International Journal of Zoology Research
ISSN: 2231-3516&E-ISSN: 2231-3524, Volume 5, Issue 1, 2016, pp.-91-95.
Available online at http://www.bioinfopublication.org/jouarchive.php?opt=&jouid=BPJ0000217

Research Article

LIVER OF PLATY *Xiphophorus maculatus* (GUNTHER, 1986) AS AN INDICATOR FOR SALINITY STRESS

JASIM BASIM M.* AND NAJIM SALAH M.

Department of Fisheries & Marine Resources, College of Agriculture, University of Basrah, Basrah, Iraq.
*Corresponding Author: Email- basim1952@yahoo.com

Received: March 03, 2016; Revised: July 21, 2016; Accepted: August 30, 2016; Published: September 06, 2016

Abstract- The liver of platy *Xiphophorus maculatus* is single lobule organs, which have no regular cords of hepatocytes and exhibited basophilic feature in fresh specimen. Platy was treated with 5&10 ppt salinity in addition to control treatment for over 70 days. Fishes were kept as dynamic specimens up to the expiry of experiment. Captive fishes showed little degenerated hepatocytes and scarcity of macrophage aggregates which increased along progressive period. In 5ppt treatment, hepatocyte density increased after 30 days, with more degenerated cells and larger slits among parenchyma as more bile stagnation. Noticeable alterations started within 10 days of exposure. There were increments in slits, spots of necrosis and black macrophage aggregates as well as increased cell density. With progressive period, there were increasing degeneration, blood infiltration and biliary stasis. The capsule remained surrounding the liver mass in all treatments, as there was an active circulation so as pancreatic tissue up to expiry of experiment.

Keywords- Platy, *Xiphophorus maculates*, Salinity, Liver, Hepatocyte, Necrosis, Macrophage, Bile.

Citation: Jasim Basim M. and Najim Salah M., (2016) Liver of Platy *Xiphophorus maculatus* (Gunther, 1986) as an Indicator for Salinity Stress. International Journal of Zoology Research, ISSN: 2231-3516 & E-ISSN: 2231-3524, Volume 5, Issue 1, pp.-91-95.

Introduction

The liver is an internal organ in the body with well-known functions of metabolism and detoxification. Most fish species have a single lobed liver, the vasculature of which is usually divided into two large circulatory regions [1] and acts as a storage organ for fats and carbohydrates (glycogen), as it has functions in blood cell destruction and blood chemistry [2]. It was shown that synthesis and degeneration of fatty acids occur mainly in the liver and several enzymes regulating these pathways show varying affinities for the different fatty acids available in this organ [3,4]. [5] found that lipid utilization in the liver of gilthead seabream is rhythmic and strongly synchronized to the light - dark cycle regardless of feeding time. In *Oreochromis niloticus*, the hepatic glycogen concentration declined with increasing saline concentration of transportation media [6]. Also, salinity acclimation can have a drastic effect on copper toxicity even in euryhaline fish, as shown by [7]. Moreover, [8] observed that transferring fish to different salinities can result in alteration in the activity of digestive enzymes.

It was noticed in *Tilapia nilotica* exposed to copper that increasing salt concentration in media significantly reduced the accumulation of copper, although accumulation in liver was higher than in muscle or gill tissues [9]. [10] stated that the liver exhibits functional complexity, as the dependence of its function on its structure, so the change in liver anatomy becomes an important consideration in the abnormal fish. The liver is considered as a good indicator of nutritional pathology due to its function in metabolizing products coming from the digestive tract.

The most common changes observed in the liver are hepatocyte vacuolization, fatty degeneration, changes in parenchyma and necrosis [11]. Histopathological changes have been integrated with the impact of various stressors as environmental conditions [12,13,3].

Platy *Xiphophorus maculatus* is a common aquarium fish capable to acclimate to variable levels of salinity, so this research was carried out to determine the effect of 5ppt &10ppt salinity levels on fish using liver as a biomarker.

Materials and Methods

Ninety individuals of platy *Xiphophorus maculatus*, 38-65mm in total length, were reread in six aquaria each containing 40liters of water and fed with an artificial diet. Two aquaria were devoted for 5ppt, two for 10ppt while another two aquaria maintained as a control treatment.

Specimens were picked randomly from each treatment after 10,30,40, 50 &70 days of exposure. Each specimen was dissected to extract the liver, which fixed in Bouin's solution, embedded in paraffin and cut into 6-µm thickness in different planes. Some sections were stained with hematoxylin - eosin and others with carmine stain [14]. Sections were viewed under x100-1000 magnification and photographed with aid of computerized camera. Cell measurements were made under resolving power of 0.2µm.

