Haemato-biochemical disorders associated with nodavirus like-agent in adult leaping mullet *Liza saliens* (Risso, 1810) in the Caspian Sea

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Abstract Betanoda virus is an emerging problem in several marine fish species in various geographic areas all over the world. In recent years, mullets stock of the Caspian Sea decreased dramatically and a betanoda virus like-agent was introduced as the cause of mullet's mortality. The main objective of the present study is to compare hemato-biochemical parameters in healthy and infected sharpnose mullets (*Liza saliens*) to betanoda virus like-agent. The adult sharpnose mullets (34 clinically affected fish + 34 apparently healthy fish) were captured from the southeast the Caspian Sea from 2012 to 2013. All of the captured fish were 4 or 5 years old. The main clinical sings of infected fish were lethargy, severe abdominal distention, abnormal swimming and hyperinflation of swim bladder. The results showed that the weight and total length of infected fish were significantly lower than the healthy fish. The results of histopathological evaluation and indirect fluorescent antibody test were confirmed the presence of a betanoda virus-like agent in infected fish. The diseased fish were severely anemic (hypochromic macrocytic anemia) and had a serious haemopoietic disorders. The anemia associated with a sever leukopenia, a significant rise of neutrophils and immature neutrophils and a significant decries of lymphocytes percentage. Total protein, albumin and total immunoglobulin levels were significantly reduced in the serum of infected fish, while the activities of aspartate aminotransferase and alanine aminotransferase

significantly increased when compared to the healthy fish. These results suggested that the feeding of the infected fish to betanoda virus like-agent were disturbed and it could be cause haemato-biochemical disorder and mortality of the fish in Iranian water of the Caspian Sea.

Keywords Nodavirus · Histopathology · *Liza saliens* · Haemogram · Biochemical changes · Caspian Sea

Introduction

Mullets have a worldwide distribution and inhabit in tropical and temperate waters and few spend their lives in freshwater [27]. Between 1930 and 1934, three mullets species including golden mullet *Liza auratus* (Risso), leaping mullet *Liza saliens* (Risso) and striped mullet *Mugil cephalus* (L.) were transplanted from Black Sea to the Caspian Sea by the Soviet authorities [7, 14] and just golden and leaping mullets are now common in the Caspian Sea [15, 32]. Mullet feed on periphyton, detritus and small invertebrates; large quantities of soil may also be found in their intestines [3].

Throughout the years, they were the most important commercial species of Caspian Sea bony fish, but since 2002, the catch of these fish has gone downwards [15, 16] because those species have faced with severe disease outbreaks causing mortality with clinical sings including lethargy, severe abdominal distention, abnormal swimming in vertical position and hyperinflation of swim bladder [42] but no etiological agent has been reported. Later, a *Nodavirus* was isolated from golden mullets and was introduced as the agent of golden mullet mortality [43, 51].

Later, the clinical sings previously mentioned in golden grey mullets also were observed in adult leaping mullets

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[8] and retino-encephalopathy associated with a nodavirus-like agent were reported of this specie [52].

Viral nervous necrosis (VNN), viral encephalopathy and retinopathy, are some of the most contagious diseases reported in many marine fish species [4, 10, 29]. The causative agent belongs to the genus *Betanodavirus*, family *Nodaviridae* [24]. In 1990, the disease was first described in Japanese parrot fish *Oplegnathus fasciatus* [49]. The main reported clinical signs of VNN are anorexia, abnormal colouration, hyperinflation of swim bladder and a whirling swimming pattern [21]. Although, nodavirus distribution is observed in many organs [28] but the main target organ for nodavirus is the central nervous system (CNS), including the brain, spinal cord and retina [19, 21, 26]. The characteristic lesions of VNN are necrosis and vacuolation of CNS and retina of the affected fish. Therefore, the clinical signs relate to the lesions present in the brain and retina, i.e. there are abnormalities of movement, swim bladder control, sight and colouration [21, 26]. It is notable that an age related susceptibility has been reported to VNN, larval and juvenile stages are more susceptible than adult fish to the disease and mortality rates of up to 100 % have been reported in larvae and juvenile fish., whereas adult fish are more resistance and survive for a longer time after infection [19, 21, 25, 26, 28].

