

Immunological study on age-dependent susceptibility to  
*Ochroconis* infection  
in marbled rockfish, *Sebastes marmoratus*

Abstract of Doctoral Thesis

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*Ochroconis* infection is a fungal disease caused by *O. humicola*. This disease has been reported in some marine fish species including marbled rockfish. Because these case reports suggest that *O. humicola* consistently infects juvenile fish, young age fish seem to be a target of the infection. The purpose of this thesis was to study the effect of age on the susceptibility and pathogenesis of the infectious disease, and to investigate the difference of immune response in marbled rockfish of different age categories.

Effect of age on susceptibility of marbled rockfish to *O. humicola* was examined by experimental challenge against juvenile fish of three age categories that showed different body length: small ( $29 \pm 2$  mm), medium ( $55 \pm 3$  mm), and large ( $74 \pm 6$  mm). The cumulative mortalities for small, medium and large fish were 100 %, 20 % and 0 %, respectively. These results demonstrated that the younger fish among juveniles were more sensitive to *O. humicola*.

For observation of inflammatory response in the different age categories, younger fish ( $52 \pm 1$  mm) and older fish ( $76 \pm 4$  mm) were intraperitoneally injected with *O. humicola*. Both fish group showed pathological symptoms in adipose tissues. In younger fish, infiltration of mononuclear leukocytes appeared at an early phase, increased the number of cells with time, and then these cells became necrotic at a late phase. The fungi spread the hyphae in the tissue with progress of pathological symptoms. In older fish, remarkable pathological symptoms were not observed, but granulomatous inflammatory response was found in some fish at a late phase. The fungal growth was limited and a few hyphae were present only in granulomatous inflammatory area. These results indicated that the quick and intense inflammation were characterized in the younger fish.

Cloning of immune relevant genes of adaptive cellular immunity that has been known to protect fungal infection was conducted for analysis of the immune activity in marbled rockfish. Four T cell marker genes and 2 cytokine genes could be identified based on the primary structure and phylogenetic analysis. The gene expression of CD4 and IFN $\gamma$  was upregulated in the spleen of younger fish ( $52 \pm 1$  mm) and older fish ( $77 \pm 2$  mm) after the intraperitoneal injection with *O. humicola*. The results showed a unique profile that the younger fish characterized quick and intense responses, but the older fish characterized slow and moderate response.

The quick and intense response of helper T-cell function (CD4 and IFN- $\gamma$  gene expression) in the younger fish probably initiates the inflammatory reaction in the adipose tissues after the infection. The immune response could be crucial for the recruitment for inflammatory cells to eliminate the fungal hyphae but this response in younger fish failed in elimination of the pathogen. The younger marbled rockfish could be characterized by operation of ineffective immune response which provokes intense inflammation resulting in tissue damage. It may be associated with the susceptibility of marbled rockfish against *O. humicola* infection.