

The innate immunity changes of the female anadromous hilsa shad, *Tenualosa ilisha*, during spawning and post spawning season

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Abstract

Tenualosa ilisha (Hamilton) is a valuable migratory fish belonging to the family Clupeidae and it is distributed in a wide area from the Persian Gulf to coasts of Pakistan and China's southern sea. The aim of the present study was to investigate the changes occurring in innate immunity parameters during upstream migration and comparing them to those during post spawning. Sixty mature female hilsa shad were obtained using gill net from Karoon River in Khorramshahr during the spawning period (July, August and September 2014) and Hendijan coasts along the Persian Gulf coasts during post spawning period (October, November and December 2014). Immediately, blood samples were collected from the peduncle vein and then parameters including WBC (White Blood Cell) count, Differential WBC Count, plasma lysozyme content and C3 and C4 complement activity were determined. Based on the results, the levels of WBC, lysozyme and C3 during the spawning period were lower than those recorded during post spawning period. However, C₄ levels were higher during spawning ($p < 0.01$). This fact revealed that some innate immunity parameters experience depression during upstream migration and spawning, while these parameters increase during the post spawning period, indicating the suppressive effects of spawning on fish immunity.

Keywords: *Tenualosa ilisha*, Lysozyme activity, Complement, Anadromous, Migration

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Introduction

Tenualosa ilisha (ilish, hilsa, hilsa herring or hilsa shad) is a species of herring family (Clupeidae) and a popular food fish in South Asia. It is found in rivers and estuaries in India, Pakistan, Bangladesh, Burma, the Persian Gulf and also in the Tigris and Euphrates Rivers in Iraq and Karoon river in Iran. The main stages of feeding and growth of this anadromous fish occurs in marine waters and spawning takes part in fresh waters of rivers. It is suggested that the main factor which evokes the migration upstream in the reproduction season is the increase of water level in rivers during spring rains (Brahmane *et al.*, 2006). The Spawning of hilsa shad is multistage and it lasts a long term from April to September (Hussain *et al.*, 1991).

However, in the current years, the capture of this fish has decreased in Khuzestan Province and it has been reported from other areas (Rahman, 1997). Also, numerous reports have been published regarding the decrease in fishing of *T. ilisha* in Shatt-Al-Arab, Iraq (Coad, 1997).

There are different studies about *T. ilisha* which have focused on some biological aspects such as diet, length, length to weight ratio (Al- Nasiri and Al- Mukhtar, 1988), fish biology and resource status in Iraq and Kuwait waters (Al- Baz and Grove, 1995).

Some biological features of this fish such as defining the spawning season, its distribution pattern, morphometric-meristic properties, fecundity, comparison of morphometric-meristic properties and frequency distribution of length in Iranian waters have also been studied (Ghafle Maramazi *et al.*, 2004). However, there is no report on the physiological or immunological aspects of this species in literatures up to now.

Hematological and immunological parameters have been recognized as valuable tools for the monitoring of fish health. Therefore, the aim of the present study was to investigate some hematological and non-specific immune system parameters and their variations in *T. ilisha* during spawning and post spawning season.

Materials and methods

A total of 60 fish were collected during the sampling months (Ten fish for each month) from Karoon River and Hendijan coast (Fig. 1) along Persian Gulf during spawning and post spawning season (July to December 2014), respectively. Blood sampling took place on the boat immediately after sampling. Fish were transferred to the lab in an ice box and biometric and hematological procedures were conducted subsequently.



Figure 1: Geographical position of sampling sites.

Gonadosomatic Index (GSI)

The sampled fish were weighed then dissected and the gonads were removed and weighed. Gonadosomatic Index was calculated using the formula below (Barber and Blake, 2006).

$$\text{GSI} = [\text{Gonad Weight} / \text{Total body Weight}] \times 100$$

Blood samples collection

Blood samples were randomly collected through the peduncle vein using a 5 mL heparinized syringe immediately after capture. Blood samples were poured into 2 mL micro tubes and immediately transferred to the fisheries laboratory in an ice box.

