IDENTIFICATION AND PREVENTION OF PARASITIC CRUTACEAN INFECTION ON MARINE CULTURED FISH: A CASE STUDY IN FISH HATCHERY OF UNIVERSITI MALAYSIA SABAH

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THIS DISSERTATION IS SUBMITTED IN FULFILLMENT OF THE REQUIREMENT TO GRADUATE AS A BACHELOR OF SCIENCE WITH HONOURS

AQUACULTURE PROGRAMME
FACULTY OF SCIENCE AND NATURAL RESOURCES
UNIVERSITI MALAYSIA SABAH

2014

PUKS 09:1

UNIVERSITI MALAYSIA SABAH

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JUDUL: Identification and prevention of parasitic crustacean infection on marine cultured fish: A case study in fish hatchery of universiti Malaysia Sabah.

IJAZAH: Sarjana Muda Sains dengan Kepujian

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I hereby declare that this is my own work and all information and sources reference have been used, they have been acknowledged.

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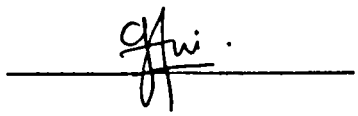

ACKNOWLEGEMENT

First of all, I would like to say thank you to the Director of BMRI, Prof. Dr. Saleem Mustafa because giving me a chance to conduct my final year project.

Next, my special appreciation goes to my final year project supervisor, Dr Faihanna Ching Abdullah for the continuous support and guidance in my project. I also would like to thank Prof. Shigeharu Senoo, who let me conducting my experiment in the fish hatchery.

I would like to thank all staff in fish and shrimp hatchery of Borneo Marine Research Institute for assisted and helping me throughout my experiment.

Finally, I would also like to thank my parent for they were always supporting and encouraging me for whole time.

ABSTRACT

The presence of unknown parasite has been detected in marine broodfish tank of fish hatchery, Universiti Malaysia Sabah. This study identified its species and elucidated the effective treatment to eliminate the parasite from culturing system. The unknown parasite was sampled and morphologically identified to the nearest genus and family and its life stage was recorded. Meanwhile, four different treatments including copper sulfate, treated freshwater, hydrogen peroxide and chlorinated tap water were tested on the parasite in order to identify the most effective treatment to eliminate them. The findings suggested that this unknown parasite was closely resembles to the *Gnathia africiana* because it has many morphology similarities with those described other author and at least 2 different life stages had been identified including the unfed larvae (zuphea) and fed larvae (praniza). This study also found that chlorinated tap water treatment was able to eliminate parasite from the culturing system with significantly in shorter time (6.95±0.47 min) compared to those observed in treated freshwater (14.43±0.81 min), hydrogen peroxide (18.88±1.08 min) and copper sulfate (49.93±19.51 min) respectively where for recovery process, finding found that in 60 and 1,440 minutes of tap water treatment, there had no recovered parasite after reintroduced with seawater. However, the infested fish can tolerate longer in freshwater (60.00±0.00 min) compared with other treatment. The findings from this study were helpful in the disease control management in aquaculture industry particularly on parasitic crustacean related diseases.

ABSTRAK

Kehadiran parasit tidak diketahui telah dikesan di dalam tangki induk ikan marin, Universiti Malaysia Sabah. Kajian ini mengenal pasti spesies dan difahami rawatan yang berkesan untuk menghapuskan mereka dari sistem pengkulturan. Parasit tidak diketahui telah disampel dan morfologi dikenal pasti genus terdekat dan keluarga dan peringkat hayatnya direkodkan. Sementara itu, empat rawatan yang berbeza termasuk sulfat tembaga, air tawar dirawat, hidrogen peroksida dan air paip berklorin telah diuji ke atas parasit untuk mengenal pasti rawatan yang paling berkesan untuk menghapuskan mereka. Anggapan dibuat di mana penemuan mendedahkan parasit yang tidak diketahui mirip dengan *Gnathia africiana* kerana ia mempunyai banyak persamaan morfologi dengan yang dinyatakan oleh penulis lain dan sekurang-kurangnya 2 peringkat hidup yang berbeza telah dikenal pasti termasuk larva belum makan (zuphea) dan larva makan (praniza). Kajian ini juga mendapati bahawa rawatan air paip berklorin berupaya untuk menghapuskan parasit dari sistem pengkulturan dengan ketara dalam masa yang lebih singkat (6.95±0.47 min) berbanding dengan mereka yang diperhatikan dalam air tawar dirawat (14.43±0.81 min), hydrogen peroksida (18.88±1.08 min) dan sulfat tembaga (49.93±19.51 min) masing-masing di mana untuk proses pemulihan mendapati bahawa dalam 60 dan 1,440 minit rawatan air paip klorin, tidak ada parasit pulih selepas parasit diperkenalkan semula dengan air laut. Walau bagaimanapun, ikan yang dipenuhi parasit boleh bertolak ansur di air tawar (60.00±0.00 min) berbanding dengan rawatan lain. Penemuan daripada kajian ini adalah membantu dalam pengurusan kawalan penyakit dalam industri akuakultur terutamanya mengenai penyakit krustasia berkaitan parasit.

