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CLEANING BEHAVIOUR AND EXPRESSION PROFILES OF IMMUNE-RELATED GENES IN THE SKIN MUCUS OF BLUESTREAK CLEANER WRASSE, *Labroides dimidiatus,* IN RESPONSE TO ITS PARSITE-INFECTED CLIENT, THE ASIAN SEABASS, *Lates calcarifer*

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Thesis Submitted in Fulfillment of the Requirement for the Degree of Master of Science in the School of Fisheries Aquaculture Science Universiti Malaysia Terengganu

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DEDICATION

This thesis is lovingly dedicated to my family and friends. A special feeling of gratitude to my loving and respective parents, Alli Bin Hassan and Patemah Bte Narawi who had been my constant source of inspiration. They have given me the drive and discipline to tackle any task with enthusiasm and determination. Without their love, support and never ending pray for my success, this research would not have been made possible. Moreover for my beloved husband, Romi Hidayah Bin Zulbahri, I do really appreciate his support and encouragement that never breaks given to me all through my research. Instead, his tolerant between our marriage life and my study had finally brought to my success in completing my research in full career. Instead, my fully strength in completing my thesis come from my son, Rizqullah Al-Hidayah Bin Romi Hidayah and Safyyullah Al-Hidayah Bin Romi Hidayah. I am immensely glad that endowed such an understanding children, whereupon I can finish my thesis writing soon.

Abstract of thesis presented to Senate of Universiti Malaysia Terengganu in fulfillment of the requirement for degree of Master of Science.

CLEANING BEHAVIOUR AND EXPRESSION PROFILE OF IMMUNE RELATED GENE IN SKIN MUCUS OF BLUESTREAK CLEANER WRASSE, *Labroides dimidiatus,* IN RESPONSE TO ITS PARASITE INFECTED CLIENT, ASEAN SEABASS, *Lates calcarifer*

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February 2014

Chairperson : Associate Professor Dr. Yeong Yik Sung, Ph.D

Member : Professor Emeritus Dr. Faizah Mohd Shaharom, Ph.D
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School : Fisheries and Aquaculture Sciences

Fish disease is one of factor that contributes to mortality in fish farming. Biology treatments were recommended to control and treat fish disease instead of chemical treatments. Biology treatments that have potential are by using cleaner wrasse Bluestreak Cleaner Wrasse, *L. dimidiatus. L. dimidiatus* act by removed parasite from other fish that called client. *L. dimidiatus* reported to have mutualism relationship with its client. Uniquely, no reports on *L. dimidiatus* had infected with parasite from its client. So this research are to determine antagonistics behaviour (Pose, bite, twitch, flee,chase and swipe) between *L. dimidiatus* and its client Asian Seabass *Lates calcarifer*, to determine immune related gene expression profile (Alkaline phosphatase, Cathepsin B, Cathepsin D dan Cathepsin L) during cleaning activities and to relate both of this research finding. So, 2 *L. dimidiatus* were placed together with infected *L. calcarifer* in 2 feet aquarium for 48 hours (2 days) to determine antagonistic behaviour. Antagonistic behaviour recorded by using video camrecorder (Canon FS100) and data analyze using MANOVA method. Moreover, *L. dimidiatus* mucus was extracted at 0, 3, 12, 24, 36 and 48 hours after infection and immune related gene expression were analyzing using Real-Time PCR method.

From research finding, antagonistic behaviour was different at all time. Antagonistic behaviour not occurred during night time due to *L. dimidiatus* passive at night time. Antagonistic behaviour increase during Day 2 because both species getting more aggressive due to low abundance of parasite at client. Moreover, AP gene expression found to increase when *L. dimidiatus* in stress condition when chased by client. Immune related gene Cathepsin B increases at low environment temperature and during *L.dimiditaus* in starvation mode. AP and Cathepsin B immune related gene were increased in extruded slime that collected during night time. Difference with Cathepsin D immune related gene expression that higher in epidermal mucus which extracted during day time. Moreover Cathepsin L immune related gene expression in *L. dimidiatus* mucus was to minimum. This research finding showed that immune related gene expression in *L. dimidiatus* have a relationship with antagonistics behaviour between *L. dimidiatus* and *L. calcarifer.* Antagonistic behaviour can be manipulated to extract target immune related gene expression that can be used to other related study.