Hematological parameters assay

Hematological parameters including, WBC count and Differential WBC Count (The percentage of Neutrophile, Lymphocyte and Monocyte), were determined according to the standard methods (Feldman, 2000). Briefly, it involves diluting blood in a diluent that lyses the red cells to remove them from view. A hemocytometer is charged with the diluted blood and nuclei are counted in the appropriate areas of the grid using a light microscope. Differential count is usually done manually by counting 100 leukocytes in the monolayer of the smear, which provides relative proportions (percentages) of WBC normally found in blood. Part of the blood samples were centrifuged (10 min, 12,000 rpm) and plasma was separated, collected and stored at -80°C until Immunological analysis.

Plasma lysozyme activity assay

Plasma lysozyme content was determined by the turbidometric assay according to the method described by Ellis (1990) with slight modifications. Briefly, aliquots of *Micrococcus lysodeikticus* suspension (Sigma) (0.375 mg/mL, 0.05 M sodium phosphate buffer, pH 6.2) was added to plasma samples at 10:1 ratio and the optical density was measured after 15 and 180 seconds by spectrophotometer (Biophotometer, Eppendorf) at 670 nm wavelength. PBS was used as the blank and results were expressed in amounts of lysozyme (g) per milligram of sample calibrated using a standard curve determined with hen egg white lysozyme (Sigma) in PBS.

Complement Assay

The immunoturbidimetric method was adopted to study Plasma complement activity (Pars Azmon Kit, Tehran, Iran). C3 and C4 in plasma samples were mixed with the antibody afforded by the kits, and then an antigen-antibody complex was produced. The optical density (OD) value was measured at 340 nm. Compared with the values of the standards from the kits, C3 and C4 contents were calculated in $\mu\text{g/mL}$.

Statistical Analysis

Hematological and immunological results were analyzed with One Way Anova variance analysis using SPSS

16.0 software. Differences between means were determined using Duncan's multiple test.

Results

Fig. 1 shows GSI in *Tenualosa ilisha* during spawning and post spawning season. Based on the results, there was a significant difference between spawning (12.5 ± 0.42) and post spawning (1.35 ± 0.15) period ($p < 0.01$).

Levels of differential leucocyte count and total leucocyte count during spawning and post spawning season are given in Figs. 2-5. Based on the results obtained from this study, white blood cells and differential leucocyte count were significantly different between spawning and post spawning season ($p < 0.01$). The differential leucocyte count of *T. ilisha* showed that the percentage of lymphocytes, neutrophils and monocytes during the spawning period was lower than that in the post spawning period ($p < 0.01$).

The levels of plasma immune parameters like Lysozyme and complement factors during spawning and post spawning period are presented in Figs. 6, 7 and 8.