LIST OF CONTENTS

CONTENT	PAGE
DECLARATION	i
VERIFICATION	ii
ACKNOWLEDGEMENT	iii
ABSTRACT	iv
ABSTRAK	v
LIST OF CONTENTS	vi
LIST OF FIGURES	viii
LIST OF TABLES	x
LIST OF SYMBOLS	xi
CHAPTER 1 INTRODUCTION	
1.1 General introduction	1
1.2 Problem statement	2
1.3 Approaches	2
1.4 Objectives	4
CHAPTER 2: LITERATURE REVIEW	
2.1 Background study of species	5
2.2 Morphology of the Gnathiidae species	6
2.3 Life Cycle of Gnathiidae species	7
2.4 The effects of parasite to host	8
2.5 Cleaner fish as a biological control of parasites	8
2.6 Chemical treatment for parasite	9
CHAPTER 3: METHODOLOGY	
3.1 Experimental Background	10
3.2 Experiment 1: Identification of parasite	10
3.3 Experiment 2: Determination of the effective treatments	
3.3.1 Treatment preparation	11
3.3.2 Experimental design	13
3.4 Experiment 3: Determination of the effective concentration	14
3.5 Experiment 4: Tolerance level of parasite	15

CONTENT		PAGE
3.6	Experiment 5: Fish tolerance toward the effective treatment	16
3.7	Statistical analysis	17
CHAPTER 4: RESULTS		
4.1	Species identification	18
4.2	Determination of the effective treatments	24
4.3	Determination of the effective concentration	25
4.4	Parasite tolerance with the effective treatments	26
4.5	Fish tolerance with the effective concentration of the effective treatment	28
CHAPTER 5: DISCUSSION		
5.1	*Gnathiaa africiana*	29
5.2	The effective treatment to kill parasites	31
5.3	The effective treatment to kill parasites on the body of infected fish	
	5.3.1 *In-vitro* treatments	33
	5.3.2 *In-vivo* treatments	33
5.4	Behavior of fish	35
CHAPTER 6: CONCLUSION & RECOMMENDATION		36
REFERENCES		37
APPENDIX A, B, C, D		41

LIST OF FIGURES

Figure number		Page
Figure 2.1	The morphology of the Gnathiidae species	5
Figure 2.2	Life cycle that showing the periods of each of the development stage	7
Figure 3.1	20 tails of parasite were inserted into the petri dish	13
Figure 3.2	The overall diagram for the treatment	13
Figure 3.3	Parasites were inserted with different concentration of treatment solution	14
Figure 3.4	The experimental design after treatment and immerse back in the seawater	15
Figure 3.5	The treatment for infected fish with a video record	16
Figure 4.1	Dorsal view of praniza. The scale bar is 10 µm.	18
Figure 4.2	Morphology of the parasite. a Segmented body, b cephalon, c pereopod, d pleon. The scale bar is 10 µm.	19
Figure 4.3	Morphology of the parasite. e Antennae, f frontal border, g pleopods, h dorsal view of praniza pleon. The scale bar is 20 µm	20
Figure 4.4	The exposure time to decease parasite in different types of treatment. The graph bar where superscript letters indicates significantly different (p>0.05) and horizontal bar indicates standard deviation.	24
Figure 4.5	The exposure time to decease parasite in different concentrations of hydrogen peroxide treatments. The graph bar with superscript letter indicates significant different (p>0.05) and horizontal bar indicates standard deviation.	25
Figure 4.6	The number of parasite (individual) deceased and recovered in treatments A; tap water, B; freshwater, C; hydrogen peroxide (800 ppm) with different exposure time.	27

