



UNIVERSITI PUTRA MALAYSIA

***DISEASE AGENTS AFFECTING THE EGGS AND ADULTS OF
HORSESHOE CRAB, *Tachypleus gigas* (Müller, 1785) AND
Carcinoscorpius rotundicauda (Latreille, 1802)
IN LABORATORY CULTURE***

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**This project report is submitted in partial fulfillment of the requirements for
the degree of Bachelor of Agriculture (Aquaculture)**

**DEPARTMENT OF AQUACULTURE
FACULTY OF AGRICULTURE
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SERDANG, SELANGOR**

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CERTIFICATION OF APPROVAL
DEPARTMENT OF AQUACULTURE
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Carcinoscorpius rotundicauda (Latreille, 1802) in
Laboratory Culture

This is to certify that I have examined the final project report and all corrections have been made as recommended by the panel of examiners. This report complies with the recommend format stipulated in the AKU4999 project guidelines, Department of Aquaculture, Faculty of Agriculture, Universiti Putra Malaysia.

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ABSTRACT

This study was conducted to identify different disease agents affecting the development of eggs and adults horseshoe crabs and to determine the effect of aeration on the horseshoe crab eggs development. *Tachypleus gigas* and *Carcinoscorpius rotundicauda* eggs used in this study were incubated using a conventional culture system. Samples with signs of infection were examined under a compound microscope and photographed. Eggs inoculations were prepared and cultured on Sabouraud Dextrose salt Agar for fungal isolation and TrypticaseTM Soy Broth (TSB) for bacterial isolation. Identification of different disease agents were conducted based on the morphology of fresh and stained mounts as well as the biochemical characters of the isolated fungi and bacteria. Adults of *T. gigas* and *C. rotundicauda* were examined similarly. This study was also conducted to determine the effect of aeration on development of *T. gigas* eggs. Eggs incubations in water medium were carried out in two treatments; with and without aeration. The eggs of *T. gigas* were infected with *Aspergillus* sp. and *A. niger*, while for *C. rotundicauda*, the eggs were infected with fungi *Aspergillus niger*, *Penicillium* sp. and *Gliocladium* sp. The eggs of *T. gigas* were infected with bacterial *Corynebacterium* sp. and *Enterococcus faecalis*, while *C. rotundicauda* eggs were infected with *E. faecalis* and *Shewanella putrefaciens*. As for adult *C. rotundicauda*, *E. faecalis* and *Bacillus cereus* were isolated from the prosoma and *S. putrefaciens* was found in the book gills. A trematode worm, identified as *Planaria* sp., was found in all parts of the body of adult *C. rotundicauda*. Percentage of hatching was found to be significantly lower ($P < 0.05$) for *T. gigas* eggs incubated with aeration as compared with those without aeration.

ABSTRAK

Kajian ini dijalankan untuk mengenalpasti agen penyakit yang mempengaruhi perkembangan telur dan belangkas dewasa, dan untuk menentukan kesan pengudaraan ke atas perkembangan telur belangkas. Telur *Tachypleus gigas* dan *Carcinoscorpius rotundicauda* yang digunakan dalam kajian ini dieram menggunakan teknik sistem kultur konvensional. Perkembangan telur dipantau setiap hari. Sampel yang menunjukkan tanda jangkitan diperhatikan di bawah mikroskop kompaun dan diambil gambar. Telur yang telah dijangkiti ini kemudian dipindahkan ke Makmal Kesihatan Ikan, Jabatan Akuakultur, Fakulti Pertanian, UPM. Inokulasi telur disediakan dan dikultur dalam Agar Sabouraud Dextrose untuk pemencilan fungus dan TrypticaseTM Soy Broth untuk bacteria. Pengenalpastian agen penyakit dijalankan berdasarkan pemerhatian morfologi dan perwarnaannya sampel, dan dengan ciri biokimia fungus dan bacteria yang dipencilkan. Pemerhatian yang sama dijalankan ke atas *T. gigas* dan *C. rotundicauda* dewasa. Kajian turut dijalankan untuk menentukan kesan pengudaraan ke atas perkembangan telur *T. gigas*. Pengeraman telur dalam medium air dijalankan dengan dua rawatan, dengan dan tanpa pengudaraan. Berdasarkan pengenalpastian agen penyakit, terdapat empat fungus yang dikenalpasti. Telur *T. gigas* dijangkiti oleh *Aspergillus* sp. dan *A. niger*, sementara telur *C. rotundicauda* dijangkiti oleh *A. niger*, *Penicillium* sp. dan *Gliocladium* sp. Empat bacteria telah dipencilkan dari telur dan belangkas dewasa. Telur *T. gigas* dijangkiti oleh *Corynebacterium* sp. dan *Enterococcus faecalis*, sementara telur *C. rotundicauda* dijangkiti oleh *E. faecalis* dan *Shewanella putrefaciens*. Untuk *C. rotundicauda* dewasa, *E. faecalis* dan *Bacillus cereus* didapati pada bahagian prosoma dan *S. putrefaciens* pada buku insang. Satu trematod yang dikenalpasti sebagai *Planaria* sp. didapati pada semua bahagian badan *C. rotundicauda* dewasa. Peratus penetasan ketara rendah ($P < 0.05$) pada telur *T. gigas* yang dieram dengan pengudaraan berbanding dengan tanpa pengudaraan.