Haemato-biochemical indices evaluation can be useful in monitoring health, finding anomalies in metabolism, identifying changes in organ function and making prognosis. Analysis of data obtained for individual diseases may provide valuable information on the specific responses in pathophysiological processes of the disease [2, 23, 34, 35, 41, 48].

For the first time, the present study provides the evaluation of hemato-biochemical disorders associated with nodavirus like-agent in the infected adult leaping mullets compared to the healthy adult leaping mullets.

Materials and methods

Fish sampling

Totally 185 leaping mullets (clinically affected fish + apparently healthy fish) were captured in the commercial fishing regions of Mazandaran province (north of Iran) from February 2012 to March 2013. Then the fish were transported as alive to laboratory of Caspian Sea Ecology Research Center (CSERC) in which the scales that were collected from the middle of the body behind pectoral fins above the hypothetical lateral line were used for age determination [15] and then 34 clinically affected fish and 34 apparently healthy fish were chosen in 4 and 5 years of age classes for further testing. Then they were transported to 6 fiberglass tank (900 L) filled with seawater (3 tanks for

clinically affected fish and 3 tanks for apparently healthy fish). Continuous aeration was provided to each tank through an air stone connected to a central air compressor. The fish were kept in the lab until 24 h after the transfer and sampling was conducted after this period.

Condition factor and blood sampling

Firstly, individual fish was anaesthetized with clove extract ($250 \mu\text{L L}^{-1}$), natural alcoholic extract of *Eugenia aromatic*, which was developed at CSERC. The total length was measured to millimeter precision and the total weight up to 0.1 g precision. The condition factor (CF) was calculated according to $\text{CF} = \text{Weight of fish (g)} / \text{Length}^3 \text{ (cm)}$ [6]. Then the blood samples were collected from caudal vein using a 2.5 mL sterile syringe. One half of each blood sample was transferred to a micro tube containing heparin anti-coagulant which was used for hematological examination, while the other half was transferred to non-heparinized micro tube and was placed at room temperature and allowed to clot for 2 h. Sera were separated by centrifugation at 1500 g for 20 min and stored at -20°C until use.

Haematology and biochemical assay

The total red blood cell (RBC: 10^6 mm^{-3}) and white blood cell (WBC: 10^3 mm^{-3}) were counted manually by haemocytometry. The haemoglobin level (Hb: g dL^{-1}) of blood was analyzed spectrophotometrically at 540 nm by the cyanomethaemoglobin method. Haematocrit (Ht %) was determined by microcentrifuge technique, using standards heparinized microhaematocrit capillary tubes (75 mm at 7000 g for 10 min) [9]. Thin blood smears slides were prepared and stained with Wright-Giemsa. A total of at least 100 leucocytes were counted under a light microscope, and the percentages of leucocyte types were calculated. The haematological indices of mean cell haemoglobin concentration (MCHC: g dL^{-1}), mean cell haemoglobin (MCH: pg) and mean cell volume (MCV: fL) were calculated using the total RBC count, Hb concentration and Ht [22]. The sera were used to evaluation of biochemical parameters. Albumin, aspartate aminotransferase (AST) and alanine aminotransferase (ALT) were estimated using commercial kits (Pars Azmoon, Tehran, Iran) and a biochemical auto analyzer instrument (Eurolyser, Belgium) [39]. Serum total immunoglobulin (Ig) levels were determined according to the method described by Siwicki and Anderson [40].

Histopathology

Brains, optical nerves and eyes from ten infected fish and seven apparently healthy fish were fixed in 10 % buffered-formalin and tissues were processed by routine method

(paraffin-imbedded) and sectioned at 5 μm prior to staining with haematoxylin and eosin (H&E) [5].