Figure number		Page
Figure 4.7	The exposure time of triplicate hybrid grouper fish in different treatments. The graph bar with superscript letter indicates significantly different (p<0.05) and horizontal bar indicates standard deviation	28
Figure 5.1	Fish breathing heavily and imbalance condition before lay down on the bottom	35

LIST OF TABLES

Table number		Page
Table 3.1	List of the concentration of each treatment	11
Table 4.1	Comparison between current species with the documented species	20

LIST OF SYMBOLS

%	Percentage
°C	Degree Celsius
cm	Centimetre
mm	Millimeter
um	Micrometer
Kg	Kilogram
mg/L	Miligram per litre
mL	Millililitre
pH	Potential of hydrogen

CHAPTER 1

INTRODUCTION

1.1 General Introduction

Parasite is an organism that exploits other organism for their nourishment destroying the host and habitats (Schulz et al., 2011). Some of the parasite can be found in the internal part or known as endoparasite and it can be found in internal tract or liver, while for the ectoparasite, it can easily found on the external area of the host such as fin, nose, and eye. Examples of the ectoparasite are lice and ticks, while for the endoparasite are roundworms, tapeworms, flukes and many others protozoans (Heckman, 2003).

Ectoparasite has different invasion strategies to infect the host such as infect the unbroken surface of the skin, invade the mouth by attached to buccal cavity and epithelial cells, also can invade the eye area. The parasite takes chances to get into the host body in order to complete the life cycle by changing the morphological and physiological that only occur when they suck blood from host or feed on the host and it will allow them to grow into the next stage of their life cycle (Hoeg *et al.*, 2005). There are two types of life cycle, direct and indirect. The differences between the life cycles are the presence of intermediate host where only indirect cycle required an intermediate host (Price, 1980). The higher rate of parasite infestation can give harm to fish and affects the host fitness in many ways (Thomas *et al.*, 1995). Therefore, it will effect fish production especially the fingerling production (Hosain *et al.*, 2008) because the present of parasite may induce the mortality to the infested fish.

1.1 Problem Statement

Recently, the existence of the ectoparasite in the culture system in UMS hatchery has been detected when fish continuously displaying unusual swimming behavior and also reduction in feed intake among the fish inside the culture systems. Through observation process, there are many unknown tiny organisms that seem to attach on the fish body and the parasite would form aggregation that grow into hairy like structure that would cause uncomfortable condition on the fish. Eventually, the fish will rub their body on the hard surface such as tank wall or net, and this is obviously it will produce lesion that may exposed to the second infection such as pathogen and bacteria and fish become sick and subsequent die.

The presence of these unknown parasites in UMS hatchery broodstock is certainly detrimental as more than 10 important species and high value marine broodstock are kept in this system and now extremely vulnerable to disease outbreak caused by parasite. Furthermore, the presence of these unknown parasites perhaps is one of the reasons why natural spawning of several important marine fish spawned is inconsistence for the past few years. In that case, it becomes the constraint in long term aquaculture production that caused loss of economic returns (Kirkim *et al.*, 2008) and also loss of protein source for human.

1.2 Approaches

Based on previous observation done in this study, the parasite is most probably an isopod crustacean that belongs to Gnathia family based on their morphological characteristics. It also an ectoparasite, but the information about this unknown parasite is insufficient, whereby the mitigation to overcome the infestation caused by this unknown parasite has not been reported. In common aquaculture practice, various types of chemical known to potentially eradicated parasites. However, improper handling and administration of these chemicals to fish often cause serious damage to fish. Apart from chemicals, freshwater has been used to kill parasite too but its efficiency is not well documented. This study was conducted to determine the suitable treatment, either chemicals (copper sulfate and hydrogen peroxide) or conventional (freshwater and chlorinated tap water) that can be use to eradicate the

parasite. The justification of choosing each of these treatments were explained as follow:

CHEMICAL TREATMENT

1. Copper sulfate (Yanong, 2004)
    - It is the commercialized chemical and many previous study already success for ectoparasites
    - The suitable chemical for the closed culture system within the concentration is 5 to 15 ppm.
    - Teleost fish can tolerate with the chemical

2. Hydrogen peroxide (Robert *et al.*, 2000)
    - Active against ectoparasites
    - Easy to dispose and environmental friendly

CONVENTIONAL TREATMENT

3. Chlorinated tap water (Unpublished) and freshwater (Pettersen *et al.*, 2006)
    - Local farmer use to treat bacteria and parasite infestation
    - Easy to get and cheaper

1.3 OBJECTIVES

Following are the objectives outlined for the study

1. To determine the species of parasite
2. To determine the effective treatment with effective concentration for parasite infestation in culture system of UMS hatchery.
3. To study the parasite and fish tolerance toward the effective treatment with effective concentration.