Proximate composition (moisture, lipid, protein and ash contents) of whole fish and liver samples were assessed according to the official methods described by [15]. Glycogen content in the same samples was determined calorimetrically according to [16].

Results

Morphology & Histology

The liver of platy *X. maculatus* was observed as one lobule organ measuring 4-6 mm length. In fresh specimens, it seems pale in color and connected to the intestine by a hepatic canal.

In histological structure, the liver appears covered by a distinct capsule. Hepatocytes are circular or ellipsoidal in shape with circular central nuclei, as some nuclei being migrated toward membrane. Parenchyma in general showed basophilic feature with cell density of approximately 4 x 10^6 cells /mm^2. There are small sinusoids filled by blood cells and lined by simple squamous epithelium as well as a number of narrow holes surrounded by simple cuboidal epithelium [Fig-1]. No slits were observed, but small spots of macrophage aggregates with yellow

color as well as some degenerated hepatocytes or with degenerated nuclei inside pale cytoplasm were observed. In general, no regular cords of hepatocytes between sinusoids were noticed.

In control treatment, after 10 days of captivity, some spherical areas formed due to degenerated cytoplasm, therefore the nuclei being closer to pink color, which reflect the presence of glycogen masses. Simple slits may be observed among middle of parenchyma, along with scarcity of macrophage aggregates mostly formed on peripheries. The remarkable phenomenon was the formation of some blocks as a ring composed of columnar hepatocytes with central nuclei [Fig-2].

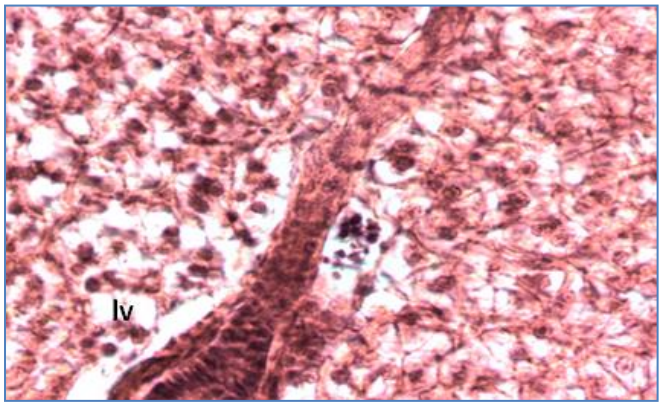

Fig-1 Cross section in parenchyma of liver illustrates bile duct (in center), lost nuclei (arrow head) and lipid vacuole (lv).X1000

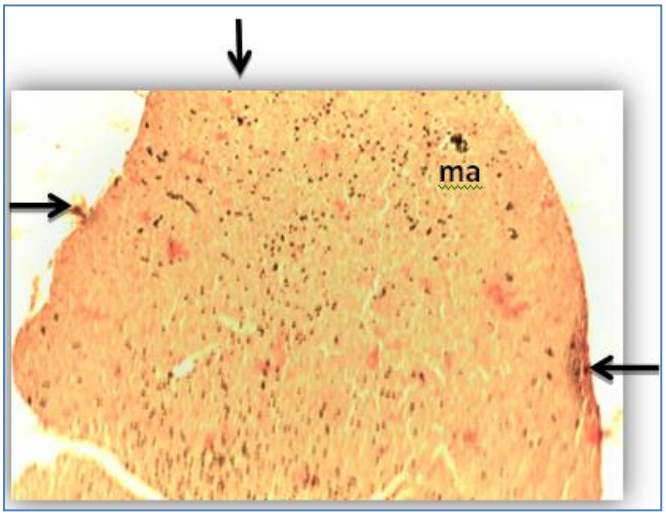

Fig-2 Cross section through parenchyma illustrates glycogen masses (arrows), simple slites (arrowhead) and macrophage aggregates (ma).X200

