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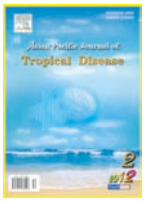

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Melanomacrophage centers aggregation in *P. lineatus* spleen as bio-indicator of environmental change

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ABSTRACT

Objective: To monitor the water quality parameters and climatic impact on the health condition of the fish through pathology study of Marine catfish spleen using Melano-macrophage centers (MMCs) aggregation as a biomarker Marine catfishes along the parangipettai coast of Tamil Nadu. **Methods:** Replicate samples were done for all the seasons along the coast. Condition factor (K) and Histopathology technique, were used to evaluate the condition. **Results:** The result revealed the Condition factor (K) of fish was minimum in winter 1.0874 and maximum in summer 1.6726. Histology show the number of MMCs aggregation in spleen 59.5 ±8.3 MMCs/mm^2 was observed in at post monsoon and 2-5 MMCs/mm^2 in summer. Anova shows significantly different at P<0.05. The MMCs aggregation excited 30/mm2 when the water temperature is below 22℃and reaches maximum at temperatures of below 20 ℃. The MMCs were correlated with water temperature shows positive correlation mean drop in water temperature may also reason for MMCs aggregation in spleen. **Conclusion:** The rise in temperature causes effect in health of the fish which was indicated in condition factor and MMCs aggregation. Animals are vulnerable to disease due to lowering of temperature and other environmental conditions. The MMCs aggregation can be used as bio-indicators for environmental.

1. Introduction

In the past 50 years, the rapid grew and still expending aquaculture field. This expansion because of increase in demand for fisheries and lose of fisheries. So there is demand for aquaculture products to compete the demand of growing world apatite. The Global environment change cause reduces the aquaculture industry. The temperature is the most important factor that affect growth and survival rate of fish[1-4]. The seasonal affective disorder (SAD) with a certain degree of humour, yet it is clear that seasonal variations can have a very real impact on over bodies and psychology[5]. So water quality management is so important that health condition of fish[6]. The condition factor (K) is influenced by age of fish, sex, season, stage of maturation, fullness of gut, type of food consumed, amount of fat reserve and degree of muscular development. In some fish species, the gonads may weigh up to 15% or more of total body weight. With females, the K value will decrease rapidly when the eggs are shed. The study of the condition factor is thus important for understanding the life cycle of fish species and contributes to adequate management of these species and, therefore, to the maintenance of equilibrium in the ecosystem[7]. Histopathology is an important biomarker in monitoring by observing the vital organs[8,9].

The spleen of cat fish is a reddish-brown, elongated thick and flattened structure, lying along the intestine and in the proximity of the pancreas, trunk-kidney[10] and the only lymph-node organ in teleost fish[11]. Spleen erythropoietic tissue involves in the synthesis of new erythrocytes and reservoir in eel primary haemopoietic organs, as spleen is the only organ in fish to trap antigens[12-14].

Melano-macrophage centers (MMCs) have four types of pigments like melanin, lipofusin, ceroid and hemosiderin[15] and present in spleen, liver and Head kidney. MMCs aggregation are potential indicators of environmental condition[16], stress full condition[17] and play role in

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cleaning and elimination of foreign particles. Histopathology of spleen shows melano macrophages center and erythrocyte destruction and synthesis and defense systems of the fishes; the lymphocytes and the macrophages[17,19-21]. This work was done as part of survey of immune organ of catfishes in parangipettai coast. Which intern provide the ecological status of the particularly area and to health condition of the fish large geographical site. This work all done to show the MMCs as biotic indicator. In this work we mainly focus on the MMCs aggregation in catfish spleen. We also compare with temperature, dissolved oxygen, total organic content to explain the MMCs aggregation in spleen.

2. Materials and methods

2.1. Sample collection

Fish were collected over a period of one year along form parangipettai south east coast of India, Tamil Nadu. (11 ° 40′ N, 79 ° 57′ E). The fish were brought to the lab and acclimatized in a 500 fiber tank for a day. Fish were randomly take in every season for further experiment (n=25).

2.2. Water quality parameters

The water samples were collected from the collection site using nansent water sampler. The analysis of different physico-chemical parameters such as water temperature, pH, dissolved oxygen, dissolved carbon dioxide, total alkalinity, on board using probe and following the method of American Public Health Association standard[22].

2.3. Condition factor

All the fish were noted for the external abnormality. Total length and body weight were recorded and were used to calculated along with the condition factor; condition factor (K)= 100 x BW (g) x FL (mm)-3, as an indicator of the general health status.

2.4. Tissues processing and histopathology

Then same size fish were scarified and the spleen was dissected out immediately. The spleen were fixed in 5% paraformaldehyde and stored at 4 ℃ until use. (Cryostat sections in detail the spleen. A thin section of 7-8 mm thickness section was made in Cryostat Leica CM1850 at -20 ℃. Sections were then collected on glass slides and fixed with 3.7% formaldehyde in 0.1 mol/L PBS (pH 7.4) for 20 min. and stained with hematoxylin and eosin (H&E) and mounted with DPH/ glycerol (1:1) and observed, photographed using Olympus microscope at 10 x10.

2.4. Aggregation of MMCs in spleen

A quantitative analysis of aggregation of MMCs in was performed in tissue section of 590 fish spleen. MMCs aggregation was measured using image analysis in light microscope fitted with imaging Sony camera measured and calculated in 10X magnification. The MMCs aggregation randomly measured as number of MMCs per mm^2. The true color image then capture as a digital image of the spleen image was then captured as a digital image. Image generated in computer mask of the MMCs in each screen enable to calculate the MMCs aggregation per screen and slide. The whole spleen was examined for the total analysis.

