ±1.21 A

*The capital letters (A,B,C) means a significant differences among the groups of *A. latue* adults.

**The small letters (a,b,c) means a significant differences among the groups of *A. latue* juveniles.

Sodium Na+

Table (6) and figure (6) showed sodium concentration (Mmol.L-1) for *A. latus* juvenile and adults plasma in the short term effect experiment of different salinities (1.5, 7.5, 15, 30 and 45 psu) at different times (6. 24, 48 and 96 hrs). Sodium concentration values correlated directly with salinity increase and it decrease with time at each salinity in both juvenile and adults of *A. latus*.

Figure (6) showed that adults of *A. latus* had highest values of sodium from juvenile at all salinities and times. The highest value of sodium concentration obtained in adults in the salinity 45 psu at the time 48 hrs which was (259.7 ± 15.87) (Mmol.L-1), it decreased to (198.18 ± 1.31) Mmol.L-1 at the end time 96 hrs of experiment. This reflect the high efficiency of adults to osmoregulate at high salinities. While in juvenile of *A. latus*, sodium concentration values correlated directly with salinity increase and it lower than that of adults in all salinities and at all times. The highest value of sodium concentration obtained in juvenile was (201.32 ± 12.1) Mmol.L-1 in the salinity 45 psu at the time 6 hrs, this value is so lower than the highest one in adults in the same salinity. This explain the insufficient of juvenile to supply sodium ions for osmoregulation activities and the fish die at this salinity. Statistical analysis for juvenile showed there were significant differences ($P < 0.05$) in sodium concentration values between the control salinity 1.5 psu and the other salinities except the salinity 7.5 psu and there were no significant differences ($P > 0.05$) in sodium concentration values between the salinities (15, 30) at the end time (96) hrs of experiment. While adults showed there were no significant differences ($P > 0.05$) in sodium concentration values between the salinities (7.5, 30) psu and (15, 30) psu at the end time (96) hrs of experiment.

Table (6): Sodium concentration (Mmol.L-1) for *A. latus* juvenile and adults plasma in the short term effect experiment of different salinities (Mean ± S.E.).

Time	1.5 psu		7.5 psu		15 psu		30 psu		45 psu	
	F	H	F	H	F	H	F	H	F	H
6hrs	132.44 ±2.41 b	157.31 ±2.40 C	136.40 ± 0.009 b	154.59 ±1.20 C	142.87 ±0.009 b	197.20 ±4.68 B	158.06 ±12.06 b	199.70 ±15.87 B	201.32 ±12.1 a	247.45 ±4.43 A
24hrs	135.84 ±4.16 b	140.02 ±4.24 C	135.28 ±4.11 b	153.94 ±5.11 BC	141.81 ±1.13 ab	174.70 ±3.05 BC	155.62 ±14.90 ab	184.48 ±1.52 B	170.81 ±12.45 a	253.61 ±24.57 A
48hrs	123.16 ±2.88 c	134.05 ±1.09 C	123.39 ± 0.45 c	149.70 ±6.74 BC	141.11 ±1.86 b	172.31 ±4.79 B	166.67 ±2.91 a	169.24 ±1.59 B	—	259.70 ±15.87 A
96hrs	97.02 ±1.09 b	123.94 ±7.72 D	100.15 ±8.25 b	149.52 ± 4.09 C	138.04 ±2.65 a	173.50 ±4.02 B	138.19 ±1.06 a	157.87 ± 5.30 BC	—	198.18 ±1.31 A

*The capital letters (A,B,C) means a significant differences among the groups of *A. latue* adults.
**The small letters (a,b,c) means a significant differences among the groups of *A. latue* juveniles.

