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A SEASONAL VARIATIONS ON IMMUNE SYSTEM OF *MUGIL CEPHALUS* (CARL LINNAEUS 1758)

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ABSTRACT
The study aimed to assess the seasonal variations in the histology of grey mullet (Mugil cephalus) in Puducherry (site I) India, and Cuddalore (site II) Tamil Nadu, India, from the period March 2023 to February 2024. Seasonal collections of tissues were done somatically, and immune organs head kidney, spleen, and associated organs gill, liver, and muscles were assessed. The local investigated parameters in the sit II area showed variations were changed than that of the site I. These investigations showed the water contamination level in site II as the biomarkers that reveal negative impacts on the histological studies, and the physical and physiological status of the fish in the monsoon at lower temperatures. Likewise in the summer season, positive impacts were greater in both sites. There was a gradual change noted in the pre-monsoon and post-monsoon periods. The histological investigations revealed that fish face immunological challenges and stress conditions in deteriorated ecosystems at monsoon and are alert to monitor the environment. The variations were mainly assessed in MMC (Melano macrophage center), RBC (Red blood corpuscles), WBC (White blood corpuscles), lymphoid cells, epithelial cells, and blood vessel changes rather than other cell organelles. Overall, this study demonstrates that M.cephalusis a biomarker species in marine environments and the anomalies were indicated to discriminate the seasons and the water quality. These are mainly impacting the immunological and physical status of the fish. More histopathological changes were observed in site II fish due to excess contamination of the coastal area.

KEYWORDS: immune system, histology, M.cephalus, summer, pre-monsoon, monsoon, post-monsoon

INTRODUCTION

The abiotic factor of temperature is the most influencing and challenging factor in the environment, it causes various effects in marine fish. However, fish can combat fluctuating temperatures (Cho et al., 2015; Rezakhani et al., 2020). Seasonal changes are most important in the environment and temperature fluctuation has predominantly influenced the immune system. Higher temperatures enhance immunity whereas lower temperatures decrease immune response (Sakthivel et al., 2012). Weather and rainfall influence aquaculture ecosystems (Cheng et al., 2013). Fish is an ideal biomarker of all stress in aquatic systems and plays a vital role in the food chain (Ameur et al., 2012).

Fish experience seasonal temperature fluctuation in their aquatic system as ectotherms. Generally, climate directs the internal heat level and the effective changes subsequently happen against their natural ecosystem. Seasonal variation may affect the histology of various organs (Gallock et al., 2005; Lewis et al., 2010). Histopathological biomarkers are marks of the impacts of different stressors like temperature variances. The reflection of the health of an organism enables, through impression the general strength of the whole population in the biological system including fish, even tissue damage may be caused because of temperature changes (Velkova et al., 2005; Salazar et al., 2011and Dash et al., 2011).

Fish is highly proteins, easy to digest, muscle deterioration, and is especially an immunity enhancer (Begum et al., 2013). Upcoming research will reveal the differences associated with the environment, season, sex, feeding behavior, and age (Norouzi and Bagheri 2015). In an aquatic ecosystem, fish are at the base of the food chain which is equally parallel to the quality and contamination of the water even today. They are noticed that store chemicals, insecticides, and heavy metals; for this reason, the contamination in their environment was excluded (Ambreen et al., 2018). Biological and physical effects are influenced by water contamination that affects and leads to aquatic and health problems (Faheem et al., 2021).

In many aquatic habitats, the histopathological changes serve as accurate biomarkers for identifying pollution levels and water quality (Nascimento et al., 2012; Abdel-Moneim et al., 2012; Van Dyk et al., 2012; Rašković et al., 2015). However, selective research examines how immune organs vary with the seasons and how those variations relate to changes in water quality. As the current study was a component of a much larger monitoring study on the Southeast Coast of Tamil Nadu, its goal was to determine whether histopathological analysis could be used for the monitoring alterations rather than to establish a strong correlation between particular water quality parameters or specific pollutants and the immune system. We utilized the native freshwater fish species M.cephalus frequently seen in marine environments (Fazio et al., 2015; Thirunavukkarasu et al., 2022). It is a significant species in Indian marine, freshwater, estuaries, benthopelagic, and catadromous. Many studies have been reported on this species even though there are very selective papers on seasonal variation in the immune system and immune response in histology. Therefore, the additional aim was to evaluate the organism for pollution monitoring in the natural environment. Hence, we can further expand our knowledge on immune responses of aquatic organisms to seasonal variations in environmental conditions and can apply this knowledge to ecological monitoring has been achieved successfully.