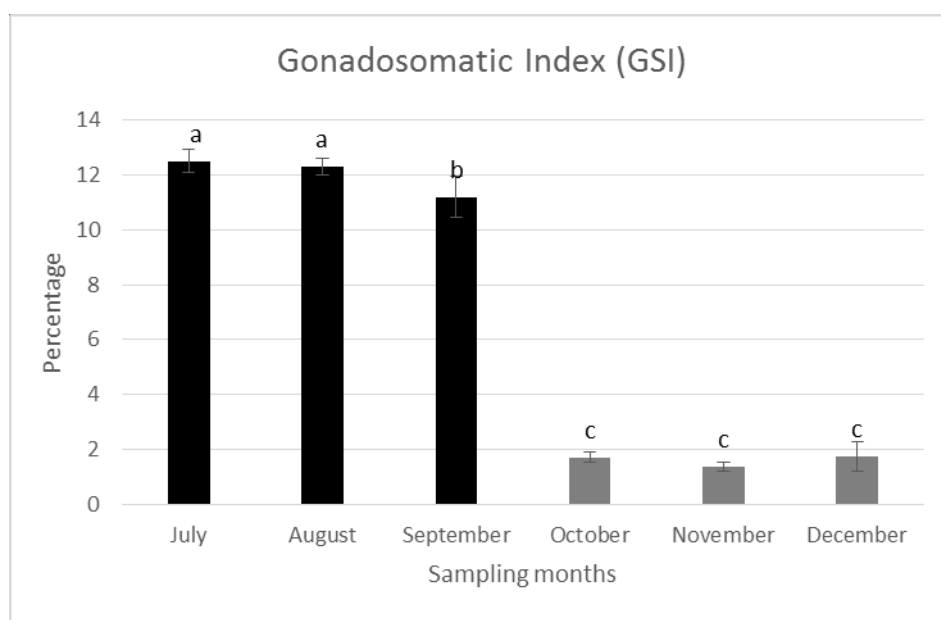


Figure 1: Gonadosomatic index (GSI) of *T. ilisha* during spawning and post spawning period. There were significant differences between samples in the spawning and post spawning period.

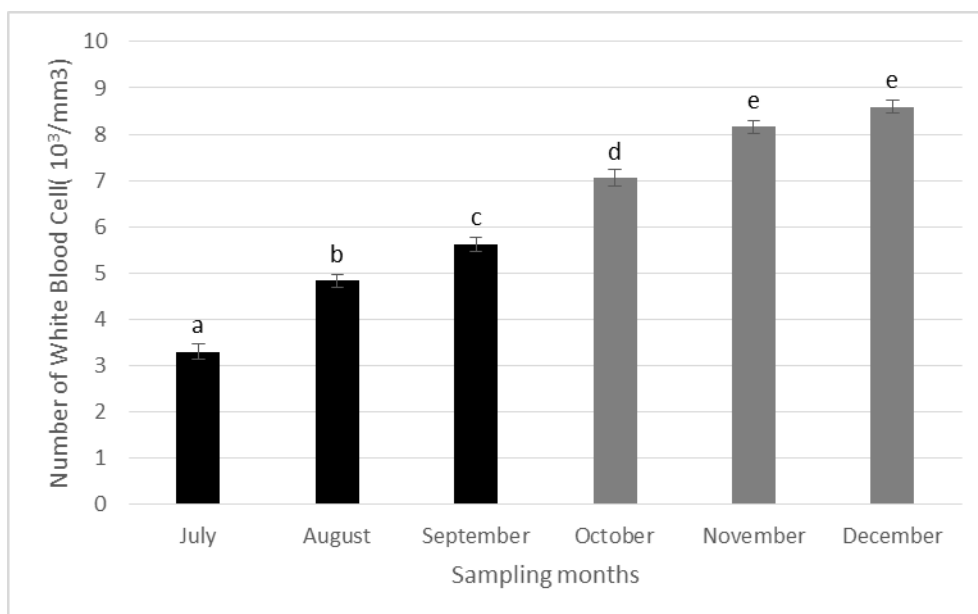


Figure 2: Changes in the number of white blood cell of the female *T. ilisha* during spawning and post spawning season. There were significant differences between all groups but the last two groups.

