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Crosstalk between Innate Immunity and Circadian Rhythm: Do fish immune defences have a sense of time?

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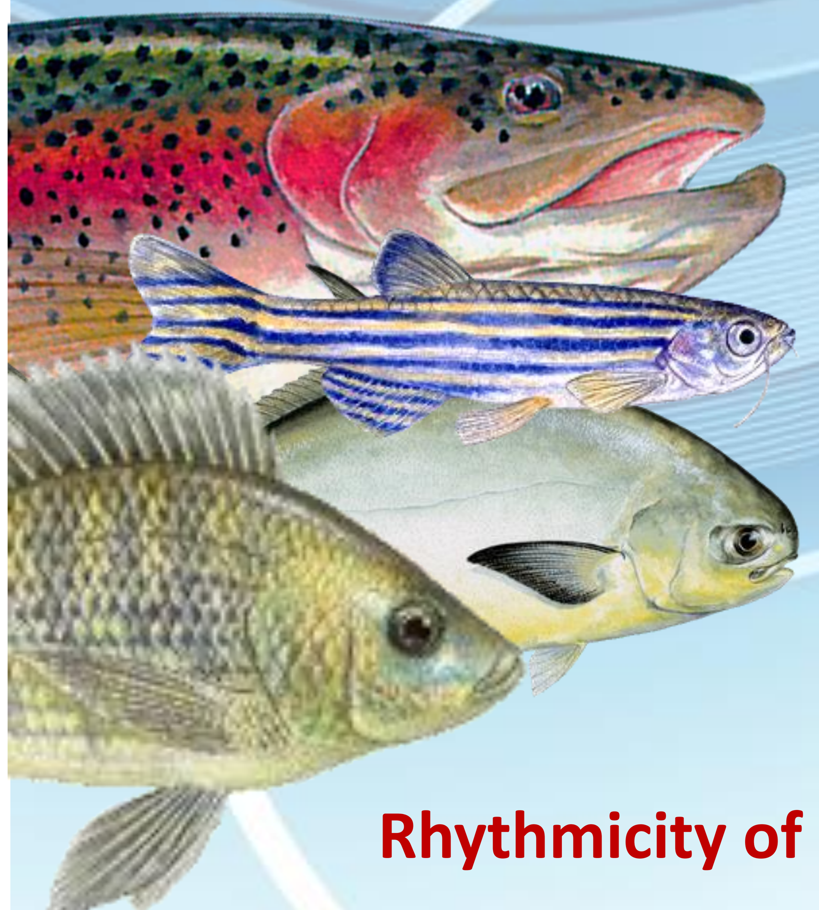


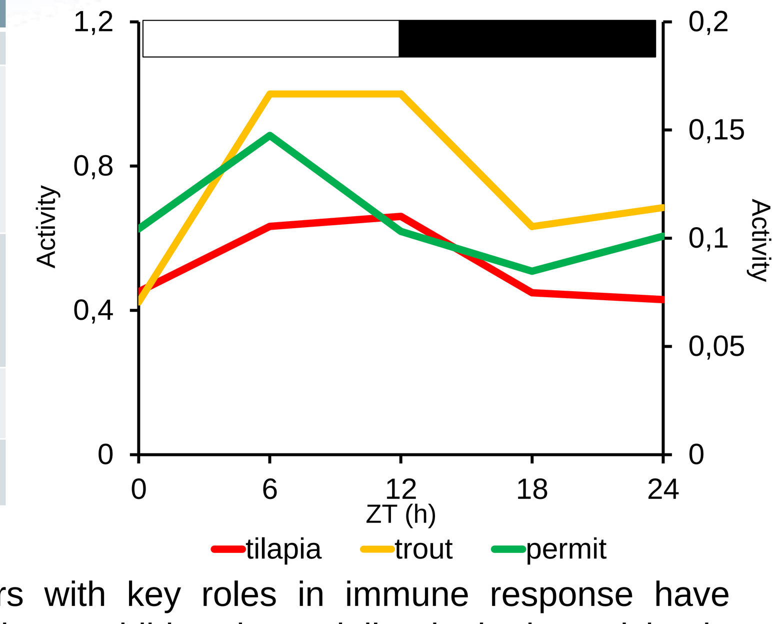
Circadian rhythms established by the endogenous clocks enable organisms to anticipate periodic and cyclic changes in their environment. This internal time-keeping system is highly conserved amongst animals and provides them with adaptive advantages as biological processes can be scheduled to occur at the most crucial times of the day. Circadian rhythm is emerging as an important regulator of immune functions. However, there is a paucity of information on the influence of this biological phenomenon in the immune mechanisms of teleost fish. A series of studies was conducted to investigate the circadian rhythms of systemic and mucosal innate immunity using model (*i.e.*, zebrafish, *Danio rerio*) and non-model (*i.e.*, permit, *Trachinotus falcatus*; rainbow trout, *Oncorhynchus mykiss;* tilapia, *Oreochromis niloticus*) fish species.

