

**PEMELIHARAAN CACINGDARAH
(*Marphysa mossambica*)
DI DALAM KURUNGAN UNTUK PEMBANGUNAN
MAKANAN HIDUP BAGI UDANG HARIMAU,
*Penaeus monodon***

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SARJANA SAINS**

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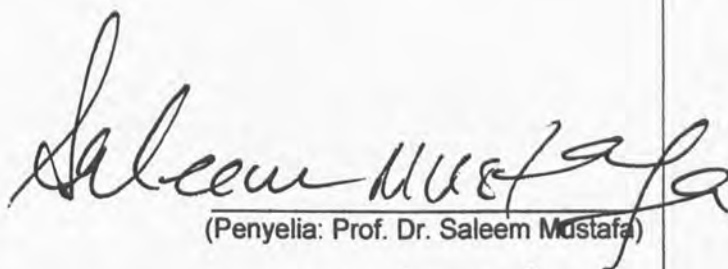


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DECLARATION

I hereby declare that this thesis contains my original research work. Sources of findings reviewed herein have been duly acknowledged.



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ABSTRACT

This study was undertaken to determine the feasibility of rearing the bloodworm, *Marphysa mossambica*, and to examine the factors that support their growth in captivity. The aim was to develop a sustainable culture system for supply of this bloodworm to shrimp farming industry as an important component of maturation diet. Marine worms are natural items of shrimp diet and a growing number of evidences points towards the role of many of their biochemical constituents, including highly unsaturated fatty acids, sterols and hormones in stimulating gonad development, improving egg quality and fertility in shrimp. In this study, samples of the bloodworm were collected from the wild at 3 sampling stations in a Sembulan coastal area in Kota Kinabalu. The salient morphological features and taxonomic characteristics of bloodworm were examined. The habitat of bloodworm was analyzed and quality of sediment was investigated. The growth parameters were estimated on a monthly basis. The experimental trials involved determination of the effect of different diets, salinities and indoor/outdoor thermal conditions on growth of the bloodworm. The trials on diets and salinities were conducted at normal room temperature (24°C – 30°C) for 4 weeks. The polychaetes were offered diets constituted of different raw materials: decomposed mangrove leaves, seaweed (*Sargassum* sp.), poultry waste and a mixture of poultry waste and decomposed mangrove leaves (50:50). The salinity experiment involved exposure of the worm to 30, 20, 15, 10 and 5 ppt. The effect of temperature was examined by exposing the test specimens to indoor (30 – 22°C) and outdoor (33 - 24°C) conditions. The contents of protein, lipid, cholesterol, water content and ash of the wild bloodworm were determined. A normal linear regression of the annual length-weight relationship was obtained and the relationship between length and weight was strong. Feeding trials yielded interesting results. Poultry waste mixed with decomposed mangrove leaves produced the best results, with a daily growth rate of 13.67 ± 2.42 mg/day compared to other treatments. Of all the salinity treatments, 10 ppt showed the best daily growth rate (18.29 ± 3.31 mg/day). The data suggested that the bloodworm, an omnivorous scavenger, thrives best when diet comprises protein/nitrogen originating from decomposition of organic matter. The salinity treatments revealed that this marine polychaete is euryhaline and can survive in the range of 5 – 20 ppt, but a brackish water environment where salinity is 10 ppt provides a better condition for growth. The indoor rearing was better for growth and survival, and the temperature at 30°C seemed to be optimum for growth and maturity. Diet, salinity and temperature are just three of the many factors that determine the success of captive rearing of the bloodworm. Quality of sediment, bacterial count, rate of water renewal and sediment reworking conditions are among the other factors that deserve serious consideration.