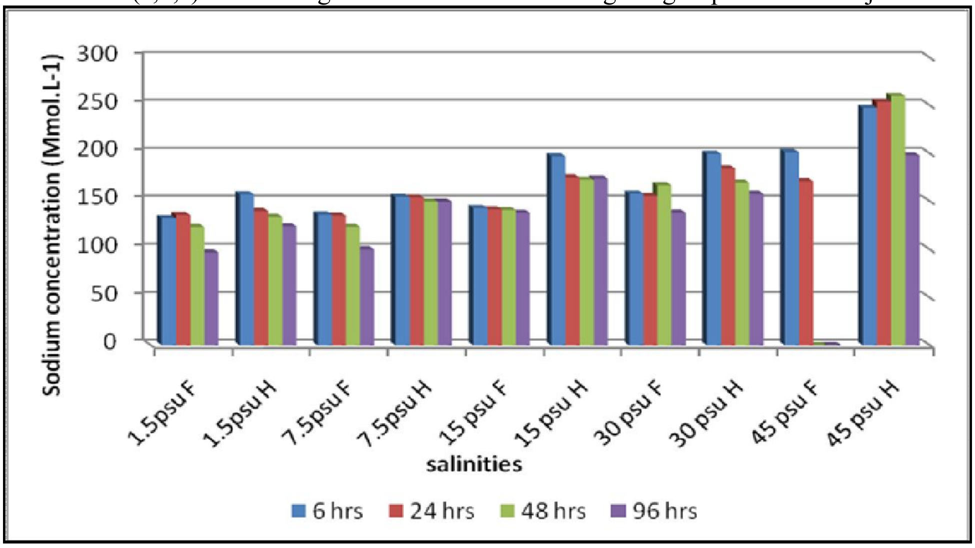

Figure (6): Sodium concentration (Mmol.L-1) for *A. latus* juvenile and adults plasma in the short term effect experiment of different salinities

Potassium K^+

Table (7) and figure (7) showed potassium concentration (Mmol.L-1) for *A. latus* juvenile and adults plasma in the short term effect experiment of different salinities (1.5, 7.5, 15, 30 and 45 psu) at different times (6. 24, 48 and 96 hrs). Potassium concentration values correlated directly with salinity increase in both juvenile and adults of *A. latus*.

Figure (7) showed that in adults of *A. latus* there were a gradual increase in potassium concentration values with time especially at the salinities (15, 30) psu, and it little decrease in other salinities at the end time 96 hrs of experiment. While in juvenile there were an increase in potassium concentration values at the first hours and then it start to decrease with time spend, except in the highest salinity 45 psu the peak value was at the time 24 hrs (15.74 ± 1.14) $Mmol.L^{-1}$. The highest values of potassium concentration in adults was (15.67 ± 0.96) $Mmol.L^{-1}$ in the salinity 45 psu at the time 48 hrs, and it's close to the peak value in juvenile , then it decrease to (13.61 ± 0.40) $Mmol.L_{-1}$ at the end time 96 hrs of experiment. So the results for potassium concentration were disorganized and don't explain a clear distinguish between adults and juvenile of *A. latus*. Statistical analysis for juvenile showed there were significant differences ($P < 0.05$) in potassium concentration values between all salinities except the salinities (7.5, 15) psu at the end time (96) hrs of experiment. While adults showed there were a significant differences ($P < 0.05$) in potassium concentration values between all salinities at the end time (96) hrs of experiment.

Table (7): Potassium concentration (Mmol.L-1) for *A. latus* juvenile and adults plasma in the short term effect experiment of different salinities (Mean ± S.E.).

Time	1.5 psu		7.5 psu		15 psu		30 psu		45 psu	
	F	H	F	H	F	H	F	H	F	H
6hrs	4.65 ±0.00 d	3.53 ±0.009 D	6.54 ± 0.3 c	4.30 ±0.002 D	10.65 ±0.004 b	5.92 ±0.26 C	11.59 ±0.004 b	7.52 ±0.11 B	13.69 ±0.64 a	11.01 ±0.60 A
24hrs	6.48 ±0.007 c	4.27 ±0.006 E	8.59 ±0.009 c	5.72 ±0.11 D	9.78 ±0.60 b	8.38 ±0.14 C	11.55 ±0.62 b	9.26 ±0.35 B	15.74 ±1.14 a	14.47 ±0.15 A
48hrs	5.76 ±0.22 c	5.37 ±0.25 D	5.82 ± 0.002 c	8.58 ±0.006 C	8.11 ±0.005 b	9.53 ±0.41 BC	9.58 ±0.64 a	11.06 ±0.35 B	—	15.67 ±0.96 A
96hrs	3.22 ±0.56 c	4.77 ±0.12 E	4.54 ±0.31 b	6.42 ± 0.31 D	5.60 ±0.008 b	10.39 ±0.23 C	7.47 ±0.36 a	12.13 ± 0.49 B	—	13.61 ±0.40 A

*The capital letters (A,B,C) means a significant differences among the groups of *A. latue* adults.
**The small letters (a,b,c) means a significant differences among the groups of *A. latue* juveniles.

