UNIVERSITI PUTRA MALAYSIA

THE EFFECTS OF FORMALIN AND SODIUM CHLORIDE ON ECTOPARASITES OF IMPORTED GRASS CARP (CTENOPHARYNGODON IDELLA CUVIER AND VALENCIENNES 1844) FINGERLINGS

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FPSS 1993 1

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By

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Thesis Submitted in Fulfilment of the Requirements
for the Degree of Master of Science in the
Faculty of Fisheries and Marine Science
Universiti Pertanian Malaysia

April 1993

ACKNOWLEDGEMENTS

I wish to thank Dr. Faizah Shaharom as my chairman, who provided guidance, assistance, support and also taught me lots of invaluable things at all times during the course of this study . My sincere thanks to Assoc. Prof. Dr. Law Ah Theem, Dr. L.H.S. Lim and Assoc. Prof. Dr. Jambari Hj. Ali on the supervisory committee who also sacrificed a lot of valuable hours, invaluable comments and helpful suggestions in my study.

I wish to express my sincere thanks to Lembaga Kemajuan Ikan Malaysia and Jabatan Perikanan Malaysia Balai Karantina Ikan Jakarta and Stasiun Karantina Ikan in Indonesia which rendered valuable help in the importation of the fish. My sincere thanks also to Balai Budidaya Air Tawar and Pasar Minggu Farm which provided the fish.

I wish to thank to Dr. G. Nash and Dr. Hassan Daud who assisted in the histopathological studies.

Thanks also to me in photography and drawing of some parasites. My thanks to all administrative staff and to all technical staff at the Faculty of Fisheries and Marine Science, UPM for their kind cooperation and assistance during the course of my study.

I also thankful to Mr. Hadi Suryanto and Mr. Liew Hoch Chark for their helps with the statistical analysis.

I wish to extend my gratitude to International Development Research Centre (Canada) and Network Aquaculture Centre in Asia (Thailand) for the sponsorships and Department of Agriculture (Indonesia) for granting me the study leave.

My deepest gratitude to my family, friends, country mates and course mates, who have supported and encouraged me throughout the course of the study and whose help are invaluable. Most of all, I give back all the Praises to God who give His Constant Guidance throughout my life and made all these possible.

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LIST OF ABBREVIATIONS

L. calcarifer : *Lates calcarifer*

M. latipinna : *Mollienesia latipinna*

M. cupanus : *Macropodus cupanus*

P. panchax : *Panchax panchax*

B. pugnax : *Beta pugnax*

M. strigatus : *Microcanthus strigatus*

A. forsteri : *Aldrichetta forsteri*

B. puntius : *Barbus puntius*

Spec. : Specimen

Nos. : Numbers

asc.piece : ascessory piece

eja.tube : ejaculatory tube

O.sucker : oral sucker

LC_{50} : Median Lethal Concentration

> : More than

< : Less than

L. : Linnaeus

LSD : Least Significant Difference

Avg : Average

repl. : replicate

h : hour

NaCl : Sodium Chloride

Fig./Figs. : Figure/Figures

DO_2 : Dissolved Oxygen

Abstract of thesis submitted to the Senate of Universiti Pertanian Malaysia in fulfilment of the requirements for the degree of Master of Science.

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April 1993

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Faculty : Fisheries and Marine Science

Four shipments of grass carp imported from Indonesia were used for this study. The grass carp (*Ctenopharyngodon idella* Cuvier and Valenciennes 1844) fingerlings were infected by the monogenean (*Dactylogyrus lamellatus* Akhmerov 1952); protozoans (*Trichodina* spp., *Trichodinella* sp., *Trichophrya* sp., *Cryptobia branchialis* Nie, *Ichthyophthirius multifiliis* Fouquet 1876 and *Piscinoodinium pillulare* Schaperclaus 1954); trematodes (metacercariae of Echinostomatidae and *Transversotrema* sp.) and a crustacean (*Argulus* sp.).

Dactylogyrus lamellatus Akhmerov 1952 obtained in this study were comparatively smaller than those previously described from USSR and Hungary. *Transversotrema* sp. was found underneath the scales of grass carp. It differed in shape, size and certain morphological details to other previously described

species. Histopathological study carried out on the gills infected by echinostomatid metacercariae showed severe swollen chondrocytes, epithelial hyperplasia, squamous metaplasia of epithelial cells, atrophy, haemorrhages, necrosis and obliteration of blood vessels.

