

HELMINTHS PARASITES OF *Nemipterus peronii* (Val) AS
INDICATOR OF METAL BIOACCUMULATION IN
COASTAL WATERS OF THE SOUTH CHINA SEA

ROSHAN MAZHAR

DOCTOR OF PHILOSOPHY
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THE SOUTH CHINA SEA

ROSHAN MAZHAR

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Degree of Doctor of Philosophy in the Institute of Tropical Aquaculture
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HAK MILIK

REPRODUKSI TANPA IZIN DILARANG

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Dedicated

October 2015

Major Advisor : Prof. Emeritus Farah Nabeel Harrison, Ph.D.

Co-Advisor : Prof. Emeritus Nur Anwar Moud Shaikh, Ph.D.

To : Prof. Emeritus, Dr. Wang Lili, Ph.D.

Address : Institute of Coastal Science

The name of ALLAH

Who subjected to you the sea so that ships may sail upon it by His command and that you may seek of His bounty; and perhaps you will be grateful.

Al-jathiyah: 14 (the crouching)

Abstract of thesis presented to the Senate of Universiti Malaysia Terengganu
in fulfillment of the requirement for the degree of Doctor of Philosophy

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Main Supervisor : Prof. Emeritus Faizah Shaharom Harrison, Ph.D.

**Co-supervisors : Prof. Emeritus Noor Azhar Mohd Shazili, Ph.D.
Prof. Anuar Hassan, Ph.D., Dr. Wong Lilian, Ph.D.**

Institute : Institute of Tropical Aquaculture

In the past two decades there has been a continuous search for bioindicators of metal pollution including helminths, leading to steep increase in reporting studies of host parasite models challenged by heavy metal exposure. The role of parasites in evaluating environmental pollution has emerged with the recognition of their excellent ability to accumulate heavy metals at much higher levels than free-living organism.

This study designed on the hypothesis that helminths parasites of notched fin thread fin bream are accumulator of heavy metals in marine ecosystem. This thesis is the first attempt to investigate the capacities of four different opportunistic helminths parasites of *Nemipterus peronii* as accumulator of metal contamination in eastern coastal waters of the South China Sea. In order to evaluate the potential of helminths of metal accumulation four independent

specific models of helminths parasites - fish systems were established and collated their metal bioaccumulation capacities with that of host tissues inhabiting mid-continental shelf region of eastern coastal waters of the South China Sea. Prior to metal analysis a detail taxonomical study was conducted to validate the identity of collected helminths parasites of bream using both morphological and molecular approaches to appraise the paucity of taxonomical information of helminths of fish collected from east coast of Peninsular Malaysia. Molecular results of 18S sequences supported the morphological findings and confirmed the acanthocephalan species *Serrasentis sagittifer* and out of three nematodes, *Anisakis pegreffii* and *Hysterothylacium reliquens* were recorded for the first time from notched threadfin bream. The most dominant parasite species was the *Anisakis pegreffii* at all sampling sites. During this study a total of 1937 notched threadfin bream were collected seasonally from November 2012 to October 2013 from Besut, Pulau Kambing, Marang and Kuantan as selected sampling sites on eastern coastline facing South China Sea. The concentration of twelve elements, were analyzed in the liver, muscle, and intestine of the host fishes, as well as in their parasites using inductively coupled plasma mass spectrometry (ICP-MS). The results showed that all essential and nonessential element's concentrations (Cd, Cr, Mn, Pb, Cd, Fe, As, Ni, Zn, Al, Sr and Cu) in parasites were significantly higher than those in the fish's tissues. *Serrasentis sagittifer* was identified as the most useful biological indicator as it accumulated all selected heavy metals in higher concentration, showing the highest bioaccumulation concentration factors (BCFs) and was found 22 times higher for