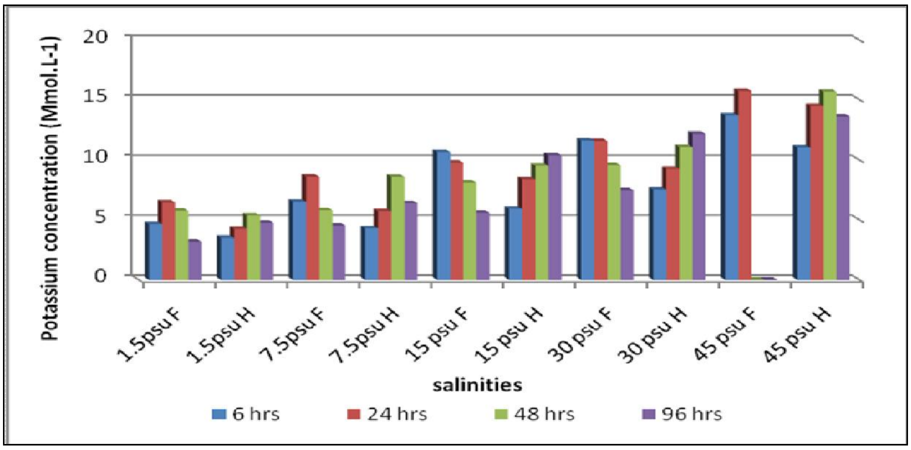

Figure (7): Potassium concentration (Mmol.L^{-1}) for *A. latus* juvenile and adults plasma in the short term effect experiment of different

Chloride Cl^-

Table (8) and figure (8) showed chloride concentration ($Mmol.L^{-1}$) for *A. latus* juvenile and adults plasma in the short term effect experiment of different salinities (1.5, 7.5, 15, 30 and 45 psu) at different times (6. 24, 48 and 96 hrs). Chloride concentration values correlated directly with salinity increase in both juvenile and adults of *A. latus*.

In figure (8) we can see that in juvenile of *A. latus* there were a gradual increase in chloride concentration values with time spend except the salinity 30 psu there were a little decrease at the end time 96 hrs of experiment. While in adults there were an increase in chloride concentration values at the first hours and then it start to decrease with time spend. This explain the high efficiency of adults to osmoregulate at the highest salinities in its short term effect. The highest values of chloride concentration obtained in juvenile was (263.53 ± 7.37) $Mmol.L^{-1}$ in the salinity 45 psu at the time 24 hrs, This reflect the high exhausted of juvenile to osmoregulate the highest salinities and the inability of them to adequate and fish die at the salinity 45 psu. Statistical analysis for juvenile showed there were significant differences ($P < 0.05$) in chloride concentration values between all salinities except the salinities (7.5, 15) psu While adults showed there were no significant differences ($P > 0.05$) in chloride concentration values between the salinities (1.5, 7.5) psu and (7.5, 15) psu at the end time (96) hrs of experiment.

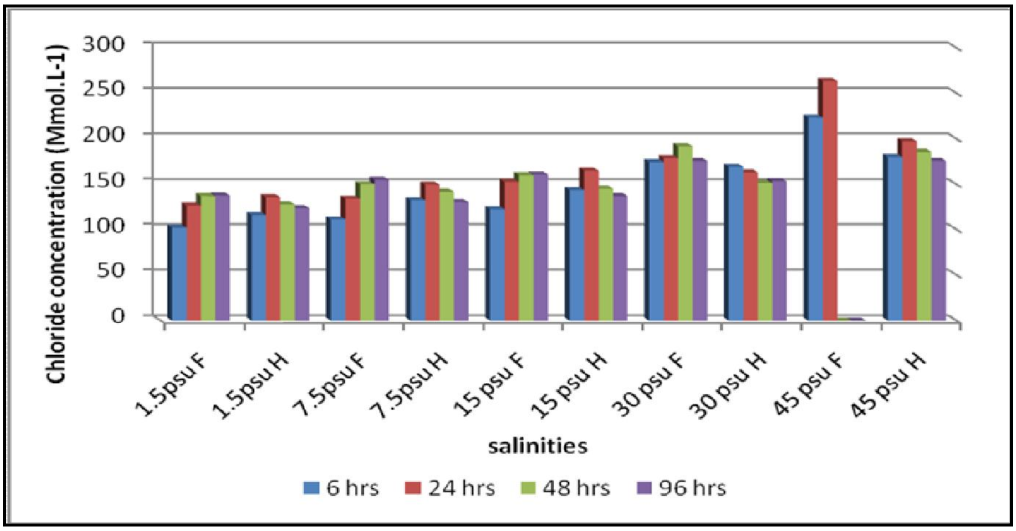

Figure (8): Chloride concentration ($Mmol.L^{-1}$) for *A. latus* juvenile and adults plasma in the short term effect experiment of different salinities.

