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# Diel variations in the selected serum immune parameters in *Oreochromis mossambicus*

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## Abstract

Almost all metabolic processes in an organism alternate through high and low activity phases with a regular periodicity of nearly 24h. These daily/diel variations are governed by factors such as light, weather conditions, availability of food or predator activity. The immune system in fish is expected to follow the same routine based on external cues from the environment which it lives. The present study was carried out to investigate such daily/diel variations in selected immune parameters such as serum lysozyme and peroxidases activity, total serum globulin level and peripheral blood leukocyte count in Oreochromis mossambicus. The fish were maintained in semi natural condition (i.e.12L:12D). The results showed significant rise in serum peroxidases and lysozyme between 0200h and 0600h of the day and serum cortisol exhibited elevated level between 2200h and 0600h. Total serum globulin exhibited peak concentration from 1400h to 1800h. Thus suggesting the possibility of rhythmic functioning of immune system in *O. mossambicus*.

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## Introduction

Almost all physiological variables investigated till date in animals and humans display biological rhythms and immune system is no exception to this phenomenon. The pineal gland via its secretory product, melatonin, influences the light–dark rhythm in most vertebrates including fish [1]. Circadian rhythms are daily variations or oscillations in behavior and physiology that are critical for maintaining homeostasis in an organism. Circadian rhythms seem to be the result of the expression of the so-called ‘clock genes’ and their synchronization by environmental and endogenous factors, mostly by light. Rhythmic variations have been reported in the activity of a number of vertebrate immune system components [2] as well. Circadian rhythmicity in circulating cells, lymphocyte metabolism and transformability, circulating hormones and other substances exerts various actions on different targets of the immune system. Immune cells such as human peripheral blood mononuclear cells have been shown to express clock genes (*hPer1*, *hPer2*, *hPer3*, *hDecl*) in a circadian manner [3]. Circadian changes in the circulation of T, B, or natural killer (NK) lymphocyte subsets in peripheral blood and in the density of epitope molecules on their surface, are related to cell reactivity to antigen exposure [4]. Changes in lymphocyte subset populations can depend on time of day-associated changes in cell proliferation in immunocompetent organs and/or on diurnal modifications in lymphocyte release and its traffic among lymphoid organs. Literature on rhythms and daily variations involved in fish immune system is scarce. The influence of a photoperiod on humoral innate immune response in gilthead sea bream, *Sparus aurata* and sea bass, *Dicentrarchus labrax* has been reported earlier [1]. Core clock genes such as *Clock*, *Period*, *Bmal*, and *Cryptochrome* have been shown to rhythmically express in zebrafish, *Danio rerio* [5]. Daily variations in immune function have direct implications for health and disease states [2]. Hence, the present study was carried out with objective of finding the possible diel variation in selected immune parameters of *Oreochromis mossambicus*. Serum lysozyme activity and serum peroxidases activity were measured to assess nonspecific immunity. Total serum globulin level was measured to assess general specific immunity in tilapia, *O. mossambicus*. These immune functions were chosen based on the relative ease in performing these assays in short time. Peripheral blood leukocyte count was also performed, as these cells are the source of the above mentioned serum immune molecules.

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### Fish

The fish, *O. mossambicus* ( $n=120$ ) weighing  $50\pm 5$ g of both the sexes were obtained from a local farmer and were maintained at ambient, uncontrolled temperature of  $28\pm 2^\circ\text{C}$  under natural photoperiod (12L:12D) in a 150l fibre reinforced plastic (FRP) tanks at a stocking rate of  $4\text{g l}^{-1}$  (grams per litre) i.e. 10 fish per tank/group. A total of twelve tanks/groups were maintained. The first set of six tanks/groups was utilized for serum based assays and second set of six tanks was used for haematological

### Results

The results showed a clear pattern of diel variation with significantly higher concentration of serum lysozyme (Fig. 1) ( $F\{5,54\}=2.56$ ,  $P<0.05$ ) and serum peroxidases (Fig.2) ( $F\{5,54\}=3.15$ ,  $P<0.02$ ) from 0200h to 0600h, and cortisol (Fig.3) exhibited significantly higher concentration ( $F\{5,54\}=23.02$ ,  $P<0.001$ ) from 2200h to 0600h. Total serum globulin exhibited significant ( $F\{5,54\}=3.08$ ,  $P<0.02$ ) peak concentration from 1400h to 1800h (Fig.4). Thus, all the above parameters showed typical robust

### Discussion

Fish can detect daily and seasonal changes in surrounding environment and adjust their physiological condition and behavior to the changes. Among the environmental factors, light and water temperature are considered to be important to fish [15]. In the present study, serum cortisol showed a typical S-Shaped rhythm with higher concentration in the morning and lower concentration during night which is similar to that of humans [16] with a peak at early morning. Earlier studies on diel variations

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...In addition, small sections of kidney and spleen were sampled and stored at  $-80^{\circ}\text{C}$  for RNA isolation. Lysozyme activities in fish serum, hepatopancreas, kidney, and spleen were determined by the method of Binuramesh and Michael (2011). Bacterial suspension of *M. lysodeikticus* was obtained by dissolving cold dried bacterial powder with 0.04 M PBS (pH 6.2 and  $\text{OD}_{520\text{nm}} = 0.3\text{--}0.5$ )....

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