**PEMELIHARAAN CACINGDARAH (*Marphysa mossambica*) DI DALAM
KURUNGAN UNTUK PEMBANGUNAN MAKANAN HIDUP
BAGI UDANG HARIMAU (*Peneaus monodon*)**

ABSTRAK

Kajian ini telah dijalankan untuk menentukan kemungkinan untuk memelihara cacingdarah, *Marphysa mossambica*, dan mengkaji factor-faktor yang menyokong pertumbuhannya di dalam kurungan. Tujuannya adalah untuk membangunkan sistem penternakan cacingdarah yang mapan bagi membekalkan komponen diet pematangan yang penting kepada industri penternakan udang. Cacing laut adalah komponen semulajadi dalam pemakanan udang and pelbagai bukti yang menunjukkan peranan unsur-unsur biokimianya, termasuk asid lemak tak tepu yang tinggi, sterol dan hormon-hormon yang merangsang perkembangan gonad, memperbaiki kualiti telur and kesuburan udang. Dalam kajian ini, contoh cacingdarah di ambil dari 3 stesen di Kampung Air Sembulan, Kota Kinabalu. Ciri-ciri morfologi yang menonjol dan ciri-ciri taksonominya telah dikaji. Analisis terhadap habitat cacingdarah dan kualiti sedimen telah dijalankan dan penganggaran parameter pertumbuhannya dibuat secara bulanan. Eksperimen-eksperimen percubaan melibatkan penentuan kesan pemakanan, saliniti and keadaan suhu luar/dalam makmal yang berbeza ke atas pembesaran cacingdarah. Percubaan ke atas pemakanan dan saliniti dijalankan pada suhu bilik (24°C – 30°C) selama 4 minggu. Cacing ini diberi makanan daripada bahan mentah yang berbeza iaitu daun pokok bakau yang direputkan, rumpai laut (*Sargassum* sp), bahagian-bahagian ayam yang tidak digunakan dan campuran bahagian ayam dan daun pokok bakau yang direputkan. Eksperimen saliniti ke atas cacaiing darah melibatkan pendedahan cacing kepada 30, 20, 15, 10 dan 5 ppt. Kesan suhu dikaji dengan mendedahkan spesimen kajian kepada keadaan di dalam (30 – 22°C) dan di luar (33 - 24°C) bilik. Kandungan protien, lipid, kolesterol, kandungan air dan abu cacing darah liar telah dianalisa. Regresi linear yang normal bagi perkaitan panjang-berat dalam setahun telah diperolehi dan menunjukkan perkaitan kuat antara panjang dan berat bagi cacing ini. Percubaan dalam kajian pemakanan menghasilkan keputusan yang baik di mana campuran bahagian-bahaian ayam dan daun pokok bakau yang direputkan memberi hasil yang terbaik berbanding dengan rawatan-rawatan yang lain dengan kadar pertumbuhan harian 13.67 ± 2.42 mg/hari. Bagi rawatan saliniti, 10 ppt menunjukkan kadar pembesaran yang terbaik (18.29 ± 3.31 mg/hari). Data-data yang diperolehi mencadangkan bahawa cacing darah, pembangkai omnivor boleh hidup dengan baik apabila diberi diet yang mengandungi protien/nitrogen yang berasal daripada pereputan bahan organik. Rawatan saliniti mendapati bahawa polycheta marin ini boleh hidup dalam julat saliniti yang besar (5-20ppt), tetapi air payau di mana saliniti adalah 10 ppt memberi persekitaran yang lebih baik bagi pertumbuhannya. Penternakan di dalam makmal adalah lebih baik dari segi pertumbuhan dan kelangsungan hidupnya dan kelihatan pertumbuhan dan kematangan adalah optimum pada suhu 30°C. Diet, salinit dan suhu hanya merupakan tiga daripada banyak faktor lain yang menentukan kejayaan penternakan cacing darah. Kualiti sedimen, kiraan, kadar pembaharuan air dan penambahbaikan keadaan sedimen adalah antara faktor lain yang memerlukan pertimbangan yang serius.

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ABBREVIATIONS

%	percentage
cm	centimeter
m	meter
mg	milligram
g	gram
°C	degree Celcius
+	plus
ml	milliliter
µm	micrometer
ppt	part per thousand
mg/l	milligram per liter



CHAPTER 1

INTRODUCTION

1.1 Research Background

Aquaculture of tiger prawn (*Penaeus monodon*) is rapidly developing in response to increasing demand. A sustained growth of 12% per year (Olive, 1999b) of the prawn industry has been recorded in tropical Indo-Pacific region. The demand of 200,000 tonnes of prawn per annum exceeds the present capacity of hatchery production and therefore wild stocks remain under the pressure of exploitation (Olive, 1999b).

