UNIVERSITI PUTRA MALAYSIA

THE MALE REPRODUCTIVE BIOLOGY OF CULTURED RIVER CATFISH, *MYSTUS NEMURUS* (CUYIER & VALENCIENNES)

ANNIE CHRISTIANUS

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By

ANNIE CHRISTIANUS

Thesis Submitted in Fulfilment of the Requirement for the Degree of Doctor of Philosophy in the Faculty of Science and Environmental Studies Universiti Putra Malaysia

October 2001

DEDICATION

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To my beloved husband,

James,

your love, encouragement and patience sustained me through, thank you.

*****

To my parents,

Evalinah & Christianus,

my brothers and sister, Petrus, Denis, Dyno & Jenita,

thank you for your love, understanding and support.

and

To my in laws,

thank you for your patience and understanding.

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Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfilment of the requirement for the degree of Doctor of Philosophy

THE MALE REPRODUCTIVE BIOLOGY OF CULTURED RIVER CATFISH, *MYSTUS NEMURUS* (CUVIER & VALENCIENNES)

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Chairperson : Associate Professor Dr. Siti Shapor Siraj

Faculty : Science and Environmental Studies

Mystus nemurus broodstock bought from Rawang, Selangor in March 1995, conditioned in the Hatchery Unit of Universiti Putra Malaysia were used for all the experiments including larvae production. In the first experiment, morphologically the testis has shown to consist of a mass of finger like structures with an anterior part accommodating clusters of spermatogenic cells and spermatozoa; and the posterior part, displaying some muscle layers and ducts, described as spermatogenic and the glandular testis, respectively. Electron microscopy showed that the morphology of the *M. nemurus* spermatozoa consist of a head measuring 1.88 ± 0.01 μm in diameter; midpiece, 1.69 ± 0.02 μm in width and flagellum, 11.42 ± 0.07μm in length.

In the second experiment, males *M. nemurus* were distinguishable only at 5-month-old and gonads started differentiating at 8-month-old. Histometric measurement of the lumen areas of 11, 12 and 13-month-old showed a significant increase ($P < 0.05$)

in sizes compared to 8, 9 and 10-month-old and a significant ($P < 0.05$) correlation with r = -0.567 between these lumen and spermatogenic areas from the initial stage of differentiation. Males were found to produce spermatozoa at 13-month-old but only attained the ability to fertilize eggs at 17-month-old.

In the third experiment, among the various physical and biochemical characteristics determined between non-treated and carp pituitary extract (CPE) treated groups, significant differences ($P < 0.05$) were detected in the milt volume, pH, osmolality, glucose, urea, total protein, sperm density and total sperm count. The significantly ($P < 0.05$) higher fertilization rate of CPE treated group showed that hormone treatment on *M. nemurus* produces better quality of milt and spermatozoa.

In the last experiment, the testosterone (T) and 11-ketotestosterone (11-KT) levels in the circulating plasma of matured and immature males *M. nemurus* measured by radioimmunoassay (RIA) and enzyme linked immunosorbent assay (ELISA), ranged from 0.25 to 2.34 and 0.75 to 4.58 ng/ml; and 0.19 to 1.57 and 0.49 to 4.19 ng/ml, respectively, with 11-KT as the dominating androgen. In the one-year duration, T and 11-KT profiles of the two groups of matured and immature showed a significant correlation ($P < 0.05$) with r = 0.814 and 0.682, respectively. Hormones profiles suggested the association of these T and 11-KT with the reproductive cycle and gonadal development of the matured and immature groups, respectively.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi syarat untuk ijazah Doktor Falsafah