Indirect florescent antibody test (IFAT)

Indirect florescent antibody test for detection of nodavirus antigen was performed on selected brain imprints (from 17 infected and 17 healthy fish) using a mouse anti-nodavirus monoclonal antibody (Mab, Aquatic Diagnostic Ltd., Scotland) according to Saksida et al. [38] and Soltani et al. [43].

Statistical analysis

Data were analyzed using SPSS (Ver. 18) and significant differences between groups were determined by a *T* test [50]. Mean values were considered significantly different at $p < 0.05$. Data are expressed as mean values $\pm$ SE.

Results

Pathological changes

Although the fish were similar in age classes (4 and 5 years), the total weight, length of the infected fish were

significantly lower than those of the healthy fish ($p < 0.05$). However the CF was not shown any significant difference between two groups ($p > 0.05$) (Table 1). On necropsy no gross lesions were observed except swim bladder enlargement (Fig. 1a, b). The gastrointestinal tract was completely empty and enlargement of gall bladder was observed (Fig. 1b, c).

The histopathology revealed vacuolization and neural degeneration of the optical nerve and granular layer of the retina (Fig. 2a, b) typical of nodavirus induced retiopathy, in clinically affected fish. The intensiveness vacuolation of the optical nerve and retina tissues varied among fish. In some cases, the optic nerve showed extensive vacuolation. However the same lesions were found lower in the brain samples.

In IFAT, positive reaction was observed in all slides which were prepared from infected fish (Fig. 3a) but no positive fluorescence reaction was observed in the slides of the healthy fish (Fig. 3b).

Haematology and biochemical assay

The results of the haematological profiles of the infected fish and those of the apparently healthy fish were presented in Table 1. Statistical analysis of data showed that the RBC

Table 1 Comparison of the weight, length, hematological and biochemical parameters of healthy and infected to nodavirus-like agent leaping mullets (*L. saliens*)

Parameters	Diseased fish		Healthy fish		<i>t</i> test	
	Mean	SE	Mean	SE	<i>t</i> value	<i>p</i>
Weight (g)	75.7	4.73	216.4	9.38	−17.677	0.001
Length (cm)	22.2	3.15	33.5	2.44	−16.471	0.003
CF	0.70	0.02	0.68	0.01	0.695	0.489
RBC (10^6 mL^{-1})	1.8	0.56	4.5	0.78	−16.172	0.001
WBC (10^3 mL^{-1})	5.2	0.38	12	0.48	−11.038	0.005
Ht (%)	20	6.39	44.2	8.26	−13.485	0.002
Hb (g dL^{-1})	5.6	0.36	13.7	0.47	−13.425	0.001
MCV (fL)	114.4	14.65	99.5	7.43	5.313	0.002
MCH (pg)	30.7	4.39	31.5	2.38	0.946	0.347
MCHC	27.6	2.06	30.9	1.52	−8.959	0.007
Lymphocytes (%)	57.6	2.77	70.8	1.69	−4.027	0.003
Neutrophils (%)	18.8	2.56	8.8	1.50	−2.145	0.036
Monocytes (%)	14.1	2.24	6.4	1.56	7.814	0.001
Myelocytes (IN) (%)	1.3	0.33	0.4	0.22	2.728	0.008
Total protein (g dL^{-1})	1.9	0.86	4.5	1.43	−8.886	0.002
Albumin (g dL^{-1})	1	0.12	2.8	0.20	−7.645	0.009
ALT (IU dL^{-1})	53.5	3.11	116.1	5.98	−9.067	0.005
AST (IU dL^{-1})	27.5	1.74	3.8	0.24	13.418	0.003
Total Ig (mg mL^{-1})	317.2	21.08	49.8	4.18	12.438	0.002