The toxicity tests of formalin and sodium chloride to grass carp fingerlings were carried out. Based on the results of the 96h-LC_{50} value, formalin at 25, 50 and 75 mg/L and sodium chloride at 6, 8 and 10 g/L in four hours were used for treatments of ectoparasitic diseases on this fish. In this study, the fish was held for 14 days in experimental tanks and treated using formalin and sodium chloride.

Formalin (25 and 50 mg/L) and sodium chloride (8 and 10 g/L) in four hour baths were found to effectively control monogenean and protozoan parasite infections.

The efficacy of both formalin and sodium chloride in this study showed almost similar results but due to the harmful effect of formalin on human and fish (influence on human tissue and oxygen depletion of water), sodium chloride was preferably used for treatment.

Abstrak tesis yang dikemukakan kepada Senat Universiti Pertanian Malaysia sebagai memenuhi keperluan untuk mendapat Ijazah Master Sains

KESAN FORMALIN DAN NATRIUM KLORIDA TERHADAP EKTOPARASIT PADA ANAK IKAN KAP RUMPUT (*CTENOPHARYNGODON IDELLA* CUVIER DAN VALENCIENNES 1844) YANG DIIMPORT

Oleh

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April 1993

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Empat pengiriman ikan dari Indonesia digunakan dalam kajian ini. Anak ikan kap rumput (*Ctenopharyngodon idella* Cuvier dan Valenciennes 1844) yang diimport ini, dijangkiti oleh monogenea (*Dactylogyrus lamellatus* Akhmerov 1952); protozoa (*Trichodina* spp., *Trichodinella* sp., *Trichophrya* sp., *Cryptobia branchialis* Nie, *Ichthyophthirius multifiliis* Fouquet 1876 dan *Piscinoodinium pillulare* Schaperclaus 1954); trematoda (Metaserkaria dari famili Echinostomatidae dan *Transversotrema* sp.) dan krustasia (*Argulus* sp.).

Dactylogyrus lamellatus Akhmerov 1952 yang ditemui dalam kajian ini adalah lebih kecil berbanding dengan spesies yang terdapat di USSR dan Hungary. *Transversotrema* dijumpai di bawah sisik anak ikan kap rumput. Trematoda ini berbeza dari segi bentuk, saiz dan perincian morfologi tertentu berbanding dengan species lain yang terdapat

dalam kajian sebelumnya. Kajian histopatologi pada insang yang dijangkiti oleh metaserkaria ekinostomatid menunjukkan kondrosit sangat membengkak, hiperplasia epitelium, metaplasia skuama pada sel epitelium, atropi, pendarahan, nekrosis dan kerosakan pada saluran darah.

Ujian ketoksikan formalin dan natrium klorida ke atas anak ikan kap rumput dijalankan. Berdasarkan kepada keputusan nilai 96j-LC_{50}, formalin pada 25, 50 dan 75 mg/L dan natrium klorida pada 6, 8 dan 10 g/L pada 4 jam pendedahan digunakan bagi merawat penyakit ektoparasit ikan ini. Dalam kajian ini, ikan disimpan di dalam tangki eksperimental selama 14 hari dan dirawat dengan formalin dan natrium klorida.

Pendedahan kepada formalin (25 dan 50 mg/L) dan natrium klorida (8 dan 10 g/L) selama empat jam adalah didapati berkesan dan disyorkan untuk mengawal jangkitan parasit monogenea dan protozoa.

Keberkesanan kedua-dua formalin dan natrium klorida dalam kajian ini adalah hampir sama, tetapi disebabkan kesan merbahaya formalin ke atas manusia dan ikan (mempengaruhi tisu manusia dan mengurangkan oksigen terlarut dalam air), maka penggunaan natrium klorida adalah lebih disyorkan bagi rawatan.

CHAPTER I

GENERAL INTRODUCTION

The importance of aquaculture is in providing high protein nutrition for the people and enhancing economies world wide. Hence, aquaculture production is a significant and rapidly-growing segment of total world wide food production (Pillay, 1976; Shireman and Smith, 1983; Brune and Tomasso, 1991).