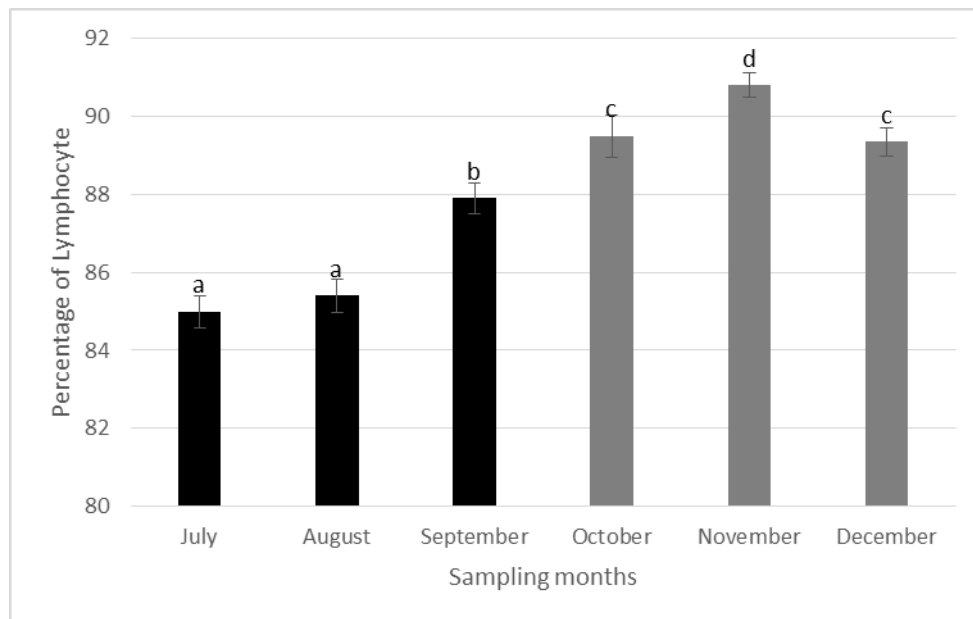


Figure 3: Changes in the percentage of female *T. ilisha*'s lymphocytes during the spawning and post spawning season. Significant differences were observed between spawning and post spawning time.

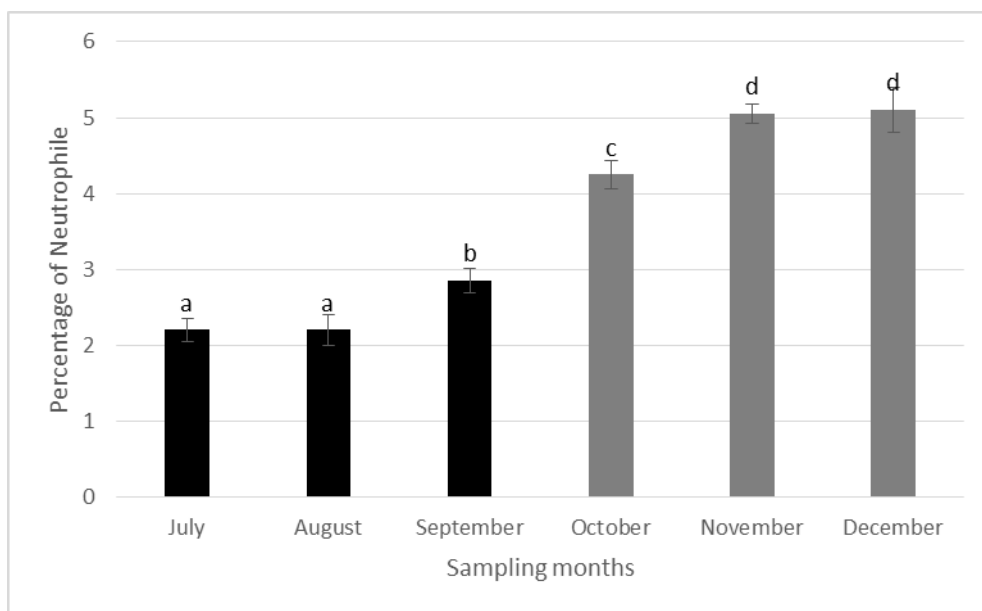


Figure 4: Changes in the percentage of female *T. ilisha* neutrophiles during spawning and post spawning season. There were significant differences between spawning and post spawning time.