SE standard error of the mean, CF condition factor, RBC red blood cells, WBC white blood cells, Ht hematocrit, Hb hemoglobin concentration, MCV mean cell volume, MCH mean cell hemoglobin, MCHC mean cell hemoglobin concentration, IN immature neutrophils, ALT alanine aminotransferase, AST aspartate aminotransferase

Fig. 1 **a** Severe abdominal distention and abnormal swimming in vertical position, **b** hyperinflation of swim bladder (SB, head arrow) and emptiness of gastrointestinal system (In, head arrow), **c** enlargement of gall bladder (GB, head arrow)

Fig. 2 **a** Extensive vacuolization of optic nerve tissue of the infected *L. saliens* (arrow). H & E. Mag. $\times 100$. **b** Section of eye tissue of the infected *L. saliens* showing vacuolation in retina (arrow). H & E. Mag. $\times 40$

and WBC counts, Hb value, Hct, and the MCHC level of the infected fish were significantly lower than those of the healthy fish ($p < 0.05$). There was no significant difference in the MCH level of the two groups ($p > 0.05$) but the MCV value of the healthy fish was significantly lower than that of the infected fish ($p < 0.05$). The results of differential leukocyte counts of the infected fish indicated that the percentage of lymphocytes significantly decreased ($p < 0.05$) while the percentage of neutrophils and myelocytes (immature neutrophils) increased ($p < 0.05$), whereas no difference in the percentage of monocytes was found ($p > 0.05$). Mean values of plasma parameters of the infected fish and the apparently healthy fish were shown in Table 1. Results of biochemical plasma profile showed that the albumin, TP and total Ig of the infected fish were significantly lower than those of the healthy fish ($p < 0.05$). Whereas the ALT and AST activities showed a significant increase in the infected fish compared to the healthy fish.

Discussion

Viral nervous necrosis can be diagnosed, at least presumptively by clinical signs, specific lesions in the brain and/or retina by light microscopy and detection of viral

antigens by IFAT [26]. The present disease in the adult sharpnose mullet closely resembles viral nervous necrosis (VNN) in clinical signs, histopathological manifestation and IFAT.

The neurotropism of the agent is remarkable but the pattern of vacuolation is different. In several reports, in light microscopy, the most vacuolization have been found in brain and retina [2, 19, 21, 26] but in this study the results of histopathology contains less lesions of brain. A study similar to the present one, were recorded by Munday et al. [25], Comps et al. [11] and Soltani et al. [43], however, the reasons are not clear for us.

Although the fish were similar in age classes, the results indicated that the infected fish were shorter and much thinner than the normal fish. However the CF was not shown significant difference between the two groups. Similarly, Johansen et al. [21] compared the growth rate of the infected 1 year Atlantic halibut *Hippoglossus hippoglossus* (L.) nodavirus and that of the non-infected fish and found that the infected fish had a significantly lower growth rate compared to the non-infected group. These findings suggest that nodaviral infection could influence fish growth.

In this study, the infected fish were so weak, the gastrointestinal system was absolutely empty and without feed remains and the gall bladder was large and filled up with

Fig. 3 **a** Immunofluorescent localization of nodavirus-like agent in the brain of infected *L. saliens* (arrow). Mag. $\times 40$. **b** Immunofluorescent negative reaction in the brain of the healthy *L. saliens* (arrow). Mag. $\times 40$

bile. The sings, which likewise, were found in the fish were feed deprivation for long-term [43, 51].

The leaping mullets have been described as bottom feeder because they feed on periphyton, detritus and small invertebrate [16]. It seems that the infected fish could not feed normally because their swim bladder enlargement and damaged retina prevented them from going into the sea bottom and see the food. During in long term starvation, the fish conserved energy for vital functions which leads to their growth stop and energy gain from proteolysis of muscles [17, 20]. This could describe the difference of the growth rate between the two fish groups.