Table (8): Chloride concentration (Mmol.L^{-1}) for *A. latus* juvenile and adults plasma in the short term effect experiment of different salinities (Mean ± S.E.).

Time	1.5 psu		7.5 psu		15 psu		30 psu		45 psu	
	F	H	F	H	F	H	F	H	F	H
6hrs	102.12 ±4.71 c	116.32 ±5.37 E	111.26 ± 2.89 c	132.71 ±1.57 D	122.74 ±4.51 c	143.83 ±0.94 C	174.93 ±12.37 b	169.47 ±0.37 B	223.06 ±21.09 a	180.44 ±0.58 A
24hrs	126.70 ±6.30 d	136.02 ±4.32 D	134.25 ±3.68 cd	149.88 ±1.70 C	152.71 ±0.00 c	165.22 ±4.85 B	179.25 ±8.68 b	163.22 ±0.005 B	263.53 ±7.37 a	197.51 ±3.71 A
48hrs	137.31 ±2.83 d	127.97 ±1.63 B	150.25 ± 2.47 c	141.96 ±0.70 B	160.21 ±3.35 b	145.45 ±1.17 B	192.14 ±1.84 a	151.79 ±5.80 B	—	185.98 ±15.66 A
96hrs	137.93 ±2.46 c	123.97 ±1.1 D	155.18 ±2.47 b	130.40 ± 1.96 CD	160.94 ±4.08 b	137.30 ±1.64 C	176.24 ±1.36 a	153.68 ± 0.88 B	—	176.41 ±6.34 A

*The capital letters (A,B,C) means a significant differences among the groups of *A. latue* adults.
**The small letters (a,b,c) means a significant differences among the groups of *A. latue* juveniles.

DISCUSSION

Fish species have attracted considerable interest in studies assessing biological and biochemical responses to environmental contaminants and stress. They are used as test organisms in aquatic toxicology and salinity variable because of their top- position in the trophic chain and their role as food for humans (30). Blood and its derivatives (serum, plasma) is the most accessible component of the body fluid system and has frequently been examined to assess physiological status (31).

Results for PCV in juvenile and adults of *A. latus* correlated directly with the salinity increase in all times of experiments, and the adults showed the highest value (60.65 ± 2.4)% in the salinity 45 hrs at 48 hrs. This is in agreement with many studies found an increase in PCV values with salinity increase like the study of (32) on *Tilapia zillii* fingerlings affected by different salinities, and the study of (33) which found an increase in the PCV to (36.17, 40.15 and 45.30%) when the salinity increased to (5, 10 and 15g/l) respectively in *Cyprinus carpio*.

The osmotic pressure correlated directly with salinity increase in both juvenile and adults of *A. latus* and the highest value of osmotic pressure obtained in adults which was (514.29 ± 2.45) mOsmol.Kg^{-1} in the salinity 45 at the end time 96 hrs of experiment. A similar results found in the study of (24) by showing that the *Siganus rivulatus* had an increase in osmotic pressure values with a salinity increase ranging from 10 psu to 50 psu.

The water content correlated reversely with salinity increase in both juvenile and adults of *A. latus* and these results in agreement with the study of (34) which found that high salinity caused alterations in the water content of the muscles of blue tilapia (*Oreochromis aureus*) which were significantly decreased in 150% SW and 200% SW. (35) was found a decrease in water content of the catfish (*Mystus vittatus*) muscles with an increase in salinity.

Total protein values correlated directly with salinity increase and it decrease with time at each salinity in both juvenile and adults of *A. latus*. This result in agreement with (36) who 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. In contrast the study of (37) found an overall downward trend in the content total protein (TP), albumin (ALB), and globulin (GLB) content of Young Chum Salmon (*Oncorhychus keta*)fish reared in the higher salinity treatment groups. The TP and GLB content in fish exposed to freshwater was significantly higher than found in fish in the 15‰ and 20‰ salinity treatment groups.

Glucose concentration values correlated directly with salinity increase in both juvenile and adults. 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 (38) the plasma glucose levels increased significantly with the increase in the environmental salinity and temperature. The results of (32) showed that the glucose concentrations in the *T. zillii* plasma had a direct proportion with salinity increase.

Sodium, chloride and potassium concentration values correlated directly with salinity increase in both juvenile and adults of *A. latus*. This results are in agreement with the study of (39) they showed that water salinity level increased to concentrations above 8 psu can have significant effect on the mineral level (sodium, potassium, calcium, phosphorus) of blood serum in grass carp. (40) also indicated that sodium and chloride concentrations increased at higher salinities than 8 psu. of cultured Eurasian perch, *Perca fluviatilis*. (41) 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 (42). A study by (43) recorded an increase in potassium ion in *Liza abu* plasma transferred from fresh water to higher water salinities. (32) study showed an increase in sodium, potassium anfd chloride ions with salinity increase in *Tilapia zillii* plasma.