As than the host muscles. Contrastingly *Paraphilometroides nemipteri*, *H. reliquens* and *A. pegreffi* showed highest BCF for Cd 10, 7.8 and 1.4 times higher than host's muscle respectively. Statistical analysis showed significantly higher concentration of all selected metals in liver, intestine and muscle of uninfected as compared to those of infected fish tissues. Cobalt displayed the largest number of strong correlation with Mn, Fe and Ni in different tissues of uninfected fish, which was found to be moderate or weak for infected fish. The present findings exhibited disparity between the accumulation capacities of parasitic nematodes and those of host tissues which might be linked to various factors like specification of microhabitats, thickness of cuticle, competition and parasite's physiology. The present findings revealed that bioavailability of specific metal which is directly linked with particular type of anthropogenic activities of ambient environment. The seasonal data showed concentrations of all selected metals were significantly higher in the helminths samples than in those in tissues of host. The metal concentrations in parasites displayed a visible seasonal templates throughout the year, while the concentrations in the fish tissues remained similar. During the northeast monsoon season concentrations of Cr, Cd, Cu, Pb, Ni, Mn, As and Fe were significantly higher in *S.sagittifer*, *H.reliquens*, and *P. nemipteri* than other seasons, while concentrations of Sr, Al and Zn recorded higher in southwest monsoon. Selenium was found in maximum level in all parasites in intermonsoon period. Furthermore, the effectiveness of all four helminths index of *N. peronii* were assessed to discriminate between polluted and non/less polluted sites located on the east coast. The analyzed data exhibited

Abstrak yang dikemukakan kepada Senat Universiti Malaysia Terengganu
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**PARASIT HELMIN *Nemipterus peronii* (Val) SEBAGAI PENUNJUK
BIOPENUMPUKAN LOGAM DI PERAIRAN PESISIR
LAUT CHINA SELATAN**

ROSHAN MAZHAR

Oktober 2015

Penyelia utama : Prof. Emeritus Faizah Shaharom Harrison, Ph.D

**Penyelia Bersama : Prof. Emeritus Noor Azhar Mohd Shazili, Ph.D.
Prof. Anuar Hassan, Ph.D., Dr. Wong Lilian, Ph.D.**

Institut : Institut Akuakultur Tropica

Sejak dua dekad yang lalu, terdapat pencarian yang berterusan bio-indikator pencemaranlogam termasuklah helminths, yang menjurus kepada peningkatan laporan kajian tentangancaman model perumah parasit terhadap pendedahan oleh logam berat. Peranan parasite dalam menilai pencemaran alam sekitar telah diketahui dengan kebolehannya untuk akumulasi logam berat pada kepekatan yang lebih tinggi daripada organisma yang hidup bebas.

Kajian ini dirancang berdasarkan hipotesis yang mana parasit helminths pada ikan kerisisirip bertakuk adalah penumpuk an logam berat dalam ekosistem marin. Tesis ini merupakan percubaan pertama untuk menyelidik keupayaan empat parasit helminths *Nemipterus peronii* yang berbeza sebagai pengakumulasi pencemaran logam di kawasanperairan Pantai Timur Laut China