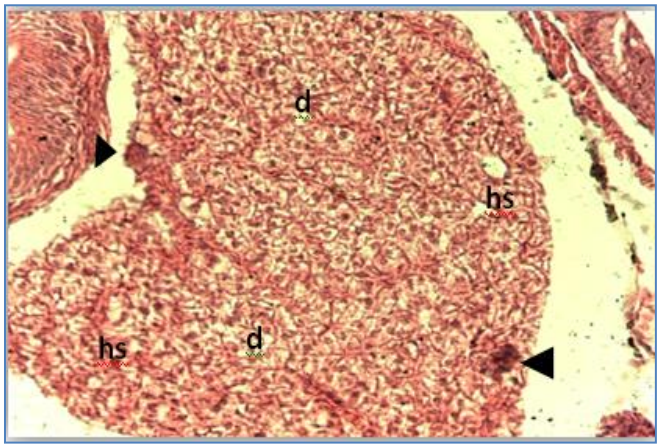

Fig-3 After 40 days in control treatment, there is hepatic steatosis (hs), larger macrophage aggregates (arrow head) and some cell with degenerated nuclei (d).X600

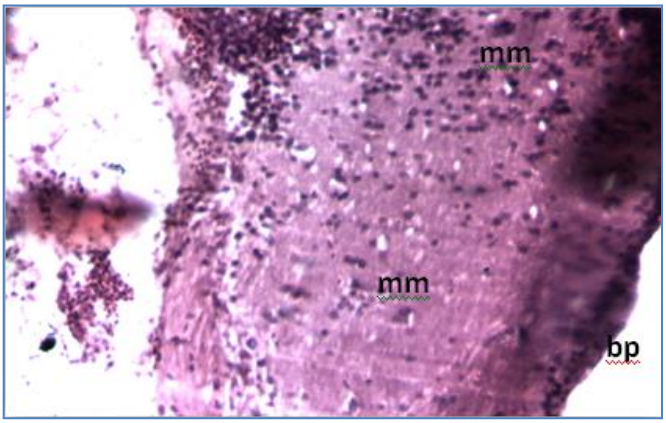

Fig-4 More degenerated hepatocytes with additional basophitic peripheries (bp) and melano-macrophages (mm). X400

After 40 days of captivity, the macrophages aggregates attained about 7µm in diameter, where some of them were shown as small black spots mostly on periphery. State of biliary stasis was clear to observe, where the hepato-pancreas seems as an active organ, although there is proportion of necrosis damaged its tissue. Simultaneously, there was a collection of large size cells characterized by pale cytoplasm, which seems as regenerated cells with density of 7.35 x 10^{7} cell /mm^2 [Fig-3].

After 50 days, no noteworthy alterations could be noticed. After 70 days of captivity, the hepatocytes being more variable in stain, where the darker ones appear smaller in size. However, the periphery showed more basophilic and more cell density than middle eosinophilic parenchymal region .Blotches of necrosis occupied greater area besides outset spots. In general, the captivity up to 70 days have no effective alterations on liver structure.

In 5ppt salinity, after 10 days of treatment, no significant alterations could be observed. After 30 days of exposure, hepatocyte density amounted 4.75x10^{6} cell/ mm^2, with more degenerated ones. Necrosis is represented by small areas, along with outset of macrophage aggregates and black irregular spots. There were large slits lined by ordinary or degenerated hepatocytes [Fig-4]. The scattered pink spots that were evident with carmine stain indicate the abundance of glycogen, where the peripheries being darker stained. However, the hepatocytes still with pale uniform cytoplasm in most parenchyma.

After 40 days of exposure, the hepatocytes density increased up to 5x10^{6} cell/ mm^2, with more migrated nuclei. There were some vacant cysts observed among parenchyma .It is apparent that bile duct still in well and active structure [Fig-5].

No manifested alterations were noticed after 50 days of exposure where cell density showed no noticeable increment, although most of them still as ordinary, circular ones, containing circular nuclei. Some hepatocytes shrunk about 2/3 0f their usual size with longitudinal, degenerated, smaller or even lost nuclei, whereas the longitudinal nuclei may be darker in stain. However, hepatocytes appear in higher density on the peripheries.

After 70 days of treatment, the noteworthy feature is a large unilocular cyst at parenchyma [Fig-6], where some macrophage aggregates exhibited a primary situation. Hepatocyte density approximated 5.25 x 10^{6} cell /mm^2, as they appear in variable cytoplasm stain where the darker marked smaller cells. There were more pale sites which characterized by increasing degenerated hepatocytes. Sinusoids, in turn, began to decrease in the number of blood cells.

In 10 pp treatment, after 10 days of exposure, the noticeable feature is increased small slits among parenchyma. Small areas of necrotized tissues in addition to primary macrophage aggregates and small black ones were observed [Fig-7]. Hepatocytes are variable in size and cytoplasmic stain, along with a proportion of degenerated cells with an estimated density reached 5 x 10^{6} cell /mm^2.

