BIOLOGICAL STUDIES ON PARASITIC ISOPOD, *Caecognathia coralliophila* (GNATHIIDAE) INFESTING CULTURED MARINE FISH

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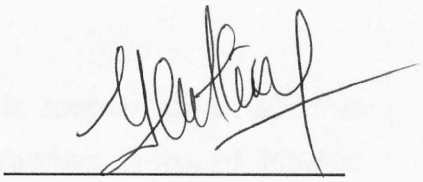

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ABSTRACT

The distribution and impacts of many parasitic isopods to natural and culture fish species have been extensively studied in many parts of the world. Nevertheless, such information is lacking in Malaysia especially the parasitic crustacean isopod from the family Gnathiidae. Recently, an infestation of the parasite occurred in one of the hatcheries in Sabah. It affected the broodstock of tiger grouper, *Epinephelus fuscoguttatus*; Napoleon wrasse, *Cheilinus undulatus* and Asian seabass, *Lates calcarifer*. Such infestation has been blamed for the series in spawning failures of the fish broodstocks in the hatchery. The parasites were collected from the broodstock tanks in that particular hatchery and subjected to a thorough systematic morphological examination by the use of light and electron microscopes. The result confirmed that the isopod parasite was belonging to the member of *Caecognathia coralliophila* (Gnathiidae). In addition to species identification, this study was also able to significantly contribute the description of new morphological features of the adult gnathiid which were never described previously, even in the holotype specimen. Investigation on its life cycle of the gnathiid was also conducted with the aim to determine which stage of the gnathiid is parasitic. It was noted that the *C. coralliophila* undergone three main parasitic larval stages which are zuphea 1 and praniza 1; zuphea 2 and praniza 2; zuphea 3 and praniza 3 before finally molted into male and female adult. All stages of zuphea were noted dependent to fish blood for survival and molting while the praniza stages were dormant. *In vitro* experiment was conducted to determine the sensitivity of the parasitic larvae to chemicals that are approved and widely used in aquaculture which included hydrogen peroxide, formalin, copper sulphate and trichlorfon. The larvae were exposed to different concentrations of each chemical for 10, 20, 30, 60 min and 24 h. The result showed that trichlorfon of 0.2 ppm concentration was able to eliminate all parasites within 24h exposure. Following the result, an *in vivo* sensitivity test of the organophosphate at concentrations 0.2 ppm and 3.2 ppm to host fish, tiger grouper (*Epinephelus fuscoguttatus*) was conducted at 24h and 60 min, respectively. It was noted that all fish survived through the trichlorfon challenge. This shows that trichlorfon can be used to treat gnathiid infestation from the member of *C. coralliophila* in marine fish aquaculture. However, one cannot rely much on the use of chemical such as trichlorfon to treat parasitic isopod infestation as it might bring other negative impacts that yet to known to the fish, consumer and environment. Hence, further studies on alternative prevention and treatment of gnathiid infestation in marine aquaculture facilities which are environmental-friendly, and harmless to fish and consumer are the way forward in the fish health and diseases management program for marine aquaculture in Malaysia.