The haematological results showed that RBC counts, Hct and Hb values of the infected fish are significantly lower than those of the normal fish and they showed a serious anemia. However, their MCV increased and MCHC decreased significantly compared to the healthy fish. On the basis of these results, the anemia is categorized as the hypochromic macrocytic type which was observed in haemopoietic disorders [36, 46] and was not found in viral diseases [35, 41, 48]. Macrocytic hypochromic anemia was seen in long term starved common carp *Cyprinus carpio* (L.) [44] and it was caused by vitamin B12 deficiency [45].

The results of this study showed a significant leukopenia which were accompanied by neutrophilia and lymphopenia in the infected fish. It has been shown that in viral and bacterial infections, leukocytosis, lymphocytosis and neutropenia are dominant results in haematologic profiles of the infected fish [23, 48]. Unlikely, the reduction of leukocytes and lymphocytes as well as the increase of neutrophils and myelocytes were seen in long time starvation [45, 46]. Vitamin C and amino acids have important roles on regulation of innate and adaptive immunity [1, 18]. Thus, it seems that the long time malnutrition disturbed the haemopoiesis of these cells and caused incompetency of the fish immunity in the infected group.

The content of TP and albumin significantly decreased in the infected fish. The same results were seen both in the infectious diseases and in the longtime starvation [20, 33–

35, 37]. The reduction of TP and albumin could be the result of amino acids deficiency and/or reduced proteins synthesis due to hepatopathy and renal failure, however, the reasons are not clear for us.

The AST and ALT concentrations in the infected group were significantly higher than the healthy fish. These are the aminotransferases that were distributed in liver and plasma whose levels are low when the animal is healthy but high in case of hepatopathy [30, 31]. To our knowledge, the liver might not be a target organ for nodavirus and it could not cause hepatic failure [19, 26], therefore, it seems that the rise of ALT and AST in infected group could be attributed to the participation of reactive oxygen species generated by long time starvation in liver which caused liver damage [47].

In this study, the total Ig levels of the infected group were significantly lower than those of the healthy group. Natural antibodies are found in high levels in the fish serum, where they provide immediate and broad protection against bacterial and viral pathogens, making these factors key components of nonspecific and specific immunity [12]. Teleost mainly produce two forms of Ig including soluble (secreted) and membrane-bound forms. The soluble form is secreted from B cells and is present in blood and other fluids, where it plays a role as an immune effector molecule [13]. Total Ig are reportedly not utilized during starvation, but it is possible that Ig production may be diminished in starved fish [12]. The low levels of total Ig in the infected fish are probably related to the reduction of lymphocytes counts and/or synthesis of proteins.

According to Munday et al. [25], Arimoto et al. [4], Grotmoll et al. [19] and Johansen et al. [21] betanoda virus caused an acute disease in larva and juvenile fish but it could cause a chronic disease in adult fish. The results of hemato-biochemical parameters showed that the agent disturbed feeding of the fish. It seems that the swim bladder enlargement and damaged retina were caused by virus agent can disrupt the normal fish feeding. Finally, it would cause that long-term starvation imposed to the fish. It is

necessary that future investigation must focus on the study of betanoda virus-like agent identification and pathogenesis, for a better understanding of infection mechanism and pathophysiology of disease.