MATERIALS AND METHODS

2.1 Sampling site

Puducherry (site I) is located at latitude 110 54' 2 N and longitude 790 49' 45 E) on the Tamil Nadu, Southeast Coast of India. The coastal area is polluted due to the discharge of agricultural fields, industrial, and domestic wastes through small canals and channels into the coastal waters. Several small industries are located near the study area, discharging their effluents continuously into the coastal area. The next site Cuddalore (site II) coastal area covers over 3678 km2 including the stretch (68km) in the coastal line. Here more industries and drainages drain exclusively in to rivers and the rivers finally drain into the Bay of Bengal (Cuddalore) daily. The present study was carried out from the southeast coast of (Lat, 11°42'N; long, 79° 46' E) Tamil Nadu.

2.2 Collection and analysis of samples

M.cephalus samples 20 to 25 were collected randomly in each season from each site (Site I; Site II). The fish was measured at a length of 10 ± 21 cm and weighed 53 ± 77.8 g. Samples were alive and transported to the laboratory using enough aeration. For acclamation, the fish were maintained in aerated tanks to minimize the stress and health conditions. All recordings followed in the laboratory.

2.3 Histology

The histopathological studies of the kidney, spleen, gill, liver, and muscle of exposed and the control fish were processed by the given method (Nelson et al., 2013). Cryosection is the frozen section cutting procedure for tissues and used in microscopic analysis. Histological techniques were followed by previous methods (Fischer et al., 2008). It is an excellent method to visualize minute cells.

2.4 Cryosection and Staining

The cryostat (Leica, CM 1850) was frozen at -200C before the procedure. All the instrumental parts were cleaned thoroughly. The sample tissue was dissected from euthanized fish, and the tissues were kept in the mould of a freezing medium (Jung Tissue Freezing Medium, Hi-Media, Puducherry) the fresh tissues were mounted in the mould. The block was embedded in an optimal cutting temperature (OCT) medium and the sides were to be checked in the holder. Leave the setup for 30 minutes at -400C so that the medium solidifies completely. Cut sections might be 5-15 µm thick in the cryo-stat section at −20°C frozen. Sectioned tissues were collected immediately using glass slides (Nelson et al., 2013). The sectioned tissues were floated in the warm water bath, to remove the freezing medium using a soft brush. A sterilized and clean slide was used for mounting the tissue at room temperature (Ravikumar et al., 2014). The tissue sectioned was kept on the slide and stained with deparaffinized xylene for five minutes. Dehydration takes place with ethyl alcohol in descending order as follows (Ochei and Kolhatkar, 2000). After the floatation process, the sample slides are stained in Hematoxylin (H) and Eosin (E). The prepared slides were preserved in the laboratory and observed under the NOVEX HOLLAND microscope and photomicrographs were taken and find out the histopathological changes in the tissues were evaluated.

RESULTS

3.1 Head Kidney

In the site I in histology the head kidney in various seasons showed changes in hematopoietic interstitial cells (HIC), melanomacphage center (MMC), white blood cells (WBC), venous sinus (VS), post cardinal vein (PV), and blood vessels (BV) bowman's capsule (BC). Scattered nucleus, swells are noted. All the changes increased in summer but not in monsoon. Loosely packed cells were noted (Figure 1 A-D).In site II, the head kidney during the summer the MMC and WBC have higher counts and swells in increased cells, and tubules whereas in monsoon it was vice-versa (Figure 2 A-D).

3.2 Spleen

M. cephalus spleen (site I) white pulp is mostly composed of lymphoid and hemoblast cells. It also includes reticular fibers, fibrocytes, and ellipsoids that develop as periarterial sheets with mononuclear cells of the phagocytic system. The mononuclear phagocytic system (MPS) cells can collect particulate matter, including old erythrocytes and effete blood cells and delimited germinal centers were not found (Figure 3 A-D). Erythrocytes and leukocytes were also increased in the spleen section in the summer season.