Tiger prawn aquaculture in Malaysia remained a small-scale traditional farming until early 1960s. Subsequently, major developments in research and increase in commercial interest led to the setting up of the first hatchery in Penang in 1975. The demand of prawn-seafood market received more interest because of higher margin of profit, biological attributes of the prawn and feasibility of culture in the country. In the year 2000, the prawn aquaculture productions increased to 14.3% from capture and culture fisheries. Sabah has the largest prawn farming area and contributed 13% of the total prawn production in 2000 (Subramaniam *et al.*, 2002).

The number of prawn hatcheries for research or commercial purposes is on the increase. Economically, the expansion has encouraged a number of associated businesses such as feed manufacturing, hatchery, storage, processing, export industry networks and contract farming involving large private enterprise in association with many small-scale prawn farmers.



The Malaysian government, universities and private agencies have been carrying out research to improve culture conditions for prawns. This is to ensure better quality and higher yield from production units to fulfil commercial demand. Nutritional management of prawn is a very important aspect of aquaculture. It is also very challenging especially in prawn farming industry where economic feasibility of feeding is a major factor in industrial level operation.

One of the major problems with captive breeding of penaeid is the inability of the diet to supply the essential nutrients that are required for rapid ovarian development. In a highly authoritative publication, Benzie (1997) has reported the outstanding performance of natural feed stuffs vis-s-vis artificial diets with respect to the spawning and larval production in prawn. Obviously, nutrition is a critical factor in managing the broodstock condition from the onset of maturation to spawning. Artificial feeding in prawn farming is linked not only to health and production but also to water quality and environmental impact. Unconsumed feed deteriorates water quality, decreases dissolved oxygen level, promotes microbial activity, and these factors collectively put stress on the captive stocks. Effluents from such farms pollute the environment where they are discharged. In this context, the role of live feed as the environment-friendly and highly nourishing diet occupies a place of prominence. The excellent performance of live feed in hatchery diet is beginning to be realized. It contributes to quality and quantity of production in aquaculture. Trials with marine polychaete (bloodworm) have demonstrated their significance in broodstock nourishment, fertility, egg quality and growth enhancement.

1.2 Polychaete Industry

The recent interest in polychaete as a fishing bait as well as livefeed provides the basis for large-scale production of these annelids. In order to sustain this pattern of economic growth in a competitive market place, a research programme designed to

increase the effectiveness of the production system is necessary. Enhancing hatchery output of prawns by the inclusion of fresh feed adds to the already high dependency of aquaculture on unsustainable resources. The programme involved investigations of a fundamental nature resulting in an interesting synergism between the knowledge requirements of developing polychaete aquaculture industry and continuing academic interests in the physiology and reproductive biology of the polychaete. Therefore, culture of bloodworm as a means of increasing the production of good quality diet for aquaculture will provide an excellent source of nutrients (Olive *et al.*, 2002).

The importance of marine baitworms has grown so enormously that these invertebrates now form the basis of a fishery. For many years, worms for commercial purposes were collected from natural population. The best known fishery of marine worms is found on the Eastern Seaboard of the USA on the coast of Maine where the marine baitworm is the fourth or fifth most valuable fishery, accounting 90% of the baitworm fisheries in the United States, and the value of landings reached US\$ 4 million annually (Cohen *et al.*, 2001). The collection of polychaete worms, however, is a form of fishing and, like any other fishery, there is a limit to the sustainable yield. In Western Europe, this fishery appeared to be stable and sustainable but in the last few years the natural supply of polychaetes has not been found to be sufficient to meet the market demand and, to a greater extent, has been perceived as a non-sustainable activity with potentially detrimental effects on the natural environment.