CHAPTER 2

LITERATURE REVIEW

2.1 Background study

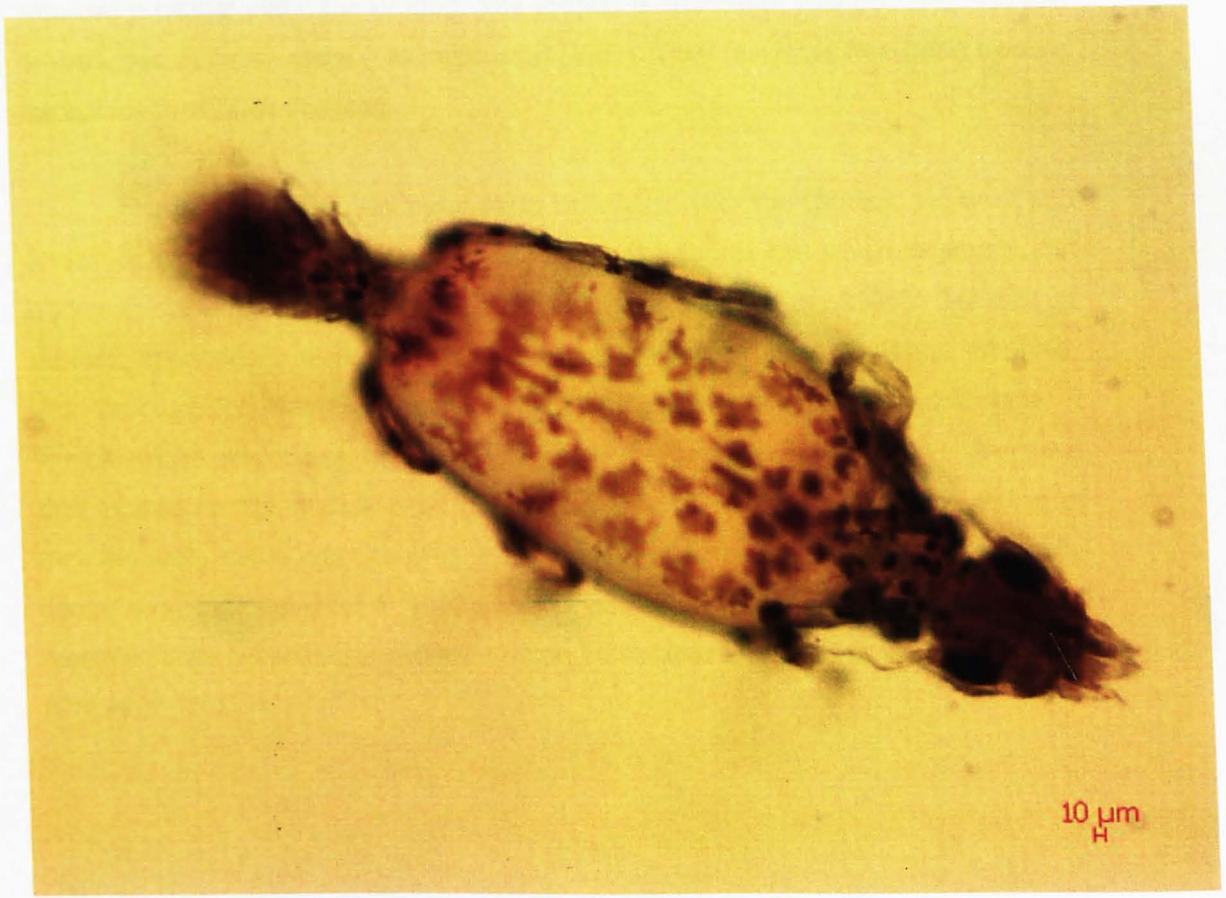

Figure 2.1 The morphology of the Gnathiidae species

Parasitic isopod is a common crustacean ectoparasite among tropical marine fish. They can live in wide range of depth such from littoral zone into the deep sea in major parts of the world ocean from polar to tropical waters. They rarely found in the freshwater and less in cold marine. There are many types of isopod parasites, and it refers to all legs being similar in size and shape (Ravinchandran *et al.,* 2010). There are two main groups of isopod with parasitic, Flabellifera and Gnathiidae. In this study, the main focus only for Gnathiidae species.