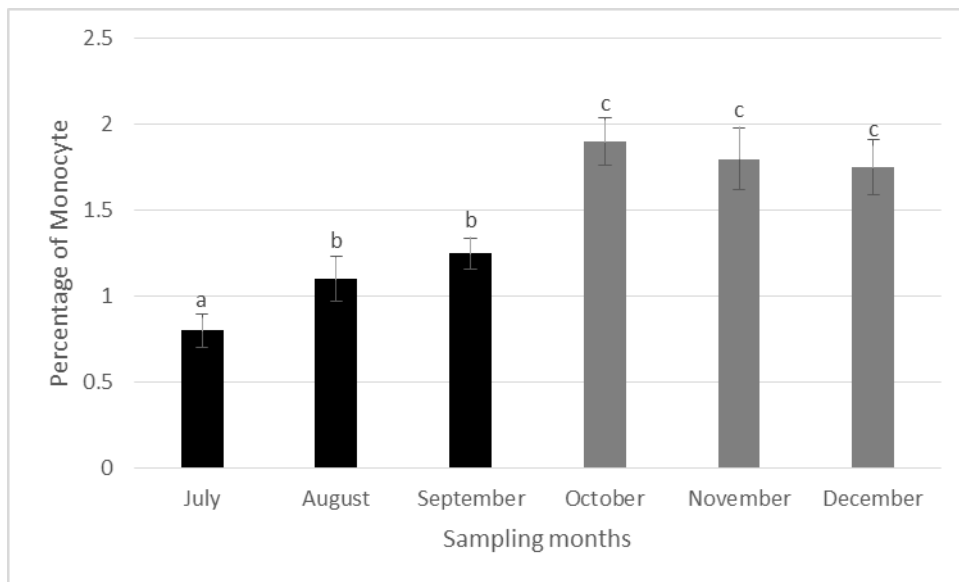


Figure 5: Changes in the percentage of female *T. ilisha* monocytes during spawning and post spawning season. Significant differences were observed between spawning and post spawning time.

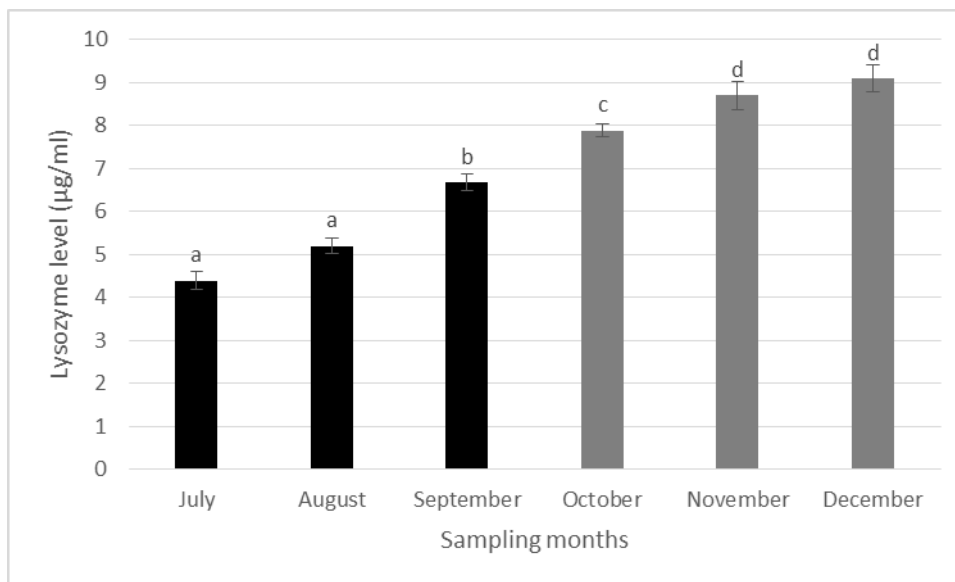


Figure 6: Changes in the levels of plasma lysozyme of female *T. ilisha* during spawning and post spawning season