ABSTRAK

KAJIAN BIOLOGI PARASIT ISOPOD, CAECOGNATHIA CORALLIOPHILA (GNATHIIDAE) YANG MENYERANG IKAN TERNAKAN MARIN

Pengagihan dan impak isopods parasit kepada spesies ikan liar dan ternakan telah banyak dikaji secara meluas di banyak tempat di dunia. Walau bagaimanapun, di Malaysia maklumat tersebut amatlah kurang terutamanya maklumat tentang parasit krustasea isopod dari famili Gnathiidae itu. Baru-baru ini, satu jangkitan parasit isopod telah berlaku di salah satu hatceri di Sabah. Ia telah memberi kesan kepada induk ikan kerapu harimau, Epinephelus fusoguttatus; ikan mameng, Cheilinus undulatus dan ikan siakap, Lates calcarifer. Jangkitan itu telah menyebabkan kegagalan induk-induk ikan di hatceri tersebut untuk membiak. Parasit dikumpulkan dari tangki induk di hatceri tersebut dan kajian untuk menentukan ciri-ciri morfologi telah dijalankan dengan sistematik dengan menggunakan mikroskop cahaya dan mikroskop elektron. Hasil kajian membuktikan bahawa parasit isopod adalah Caecognathia coralliophila (Gnathiidae). Selain daripada pembuktian spesis, kajian ini juga telah menyumbang kepada penyempurnaan penghuraian ciri-ciri baru terhadap gnathiid dewasa yang telah dihuraikan sebelum ini dalam spesimen holotype. Penyelidikan untuk menentukan kitar hidup parasite juga telah dijalankan untuk memastikan peringkat gnathiid yang merupakan parasit. Telah dikenalpasti bahawa C. Coralliophila akan malalui tiga peringkat parasit larva utama iaitu zuphea 1 and praniza 1; zuphea 2 and praniza 2; zuphea 3 dan praniza 3 sebelum perubahan terakhir menjadi jantan dan betina dewasa. Semua peringkat zuphea akan bergantung dengan darah ikan untuk hidup dan molting manakala praniza sentiasa dalam tahap yang tidak aktif. Eksperimen in vitro telah dijalankanuntuk menguji sensitiviti parasit terhadap bahan kimia yang termasuk formalin, kuprum sulfat, hydrogen peroxide dan trichlorfon. Larva didedahkan kepada kepekatan yang berbeza untuk setiap bahan kimia untuk masing-masing selama 10, 20, 30, 60 min dan 24 jam. Keputusan menunjukan trichlorfon pada kepekatan 0.2 ppm dapat membunuh semua parasit dalam jangka masa pendedahan selama 24 jam. Selepas keputusan ditentukan, ekperimen in vivo telah dijalankan terhadap perumah, ikan kerapu harimau, (Epinephelus fuscoguttatus) dengan menggunakan kepekatan 0.2 ppm dan 3.2 ppm dan masing-masing dijalankan selama 24 jam dan 60 minit. Semua ikan perumah dapat bertahan dalam sepanjang masa kajian. Ini telah membuktikan bahawa trichlorfon dapat digunakan untuk rawatan dalam akuakultur marin untuk membasmi infestasi C. Coralliophila. Walaubagaimanapun, kita tidak sepatutnya begantung dengan sepenuhnya kepada penggunaan bahan kimia seperti trichlorfon untuk merawat infestasi gnathiid kerana impak negatif terhadap ikan, pengguna dan alam sekitar yang masih tidak diketahui. Oleh sebab itu, kajian terhadap pencegahan alternatif dan rawatan alternatif terhadap infestasi gnathiid dalam fasiliti akuakultur marin pada masa hadapan merupakan salah satu hala tuju bagi pengurusan penyakit dan kesihatan ikan di Malaysia.

LIST OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

7 days duration. A: replicate 1 (0.2ppm); B: replicate 2 (0.2 ppm); D: replicate 1 (3.2 ppm); E: replicate 2 (3.2 ppm); C1: control replicate 1; C2: control replicate 2

LIST OF ABBREVIATIONS

FAO	Food and Agriculture Organization
FRI	Fisheries Research Institute
GDP	Gross Domestic Product
HDMS	Hexamethyldisilazane
RM	Ringgit Malaysia
SEAFDEC	Southeast Asian Fisheries Development Center
SEM	Scanning Electon Microscope
USA	United States of America

LIST OF SYMBOLS

%	Percentage
°	Degree
'	Minutes
"	Seconds
°C	Degree Celcius
$\bar{x}$	Mean
±	Plus minus
µm	Micrometer
cm	Centimeter
et al.	And others
g	Gram
L	Liter
m	Meter
min	Minute
mm	Milimeter
n	Number of population
ppm	Parts per million
SD	Standard Deviation
sp.	Species

LIST OF APPENDICES

CHAPTER 1

GENERAL INTRODUCTION

1.1 Aquaculture in Malaysia

Aquaculture is an industry of farming aquatic organisms. In Malaysia, this sector is undeniably important, as its contribution to the national economic growth is significant (Table 1.1). In 2013, fish production from the fisheries sector has contributed 1.1% or RM 7.91 billion to the nation Gross Domestic Product (GDP) (DoF, 2013).

