OOCYTE FINAL MATURATION, EGG DEVELOPMENT AND EFFECTS OF STRIPPING TIMINGS ON EGG QUALITY IN *Pangasius hypophthalmus* AND *Clarias gariepinus*

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BORNEO MARINE RESEARCH INSTITUTE
UNIVERSITI MALAYSIA SABAH

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DECLARATION

The materials in this thesis are original except for quotations, excerpts, summaries and references, which have been duly acknowledged.

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ABSTRAK

KEMATANGAN AKHIR OOSIT, PERKEMBANGAN TELUR DAN KESAN MASA PELURUTAN TERHADAP KUALITI TELUR Pangasius hypophthalmus DAN Clarias gariepinus

Patin, *Pangasius hypophthalmus* dan Keli Afrika, *Clarias gariepinus* popular dalam industri akuakultur kerana beberapa sebab: kadar tumbesaran yang tinggi, tabiat pemakanan omnivor, rintang terhadap penyakit dan toleransi terhadap kepekatan oksigen yang rendah. Kedua-dua spesies ini merupakan spesies keli yang paling banyak dikultur di Asia Tenggara. Walaupun benih-benih ikan ini diperolehi melalui aruhan hormon, pengeluaran benih secara besar-besaran masih menjadi penghalang disebabkan kadar persenyawaan dan penetasan yang rendah. Kekurangan maklumat berkaitan ciri-ciri biologi oosit dan perkembangan telur spesies ini membantutkan usaha pengkulturannya. Penyelidikan yang bersifat aplikasi industri ini dijalankan untuk menentukan masa ovulasi dan tempoh pelurutan bagi mendapatkan telur berkualiti tinggi dan seterusnya mengelurakan benih secara besar-besaran. Ovulasi dan kematangan oosit diaruh dengan dua dos suntikan hormon korionik gonadotropin manusia (500 dan 1500 IU/kg pada selang masa 14 jam) bagi *P. hypophthalmus*, dan satu dos Ovaprim (0.5*ml*/kg) bagi *C. gariepinus*. Oosit dipantau secara berterusan melalui teknik kanulasi. Peringkat-peringkat perkembangan telur diperhatikan di bawah mikroskop. Selepas ovulasi, telur dilurut dan disenyawakan pada selang masa satu jam. Kadar persenyawaan dan penetasan yang dilurut pada selang masa berlainan dikira bagi menentukan kualiti telur. Kematangan akhir oosit dibahagikan kepada enam peringkat. Kematangan akhir pada *P. hypophthalmus* ialah pada peringkat I dan peringkat III pada *C. gariepinus* sebelum rawatan hormon, telur dilepaskan pada peringkat VI. Tempoh pelurutan adalah bergantung kepada suhu, di mana ianya menurun apabila suhu air meningkat. Kadar persenyawaan dan penetasan menurun dengan masa selepas ovulasi. Bagi tiga ekor betina *P. hypophthalmus* (1.8-3.3 kg), min kadar persenyawaan adalah 92.4% pada 0 jam selepas ovulasi (SO) dan 15.7% pada 8 jam SO; dan min kadar penetasan juga menurun dengan masa iaitu 80.6% pada 0 jam SO dan akhirnya 0% pada 6 jam SO. Bagi tiga ekor betina *C. gariepinus* (0.6-1.3 kg), min kadar persenyawaan ialah 92.2% pada 0 jam SO dan 40.6% pada 6 jam SO; dan min kadar penetasan ialah 83.7% pada 0 jam SO dan akhirnya menurun kepada 0% pada 6-8 jam SO. Kajian ini menyimpulkan bahawa kadar persenyawaan dan penetasan yang tinggi boleh dicapai dengan menentukan masa optima bagi pelurutan telur serta menjalankan pengawasan ke atas kematangan oosit akhir, yang dianggarkan berlaku pada 7 jam 30 min-14 jam 30 min selepas rawatan hormon kedua pada suhu di antara 26.0-33.5°C bagi *P. hypophthalmus* dan 7-8 jam selepas rawatan hormon pada suhu 27.0-28.0°C bagi *C. gariepinus*. Kajian ini juga mendapati bahawa telur yang berkualiti (lebih dari 50% min kadar penetasan) boleh diperolehi dengan melakukan pelurutan dan persenyawaan dalam masa 2 jam selepas ovulasi bagi *P. hypophthalmus* dan 3 jam bagi *C. gariepinus*.