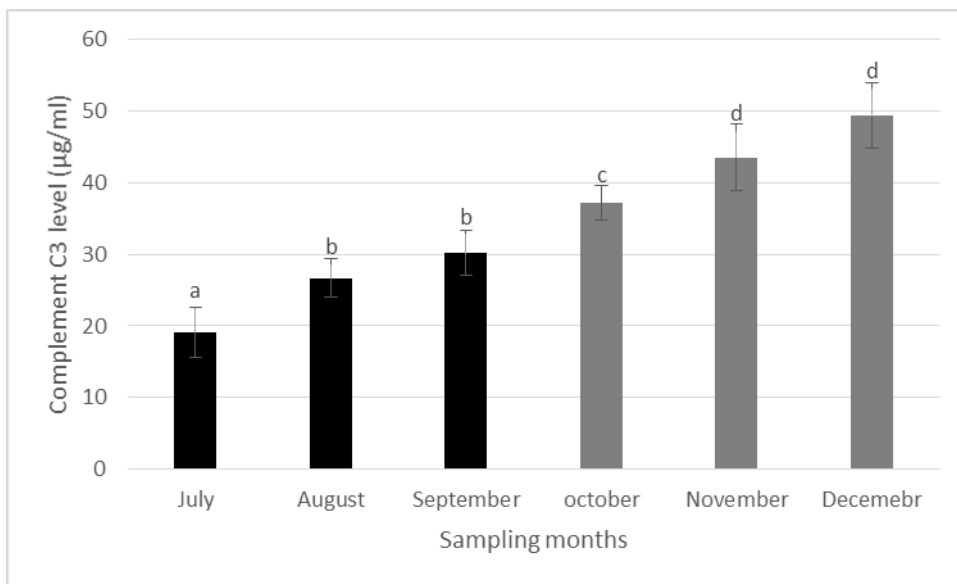


Figure 7: Changes in the levels of plasma Complement C3 of female *T. ilisha* during spawning and post spawning season

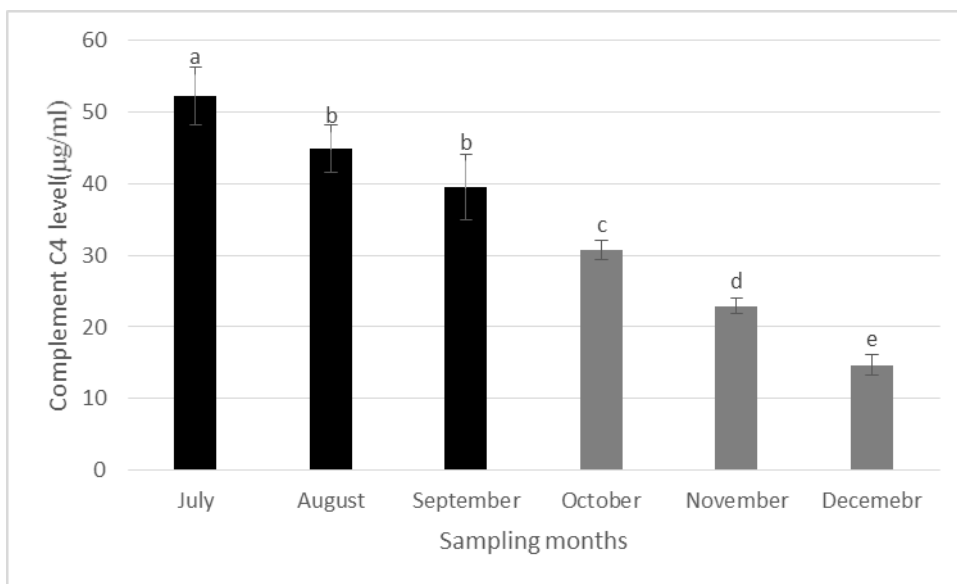


Figure 8: Changes in the Complement C4 levels of female *T. ilisha* during spawning and post spawning season

All factors are significantly different between spawning and post spawning period ($p < 0.01$). The levels of lysozyme and C3 during the spawning period were lower than that during the post spawning period. However C4 level was higher during the post spawning period ($p < 0.01$).

Discussion

During upstream migrations when fish lay their eggs in a safe place away from their predators, a complex interaction happens inside the fish which affects the physiology of the fish and the immune system as well (Cuesta *et al.*, 2007).

Some fish species stop feeding or their food intake drastically reduce during their spawning migration and endure a lot of hardship to swim up the rivers (Quereshi, 1968). Also we should consider the hormonal fluctuations during the spawning time which can have significant interactions with the immune system (Cuesta *et al.*, 2007). All these challenges have a negative effect on the general health status of the fish.

According to studies conducted by Querishi (1968), Pillay and Rosa (1963), Rajyalakshmi (1973) and Rahman (1996), it has been clear that *T. ilisha* stops feeding when it starts its migration upriver for spawning and endures starvation and semi- starvation periods. In addition, we opened up the

fore gut of our samples looking for any evidence of feeding, but there was no sign of feeding inside the digestive tract. During the entire upstream migration time, the fish uses the fat reserves in its body as an alternative source of energy. Clearly starvation during spawning period has some negative effects on the immune system which were emphasized in the following researches. Martin *et al.* (2010), showed that genes encoding a number of secreted immune related proteins decreased in expression following starvation, including serum amyloid A, complement factor B and serotransferrin. Also in a study conducted by Caruso *et al.* (2011), short term starvation showed a significant suppression on non-specific immune parameters of European sea bass (*Dicentrarchus labrax*) and black spotsea bream (*Pagellus bogaraveo*).