2.1 Morphology of Gnathiidae species

The gnathiidae isopods differ in morphology when compared to others isopod. They had a flattened body with their head has five thoracic and six abdominal segments. It also has a caudal plate and uropod. The uropod is short with rounded and also has smooth and plumose setae (Gianneto *et al.*,2003). They resemble free-living isopods except for their hook-like legs.

The parasite body has three parts that fused with the thoracic segment and known as cephalothorax, thorax and abdomen. The thorax has seven segments and abdomen six where it often fused into two to five (Smit *et al.,* 1999). One pair of thoracic appendages modified into mouthparts, and only last pairs is not modified. The structure for larvae and adult stage is different where for the adult male, it exhibit sexual dimorphism and have well developed mandibles or horn like structures that located on top of their head (Ota *et al.,* 2012) and together with expanding of their digestive caeca and billowing in the peroen and also their testes which in white dorsal stipe also developed (McKiernan *et al.,* 2005). While for females, they just resemble their juvenile stage with swollen pereonites that used to brood their eggs (Ota *et al.*, 2012)

2.2 Life Cycle of Gnathiidae species

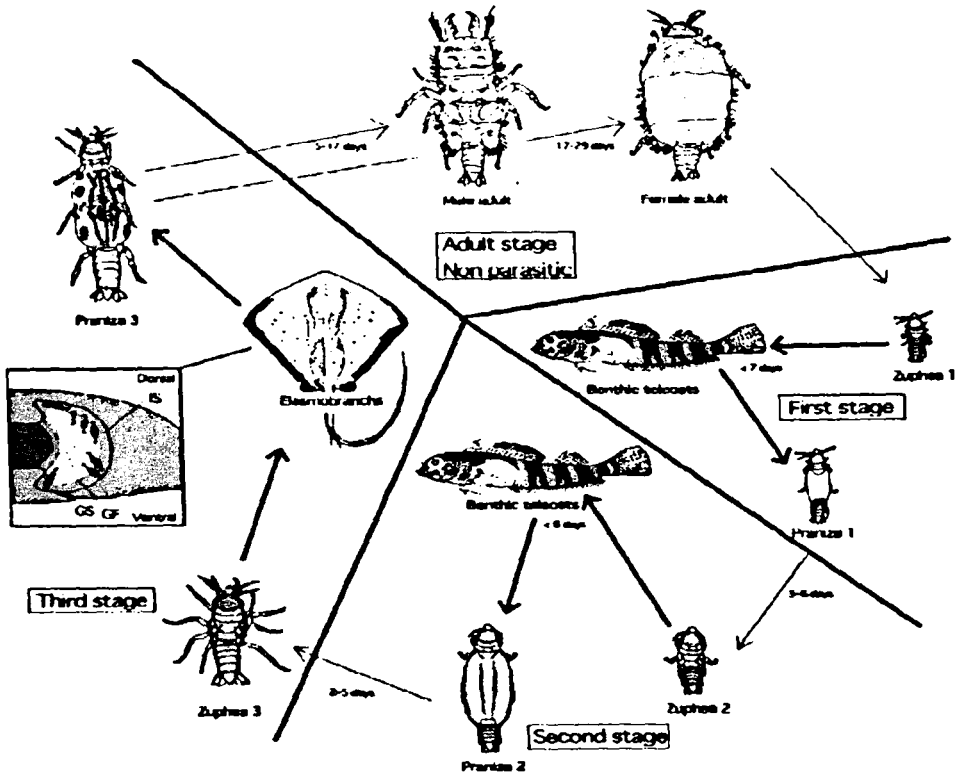

Figure 2.2 Life cycle that showing the periods of each developmental stage

Figure 2.2 is the complete life cycle of the one of Gnathiidae species, *Gnathia trimaculata*. There are four stages that include three non-adult adult stages and one adult stage. The gnathiidae is known as temporary parasite because they are parasitic during juvenile stages, and become non-parasitic when turn to adult stage (Heckmann, 2003). The non-adult stage is consists of zuphea or unfed phase and praniza or fed phase (Ota *et al.*, 2012). In order to become the praniza stage, the zuphea stage larvae suck blood from their host until their body becomes swollen and leave their host to rest in the sea bottom for several days to digest their meals before they molt to the next stage. This cycle is repeated until they become adult. When they turned to mature adult, they will just rest on the sea bottom (Ota *et al.*, 2012) or inside their habitat such as dead barnacle, coral and sponges. During this stage, the adult will not feed on blood or fish tissue, thus they do not undergo molt process (Ota *et al.*, 2012). They will reproduce immobile larvae without feed on blood or fluid for continuing their cycle and the complete life cycle will take around two months. The distribution of parasite on host body is related with their parasitic

stage. It can be found on dorsum, on dorsal, pectoral and caudal fins, gill of the infected fish (Gianneto *et al.*, 2003), but rarely attached to the body surface near the gill slits (Ota *et al.*, 2012).

2.3 The effects of parasite to host

Effects of parasite to host is related with the body characteristics of the parasite itself that can give harm to the host. The mouth has form a cone with maxilliped that tear at the flesh and tiny pointed mandibles that pierce into the tissue to penetrate the blood vessels (Hoeg *et al.*, 2005). It can cause damage with their biting and sucking mouthpart including pressure necrosis of gill tissue limiting respiratory surface or cause damage to host tissue (Heupel and Bennet, 1999) and growth retardation (Heckmann R., 2003; Ravi and Rajikumar, 2007). When they parasites occupy the entire of the host, it may produce pressure on the gill surface and thus affecting the efficiency of respiration.