The demand of polychaetes in prawn industry has lead to emergence of commercial-scale bloodworm farming. Extensive tests and trials involving polychaetes are being carried out in many countries. University of Newcastle Upon Tyne has even developed a systematic research programme for the aquaculture of marine worms. The premium quality of marine worms are bred and grown for use in

aquaculture, angling and scientific research. The objective is to create a system of production that is commercially profitable and independent of natural population of broodstock and larvae. The use of polychaetes as bait in sea angling creates an economic demand for marine worms. Several families such as Arenicolidae, Eunicidae, Glyceridae, and Nephtyidae, have been exploited worldwide in sport and commercial fishing (Fidalgo e Costa, 1999). The most common species of marine polychaetes sold as fishing bait are *Nereis virens* (pileworm), *Glycera dibranchiata* (bloodworms) and *Arenicola marina* (lugworms). The value of European baitworm market is estimated to be about 200 millions ECU (Olive, 1999a). In Australia, captive breeding technique for bloodworm has been successfully developed and the commercial-scale supply to prawn aquaculture has started.

1.3 Live Feed for Prawn Culture

Some of the marine invertebrates are well known as natural sources of critically important nutrients for prawns in captivity. Broodstock are usually fed with natural seafood items such as squid, mussels, clams and polychaetes together with formulated pellet diets for successful maturation. Penaeids can consume polychaetes throughout all stages of their juvenile and adult life (Nunes *et al.*, 1997; Stoner and Zimmerman, 1998), but grazing rates are thought to progressively increase with size (Nunes and Parson, 2000). The potential use of polychaete as a dietary source of protein, lipid and amino acids for prawn aquaculture is important especially at a time when there is much interest in the uses of alternatives to fish meal and fish oils. Bloodworm is one such prey food item that contains several hitherto unidentified ingredients that promote maturation in penaeids. There are, however, some indications that polyunsaturated fatty acids (PUFAs) in the bloodworm might have a role in the maturation process. Investigations on chemically active substances that trigger maturation are therefore important.

The expansion of prawn aquaculture industry needs sufficient quantity of seed to support the farmers. The broodstock management including fertility and egg quality is vitally important. The quality of eggs is linked to viable larvae. Early research demonstrated the importance of fresh or fresh-frozen seafood such as polychaetes, squid and fish in the maturation diets of prawn. High quality natural feeds have been found to support maturation and breeding of prawns. PUFAs and possibly other hormonally active compounds in bloodworm may be bioactive products for maturation diets. The quality of bloodworm varies seasonally within the same area. Therefore, bioencapsulation is considered for increasing the consistency in the performance of diet and preventing fouling of the environment (Annita, 2003).

Bloodworm has been recognised as important prey item for several penaeid species (Boddeke, 1983; Nunes *et al.*, 1997). Their increasing demand is fast depleting natural stocks. A common species of bloodworm, *Marphysa mossambica*, is known to provide good nutrition for prawn maturation. This bloodworm lives in sand and mud in the subtidal and intertidal zones of coastal areas, especially along mangroves in many parts of Sabah. It is generally more abundant in the middle portion of tidal flats. Bloodworm abundance in a prawn grow-out farm reflects its carrying capacity for stocking. However, for a quantitative data on a farm's capacity for biomass yield, more studies are required for setting up guidelines for a semi-intensive culture system.

1.4 Objectives and Approach

The role of biochemical constituents in bloodworm in reproductive cycle of prawns and the biological incapacity of prawn to endogenously synthesize these chemical substances have motivated the interest in development of feed comprising bloodworm. Efforts to ensure the reproductive success of the prawn broodstock have focused almost entirely on the maturation of female spawners. Early investigations

(Annita *et al.*, 2004a; Annita *et al.*, 2004b) on egg quality of tiger prawn have shown that the bloodworm performs better than artificial diets. Nutrition is one of the critical factors in managing the broodstock condition from the maturation to spawning. Several findings have shown that the accumulation of certain nutrients in the ovary is critically important for egg quality.