The spleen section (Site II) shows tightly packed MMC during summer and pre-monsoon, the WBC and MMC were densely packed likewise in monsoon and post-monsoon the cells were loosely packed (Figure 4 A-D).

3.3 Gill

During (Site I) summer the dense epithelial cells and lamellae are noted and changes occur in monsoon in epithelial tissues (Figure 5 A-D). All fishes in monsoon tarred and fused lamellae and fused epithelial cells and some vacuoles are noted. Histology of the gill (Site II) in various seasons showed normal primary and secondary lamellae, epithelial cells, and filaments. In summer the epithelial cells had higher counts whereas in monsoon it was lower (Figure 6 A-D).

3.4 Liver

In liver (Site I) the MMC, sinusoids, and hepatocytes were higher (Figure 7 A-D). The red pulp was increased during summer in all the fishes. Whereas in(Site II) fish, it was observed that the hepatocytes were polygonal in shape, MMC, and sinusoids, and some of them had distinct nuclei during summer but were reduced in monsoon (Figure 8 A-D).

3.5 Muscle

Histological examinations(Site I) focused on summer the muscle bundle and connective tissues are clear structure whereas changes in the fleshy storage in pre-monsoon, in monsoon within the skeletal musculature that occurs loose in the muscles, and some spaces were observed in muscle fibers in post-monsoon (Figure 9 A-D). In (Site II) the fleshy part was higher to store the lipids to accommodate the stress condition during monsoon and the closely packed bundles observed, and it was the quite opposite in summer composed of skeletal muscle lateral muscle layers such as muscle fiber and connective tissue (Figure 10 A-D).

Based on the findings, histological alterations showed maximum positive variations in summer, it showed negative variations in monsoon in both sites. In pre-monsoon and post-monsoon seasons the results were more likely to fluctuate according to the water temperature condition and stress. Hence the report indicates the seasons mainly impact the immune response action in all two sites.

DISCUSSION

Numerous biological processes, including immunology, behavior, metabolism, feeding habits, and reproduction in fish, might be impacted by seasonal changes. Many environmental factors impact fish immunity, with water temperature being considered the primary influence. The study demonstrates that the seasonal influence is the main factor than that of water temperature. The biological response and stress modulate the climate (Saha et al., 2002; Herrero et al., 2018). It has been discovered that photoperiod, water temperature, dissolved oxygen, salinity, and food consumption, quality of water have the greatest effects on fish blood levels of biochemical variables. The WBCs in our research were lower during the monsoon season and greater throughout the summer. These findings are consistent with those in the study of previous methods that investigated the rainbow trout Oncorhynchus mykiss L. immune response in commercial ponds (De Pedro et al., 2005; Morgan et al., 2008) which stated that leucocyte counts Tinca tinca L. were considerably lower in spring and winter compared to summer and fall. Seasonal variations are also observed in the circulation levels of various blood cells, such as the erythrocyte count, leucocyte count, and percentage of hematocrit values. Fish have a nonspecific immune response that consists of systemic inflammation or cellular components in phagocytic action and humoral elements that are mostly the complement system. Various accounts stated that innate immune system components are highly influenced by temperature-independent, although lower water temperatures have been shown to inhibit the acquired immune system, as seen by changes in lymphocyte activity and antibody production (Magnadóttir, 2006).