Aquaculture production in this region has exhibited a remarkable growth, and by 1978 had reached four million tonnes or 70 % of world production (Bergin, 1986). Total production of aquaculture in Malaysia in 1986 was 51,644 tons valued at U.S $15.8 million (Malaysia, 1987). While fish production in Indonesia in 1989 had increased up to 3,680,420 tons with an average rate of 5% per year (Indonesia, 1990).

The disease problems in fish culture frequently occurred as a result of the lack of a controlled system in the farms either at broodstock level or fry and fingerling stages. Parasites and other fish disease agents are the main cause of disease problems in cultured fish. They could either have already occurred in the farm or brought into the farm by an introduced fish.

Several cases of parasites and diseases from imported fish have been studied in the region: *Sanguinicola armata* was found on bighead carp and grass carp fingerlings imported into Malaysia from Taiwan (Anderson and Shaharom-Harrison, 1986); *Lernaea cyprinacea* was introduced with imported carps from Japan into Indonesia (Djajadiredja *et al*, 1983). Such introductions indicate that imported fish need to be quarantined. A quarantine system could become a supporting factor in the development and intensification of aquaculture activities (Shariff, 1987; Arthur and Shariff, 1991).

Grass carp, *Ctenopharyngodon idella* Cuvier and Valenciennes is the most widely cultivated species in fish culture in the region (Pillay, 1976) and it fetches a high price in the market in Southeast Asian countries (Nikolsky, 1963; Shireman and Smith, 1983). Grass carp is susceptible to diseases and according to Wu (1971), the fish farmers in Taiwan had reduced profits each year caused by high mortality in cultured grass carp.

Parasite infections cause pathological changes in the structure of an infected organ. There are few studies in the histopathological changes of the gills infected by encysted metacercariae. According to Eller (1975), encysted metacercariae of trematodes in the gill tissue caused mechanical injury to gill tissue which was represented by a mild hyperplasia of lamellar epithelium.

In this study, formalin and sodium chloride were used as chemical treatments for ectoparasites found in imported grass carp. These chemicals were chosen because of low cost, easy availability and relatively safe (Hoffman and Meyer, 1974; Kabata, 1985; Ferguson, 1988; Plumb, 1992). Formalin and sodium chloride are recommended as therapeutans for parasites and have been approved by the US Food and Drug Administration (Herwig, 1979; Schnick *et al*., 1986; Post, 1987; Cross and Needham, 1988; Alderman, 1988). Therefore the objectives of the present study are as follows :

1. To study the morphology of some ectoparasites found on the imported grass carp (*C. idella*).
2. To determine the median lethal concentration of formalin and sodium chloride to grass carp (*C. idella*) fingerlings so that the effectiveness of formalin and sodium chloride as chemotherapeutants against ectoparasites on grass carp (*C. idella*) fingerlings can be evaluated.
3. To study the prevalence and intensity of ectoparasites on grass carp (*C. idella*).
4. To study the histopathological changes on the gills of grass carp (*C. idella*) infected by metacercariae.

CHAPTER II

REVIEW OF RELATED LITERATURE

Fish Disease Problems In Aquaculture

Parasitic Infections in Fish Culture

As the fish culture activities have been increasing, the fish disease problems appear frequently. The parasites and diseases of fish culture constitute one of the most important problems confronting the modern fish culturist (Davis, 1967).

The major diseases associated with fish culture in this region may be divided into two main groups, namely protozoan diseases and microbial diseases (Leong, 1987). However, other causal agents like parasitic worms (monogeneans, trematodes, cestodes, nematodes and acanthocephalans); crustaceans; leeches and mollusc can also be disadvantageous in the fish culture system and may decrease the aquaculture production (Nikolsky, 1963; Davis, 1967; Hoffman, 1976; Needham and Wootten, 1978; Kabata, 1985).