Table 1.1: Fisheries production in Malaysia in the year 2007 to 2013

Year	Fisheries production (mill tonnes)	Value (RM mill)	Aquaculture Production (Tonnes)	Value (RM mill)	Seed Production (mill pieces)
2013	2,018.75	11,466.53	530,205.04	2,688.71	16,379.14
2012	2,111.66	11,440.31	634,376.32	2,758.10	22,176,96
2011	1,905.31	10,620.97	526,507.41	2,505.37	3,904.11
2010	2,014.53	9,495.28	581,048.41	2,798.74	1,853.86
2009	1,870.00	8,683.81	472,306.44	2,321.97	2,922.84
2008	1,753.31	7,406.29	354,427.55	1,740.05	1,482.61
2007	1,654.22	6,467.40	268,514.21	1,393.35	3,269.48

Source: (Department of Fisheries 2007, 2008, 2009, 2010, 2011, 2012, 2013)

Mat Diah *et al.* (2013) reported that the most important fresh water aquaculture species are Javanese carp, common carp, grass carp, big head carp, giant freshwater prawn, black tilapia, red tilapia, river carp, freshwater catfish, goby, eel, river catfish and giant snakehead. Whereas the main species cultured in brackish and marine water aquaculture are Asian seabass, mangrove red

snapper, milkfish, grouper, golden snapper, white shrimp, tiger prawn, mud crab, red tilapia, red snapper, cockles, mussels, oysters and seaweeds.

1.2 Challenges in Aquaculture Industry in Malaysia

Southeast Asian Fisheries Development Center (SEAFDEC) (2012) reported that many countries in the Southeast Asian region have been trying to integrate aquaculture into rural development to address poverty, food insecurity, nutritional deficiencies, insufficient livelihood alternatives, limited human skills and environmental degradation that drag economic growth and hinder improvement of societal welfare in the rural communities. Governments in the region are targeting on small-scale and subsistence-oriented farming as the means to improve the livelihood of the rural communities (Ahmed and Lorica, 2002).

Malaysia as one of the countries that produces a great number of aquaculture products is also facing challenges where weak legislation and enforcement have increased the burdens of farmers (Othman, 2008). Moreover, Othman (2008) has also pointed out many problems faced by farmers, which include low seed production, small-size farm and low investment capital. Furthermore, many of the farmers are lacking in aquaculture knowledge. Nevertheless, Malaysia is not alone in facing these problems (SEAFDEC, 2012; Ahmed and Lorica 2002). The increasing demand in the production from the aquaculture industry has brought many problems. Generally, aquaculture farms worldwide are facing disease problem which constrain the production and the expansion of the industry (Grisez and Tan, 2005).

1.2.1 Weak Legislation and Enforcement

Diseases are able to spread through the export and import of the aquaculture products (Perera *et al.*, 2008; Walker and Winton, 2010). The prevention of spread of the pathogens often limited by the poor management or enforcement of the local authorities to implement an effective biosecurity measure for the quarantine and/or a well regulated international trans-boundary movement of live aquatic animals (Yoshimizu, 1996).

In Malaysia, fisheries activities are governed under the Fisheries Act 317 (1985), but there is only one regulation which governs the marine culture system operating in the sea and lagoons, namely the Fisheries (Marine Culture System) Regulations (1990) (Mat Diah *et al.*, 2013). Malaysian Standard (MS 1998:2017) prescribes a generic code of practice for aquaculture farm operators to promote Good Aquaculture Practice (GAqP) for sustainable industry that is environmentally sound, socially acceptable and economically viable to ensure quality produce that is safe for human consumption and/or other utilisation.

1.2.2 Production scale

The activity of marine fish culture has existed for some time, yet the development is however, slow and less dominant in comparison to the shrimp and seaweed culture. The industry of marine finfish is still in the concept of traditional farming, in which the production comes from small to medium sized open floating net-cages (Othman, 2008).

Malaysian government has been very involved in the expansion of aquaculture industry. Many aquaculturists have been engaged in small projects by the encouragement of the government bodies to improve the livelihood. These projects are aimed to help poor fishermen to improve their livelihood and provide sufficient affordable protein source. Among the projects initiated are: bivalve molluscs culture in coastal areas, seaweed culture and tilapia farming in earthen ponds in the inland (FAO, Malaysia, 2016).

1.2.3 Aquaculture knowledge

Research and development is important for a sustainable aquaculture system, thus research institutes such as the Fisheries Research Institute (FRI), Department of Fisheries and local universities often carry out training programs focusing on the fundamental aspects of aquaculture which include gonadal maturation of fish, fish breeding techniques, fish seed production, nutrition, fish disease and production technology (FAO, Malaysia, 2016) every year. These are aimed to provide sufficient fundamental knowledge to the farmers.