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LIST OF SYMBOLS

°C	Degree Celsius
ppm	part per million
ppt	part per thousand
mm	millimeter
ml	milliliter
mg/L	milligram/ litre
mg/kg	milligram/ kilogram
g	gram
min	minute
h	hour
sec	second
%	percent
™	trademark
TSA	Trypticase™ Soy Agar
TSB	Trypticase™ Soy Broth
DO	Dissolved oxygen

CHAPTER 1

INTRODUCTION

Horseshoe crab knows is a living fossil. Based from fossil record and fossil discoveries, horseshoe crabs already lived during late Ordovician Period about 445 million years ago (Rudkin and Young, 2009). Horseshoe crabs are not gregarious arthropods (Sekiguchi and Shuster, 2009) and takes about 9-12 years to mature (Shuster and Sekiguchi, 2003; Faizul *et al.*, 2011). Horseshoe crab is an animal having jointed body and limbs, belong to the phylum Arthropoda. Three major classes of Arthropods are Crustaceans (such as shrimps, lobsters and crabs), Insects (such as beetles and ants) and Arachnids (such as scorpions, spiders, ticks and mites) (Li, 2008). Class Merostomata of horseshoe crab means "legs attached to the mouth".

In the world, four remaining species of horseshoe crab are *Carcinoscorpius rotundicauda* (Latreille, 1802), *Tachypleus tridentatus* (Leach, 1819), *Tachypleus gigas* (Muller, 1785) and *Limulus polyphemus* (Linnaeus, 1758) (Sekiguchi, 1988; Pocock, 1902; Brusca and Brusca, 1990). All four species are classified as data deficient and near threatened (IUCN, 2010). Three species of horseshoe crab found in Southeast Asia are *C. rotundicauda*, *T. tridentatus* and *T. gigas*. The population of these three species of horseshoe crab is decreasing locally and regionally (Shakiba *et al.*, 2009) and diminishing in Japan (Itow, 1993; Botton, 2001), China and Taiwan (Chen *et al.*, 2004), Malaysia (Christianus and Saad,

2007) and Hong Kong (Morton, 1999; Chiu and Morton, 2003). The last species is *L. polyphemus* (American horseshoe crab) inhabit in the East Coast of North America (Sekiguchi, 1988). *Limulus polyphemus* also known as the “walking museum” because carries many mobile and sessile on its carapace (Tan *et al.*, 2011).

Horseshoe crabs are known to have high fecundity but high larval and eggs mortalities (Loveland *et al.*, 1996), prefer inhabit in shallow marine water and intertidal areas (Sekiguchi and Shuster, 2009). The average size of male is always smaller than female (Chatterji *et al.*, 1994) and horseshoe crab must molt to grow. The color of live horseshoe crab is greenish grey (Yamasaki, 1988). *Tachypleus gigas* have triangle-tailed (Kanchanapongkul, 2008).

Until now, there are very few documented reports on the disease agents causing infection for horseshoe crabs. Disease agents can affect development and growth of eggs, larvae and juvenile of horseshoe crabs. These disease agents include fungi, viruses, bacteria and parasites. Those organisms are commonly found in natural environment such as water, soil, animals and plants and can be beneficial for life or may be dangerous. Some biological agents cannot be seen by naked eye and can reproduce very rapidly in short time. *Tachypleus gigas* larvae were more often found with fungus infection in conventional method as compared to recirculating aquaculture system (Faizul *et al.*, 2011).

Thus, the objectives of this study were:

1. Isolation and identification of different disease agents infecting the eggs and adults of horseshoe crabs.
2. Determining the effect of aeration on the horseshoe crab eggs development.



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