Protozoan parasites caused great damage to fish culture in European and Asian countries because of their pathogenicity (Bauer *et al.*, 1973; Kabata, 1985). *Cryptobia branchialis* was implicated in the mortality of

cultured carp in eastern Europe (Bauer *et al.*, 1973). Parasites like *Ichthyophthirius multifiliis*, *Dactylogyrus* spp., *Oodinium* sp., *Gyrodactylus* sp., and *Lernaea cyprinacea* are known to cause great losses to fish culture in North America (Hoffman, 1970).

There were similar cases in the tropical countries. In the 1950's, *Lernaea cyprinacea* Linnaeus, 1958 reduced fry production of common carp, *Cyprinus carpio* Linnaeus and Java carp, *Puntius gonionotus* Bleeker in Java, Indonesia (Djajadiredja *et al.*, 1983).

During the 1970's, two myxosporidians, *Myxobolus* sp. and *Myxosoma* sp. caused heavy losses of common carp fingerlings in West Java (Djajadiredja *et al.*, 1983). Then in late 1980, a large number of fish died also in west Java and Sumatera from an outbreak of bacterial disease (*Aeromonas* sp.).

Protozoans (*Ichthyophthirius multifiliis*, *Chilodonella* spp., *Trichodina* spp.); monogeneans (*Dactylogyrus* spp., *Gyrodactylus* spp.), branchiuran (*Argulus* spp.) and copepod (*Lernaea* spp.) were common on aquarium fishes in Malaysia (Shariff, 1980). Ectoparasitic protozoans caused irritation of skin and gills, paleness of colour, hyperaemia of skin, bristle scales, increased mucus production and frayed fins (Reichenbach-Klinke, 1973; Kabata, 1985).

In a catfish farm in Pansoly Laguna, Philippines, 1,000 out of 5,000 fishes died in two days because of monogenean infections (Velasquez, 1975b). Monogenean caused irritation of skin and gills. The families Dactylogyridae and Gyrodactylidae are the most common gill and skin parasites (Reichenbach-Klinke, 1973). The genus *Dactylogyrus* attack the gills and cause increased breathing frequency in fish (Duijn, 1973). The gill lamellae became inflamed and swollen as a result of the attachment of the worms. Mucus production is increased and gills are discoloured. Parts of the gill sheet are covered with a cloudy film, consisting of slime and destroyed epithelial cells (Paperna, 1980; Kabata, 1985). Focal erosion and extensive hyperplasia due to *D. extensus* and *D. anchoratus* infections in carp cause morbidity and mortality (Paperna, 1980).

The fish trematodes commonly occur as metacercarial stages. The cercariae of *Cryptocotyle lingua* Creplin 1825 which settled and encysted underneath the fish skin, cause black spots in the skin (Grabda, 1991). *Prototransversotrema steeri* Angel 1969, a trematode found on the scale of *Mugil cephalus* causing initiation of some lesions (inflammation and haemorrhage) was observed to be associated with red spot disease (vibriosis) (Rodgers and Burke, 1988).

The genera *Diplostomum* and *Clinostomum* encyst in the gills and skin of tropical fish. Encysted trematodes in

the gills cause irritation of the epithelium, increased mucus production and leads to increase in thickness of respiratory exchange surface, as a consequence of which the gill function is impaired and respiration inhibited (Duijn, 1973; Eller, 1975). Encysted metacercariae in the gills of fish also cause severe histopathological changes (Grabda, 1991).

Choice of Fish

Grass carp (*Ctenopharyngodon idella*) Cuvier and Valenciennes was the chosen species for the present study. This freshwater fish has been cultured during the last two decades and it is widely accepted by Malaysian (Ong, 1968; Ji, 1976; Mohsin and Ambak, 1983; Ong, 1988; Ang, 1990).

The grass carp has been the subject of considerable research in recent years because of its ability to utilize aquatic vegetation (Shireman et al.,1977; Riley, 1978; Meske, 1985). On the other hand, grass carp is susceptible to numerous diseases and parasitic infections (Wu, 1971; Shireman and Smith, 1983). A number of parasitic infections on grass carp (*C. idella*) has been recorded in some countries (Riley, 1978; Shireman and Smith, 1983; Ebregt, 1985); from the data, over forty species of parasitic protozoa; over twenty species of monogeneans and trematodes, about seven cestodes, nine nematodes and a few crustaceans have been reported from grass carp.