BIOLOGI PEMBIAKAN IKAN DURI SUNGAI JANTAN, *MYSTUS NEMURUS* (CUVIER & VALENCIENNES) YANG DIKULTUR

Oleh

ANNIE CHRISTIANUS

Oktober 2001

Pengerusi : Profesor Madya Dr. Siti Shapor Siraj

Fakulti : Sains dan Pengajian Alam Sekitar

Induk *Mystus nemurus* dibeli dari Rawang, Selangor pada Mac 1995, disesuaikan di Unit Penetasan Universiti Putra Malaysia telah digunakan untuk semua eksperimen termasuk penghasilan larva. Dalam eksperimen pertama, testis secara morfologinya telah menunjukkan keadaan sebagai satu jasad dengan struktur jejari terdiri daripada bahagian anterior yang mengandungi kumpulan sel-sel spermatogenik dan spermatozoa, dan posteriornya, menunjukkan kehadiran lapisan-lapisan otot dan duktus, masing-masingnya digelar sebagai testis spermatogenik dan glandular. Mikroskopi elektron menunjukkan morfologi spermatozoa *M. nemurus* mempunyai satu kepala yang bergarispusat 1.88 ± 0.01 µm, 1.69 ± 0.02 µm lebar bahagian tengah, dan 11.42 ± 0.07µm panjang flagellum.

Dalam eksperimen yang kedua, *M. nemurus* jantan hanya dapat dikenalpasti pada umur 5 bulan dan gonad memulakan pembezaan pada umur 8 bulan. Pengukuran histometrik bahagian lumen pada ikan yang berumur 11, 12 dan 13 bulan

menunjukkan peningkatan saiz yang bererti ($P < 0.05$) berbanding dengan umur 8, 9 dan 10-bulan dan satu korelasi yang bererti ($P < 0.05$) dengan r = -0.567 di antara bahagian lumen dan spermatogenik daripada peringkat awal pembezaan. Ikan jantan menghasilkan spermatozoa pada umur 13 bulan tetapi hanya mencapai kemampuan untuk mensenyawakan telur pada umur 17 bulan.

Dalam eksperimen yang ketiga, di antara ciri-ciri fizikal dan biokimia yang telah ditentukan di antara kumpulan-kumpulan tanpa-rawatan dan dengan rawatan ekstrak pituitari ikan kap (CPE), perbezaan yang bererti ($P < 0.05$) telah dikesan pada isipadu air mani, pH, osmolaliti, glukosa, urea, jumlah protein, densiti sperma dan jumlah kiraan sperma. Kadar persenyawaan yang bererti ($P < 0.05$) lebih tinggi pada kumpulan dengan rawatan CPE dengan menunjukkan rawatan hormon ke atas *M. nemurus* menghasilkan kualiti air mani dan sperma yang lebih baik.

Dalam eksperimen yang terakhir, takat testosteron (T) dan 11-ketotestosteron (11-KT) dalam edaran plasma *M. nemurus* jantan yang matang dan belum matang diukur dengan "radioimmunoassay" (RIA) dan "enzyme linked immunosorbent assay" (ELISA), masing-masing di antara 0.25 hingga 2.34 dan 0.75 to 4.58 ng/ml; dan 0.19 hingga 1.57 dan 0.49 hingga 4.19 ng/ml, dengan 11-KT sebagai androgen yang dominan. Dalam jangka masa setahun, profil T dan 11-KT dalam kedua-dua kumpulan matang dan belum matang menunjukkan satu korelasi yang bererti ($P < 0.05$) dengan r = 0.814 and 0.682 masing-masing dengan. Profil hormon menunjukkan terdapatnya pertalian diantara T dan 11-KT dengan kitar pembiakan dan perkembangan gonad kumpulan ikan matang dan belum matang, masing-masing.

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I certify that an Examination Committee met on the 30th October 2001 to conduct the final examination of Annie Christianus on her Doctor of Philosophy thesis entitled "The Male Reproductive Biology of Cultured River Catfish, *Mystus nemurus* (Cuvier & Valenciennes)" in accordance with Universiti Pertanian Malaysia (Higher Degree) Act 1980 and Universiti Pertanian Malaysia (Higher Degree) Regulations 1981. The Committee recommends that the candidate be awarded the relevant degree. Members of the Examination committee are as follows:

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This thesis submitted to the Senate of Universiti Putra Malaysia has been accepted as fulfilment on the requirement for the degree of Doctor of Philosophy.