In tropical regions, rainfall is the most significant cyclic phenomenon. It brings about significant changes in the hydrographical features of the marine and estuaries. In this investigation, the maximum rainfall data are kept during the monsoon month (Dinakaran et al., 2023).According to the current study, fish living Southeast Coast of India displayed some histological symptoms, including loosened muscle bundles and a noticeable gap between tissues. Changes in the fish's tissues that were taken from the sites may have contributed to water contamination. The author (Jeffree et al., 2006), recent investigation demonstrated that the histological alterations in fish muscles may be caused by contaminants by season ingested from the surroundings. The study's histological evaluation supports the buildup of pollutants in the muscles since it shows some degree of muscle fibers. Fish may have histological changes, such as the degeneration of muscle bundles with specific focus locations (Kaoud and El-Dahshan, 2010). The current investigation demonstrated that fish may have histological variation (Al-Halani, 2018). The activities of immunity are produced highly by immune organs during monsoon due to the invasion of various pathogens. Therefore, the monsoon season is a susceptible infectious season due to the reduction of WBC cells (Esteban et al., 2015). The fish increase melanomacrophage centers in the monsoon trying to combat the stress while it is vice-versa (Raina et al., 2015).The present report revealed the two sites of histopathological changes in the immune system and immune response. The tissues were altered and the muscle may show excessive uptake of pollution. The pollution will denature the environment and fluctuate the seasons (Al-Halani, 2018).

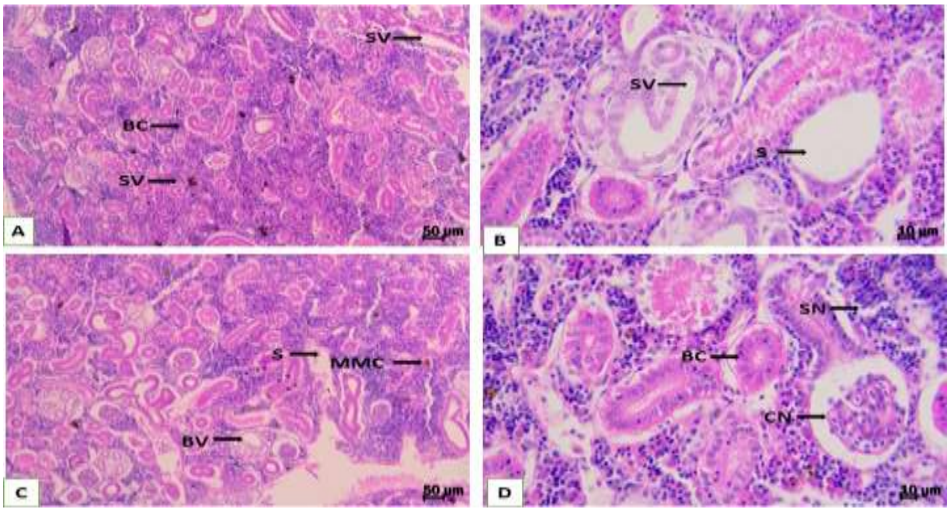

Figure 1. M.cephalus (Site I) Head kidney (H&E 10x, 40x); Scale bar = 10, 50 µm;Photomicrograph showing histological features of the head kidney in various seasons; Summer (A); Pre-monsoon (B); Monsoon (C); Post-monsoon (D). BC – bowman's capsule; MMC – melano macrophage center; SV – sinus venosus; S – swells & space; WP - white pulp; RP- red pulp; SN – scattered cell; C - cluster nucleus

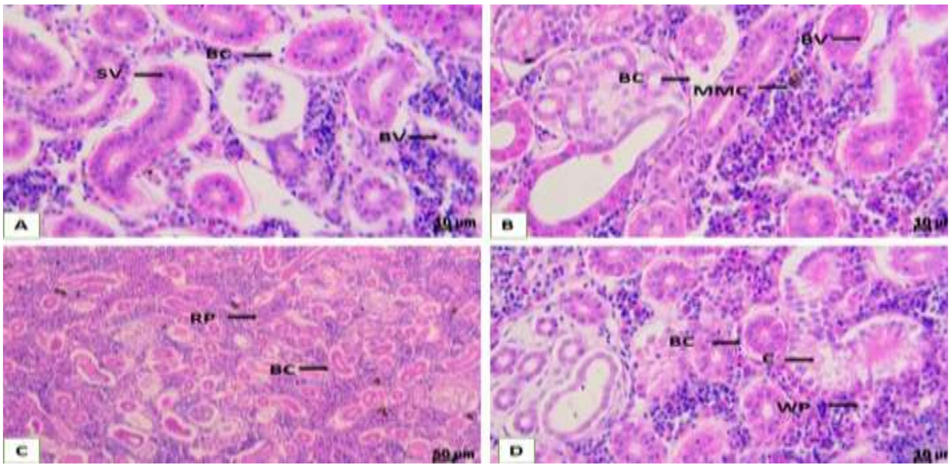

Figure 2. M.cephalus (Site II) Head kidney (H&E 10x, 40x); Scale bar = 50, 25 µm; Photomicrograph showing histological features of the head kidney in various seasons; Summer (A); Pre-monsoon (B); Monsoon (C); Postmonsoon (D). BC – bowman's capsule; S - space; WP - white pulp; SN – scattered nucleus