After 40 days of treatment, there were more and larger slits besides more spots of black macrophage aggregates. With progress period, more degenerated hepatocytes or migrated nuclei have been established. Simultaneously, there were decreasing blood cells inside dilated sinusoids or blood vessels [Fig-8].

After 50 days of exposure, the histological alteration being more evident where

macrophage aggregates accumulated on peripheries as black spots. State of blood infiltration, along with biliary stasis was demonstrated [Fig-9]. After 70 days, some histological sections revealed more eosinophilic tissue where cell density declined to 3 x 10^6 cell/mm^2 due to the higher rate of degenerated cells among parenchyma. There was an augmented necrosis as well as an increased alteration among hepatocytes [Fig-10]. The pancreatic tissue still appears to surround a blood vessel, which points out its function although some necrosis was still seen, while the bile stagnancy became more evident [Fig-11]. Mass of smaller size and deeper stain of hepatocytes was observed around sinusoid that refers to a state of atrophy. However, sinusoids contain blood cells that denote continuity of circulation.

Zonal architecture in platy liver is not apparent, so the lesions distribution, in turn, tend to be without distinct zonal pattern [Fig-12]. In general, it is visible that the capsule is the most resistance element within liver structure against salinity stress [Fig-9]

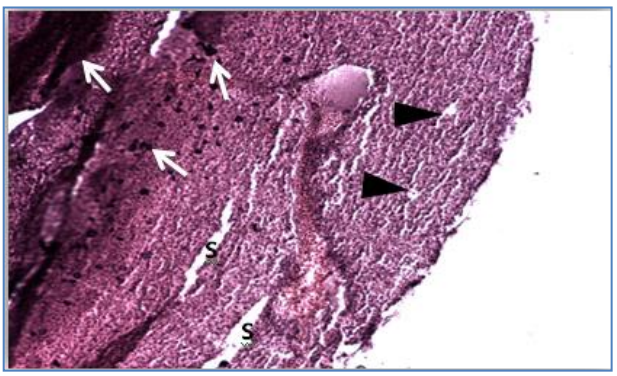

Fig-8 More and larger slits (s), more black macrophages (arrows), with more degenerated hepatocytes (arrowhead).X200

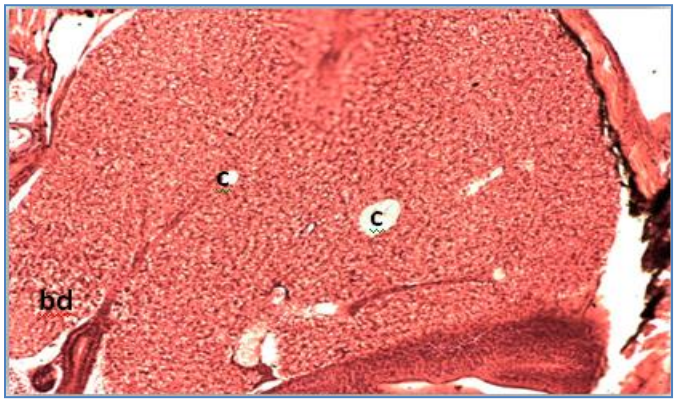

Fig-5 Vacant cysts among parenchyma (c) where the bile duct a well active structure (bd).X200

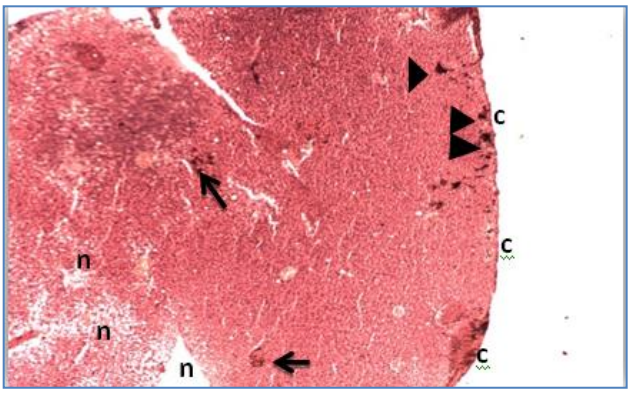

Fig-9 Accumulation of black spots on peripheries (arrow head) with more macrophage aggregates (arrows) and necrosis (n) in median of parenchyma. The capsule (c) surrounds liver mass.X100