AINI IDERIS, Ph.D.,
Professor,
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Date:

DECLARATION

I hereby declare that the thesis is based on my original work except for quotations and citations, which have been duly acknowledged. I also declare that it has not been previously or concurrently submitted for any other degree at Universiti Putra Malaysia or other institutions.

Annie Christianus
Date: 12th. November 2001

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LIST OF ABBREVIATIONS

ANOVA	=	Analysis of Variance
CL	=	cytoplasmic layer
cm	=	centimeter
CPE	=	Carp Pituitary Extract
C & V	=	Cuvier and Valenciennes
EIA	=	Enzyme immunoassay
ELISA	=	Enzyme linked immunosorbent assay
ER	=	endoplasmic reticulum
EU	=	Ellman's Unit
FSI	=	Fatsomatic index
GSI	=	Gonadosomatic index
H & E	=	Haematoxylin and Eosin
IRPA	=	Intensified Research on Priority Areas
11-KT	=	11-ketotestosterone
M	=	Molar
ml	=	milliliter
mOsmol/kg	=	milliosmol/kg
mM	=	millimolar
ng	=	nanogram
nm	=	nanometer
nmol	=	nanomol
NS	=	No significant difference

pg	=	picogram
PS	=	primary spermatocyte
S.E.	=	Standard error
SEM	=	Scanning Electron Microscopy
SPC	=	Spermatocyte
SPG	=	Spermatogonium/spermatogonia
SPD	=	Spermatid
SPZ	=	Spermatozoa
SS	=	Secondary spermatocyte
T	=	testosterone
TEM	=	Transmission Electron Microscopy
TL	=	Total length
µl	=	microliter
µg	=	microgram
µm	=	micrometer
w/w	=	weight/weight

CHAPTER I

INTRODUCTION

Background of the Study

Aquaculture practices in Malaysia is still at its infant stage. In 1996, capture fishery which mainly derived from marine landings recorded up to 1.2 million metric tonnes production while aquaculture (inclusive of finfish, cockle, mussel and oyster) produced a little bit more than 0.1 million metric tonnes (Annual Fishery Statistics, 1996). Information gathered from the fishery reports from 1971 to 1996 (Figure 1.1) by the Department of Fisheries Malaysia showed that the progress in finfish culture is rather slow but gaining momentum with the increase in its yield. This information indicates that aquaculture has its potential to become a large industry, perhaps not in the next five years but possibly in the next ten to twenty years.

Statement of Problems

Eleven species of the mystid found in Malaysian waters are *Mystus nemurus*, *M. nigriceps, M. planiceps, M. micracanthus, M. wyckii, M. gulio, M. wolffii, M. bimaculatus* (Lim *et al.*, 1993), *M. baramensis, M. vittatus* (Mohsin and Ambak, 1983) and *M. cavasius* (Smith, 1945). Until now *M. nemurus* is the most popular amongst fish consumers and aquaculturist, however, the extent of its culture is very much restricted due to the inconsistent supply of fry, which is dependant on the wild and hatchery productions. Mass production in the hatchery is still unstable since this