With the emergence of bloodworm as a feed ingredient, scientific data are necessary to provide a basis for their large-scale farming to support the aquaculture industry. This study was undertaken to fill the vacuum in knowledge on biology and living conditions of the bloodworm. The objective of this research was to determine the optimal rearing conditions of the bloodworm, such as the different diet, salinity and temperature on survival and growth. In the end of this project I assumed that the data collected on these aspects of bloodworm, *Marphysa mossambica*, could be useful for its rearing in captivity. Hopefully, the data generated as a result of these studies will stimulate interest in developing economically viable culture of the bloodworm in Malaysia for supply of a high quality maturation diet to prawn.

CHAPTER 2

LITERATURE REVIEW

2.1 Introduction

Polychaetes are primarily marine, dioecious and multi-segmented annelids with parapodia and setae arranged in distinct bundles or fascicles and gonads exiting via simple ducts (Arnold and Birtles, 1989). They are a large and extremely diverse group. Most of them are 2 to 10 mm in diameter and less than 10 cm long (Rupert and Barnes, 1994) but some interstitial species could reach more than 3 meters (Rouse, 2000). About 13,000 polychaete species have been described but there could be as many as 25,000 to 30,000 species where the highest numbers of polychaete species were found in soft sediments (Hutchings and Fauchald, 2000).

Several systems of polychaete classification have been used during the twentieth century (Quatrefages, 1865; Fauvel, 1923, 1927, 1953; Uschakov, 1955; Day, 1967; Hutchings and Glasby, 2000). Scheme of classification suggested by Fauchald (1977) had defined 17 orders based on features of the anterior end. However, Pettibone (1982) proposed 25 orders but the approach was different in morphological structures than that used by Fauchald (1977). Despite the differences, these schemes of classification remain valid.

2.2 Morphology and Ecology

Polychaetas are coelomate and metamerically segmented. The coelom (body cavity) is lined by mesoderm. Metameric segments involve a serial repetition not only of muscle bundles, associated nerves and blood vessels in the body wall, but also



internal organs such as gonads or excretory organs (Arnold and Birtles, 1989). Generally, the polychaetes are perfectly segmented with identical cylindrical body segment, well developed sense organs and numerous setae. Most have well-developed, paired, paddle-like appendages called parapodia (Rupert and Barnes, 1994). Each parapodium has two finger-like gills where gases are exchanged through their body fluid. Polychaetes usually have a well-developed head, often complete with distinct eyes, antennae, and sensory palps.

The most distinguishing feature of polychaetes is the presence of parapodia, the paired, lateral appendages extending from body segments (Rupert and Barnes, 1994). These polychaetes crawl over surfaces using well-developed parapodia and setae. The most commonly seen polychaetes are the mobile forms that live amongst algae, under stones or crawl over the sediment. These worms are equipped with eyes and other sensory structures. Members of the Eunicida and Phyllodocida, the two groups in which jaws have evolved, use them to either seize live animals (carnivores), tear off pieces of algae (herbivores) or to grasp dead and decaying matter (scavenger) (Rouse, 2000).

Based on morphology and life habits, the polychaetes have been divided into the groups of sedentaria or errantia (free moving) (Ushakov 1955; Arnold and Birtles, 1989). The sedentary species usually live in stabilized burrows, galleries or tubes while some errant species are strictly pelagic, crawl beneath rocks, burrow actively in sand and mud, and some occupy stationary tubes. The errantia species are predatory and inhabit galleries of tunnels in soft sediments. Lying in wait at the bottom of a loop, the worm uses its four tiny antennae to detect the surface movements of its prey. It slowly moves to the opening of the burrow and then seizes the prey with its pharynx (Rupert and Barnes, 1994). Within the polychaeta there is

an enormous diversity of reproductive and developmental modes. At least a quarter of the polychaete families are known to have more than one mode of fertilisation and larval development (Wilson, 1991). Asexual reproduction is known in some polychaetes, but most species reproduce sexually, and a majority of species are dioecious. The gonads of polychaetes are usually distinct organs and they are generally found in the connective tissues associated with such structures as septa, blood vessels, and the lining of the coelom (Rupert and Barnes, 1994).