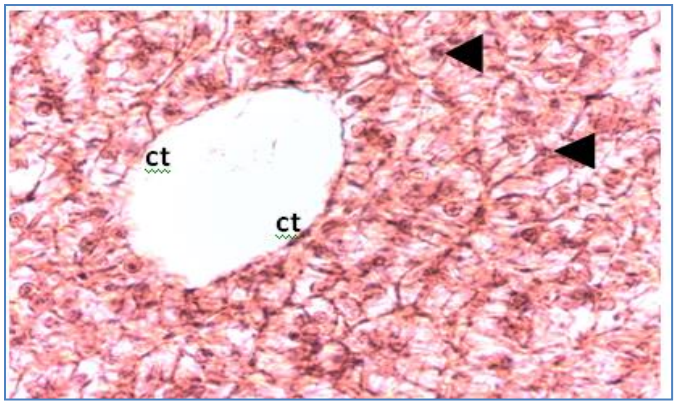

Fig-6 Large unilocular cyst lined by connective tissue (ct) and some degenerated (arrow head) or migrated (arrow head) nucleus .X1000

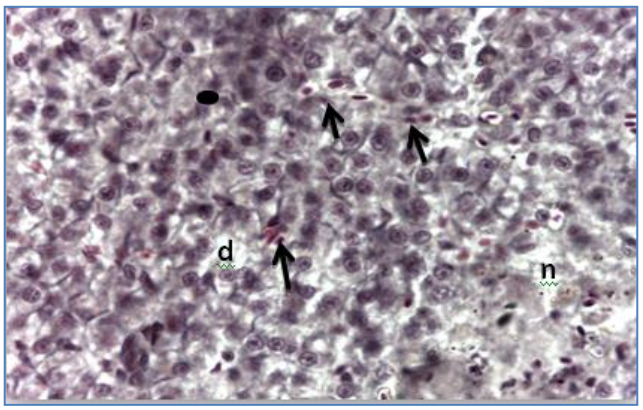

Fig-10 State of blood infiltration (arrows), necrosis (n) or degenerated nucleus (d) and biliary stasis (.) .X1000

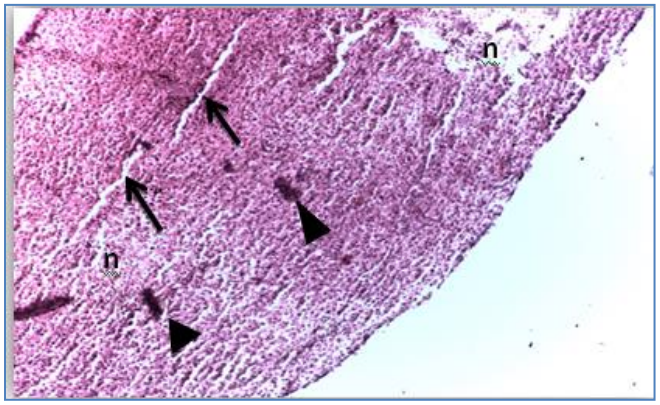

Fig-7 Cross section indicates small slits (arrows), areas of necrosis (n) and black macrophages (arrowhead) .X200

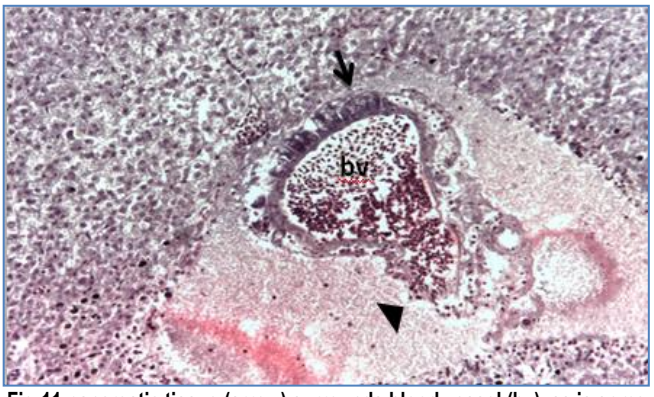

Fig-11 pancreatic tissue (arrow) surrounds blood vessel (bv), as is some degeneration (arrowhead).X400

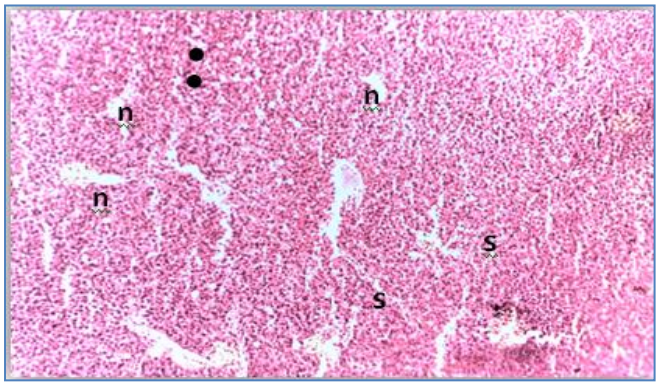

Fig-12 Absence of zonal architecture among liver mass .s, sinusoid, n, necrosis, (.), lipid vacuole .X200