3. Results

The condition factor (CF) for *P. lineatus* was decrease from 1.6598 to 1.0892 during monsoon to post monsoon (October 2011 to January 2012). The Anova show the significant with water temperature and CF of fish. The CF value of individual wild fish was examined show the fishes were at good health. The randomly selected (n=25) fish were used for histological observation in every season. A total 250 fishers were examined for the study. The observation shows the degree of MMCs aggregation were significantly different in all the seasons and shows positively correlated with water temperature. The standard mean number of MMCs aggregation were found to be maximum at 59.5±8.3 MMCs/mm^2 during the period Post monsoon, and 2-5 MMCs/mm^2 were minimum level observed during summer (Figure 2). Number of MMCs aggregation was high in when the water temperature below 20 ℃ during of October 2011 to January 2012 and minimum of 2-5 MMCs/mm^2 was observed in at water temperature at 30 ℃ in summer (Figure 3). The statistical analysis shows the MMCs aggregation were significantly different with water temperature at P<0.001.

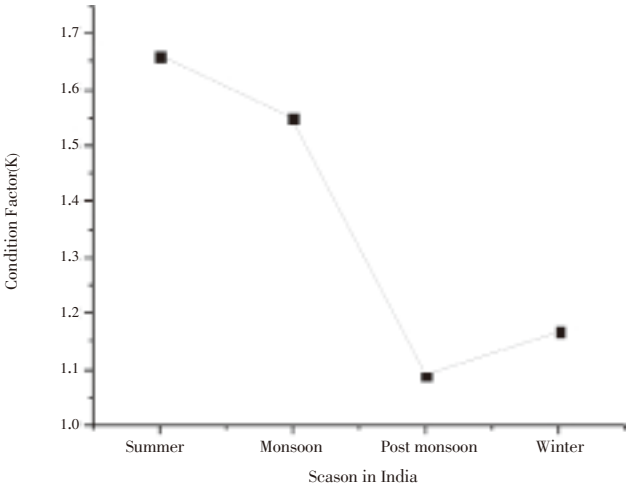

Figure 1. Condition factor (*K*) for *P. lineatus* different seasons

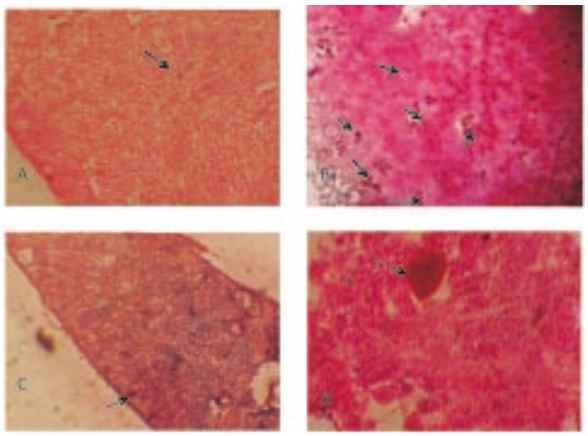

Figure 2. Histology of spleen.
A: in July to 2011 to September 2011. Shows clear white pulp and red pulp and MMCs (arrow); B: in October 2011 to December 2011. Shows large number of MMCs aggregation (arrow); C: in April 2012 to June 2012 shows clear white pulp and red pulp and MMCs (arrow); D: in January 2012 to March 2012 Shows large number of MMCs aggregation (arrow).

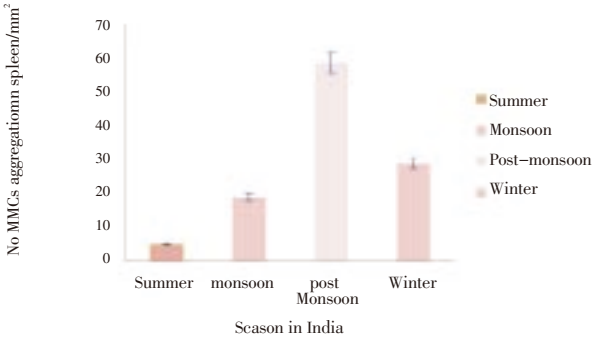

Figure 3. Mean MMCs aggregation during different seasons.

4. Discussion

These studies mainly focus on the variation in number of splenic MMCs aggregation to monitor the health condition of the fish in response to environmental changes. This hypothesis is based on the histological study of MMCs aggregation in fish spleen. The result show that the condition factor of the fish varies in season. K was 1.0874 during post monsoon, when the water temperature is below 20 ℃. Similar result was reported by other authors; as the fluctuation of CF due to change in environment feeding[23,24] and breeding cycle[25-27]. However, that the presence of MMCs is not entirely indicative of contaminant exposure. The MMCs aggregation was higher in the period of post monsoon and winter. This may due to the environmental change in that particular geographical area. The similar report was noted by[20] that number of MMCs was varied due to environmental change. Other publication shows the number of MMCs aggregation due to exposure of fish to environmental contamination john. Spleen has been used as indicator organs of environmental contamination and changes. The associated MMCs aggregation in spleen has reported by many authors in vary conditions[27-35]. Metabolic stress, high stressful condition[36,10], the number of MMCs in Ohrid trout were also due to the reproductive cycle. So this work explains the MMCs aggregation to environmental condition changes. And this work implies that MMCs aggregation was used as bio indicator of environmental changes.

Conflict of interest statement

We declare that we have no conflict of interest.

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