Rhythmicity of innate immune factors

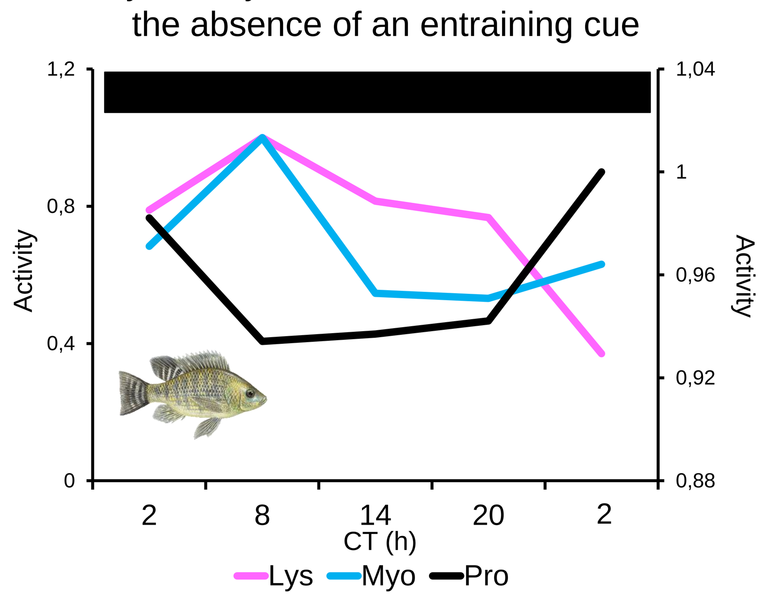
1. Identified immune defences with daily rhythmic activity

Species	Rhythmic defence factors		
	Serum	Skin mucus	Gut
Permit	globulin, myeloperoxidase, alkaline phosphatase, glutathione peroxidase	alkaline phosphatase, glutathione peroxidase	NA
Tilapia	alkaline phosphatase, lysozyme, myeloperoxidase, protease	None	NA
Trout	anti-protease, myeloperoxidase	NA	NA
Zebrafish	NA	NA	hepcidin, lysozyme

2. Myeloperoxidase – a defence factor with conserved rhythmicity in teleost fish?



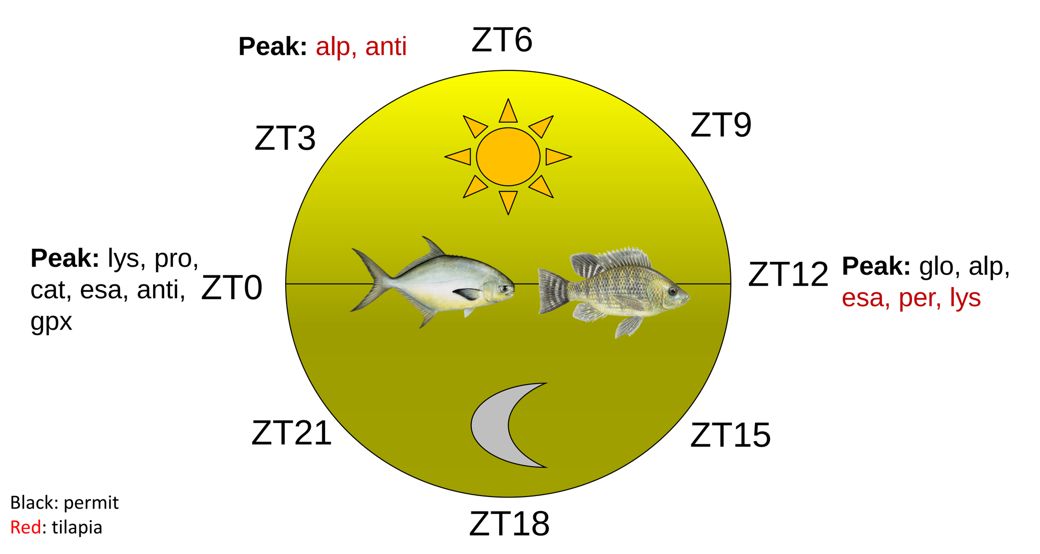
3. Rhythmicity of innate immune defences in the absence of an entraining cue