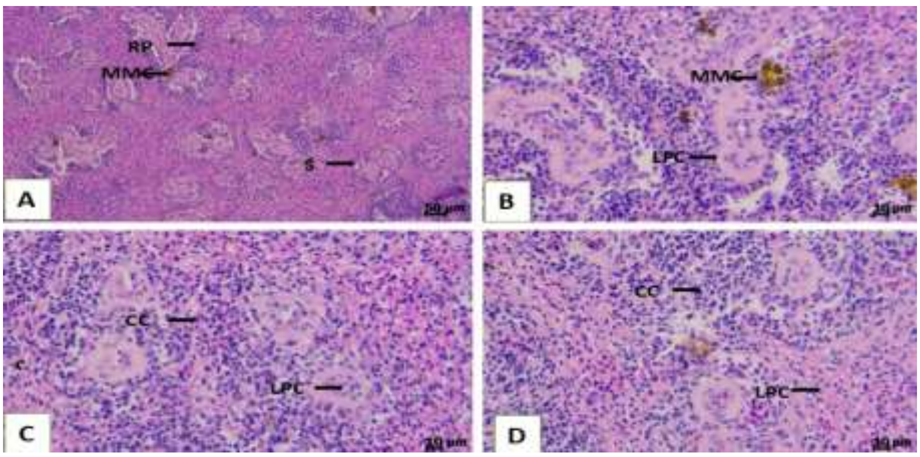

Figure 3. M.cephalus spleen (Site I); (H&E 10x, 40x); Scale bar = 10, 50 µm; Photomicrograph showing histological features of the spleen in various seasons; Summer (A); Pre-monsoon (B); Monsoon (C); Post-monsoon (D). MMC – melano macrophage center; WP - white pulp; RP- red pulp; LPC – loosely packed cell; N – nucleus; CC – clustered cells

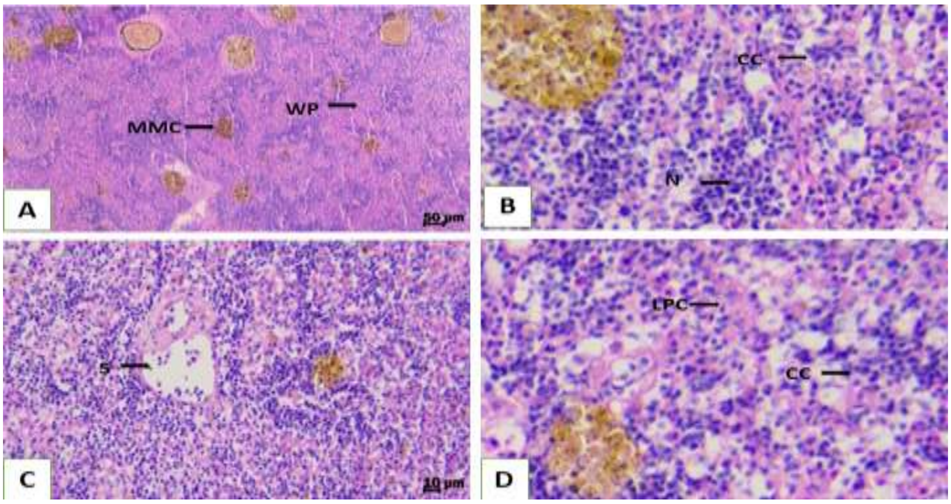

Figure 4. M.cephalus (Site II) spleen; (H&E 10x, 40x); Scale bar = 10, 50 µm.; Photomicrograph showing histological features of the spleen in various seasons; Summer (A); Pre-monsoon (B); Monsoon (C); Post-monsoon (D) MMC – melano macrophage center; WP - white pulp; RP- red pulp; LPC – loosely packed cell; N – nucleus; CC – clustered cells