The marine polychaetes are diverse in their habits and niches. Most polychaetes burrow into the surface layers of a range of sediments from fine muds to coarse gravel or live in the tubes, in both intertidal and subtidal environments. Some species live in live coral, under a rock and inside shells of oysters and other bivalves. Some species occur in the more saline sections of estuaries and others tolerate the lower, fluctuating salinities. The nereidid lives in a range of salinity while *Manayunkia* sp. inhabits the hypersaline ephemeral lakes of Western Australia (Hutchings, 2000).

Polychaetes are an important component of benthic community. This group is rich in species diversity, and resilient enough to face adverse effects of pollution and natural perturbations (Tomassetti and Porrello, 2005). Polychaetes that occur at high densities throughout the initial stages of their cycle are most predominant benthic macrofauna. Some of these species (*Capitella* sp. and *Nereis* sp.) have been suggested as indicator of anthropogenic pollution of marine and brackish water environments (Nunes and Parson, 2000). The polychaetes that live mainly in the interstitial spaces in burrows or tubes, or move freely in the sediment layers continually modify the structure of the sediment by mixing, sorting and aggregating small particles into pellets and by pumping water into and out of the bed.

2.3 Taxonomy and Biology of Bloodworm

This species was described by Chris Glasby of the Natural Sciences Museum and Art Gallery of the Northern Territory, Darwin, Australia. Earlier, it has been described as *Eunice mossambica* and *Nauphanta mossambica* (Fauchald, 1987). The occurrence of *M. mossambica* has been reported from the Africa and Indo-Pacific regions. In South-East Asia, it has been reported from Singapore and Malaysia (Monro, 1931; Tan and Chou, 1993) but no information on the biology of this species has been published from this region. A recent classification of polychaetes by Rouse and Fauchald (1997) gave the variability in results from the different coding methods is used in this study (Table 2.1).

Table 2.1. Taxonomy of *M. mossambica*

Kingdom Animalia
Phylum Annelida
Class Polychaeta
Subclass Palpata
Order Aciculata
Suborder Eunicida
Family Eunicidae
Genus <i>Marphysa</i>
Species <i>Marphysa mossambica</i>
Common name Bloodworm
Local name Punpun

The name Eunicida refers to a group having a ventral eversible pharynx with a complex jaw apparatus consisting of ventral mandibles and dorsal maxillae. Eunicidans are found worldwide in the intertidal zone to great depths. The family Eunicidae is one of the oldest polychaete families and hence its early history is beset with nomenclatorial problems. The eunicids consist of up to 1500 segments and range from less than 10 mm to 6 m in length (Paxton, 2000). They may be free-living, tubicolous or burrowers in a wide range of habits including crevices and under rocks on rocky shores, in sand or mud and dead coral. Some species are referred to as bloodworms for their well-developed parapodial branchie that are often blood-red (Beesley *et al.*, 2000).

Generally, the eunicids have mixonephridia with a simple excretory canal and a large coelomostome that also functions as a genital funnel (Goodrich, 1945). Eunicids are dioecious and show no sexual dimorphism. According to Jamieson and Rouse (1989), the type of spermatozoa of *Marphysa* species are the ect-aquasperm, typical of broadcast spawners. Most reports of reproduction in the eunicidae comprise observations of swimming reproductive worms or eggs in gelatinous masses. The eggs are shed by rapture of the body wall (Rouse, 2000). A close relative, *Palolo viridis*, is known to spawn once a year and its spawning is linked with lunar phase.

The mechanism of fertilization in bloodworm is not known. However, most eunicids are schizogamous epitokes (Rouse, 2000). This mechanism arises by modification and separation from posterior end of the worm. This mechanism appear in adult of *P. viridis* which was the posterior part was modified and fills with gametes. At the spawning time, the worm leaves its burrow, and the posterior end (the epitokes) detaches and dies, while the anterior part regenerates (Fauchald, 1992). For several species of eunicids the planktonic atrochal larvae have been known to

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