[Table-1] indicates that the captivity resulted in slight increment of whole body moisture. Salinity resulted in moisture decline up to 1.81 % in 5 ppt after 70 days in comparison with control. The 10 ppt treatment, as the longer period, caused a higher decrease reached 5.80% after 70 days in comparison with control. The higher salinity gave rise to more decline in whole body moisture.

The protein ratio decreased with time in control treatment to 14.20% after 70 days with a decrease rate of 10.07%. In contrast, salinity resulted in an increase in protein ratio by 9.43% in 5ppt treatment comparing with 25.30% in 10 ppt after 70 days. Hence, it is apparent that the above ratio accompanied closely the respected salinity levels.

Lipid ratio showed some fluctuation at 10 ppt treatment where there was more decrease than that found at 5ppt up to 50 days of exposure. Ash ratio, in general, was higher in respect to salinity level or period progression. Glycogen amount declined along captivity period, as it declined more obviously at 10 ppt (32.38-46.56%) which reflect the depletion of energy reserves in fish [Table-2].

Biochemical analysis

Table-1 *Whole fish composition (%) and muscle glycogen content (mg/g)*

	1			2			3		
	Control	5ppt	10ppt	Control	5ppt	10ppt	Control	5ppt	10ppt
Moisture	79.13	78.22	76.15	80.41	79.64	76.02	80.65	79.19	75.98
Protein	15.79	16.55	18.65	15.03	15.68	19.57	14.2	15.68	19.01
Lipid	3.53	3.61	3.21	3.13	3.19	2.42	3.48	3.41	2.92
Ash	1.55	1.62	1.99	1.43	1.49	1.99	1.67	1.72	2.09
Glycogen	1.960	1.919	1.063	1.889	1.880	1.012	1.759	1.770	1.191

Table-2 *Proximate composition (%) and glycogen content (mg/g) of fish liver*

	1			2			3		
	Control	5ppt	10ppt	Control	5ppt	10ppt	Control	5ppt	10ppt
Moisture	71.88	70.93	66.15	69.91	69.13	64.17	70.82	70.17	61.35
Protein	18.21	19.65	24.23	20.08	21.58	26.8	19.26	20.5	29.98
Lipid	9.14	8.52	7.89	8.95	8.14	7.01	8.74	8.11	6.20
Ash	0.77	0.90	1.73	1.06	1.15	2.02	1.18	1.22	2.47
Glycogen	32.025	31.425	27.135	29.103	28.075	22.575	29.925	28.807	16.575

Discussion

The histological alterations in liver structure of platy, due to salinity, indicate a significant impact of treatment. Liver cells diminished in size, particularly at the periphery resulting in a higher cell density. Moreover, the degeneration, which is found mostly midmost of liver, can be related to cell death. Some hepatocytes have migrated nuclei toward cell membrane, where they were greater at more impaired tissue. The same was observed in liver of African catfish besides an apparent steatosis and intense lipid accumulation [4]. The bleeding among intercellular gaps is another alteration due to sinusoid degeneration. [17] observed such phenomena in *Labeo rohita* after 30 days of exposure to NaCl.

Necrosis is one type of lesions that could be recognized at liver tissue [18,19] in addition to nuclear degeneration, irregular- shaped nuclei, macrophage aggregates and bile stagnation [20]. All of the above-mentioned alterations were noticed in the current study, in variable levels, which clearly indicate salinity stress. Increased macrophage aggregates are correlated to increased alterations expressed as different lesions. Therefore, it is possible that such pattern reflects its function as repositories for products of cell membrane and erythrocytes breakdown [21]. [20] observed an increase in macrophage density with important hepatic lesions in *P. lineatus*. Macrophage aggregate, in turn, is considered as the most commonly recorded lesion in the liver of *R. holubi* from polluted estuaries [22]. [23] found that histological alterations increased in the liver of *Rutilus* exposed to higher concentrations of LAS detergent. [24] observed an increase in melano-macrophage centers in hepatic tissue of *P. squamossissi* exposed to higher impact levels. Bile stagnation, which augmented along with salinity level or period of exposure, demonstrates that it was less or not being secreted, and so, the liver of examined fish being less active or undergoes metabolic problem. However, the capsule remained attached to liver surface up to termination of the three treatments, despite the histological changes noticed through other structures. This may refer to a property of platy liver. [25] mentioned that the capsule in catfish *Clarias* infected with metacercaria was loose and cloudy.