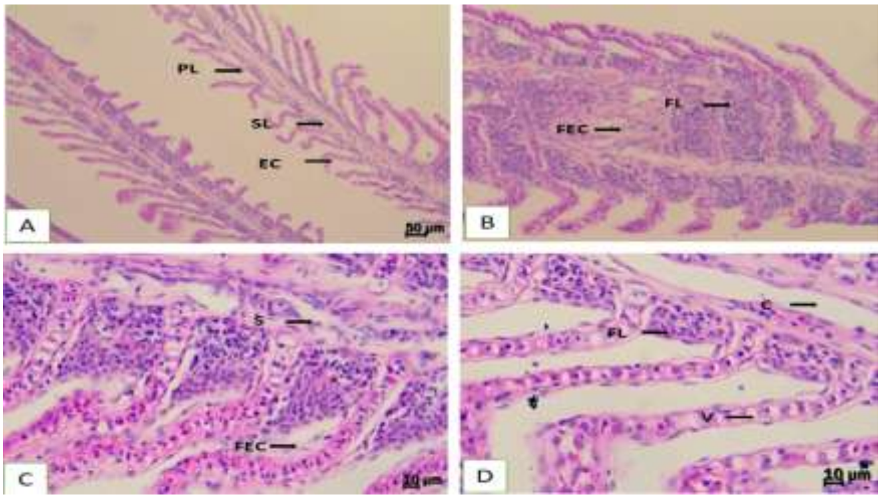

Figure 5. M.cephalus (Site I) gill; (H&E 10x, 40x); Scale bar = 10, 50 µm; Photomicrograph showing histological features of the gill in various seasons; Summer (A); Pre-monsoon (B); Monsoon (C); Post-monsoon (D). PL – primary lamellae; SL – secondary lamellae; EP – epithelial cells; FL – fused lamellae; CC- clustered cells; V – Vacuoles; FEC – fused epithelial cells

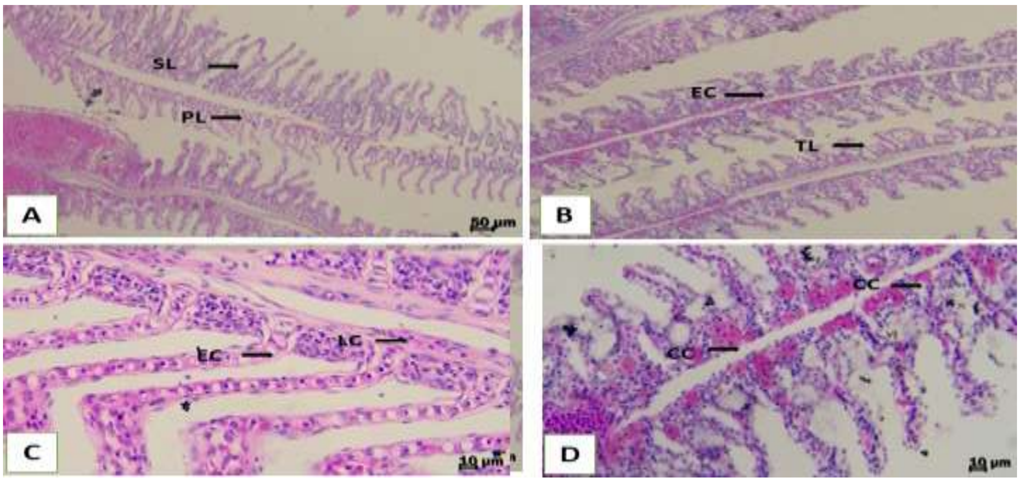

Figure 6. M.cephalus (Site II) gill; (H&E 10x, 40x); Scale bar = 10, 50 µm; Photomicrograph showing histological features of the gill in various seasons; Summer (A); Pre-monsoon (B); Monsoon (C); Post-monsoon (D). PL – primary lamellae; SL – secondary lamellae; EP – epithelial cells; CC- clustered cells