Results for all parameters (PCV, osmotic pressure, water content, total protein, glucose, sodium, potassium and chloride) showed a clear ability of adults to osmoregulate and adapt the high salinities that reach to 45 psu and explain why juvenile die at the salinity 45 psu after 48 hrs of experiment by showing the insufficient of juvenile to osmoregulate and the high exhausted of body activities in this salinity. Also statistical analysis for juvenile (in reverse of adults) showed a significant differences ($P < 0.05$) between salinities at the end time 96 hrs of experiment, which explain the unstable condition of juvenile at the end time 96 hrs of the short term effect experiment of different salinities. This may explain the nature and the life habit of this species and why larvae of *A. latus* drift and move to estuaries into the river side after the adults spawning in the coastal waters in the sea (21; 44; 45). So the larvae move to the river not only for feeding, it also can't tolerate the high salinities in the sea which may reached up to 50 psu in the shallow parts of the Arabian Gulf (46; 47).

Many studies showed the euryhalinity of *A. latus*. (48) mentioned that *A. latus* is a euryhaline teleost capable of living in environments with salinities ranging from 2‰ to 60‰. (21) said that the Adults spawn in coastal waters in the sea and the larvae move to estuaries. (49) mentioned that *A. latus* occurs in shallow coastal waters and enters river mouths and estuaries in depth range to about 50 m. Like many other sparids, this fish is a protandrous hermaphrodite and usually inhabits relatively a wide biogeographic range but is specially found in warm shallow and coastal waters, often entering river mouths and estuaries (44; 45). The study of (50) confirmed that *A. latus* was able to tolerate direct exposure to salinities ranging from 5 ‰ to 60 ‰ without showing mortalities, also these fishes were also able to tolerate gradual decrease in salinity from 42 ‰ to 1 ‰ within 10 days and successfully show temporary adaptation to freshwater without any mortality.

CONCLUSIONS

The results indicated that the salinity increase caused an increase in plasma PCV, osmotic pressure, (sodium, potassium and chloride ions), total protein and glucose. Also, a decrease in water content of muscles of juvenile and adults of *A. latus*. The time of 96 hrs was not enough for juvenile to reach the stable condition at high salinities, while adults showed more constancy at the end time of experiment in all salinities.

دراسة مقارنة لبيان تأثير ملوحات مختلفة على بعض المؤشرات الكيموحيوية في بلازما دم صغار و بالغات اسماك الشانك *Acanthopagrus latus*

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الخلاصة

اجريت تجارب مختبرية على صغار بالغات اسماك الشانك *Acanthopagrus latus* لبيان ومقارنة تاثير النقل المباشر من ملوحة السيطرة 1.5 psu الى ملوحات مختلفة (1.5, 7.5, 15, 30, 45 psu) للفترات الزمنية (6, 24, 48, 96 hrs)كتأثير قصير الامد. تم اختبار بعض الدلائل الفسلجية (مكداس الدم، الضغط الازموزي، محتوى الماء، سكر الدم، البروتين الكلي و ايونات الصوديوم والبوتاسيوم والكلوريد) في بلازما دم الاسماك في كلا المرحلتين العمريتين.

اظهرت النتائج في كل الدلائل الفسلجية المقدرة الواضحة في البالغات للتنظيم الازموزي والتكيف لمدى واسع من الملوحات من 1.5 psu الى 45 psu واوضحت النتائج كذلك سبب موت الصغار في الملوحة 45 psu بعد ٤٨ ساعة من النقل بإظهار عدم قابلية الصغار للتنظيم الازموزي والاستنزاف العالي لفعاليات الجسم في تلك الملوحة.

وضحت النتائج ان زيادة الملوحة ادت الى زيادة في قيم مكداس الدم والضغط الازموزي وايونات الصوديوم والبوتاسيوم والكلوريد ومحتوى البروتين والكلوكوز في بلازما الدم ونقصان في محتوى الماء في العضلات في

صغار بالغات اسماك الشانك، وان الوقت ٩٦ ساعة غير كافي للوصول الى حالة الاستقرار في الصغار عند كل الملوحات، بينما اظهرت البالغات حالة اكثر استقرارا عند نهاية التجربة في كل الملوحات.

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