Glycogen decline at liver is an evident indictor of stress and could be realized along pale areas among liver parenchyma or biochemical analysis. Samples from 10 ppt treatment after 40 days or that from 5 ppt after 70 days revealed more decline in glycogen content indicating utilization by fish thus sparing protein as found by biochemical analysis. Fish exposed to higher salinity has to expend additional metabolic cost for osmotic regulation, in particular the platy, which prefers freshwater. Moreover, the pale sites include degenerated hepatocytes, which were more abundant at 10 ppt specimens, as well as, after longer period of exposure. This seems in coincidence with results from biochemical analysis.

The glycogen still over concentrated at the peripheries, the common phenomenon observed in several fish species [26]. [6] noticed a decline in hepatic glycogen concentration of *O. niloticus* with increasing salinity. [27] considered the glycogen loss from catfish liver as a degenerative change. In relation to period,[28] mentioned that the intensity of changes due to toxic effects of Cd and Zn on liver of *O. mossambicus*, was influenced by the extent of period, although there were similar histological changes in specimens exposed to both 5 and 10 % concentrations of the metals. Platy appear to be similar at salinity treatments, on contrary for control treatment, showed some decrease in glycogen content along captivity. [1] stated that captive fish tend to be higher in hepatocytes glycogen along with artificial feeding and housing conditions. On the other hand, [29] observed decreased glycogen deposition in the liver with increased level of dietary meal in gilthead seabream *Sparus aurata*.

Protein reduction was found in control specimens of platy with progressive captivity in contrast to the saline medium with concentration. This contradicts with *T. mossambica* which showed a considerable decrease with salinity interpreted by [30] by the role of free amino acids in osmotic intracellular regulation or protein breakdown. However, this difference may be due to tilapia adaptation to seawater, therefore water content did not vary significantly in liver tissue at various strengths of seawater, where it decreased by only 5.80% in platy after 70 days at 10 ppt. The marine fish sturgeon *Acipencer* showed an increment in hepatic protein but a

loos in plasma proteins during acclimation to 35 ppt salinity [31] showing that the osmo-regulatory processes cause major physiological changes in sturgeon. Platy, in this situation, was different from yellow perch, which have no significant liver alterations in salt treatment, and this may be a function of gills sensitivity [32]. Still, platy seems more tolerant than some species such as zebra fish which underwent increased mortality at 11 ppt salinity [33].

Healthy wild and captive fish can store large amounts of lipid in their livers, thus it seems difficult to determine how should be the lipid vacuolation considered excessive and harmful [1]. Decline in lipid contents of platy at 10 ppt, where the liver underwent more alterations, may represents a common response to salinity since the platy expends more energy for osmo-regulation. Transferring fish to different salinities can result in alteration in the digestive enzyme activity [34], thus may affect deposition of lipids.

Conclusion

Salinity significantly affected liver structure in different trends. Hepatocytes increased positively in density, particularly on the peripheries along with salinity concentration or exposure period, whereas the necrotized tissue is more abundant midmost of liver, yet, the capsule states as resistant portion. Liver being less active due to salinity exposure, as concluded through bile stagnation or increment in macrophage aggregates. Glycogen content declined due to captivity or salinity. Protein decreased in control treatment while salinity resulted in its elevation.

Acknowledgement: Authors Thankful to Department of Fisheries & Marine Resources, College of Agriculture, University of Basrah, Basrah, Iraq.