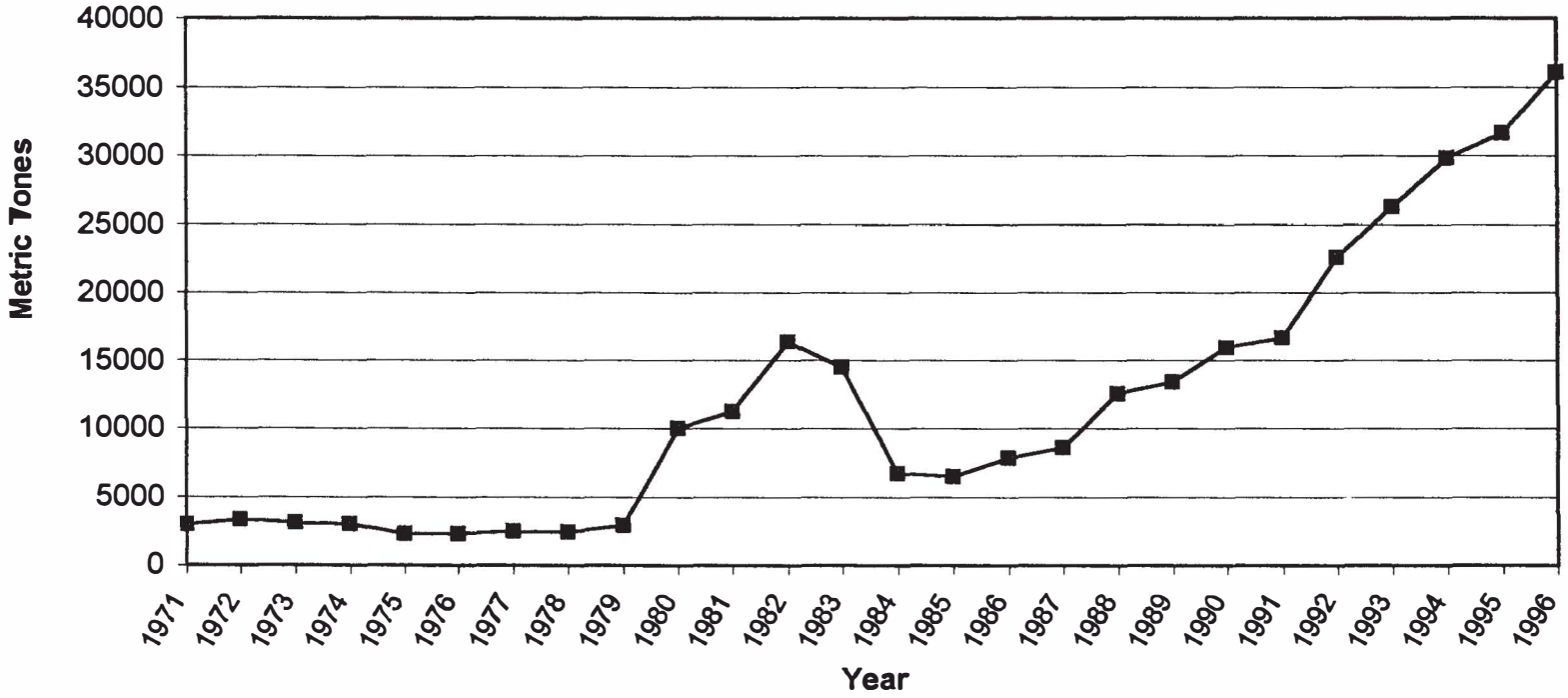

Figure 1.1: Freshwater finfish production in Malaysia from 1971 to 1996.

species is not reliable to spawn naturally in captivity The main constraint so far has been in the propagation technique, even though achievement has been made through induced breeding, fertilization rate, however, is relatively low. The females are found to be able to produce abundance of ripe eggs, unfortunately is not the case for the male gametes. Information on the reproductive biology of wild *M. nemurus* (Khan *et al.*, 1990) is very limited to give an insight on the development and maturity of male gonad which is important in order to improve the propagation technique of this species. Little information is available on the reproductive biology (Khan *et al.*, 1990) and induced breeding attempt (Thalathiah *et al.*, 1988) of *M. nemurus* is apparently insufficient to ensure the success and consistent supply of its fry.

Significance of the Study

It is essential that selected broodstocks are able to produce high quality milt and eggs to ensure the production of high quantity, quality and viable fry. The female *M. nemurus* was found to readily induce for egg production (Somga, 1996), which is not shown by the male. Activation of male to produce milt through hormone induction did not guarantee the production of high quality sperm. Hence, at puberty, though the testes are able to produce sperm, there is no definite clue to its ability to fertilize eggs. The ability to fertilize eggs is categorised as one of the factors in milt quality that influence the hatching rate (Aas *et al.*, 1991). To evaluate milt quality, factors like sperm density and motility have to be taken into serious consideration because they undoubtedly not only influence the fertilization rate but also affect the