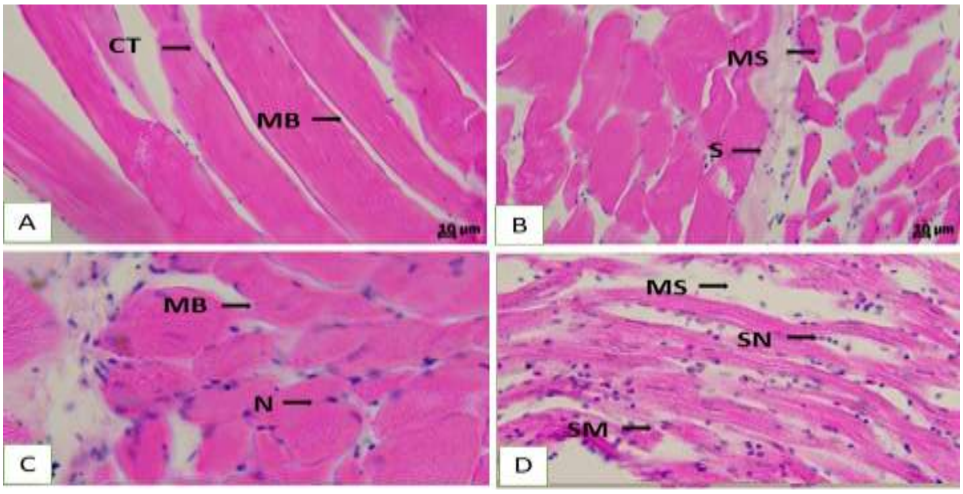

Figure 7. M.cephalus (Site I) liver; (H&E 10x, 40x); Scale bar = 10, 50 µm; Photomicrograph showing histological features of the liver in various seasons; Summer (A); Pre-monsoon (B); Monsoon (C); Post-monsoon (D). MMC – melano macrophage center; WP - white pulp; RP- red pulp; LPC – loosely packed cell; N – nucleus; CC – clustered cells

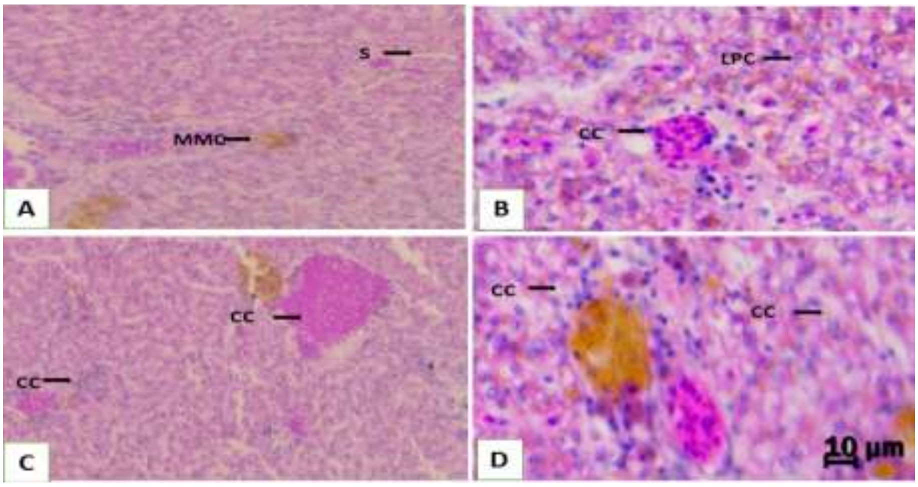

Figure 8. M.cephalus (Site II) liver; (H&E 10x, 40x); Scale bar = 10, 50 µm; Photomicrograph showing histological features of the liver in various seasons; Summer (A); Pre-monsoon (B); Monsoon (C); Post-monsoon (D). MMC – melano macrophage center; WP - white pulp; RP- red pulp; LPC – loosely packed cell; N – nucleus; CC – clustered cells