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References

- Adham KG, Hashem HO, Abu-Shaban MB, Kamel AH. Vitamin C deficiency in catfish *Clarias gariepinus*. *Aquac Nutr*. 2000;6:129–39.
- Amend DF, Smith L. Pathophysiology of infectious hematopoietic necrosis virus disease in rainbow trout: hematological and blood chemical changes in moribund fish. *Infect Immun*. 1975;11:171–9.
- Amini F. Biology of mullet species and their adaptation to fresh water. In: Proceedings of the national conference on fisheries resource management of the Caspian Sea, Babolsar, Iran; 1989. p. 73–9 (In Persian).
- Arimoto M, Mori K, Nakai T, Muroga K, Furusawa I. Pathogenicity of the causative agent of viral nervous necrosis disease in striped jack, *Pseudocaranx dentex* (Bloch and Schneider). *J Fish Dis*. 1993;16:461–9.
- Bancroft JD, Stevens A. Theory and practice of histological techniques. Edinburgh: Churchill Livingstone; 1990. p. 740.
- Bekcan S, Dogankaya L, Cakirogullari GC. Growth and body composition of European catfish (*Silurus glanis*) fed diet containing different percentages of protein. *Israeli J Aquac Bamid-geh*. 2006;58:137–42.
- Berg LS. Freshwater fishes of the U.S.S.R. and adjacent countries, 4th edn. Israel Program for Scientific Translations Ltd., (Russian version published in 1949), Jerusalem; 1965. p. 510.
- Binaii M, Ghiasi M, Pourgholam R, Zorriehzahra J, Saidi AA, Behrozi S. Comparative evaluation of hematological factors of healthy and suspected to viral nervous necrosis mullets in Mazandaran province. In: Proceedings of the 16th Iranian Veterinary Congress, Tehran, Iran; 2010. p. 200–1 (In Persian).
- Blaxhall PC, Daisley W. Routine haematological methods for use with fish blood. *J Fish Biol*. 1973;5:771–81.
- Boonyaratpalin S, Supamattaya K, Kasornchandra J, Hoffman RW. Picorna-like virus associated with mortality and a spongiosis encephalopathy in grouper *Epinephelus malabaricus*. *Dis Aquat Org*. 1996;26:75–80.
- Comps M, Trindade M, Delsert C. Investigation of fish encephalitis viruses (FEV) expression in marine fishes using DIG-labelled probes. *Aquaculture*. 1996;143:113–21.
- Cuesta A, Meseguer J, Esteban MA. Total serum immunoglobulin M levels are affected by immunomodulators in seabream (*Sparus aurata* L.). *Vet Immunol Immunopathol*. 2004;101:203–10.
- Davis CR, Marty CG, Adkison MA, Freiberg EF, Hedrick RP. Association of plasma IgM with body size, histopathologic changes and plasma chemistries in adult Pacific herring *Clupea pallasii*. *Dis Aquat Org*. 1999;38:125–33.
- Dmitriev AN. Mullet in the Iranian waters of Caspian. *Priroda* 1964;12:74–5 (In Russian).
- Fazli H, Ghaninejad D, Janbaza AA, Daryanabarda GR. Population ecology parameters and biomass of golden grey mullet (*Liza auratus*) in Iranian waters of the Caspian Sea. *Fish Res*. 2008;93:222–8.
- Fazli H, Daryanabard GH, Abdolmaleki S, Bandani GA. Stock assessment and management implications of golden grey mullet (*Liza aurata* Risso, 1810) in Iranian waters of the Caspian Sea. *J Appl Ichthyol*. 2013;29:431–6.
- Gillis TE, Ballantyne JS. The effect of starvation on plasma free amino acid and glucose concentration in lake sturgeon. *J Fish Biol*. 1996;49:1306–16.