Author Contributions: Both Authors Equal Contributions

Conflict of Interest: None declared

References

[1] Wolf J.C. and Wolfe M.J. (2005) *Toxicologic Pathology*, 33, 75-85.
[2] Lagler K.F., Bardach J.E., Miller R.R., and Passino D.R.M. (1977) Ichthyology: John Wiley & Sons.
[3] Genten F., Terwinghe E. and Danguy A. (2009) Atlas of fish histology: Science publishers, Enfield (NH).
[4] Babalola T.O., Apata D.F., Omotosho J.S. and Adebayo M.A. (2011) *Food and Nutrition Sciences*, 2, 11-21.
[5] Paredes J.F., Vera L.M., Martinez-Lopez F.J., Navarro I. and Sanchez-Vazquez F.J. (2014) *Chronobiology International*, 31(15), 613-626.
[6] Orji R.C.A. (1998) Effect of transportation stress on hepatic glycogen of *Oreochromis niloticus*. *ICLARM*, July, 20-22.
[7] Adeyemi J.A. and Klerks P.L.(2012) *Environmental Toxicology and Chemistry*, 31(7), 1573-1578.
[8] Moutou K.A., Panagiotaki P. and Mamuris Z. (2004) *Aquaculture Research*, 35, 912-914.
[9] Karakoc M.(1999) *Turkish Journal of Zoology*, 23, 299-303.
[10] Barroso J.B., Peragon J., Garcia-Salguero L., de la Higuera M. and Lupianez J.A.(2001) *The international Journal of Biochemistry & Cell Biology*, 33, 785-796.
[11] Raskovic B.S., Stankovic M.B., Markovic Z.Z. and Poleksic V.D. (2011) *Journal of Agricultural Science*, 56(1), 87 -100.
[12] Schwaiger J., Wanke R., Adam S., Pawert M., Honnen W. and Triebskorn R. (1997) *Journal of Aquatic Ecosystem stress and recovery*, 6(1), 75-86.
[13] Belge Kurutas E., Sahan A. and Altun T. (2009) *Turkish Journal of Biology*, 33, 275-282.
[14] Briggs D.(2000) Histology, A laboratory Manual: Smith College, Northampton.
[15] AOAC (2003) Official methods of analysis: Association of Official analytical Chemists. 17th ed.
[16] Krishnaswamy S. and Srinivassan V.V. (1961) *Current Science*, 30, 353-358.
[17] Bhatkar N.V.(2011) *Journal of Applied Sciences and Environmental Management*, 15(2), 331-336.
[18] Bhuiyan A.S., Nesa B. and Nessa Q. (2001) *Pakistan Journal of Biological Sciences*, 4(10),1288-1290.
[19] Velmurugan B., Selvanayagam M., Cenqiz El. and Vnlu, E. (2007) *Journal of Environmental Science & health* B, 42(2), 157-163.
[20] Camargo M.M.P. and Martinez C.B.R. (2007) *Neotropical Ichthyology*, 5(3), 327-336.
[21] Herraez M.P. and Zapata A.G. (1991) *European Journal of Morphology,* 29 (2), 89-102.
[22] Richardson N., Gordon A.K., Muller W.J., Pletschke B.I. & and Whitfield A.K. (2010) *Water South Africa*, 36(4), 407 -415 .
[23] Naeemi A., Jamili S., Shabanipour N., Mashinchian A. and Shariati F.S. (2013) *Iranian Journal of Fisheries Science*, 12(4), 887-897.
[24] Viana A.P., Fredou F.L., Montes C. daS. and Rocha R.M. (2013) *Maringa*, 35(3), 395-401.
[25] Onuoha E.O. (2010) *Animal research international*, 7(1),1151-1155.
[26] Love, M. (1980) The Chemical biology of fishes: Academic Press. Scotland.
[27] Hobbie K.R., DeAngelo A.B., George M.H. and Law J.M. (2012) *Veterinary Pathology*, 49(2), 372-385.
[28] Dyk J.C.van, Pieterse G.M. and Vuren J.H.J.van (2007) *Ecotoxicology and Environmental Safety*, 66(3), 432-440.
[29] Robaina L., Izquierdo M.S., Moyano F.J., Socorro J., Vergara J.M., Montero D. and Fernandez-Palacios H. (1995) *Aquaculture*, 130 , 219-233.
[30] Venkatachari S.A.T. (1974) *Marine Biology,* 24, 57-63.
[31] Martinez-Alvarez R.M., Hidalgo M.C., Domezain A., Morales A.E., Garcia-Galleg M. and Sanz A. (2002) The *Journal of Experimental Biology*, 205, 3699-3706.
[32] Nero V., Farwell A., Lee L.E.J., Van Meer T., Mackinnon M.D. and Dixon D.G. (2006) *Ecotoxicology and Environmental Safety*, 65(2), 252-264.
[33] Almeida D.V., de Martinez G.M.C., de Azevedo F.M., Lanes C.F., Bianchini A. and Marins L.F. (2013) *Transgenic Research*, 22(1), 75-88.
[34] Tsuzuki M.Y., Sugai J.K., Maciel J.C., Francisco C.J. and Cerqueira V.R. (2007) *Aquaculture*, 271, 319-325.