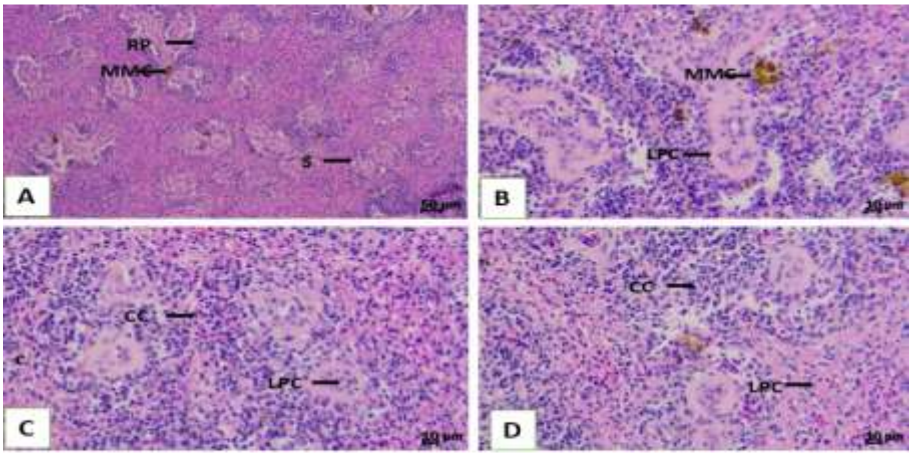

Figure 9. M.cephalus (Site I) muscle; (H&E 10x, 40x); Scale bar = 10, 50 µm; Photomicrograph showing histological features of the muscle in various seasons; Summer (A); Pre-monsoon (B); Monsoon (C); Post-monsoon (D). MB – muscle bundle; CT – connective tissue; MS – muscular space; N – nucleus

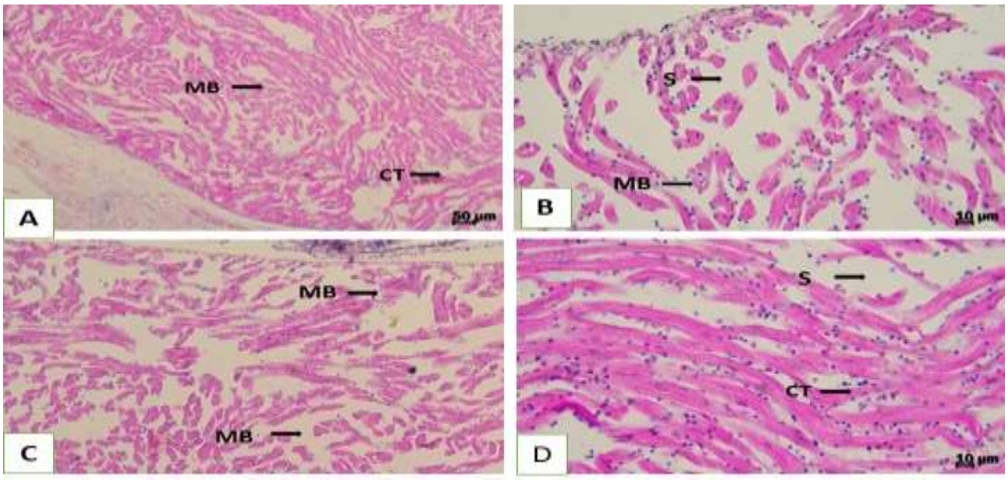

Figure 10. M.cephalus (Site II)muscle; (H&E 10x, 40x); Scale bar = 10, 50 µm; Photomicrograph showing histological features of the muscle in various seasons; Summer (A); Pre-monsoon (B); Monsoon (C); Post-monsoon (D). MB – muscle bundle; CT – connective tissue; S – space

CONCLUSION

The present study demonstrates the two important sites in the Tamil Nadu coast and the water contamination deteriorates the seasons and the environment. The variations in histology and immunology strongly state the seasons have an impact on the immune system of fish. Based on the findings, M.cephalus showed maximum variations in summer. In pre-monsoon and post-monsoon seasons the results were more likely to fluctuate according to the physicochemical condition. Hence the report indicates the seasons mainly impact the histology and immune response of the fishes. This study was undertaken and reported that seasons have a prominent effect on the histology of selected species. So, the seasons were taken to monitor and consider the health and immunity status of teleost fish. Likewise, fish histology showed various alterations are also considered to interpret fish health. For further studies, our report suggested that the seasons are taken into consideration to monitor water changes.

DECLARATION OF COMPETING INTEREST

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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