- Grohmann U, Bronte V. Control of immune response by amino acid metabolism. *J Fish Biol*. 2010;236:243–64.
- Grotmoll S, TotlandK GK, Thorud K, Hjeltne BK. Vacuolating encephalopathy and retinopathy associated with a nodavirus-like agent: a probable cause of mass mortality of cultured larval and juvenile Atlantic halibut *Hippoglossus hippoglossus*. *Dis Aquat Anim*. 1997;29:85–97.
- Hung SSO, Liu W, Li H, Storebakken T, Cui Y. Effect of starvation on some morphological and biochemical parameters in white sturgeon, *Acipenser transmontanus*. *Aquaculture*. 1997;151:357–63.
- Johansen R, Grove S, Svendsen AK, Modahl I, Dannevig BH. A sequential study of pathological findings in Atlantic halibut, *Hippoglossus hippoglossus* (L.), throughout one year after an acute outbreak of viral encephalopathy and retinopathy. *J Fish Dis*. 2004;27:327–41.
- Lee RG, Foerster J, Jukens J, Paraskevas F, Greer JP, Rodgers GM. Wintrobe's clinical hematology. 10th ed. New York: Lippincott, Williams & Wilkins; 1998. p. 1484.
- Martins ML, Mourão JLP, Amaral GV, Vieira FN, Dotta G, Jatobá AMB, Pedrotti FS, Jerônimo GT, Buglione-Neto CC, Pereira JG. Haematological changes in Nile tilapia experimentally infected with *Enterococcus* sp. *Braz J Biol*. 2008;68:657–61.
- Mori K, Nakai T, Nagahara M, Muroga K, Mekuchi T, Kanno T. A viral disease in hatchery-reared larvae and juveniles of redspotted grouper. *Fish Pathol*. 1991;26:209–10.
- Munday BL, Langdon SJ, Hyatt A, Humphrey JD. Mass mortality associated with a viral-induced vacuolating encephalopathy and retinopathy of larval juvenile barramundi, *Lates calcarifer* Bloch. *Aquaculture*. 1992;103:197–211.
- Munday BL, Kwang J, Moody N. Betanodavirus infections of teleost fish: a review. *J Fish Dis*. 2002;25:127–42.
- Nelson JS. Fishes of the world. 4th ed. New York: Wiley; 2004. p. 624.
- Nopadon P, Aranya P, Tipaporn T, Toshihiro N, Takayuki K, Masashi M, Makoto E. Nodavirus associated with pathological changes in adult spotted coralgroupers (*Plectropomus maculatus*) in Thailand with viral nervous necrosis. *Res Vet Sci*. 2009;87:97–101.
- Office International des Epizooties. Viral encephalopathy and retinopathy. In: Manual of diagnostic test for aquatic animals, OIE, Paris; 2003. p. 136.
- Park IS, Hur JW, Choi JW. Hematological responses, survival, and respiratory exchange in the olive flounder, *Paralichthys olivaceus*, during starvation. *Asian Australas J Anim Sci*. 2012;25:1276–84.
- Pascual P, Pedrajas JR, Toribio F, López-Barea J, Peinado J. Effect of food deprivation on oxidative stress biomarkers in fish (*Sparus aurata*). *Chem Biol Interact*. 2003;145:191–9.
- Patimar R. Some biological aspects of the sharpnose mullet *Liza saliens* (Risso, 1810) in Gorgan Bay-Miankaleh Wildlife Refuge (the Southeast Caspian Sea). *Turk J Fish Aquat Sci*. 2008;8:225–32.
- Pérez A, Cardenete G, Hidalgo MC, Gracia-Alcázar A, Abellán E, Morales AE. Metabolic adjustments of *Dentex dentex* to prolonged starvation and re-feeding. *Fish Physiol Biochem*. 2012;38:1145–57.
- Řehulka J. Aeromonas causes severe skin lesions rainbow trout (*Oncorhynchus mykiss*): clinical pathology, haematology and biochemistry. *Acta Vet Brno*. 2002;71:351–60.