Total WBC and differential count of leukocytes showed a decrease during spawning time while it increased after spawning. The explanation available for such a change is that during spawning, fish witness a mount in sex steroids like testosterone (T) and 17 β -estradiol (E2) (Asadi *et al.* unpublished data, 2014). As in many other studies, sex steroids have suppressive effects on the leukocyte levels. The steroids cause reduction in antibody secreting cells and low plasma antibody levels (Hou *et al.*, 1999a,b). Many reports show that

sex steroid hormones suppress antibody secreting cell numbers and antibody secretion at least in salmonid fish, which are susceptible to many diseases during the spawning season. For example, administration of testosterone or other androgens reduced antibody producing activity of lymphocytes from the anterior kidney of chinook salmon *Oncorhynchus tshawytscha* (Slater and Schreck, 1993). Hou *et al.* (1999c) reported that testosterone reduces the number of antibody-producing cells in rainbow trout. They also reported that high concentrations of estradiol-17 β (E2) significantly reduced the number of antibody-producing cells and antibody production by lymphocytes.

Fish humoral immunity is affected by sexual maturation and spawning, by the interaction of sexual hormones with the immune system (Harris and Bird, 2000). In salmonid species, decreased bactericidal activity and increased frequency of infection have been reported during sexual maturation (Iida *et al.*, 1989; Pickering and Christie, 1980; Richards and Pickering, 1978). Sexual maturation also coincided with lowered plasma lysozyme activity and decreased numbers of total leukocytes and antibody-producing cells in salmonids (Pickering and Pottinger, 1987; Maule *et al.*, 1996). Lysozyme plays an important role in the non-specific immune response of fish and it has been found in mucus, serum and

ova of fish (Magnadottir, 2006) and acts as a potent non-specific immune factor against parasitic and bacterial infections (Wang and Zhang, 2010). In this study, a significant variation in the lysozyme level was found during the reproduction phase. Lysozyme level during the spawning period was lower than that during the post spawning period. So the decreased level of lysozyme in the spawning period can also be related to the low levels of neutrophils and monocytes which are the most important agents for the secretion of lysozyme into the blood.

The complement system in fish consists of three activation pathways (Classical, Alternative and Lectin), which participate in general immune responses such as inflammation, respiratory burst, cell lysis or antigen presentation. Within the complement cascade, C3 complement factor is a key element, which is regulated via one of the three existing pathways (Boshra *et al.*, 2006).

In this study, C3 levels during spawning were lower than that in the post spawning period while during post spawning, C3 levels increased significantly. Sexual maturation and spawning activity have reversed effects on the complement activity. Also Wenger *et al.* (2011) reported that E2-treated trout had a lower capacity to activate their immune system against bacterial infection. Moreover, Cuesta *et*

al. (2007) reported that treatment with E2 enhanced complement activity 1 day post-injection while decreased it after 3 and 7 days.

Evidence for the complement system as a target of estrogen action is well established in mammals. For instance, Sundstrom *et al.* (1989) demonstrated that estrogen triggers the synthesis of C3 factor in the epithelial cells in the rat uterus. Kuivanen *et al.* (1989) also identified two estrogen-responsive proteins in rat uterus as structurally related to C3 complement component. So it seems that the decrease of C₃ during the spawning period is related to hormonal interaction with the immune system which resulted in the decrease in C₃ during reproduction, while the rise in C₃ after spawning can be due to the fall in the sexual hormone levels. Higher levels of C₄ in the spawning period may be a result of changing in the complement activation pathway from alternative to classic which may be a fish adapting to its new environment, but this thesis needs to be studied further.

Generally, the results showed that *T. ilisha* exhibits a depression in some hematological and immunological parameters during its upstream migration and it could be as a result of hormonal and environmental effects. However to make a better image of the fish physiology during spawning and post spawning seasons, more research

needs to be conducted to show other aspects of the fish physiology and its relation to its reproduction season.

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