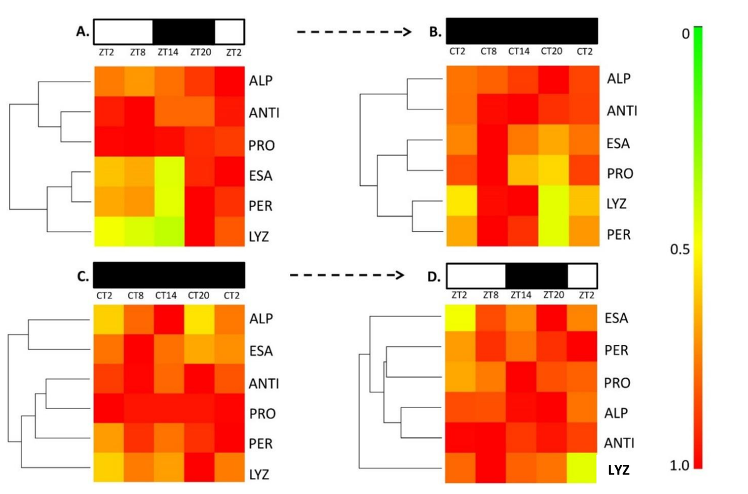
Defence factors with key roles in immune response have been identified to exhibit robust daily rhythmic activity in permit, tilapia, trout and zebrafish reared under 12L:12D (LD) photoperiod (**1**). Myeloperoxidase, a lysosomal protein with antimicrobial activity, displayed rhythmic activity in tilapia, trout and permit (**2**). This suggests that myeloperoxidase is likely a defence factor with conserved rhythmicity amongst teleost fish. Further, myeloperoxidase demonstrated a photopic profile as acrophases in all three fish were identified during the light phase. Lysozyme, myeloperoxidase and protease remained rhythmic under total darkness in the serum of tilapia (**3**). This indicates that the rhythmicity of these defence factors is endogenously driven because it can persist even without an external entraining signal, *i.e.* LD cycle.

Photic plasticity and temporal sensitivity of innate immunity

4. Evidence of circadian partioning of immune response



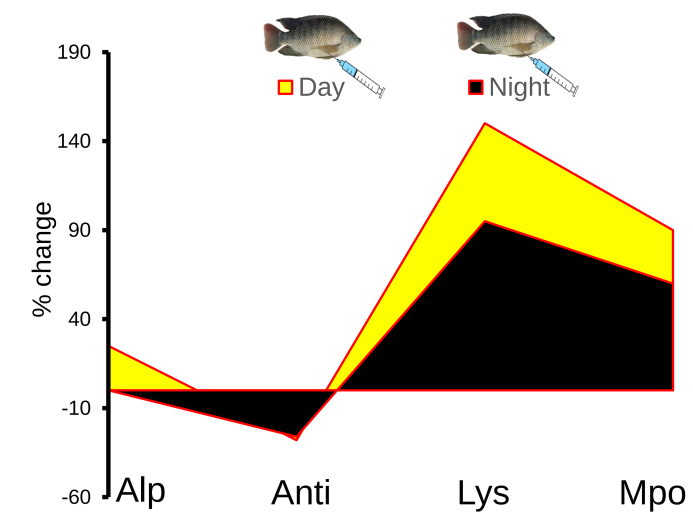
5. Immune defences adapt to varying photic conditions



ALP, alkaline phosphatase; ANTI, anti-protease; CAT, catalase; ESA, esterase; GLOB, globulin; GPX, glutathione peroxidase; LYS, lysozyme; MPO, myeloperoxidase, PRO, protease

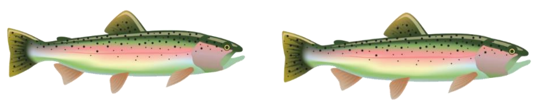
The concept of circadian partitioning is characterised by a state when the organism has enhanced immunological activity and a state when the responses are repairing and regenerating. This was observed in the daily immunological profiles of permit and tilapia, but only in serum (**4**). The humoral immune responses under various light conditions revealed that each photic environment had a distinct daily immunological profile in tilapia (**5**). The biphasic profile became less ostensible in light regimes other than LD suggesting that constant LD cycle is likely essential in establishing the fidelity of circadian partitioning. It was observed that modulation of humoral defence factors following endotoxin challenge markedly demonstrated temporal sensitivity in tilapia (**6**). Striking humoral immune responses were observed when bacterial endotoxin challenge was conducted during the light phase of the LD cycle.