35. Řehulka J. Haematological analyses in rainbow trout *Oncorhynchus mykiss* affected by viral haemorrhagic septicaemia (VHS). *Dis Aquat Org*. 2003;56:185–93.
36. Rios FS, Oba ET, Fernandes MN, Kalinin AL, Rantin FT. Erythrocyte senescence and haematological changes induced by starvation in neotropical fish Traira, *Hoplias malabaricus* (Characiformes, Erythrinidae). *Comp Biochem Physiol*. 2005;140:281–7.
37. Sakamoto S, Yone Y. Effect of starvation on hematological characteristics and the content of chemical components and activities of enzymes in blood serum of red sea bream. *J Fac Agric Kyushu Univ*. 1978;23:63–9.
38. Saksida SM, Thorburn MA, Speare DJ, Markham RJF, Kent ML. A field evaluation of an indirect immunofluorescent antibody test developed to diagnose plasmacytoid leukemia in Chinook Salmon (*Oncorhynchus tshawytscha*). *Can J Vet Res*. 1999;63:107–12.
39. Shahsavani D, Mohri M, Gholipour Kanani H. Determination of normal values of some blood serum enzymes in *Acipenser stellatus* Pallas. *Fish Physiol Biochem*. 2010;36:39–43.
40. Siwicki AK, Anderson DP. Nonspecific defense mechanisms assay in fish: II. Potential killing activity of neutrophils and macrophages, lysozyme activity in serum and organs and total immunoglobulin level in serum. In: Siwicki AK, Anderson DP, Waluga J, editors. *Fish disease diagnosis and prevention methods*. Poland: Olsztyn; 1993. p. 105–12.
41. Smith PA, Larenas J, Contreras J, Cassigoli J, Venegas C, Rojas ME, Guajardo A. Infectious haemolytic anaemia causes jaundice outbreaks in seawater-cultured coho salmon, *Oncorhynchus kisutch* (Walbaum), in Chile. *J Fish Dis*. 2006;29:709–15.
42. Soltani M, Rehanndeh M. Study of the bloat in golden grey mullet in the Caspian Sea. *J Fac Vet Med Tehran Univ*. 2001;56:1–3 (**In Persion**).
43. Soltani M, Ghasemi M, Sharifrohani M, Sharifpour I, Zorrieh Zahra J. Isolation and identification of betanodavirus causing mass mortalities in golden grey mullet (*Liza auratus*) in the Caspian Sea. *Iran J Vet Res*. 2010;4:201–8.
44. Sopińska A. Effect of physiological factors, stress and disease on hematological parameters of carp, with particular reference to leukocyte pattern. II. Hematological results of stress in carp. *Acta Ichthyol Piscia*. 1984;16:121–39.
45. Stoskopf MK. *Fish medicine*. Philadelphia: W. B. Saunders Company; 1993.
46. Tm C, Dove ADM, Arnold JE. Hematologic disorders of fish. *Vet Clin Anim*. 2008;11:445–64.
47. Uribe C, Folch H, Enriquez R, Moran G. Innate and adaptive immunity in teleost fish: a review. *Vet Med*. 2011;10:445–62.
48. Waagbø R, Sandnes K, Espelid S, Lie Ø. Haematological and biochemical analyses of Atlantic salmon *Salmo salar* L., suffering from cold water vibriosis (Hitra disease). *J Fish Dis*. 1988;4:417–23.
49. Yoshikoshi K, Inoue K. Viral nervous necrosis in hatchery—reared larvae and juveniles of Japanese parrot fish *Oplegnathus fasciatus*. *J Fish Dis*. 1990;13:69–77.
50. Zar JH. *Biostatistical analysis*. New Jersey: Prentice-Hall; 1994. p. 662.
51. Zorriehzahra MJ, Nakai T, Sharifpour I, Gomez DG, Chi CS, Soltani M, Hassan HMD, Sharifrohani M, Saidi AA. Mortality of wild golden grey mullet (*Liza auratus*) in Iranian waters of the Caspian Sea, associated with viral nervous necrosis-like agent. *Iran J Fish Sci*. 2005;4:43–58.
52. Zorriehzahra MJ, Nazari A, Ghasemi M, Ghiasi M, Haghighi Karsidani S, Giuseppe B, Mohd Daud HH. Vacuolating encephalopathy and retinopathy associated with a nodavirus-like agent: a probable cause of mass mortality of wild Golden grey mullet (*Liza aurata*) and Sharpnose grey mullet (*Liza saliens*) in Iranian waters of the Caspian Sea. *J Virus Dis*. 2014;25(4):430–6.