Besides that, the infested fish become fatigue and could not swim fast and the oxygen demand increases with increasing swimming speeds in fish (Nilsson *et al.*, 2007). From Ravi and Rajikumar (2007), the infested fish have extreme pale gills that indicate to anemia and the gill rakers were lost. As the matter of fact that the parasite infestation may lead to death, it will also give affect on the host or fish normal growth and relatively degeneration of sexual organs (Ravinchandran *et al.*,2010). If heavy infestations of the parasite on the juvenile fish, it may have the potential to kill small fingerlings.

2.4 Cleaner fish as a biological control of parasites

Cleaner fish is one of the methods to control the parasite load in the culture system. The biological relation between the host fish with parasite attached on the body and the cleaner hunger levels (Becker, 2005) make a mutualistic where both of the partners can get benefit where the cleaner get parasite as their feeds and host fish can be free from the parasite attached on the body. This method is very environmental friendly where it plays the prey and predator relationship has been

effective in reducing the parasite that infested the fish on a commercial scale (Cowell *et al.*,1993).

However, there is a problem that associated with this relation because usually the host fish will eat the cleaner due to small in size. Beside that, the cleaner can missed the parasite on fish body because the parasites are mobile and also they can get the infection during the attachment. The parasite will make a wound and leads to the second infection that caused by bacteria. Furthermore, this method is not suitable to be applied in term of cost because in the UMS culture system, the infected fish is among the broodstock fish, thus the cleaner fish density will be increased and it also will increase the cost size due to the large size of broodfish. Other than that, UMS culture system used coral as the water filteration where the coral act as the place for parasite to live in. Therefore, parasite loads in culture system will not be eradicated by using the cleaner fish because the cleaner cannot pass through the corals.

2.5 Chemical treatment for parasite

From previous study, there are several chemicals that have been used to kill the ectoparasite in order to overcome the parasite infestation problem. The application of chemicals by immersion and also the addition of antibiotic methods to prevent the infestation are the normal practice among local farmers (Supriyadi and Rukhyani, 1996). Based on Herwig (1979), for treatment with chemical, it used should be relatively insoluble in water, act on contact, and either be denser than water to prevent the infected fish from rubbing it off. Yanong (2003) also supported, the concentration of the chemical used must not give effect to the biological organism if the culture system used biofilter.

CHAPTER 3

METHODOLOGY

3.1 Experimental Background

Experiments were conducted in fish hatchery of Universiti Malaysia Sabah (UMS Hatchery), particularly in broodstock recirculation system (RAS) tank. This study consisted of 5 experiments included 1) Identification of parasite, 2) Determination of effective treatment, 3) Determination of effective concentration, 4) Tolerance level of parasite, and 5) Tolerance level of fish, respectively. Experiments were conducted from February to April 2014.