6. Immune response to bacterial endotoxin is gated by the time of the day



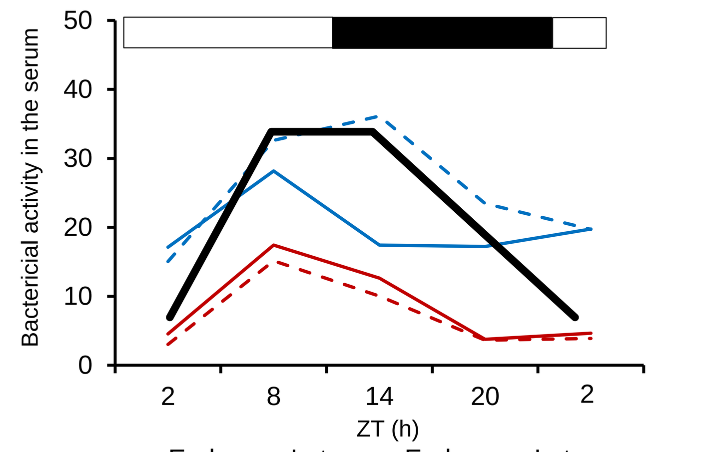
Interplay between rhythmic serum-mediated bacterial killing activity and oscillating defence factors

The emergence time of salmonid is thought to be related to their stress-coping style (SCS): individuals emerging early are more proactive while those emerging late are thought to exhibit a reactive SCS. Proactive SCS is believed to be more resistant to diseases, however, too little is known about the immunological robustness amongst emergence fractions, especially those that have already been subjected to domestication.



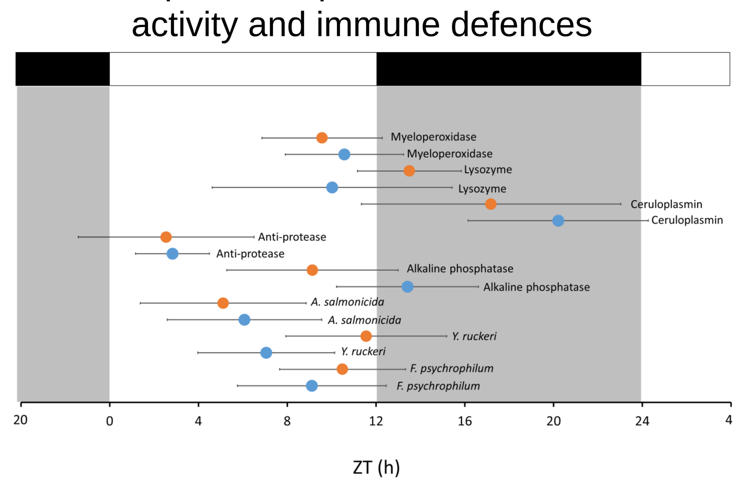
Serum-mediated bacterial killing activity against *Flavobacterium psychrophilum* and *Yersinia ruckeri* demonstrated daily rhythms in both emergence fractions of rainbow trout, where the peak of activity was identified during the light phase (**7**). Moreover, several serum defence factors manifested variations during the LD cycle, where anti-protease and myeloperoxidase activities exhibited significant daily oscillation. The two emergence fractions displayed nearly comparable immunological profiles. The peaks of activity of alkaline phosphatase, anti-protease, lysozyme and myeloperoxidase were identified during the light phase and corresponded with the period when serum bactericidal activity was also at its highest (**8**). The daily dynamics of bactericidal activity and immune defence factors displayed positive correlation, particularly between rhythmic myeloperoxidase and, *F. psychrophilum* and *Y. ruckeri*.

7. Daily dynamics of bacterial killing activity



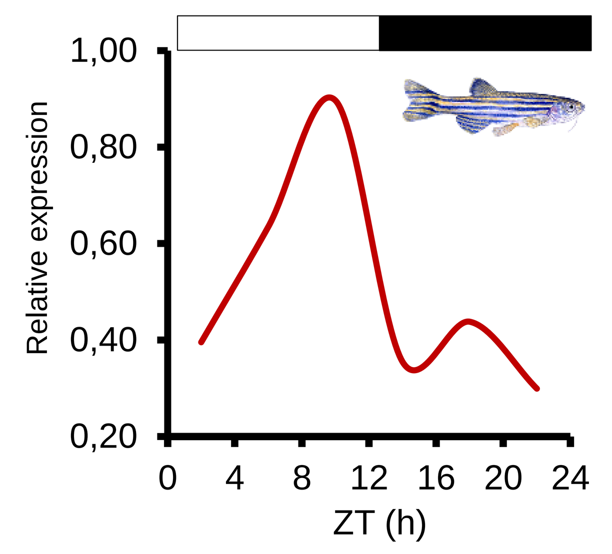
Red: against *F. psychrophilum*; Blue: against *Y. ruckeri*;
Black: myeloperoxidase