Abstrak tesis yang dikemukakan kepada Senat Universiti Malaysia Terengganu sebagai memenuhi keperluan untuk Ijazah Sarjana Sains.

TINGKAH LAKU PEMBERSIHAN DAN PROFIL PENGEKSPRESSAN GENE BERKAITAN KEIMUNAN PADA MUKUS KULIT BLUESTREAK CLEANER WRASSE, *Labroides dimidiatus,* YANG BERTINDAK BALAS TERHADAP KLIEN YANG DIJANGKITI PARASIT, IKAN SIAKAP ASIA, *Lates calcarifer*

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Penyakit ikan merupakan salah satu faktor yang menyumbang kepada kematian dalam penternakan ikan. Rawatan biologi disarankan utuk mengawal dan merawat penyakit ikan ini berbanding penggunaan rawatan kimia. Rawatan biologi yang berpotensi adalah penggunaan ikan pembersih Bluestreak Cleaner Wrasse, *L. dimidiatus. L. dimidiatus* membuang parasit pada ikan lain yang dikenali sebagai klien. *L. dimidiatus* dilaporkan mempunyai hubungan mutulisma dengan kliennya. Uniknya, tiada laporan *L. dimidiatus* dijangkiti oleh parasit daripada kliennya. Oleh itu, kajian ini dijalankan untuk mengkaji tingkah laku antagonistik (bergaya, menggigit, renggutan, melarikan diri, mengejar dan menyapu) di antara *L. dimidiatus* dan kliennya ikan Siakap Asia, *Lates calcarifer*, mengkaji profil pengekspresan gene berkaitan keimunan (Alkaline phosphatase, Cathepsin B, Cathepsin D dan Cathepsin L) semasa aktiviti pencucian dan seterusnya mengaitkan perhubungan kedua-dua hasil kajian. 2 ekor *L. dimidiatus* di tempatkan bersama seekor *L. calcarifer* yang telah dijangkiti selama 48 jam (2 hari) untuk mengkaji tingkah laku antagonistik. Tingkah laku antagonistik dirakam menggunakan video camrecorder (Canon

FS100) dan dianalisis menggunakan kaedah MANOVA. Manakala pula, mucus *L. dimidatus* diekstrak pada 0, 3, 12, 24, 36 dan 48 jam selepas *L. dimidiatus* di tempatkan bersama klientnya. Profil pengekpresan gene berkaitan keimunan diperolehi dengan kaedah Real-Time PCR Hasil kajian didapati, tingkah laku antagonistik menunjukkan ada perbezaan pada sepanjang masa. Tingkah laku antagonistik ini adalah penting untuk tingkah laku pencucian yang sempurna. Tingkah laku antagonistic tidak berlaku pada waktu malam. Di mana, *L. dimidiatus* ada pasif pada waktu malam. Tingkah laku antagonistic meningkat pada hari kedua kerana kedua-dua spesis menjadi semakin agresif kerana berkurangnya taburan parasit. Manakala pula, pengekspresan gene AP didapati meningkat apabila dalam keadaan tertekan akibat dikejar oleh kliennya. Tindak balas keimunan pengekspresan gene Cathepsin B pula meningkat pada suhu persekitaran yang rendah dan semasa *L. dimidatus* kelaparan. Pengekspresan gene AP dan Cathepsin B adalah meningkat dalam lendir tersemperit yang dihasilkan pada waktu malam. Berbeza dengan pengekspresan gene Cathepsin D yang tinggi dalam mukus epidermal yang dihasilkan pada waktu siang. Manakala pengekspresan gene Cathepsin L adalah sangat minimum dalam mucus *L. dimidiatus*. Hasil kajian ini menunjukkan bahawa tindak balas keimunan dalam *L. dimidiatus* berkait rapat dengan tingkah laku antagonistik di antara *L. dimidiatus* dan *L. calcarifer*. Tingkah laku antagonistic ini boleh dimanipulasikan untuk mendapat pengekpresan gene berkaitan keimunan yang dikehendaki untuk kegunaan kajian lain yang berkaitan.