3.2 Experiment 1 Identification of parasite

Parasites were collected from broodstock RAS tank by using 250 µm of plankton net. Parasite collection was carried out only in the morning by taking parasite preference swim to the water surface during the presence of sunlight. Collected parasites were kept into 2 forms, 1) fresh: for direct observation by naked eye and 2) fixed in 10 % formalin for microscopy observation. In this experiment, several parameters were highlighted include 1) general morphology, and 2) stage differentiation. Parasite species identification was made based on its general morphology and comparisons with other documented parasite for further clarification in order to provide the basic morphology of the infested parasite in UMS culture system.

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CHAPTER 2

LITERATURE REVIEW

2.1 Background study

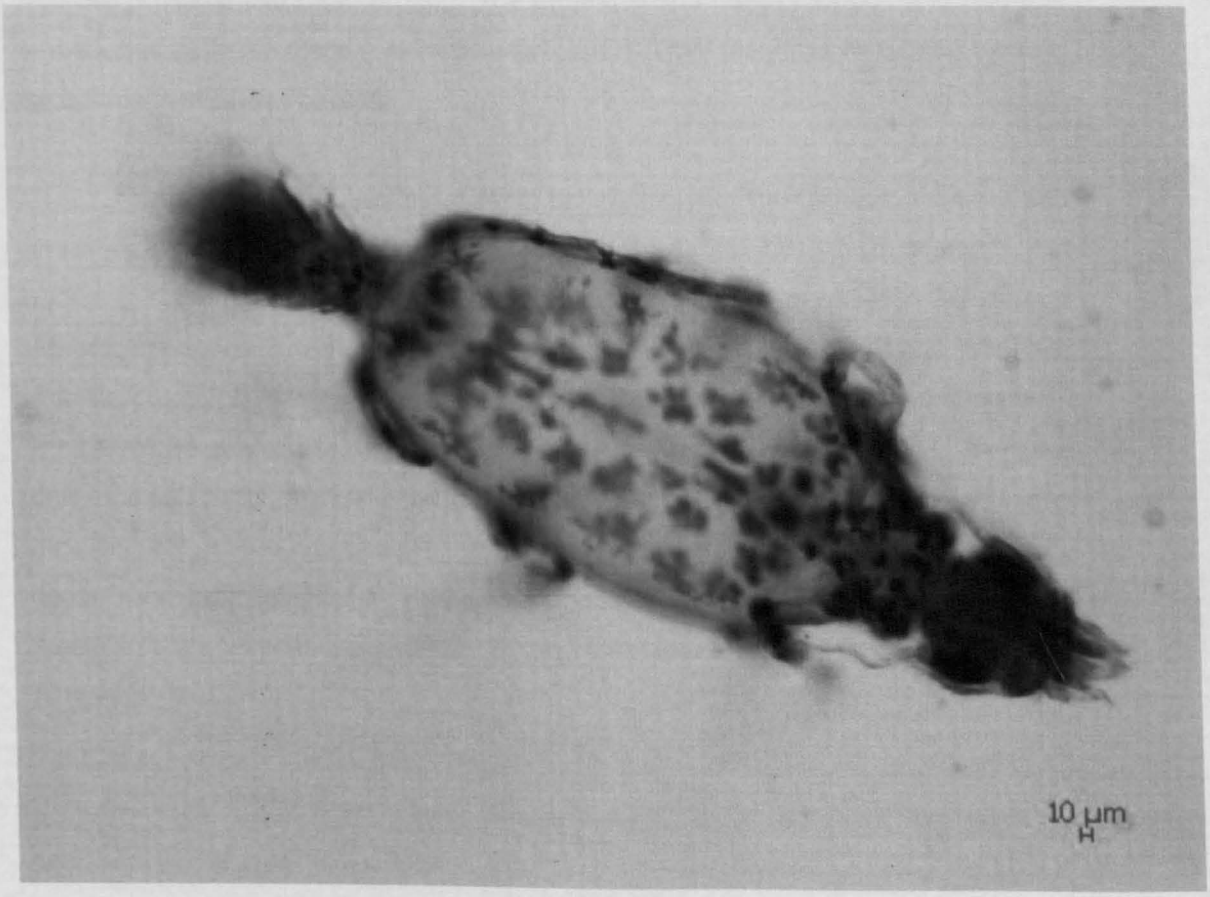

Figure 2.1 The morphology of the Gnathiidae species