8. Acrophase map of serum bactericidal activity and immune defences

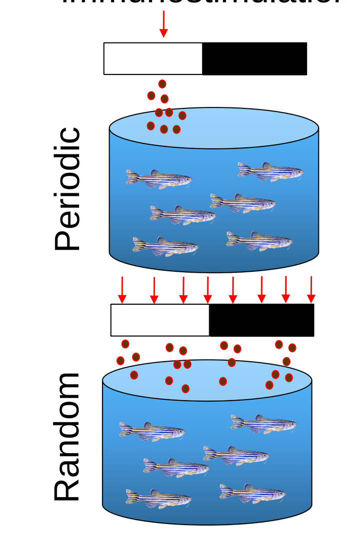


Chronoimmunonutrition: time-dependent nutritional modulation of gut mucosal immunity

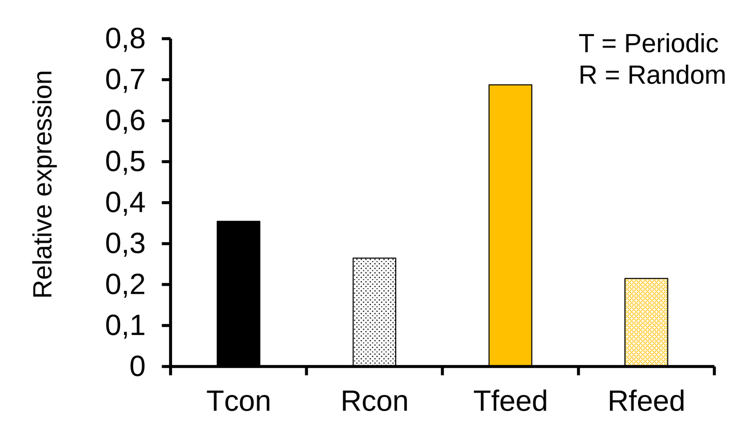
9. Lysozyme expression is rhythmic in the gut



10. Time-dependent dietary immunostimulation

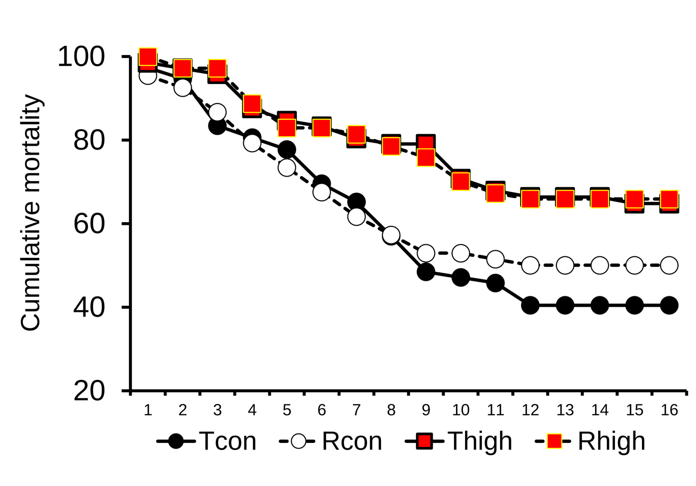


11. Lysozyme is responsive to time-dependent immunostimulation



Lysozyme is one of the genes with rhythmic activity in zebrafish gut (**9**). The observed rhythmicity of gut mucosal defences led us to hypothesise that the timing of immunostimulation has a significant contributory impact, hence, temporal mucosal immunomodulation through diets was performed (**10**). Feed with phytoimmunostimulant was delivered either periodically or randomly. Periodic delivery was scheduled when majority of the defence genes in the gut were at their peaks of activity. Lysozyme expression responded to time-dependent feeding (**11**). Groups that received the phytoimmunostimulant had improved disease resistance against *Streptococcus iniae*, regardless of the time of feed delivery (**12**).

12. Improved disease resistance



The results provide interesting insights into the crosstalk between immunity and circadian rhythms in fish. The temporal control in the array of defence molecules plays a key role in providing immunological robustness at different periods of the day and under different photic conditions. Lastly, the results may provide practical knowledge in studies about immunomodulation and pathogen challenge by taking into account the strong temporal regulation of fish innate immunity.

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