Selatan. Untuk menilai potensi helminthes terhadap akumulasi logam, empat model spesifik bebas bagi sistem parasit helminthes-ikan telah diwujudkan dan dikumpulkan mengikut keupayaan bioakumulasi logam dalam tisu perumah yang menghuni kawasan pertengahan pelantar benua di perairan timur Laut China Selatan. Sebelum analisis logam dijalankan, satu kajian taksonomi yang menyeluruh dijalankan untuk mengesahkan identiti parasit helminths dalam ikan kerisi dengan menggunakan kedua-dua pendekatan morfologi dan molekul untuk mengatasi kekurangan pengetahuan taksonomi tentang helminthes dalam ikan yang didapati daripada Pantai Timur Semenanjung Malaysia. Keputusan molekul daripada 18 jujukan menyokong penemuan morfologi dan mengesahkan bahawa acanthocephala daripada spesies *Serrasentis sagittifer* dan daripada tiga nematode, *Anisakis pegreffii* dan *Hysterothylacium reliquens* direkodkan buat kali pertama daripada ikan kerisi sirip bertakuk. Spesies parasit yang paling dominan adalah *Anisakis pegreffii* yang dijumpai di semua lokasi pensampelan. Sepanjang kajian ini, sejumlah 1937 ekor ikan kerisi sirip bertakuk didapati sepanjang musim bermula November 2012 hingga Oktober 2013 dari Besut, Pulau Kambing, Marang dan Kuantan yang dipilih sebagai lokasi pensampelan di pesisiran Pantai Timur mengadap Laut China Selatan. Kepekatan dua belas unsur, dianalisa dalam hati, otot, dan usus ikan perumah dan juga parasit mereka dengan menggunakan Plasma Induktif yang dilengkapi oleh Spektrometer Jisim (ICP-MS). Keputusan yang didapati menunjukkan bahawa kepekatan unsur perlu dan tidak perlu (Cd, Cr, Mn, Pb, Cd, Fe, As, Ni, Zn, Al, Sr dan Cu) dalam parasit ternyata lebih tinggi daripada dalam tisu ikan. *Serrasentis sagittifer* terbukti

merupakan monitor biologi yang paling berguna disebabkan ianya berupaya akumulasi kesemua logam berat yang menunjukkan faktor kepekatan bioakumulasi (BCFs) yang tertinggi iaitu 22 kali lebih tinggi bagi unsur As berbanding tisu perumah. Secara perbandingannya, *Paraphilometroides nemipteri*, *H. reliquens* dan *A. pegreffi* menunjukkan BCF yang tertinggi bagi Cd, masing-masing 10, 7.8 dan 1.4 kali lebih tinggi berbanding otot perumah. Analisis statistik menunjukkan kepekatan bagi kesemua logam terpilih adalah ternyata lebih tinggi dalam hati, usus dan otot yang tidak dijangkiti berbanding dengan tisu ikan yang dijangkiti. Kobalt menunjukkan korelasi yang kuat dengan Mn, Fe dan Ni dalam tisu yang berbeza bagi ikan yang tidak terjangkit, yang mana ianya didapati peringkat sederhana atau lemah bagi ikan yang terjangkit. Penemuan terkini mempamerkan ketidakimbangan di antara keupayaan akumulasi nematod parasit dengan tisu perumah yang mana mungkin dihubungkan dengan pelbagai faktor seperti spesifikasi microhabitat, ketebalan kutikel, persaingan dan fisiologi parasit. Penemuan terkini mendedahkan bahawa bioketerdapatan bagi logam spesifik yang berhubungkait secara terus dengan jenis aktiviti antropogenik di persekitaran sekeliling. Data bermusim menunjukkan kepekatan bagi semua logam terpilih adalah ternyata lebih tinggi dalam sampel helminths berbanding dalam tisu perumah. Kepekatan logam dalam parasit mempamerkan templat bermusim yang ketara sepanjang tahun, sementara kepekatan dalam tisu ikan kekal sama. Sepanjang musim Monsun Timur Laut kepekatan Cr, Cd, Cu, Pb, Ni, Mn, As dan Fe ternyata lebih tinggi dalam *S. sagittifer*, *H. reliquens*, dan *P. nemipteri* memandangkan

kepekatan bagi Sr, Al dan Zn direkodkan lebih tinggi pada Monsun Barat Daya. Selenium dikesan pada tahap maksimum dalam semua parasit pada jangkamasa antara monsun. Tambahan pula indeks keberkesanan bagi kesemua empat helminths *N.peronii* telah dinilai untuk mendiskriminasi antara tapak yang tercemar dan tidak/kurang tercemar yang terletak di pantai timur, data yang dianalisa menunjukkan kepekatan tinggi yang konsisten bagi kebanyakan logam berat terpilih dalam sampel helminths yang didapati dari Besut berbanding lokasi lain sementara yang didapati dari Marang merekodkan kepekatan logam yang paling sedikit.