ABSTRACT

OOCYTE FINAL MATURATION, EGG DEVELOPMENT AND EFFECTS OF STRIPPING TIMINGS ON EGG QUALITY IN Pangasius hypophthalmus and Clarias gariepinus

River catfish, Pangasius hypophthalmus and African catfish, Clarias gariepinus have become popular in the aquaculture industry due to several reasons: high growth rate, omnivorous feeding habits, strong resistance to disease and tolerance to low dissolved oxygen level. These two species are now major cultured catfishes in the Southeast Asia. Although their seeds have been produced by hormone-induced reproduction, mass seed production has remained a major constraint due to low fertilization and hatching rates. Important information on basic biological features of oocytes and eggs development of these species is still lacking and could limit aquaculture development. The present industrial application research study was conducted to understand the optimum ovulation time and duration for stripping of good quality eggs of these two morphologically different species with the objective of improving mass seed production. Oocyte final maturation and ovulation were induced by two human chorionic gonadotropin injections (500 and 1500 IU/kg at a 14-h interval) in P. hypophthalmus, and a single dose of Ovaprim (0.5 ml/kg) in C. gariepinus. Oocytes were continuously monitored by cannulation. The stages of egg development were observed under a microscope. The eggs were stripped and fertilized at hourly intervals after ovulation. The fertilization and hatching rates were observed from the eggs stripped at different times to determine the egg quality. The different grades of oocyte final maturation were divided into six grades. The oocyte final maturation was at grade I in P. hypophthalmus and III in C. gariepinus prior to hormone treatment and the fish released eggs at grade VI. The egg stripping time was water temperature dependent, which decreased with increasing water temperature. The fertilization and hatching rates of eggs stripped at different times were decreased with the time after ovulation. In six females of P. hypophthalmus (1.8-3.3 kg), the mean fertilization rates were 92.4% at 0 h after ovulation (AO) and 15.7% at 8 h AO; and the mean hatching rate was 80.6% at 0 h AO and finally decreased to 0% at 6 h AO. In six females of C. gariepinus (0.6-1.3 kg), the mean fertilization rates were 92.2% at 0 h AO and 40.6% at 6 h AO; and the mean hatching rate was 83.7% at 0 h AO and finally decreased to 0% at 6-8 h AO. This study concludes that high fertilization and hatching rates can be achieved by determining the optimum timing for egg stripping by monitoring oocyte final maturation, which was estimated at 7 h 30 min-14 h 30 min after the second hormone treatment at 26.0-33.5°C in P. hypophthalmus and 7-8 h after the hormone treatment at 27.0-28.0°C in C. gariepinus. Above study also concludes that good quality eggs (more than 50% hatching rate on average) can be achieved by stripping and fertilization of the eggs within 2 h AO in P. hypophthalmus and 3 h AO in C. gariepinus.

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LIST OF ABBREVIATIONS

%	percentage
&	and
ºC	degree celsius
AF	after fertilization
AO	after ovulation
AH	after hatching
BH	body height
BR	body round
BW	body weight
cm	centimetre
DA	dopamine antagonists
Df	degree of freedom
DO	dissolved oxygen
DOCA	deoxycorticosterone acetate
ed.	Edited, edition, editor
et al.	and others, and the rest
F	female
FAO	*Food and Agriculture Organisation*
FL	fork length
FRP	fibreglass reinforced plastic
FSH	follicle stimulating hormone
g	gram
GDP	gross domestic product
GnRH	gonadotropin releasing hormone
GnRHa	gonadotropin releasing hormone analogue
GtH	gonatdotropin hormone
GV	germinal vesicle
GVBD	germinal vesicle breakdown
H	hour
HCG	human chorionic gonadotropin
HDPE	high density polyethylene
HL	head length

ICLARM	*International Center for Living Aquatic Resources Management* (Now named the *World Fish Centre*)
IU	international unit
Kg	kilogram
l	litre
LIFD	low-income food-deficit
LH	luteinizing hormone
LHRH	luteinizing hormone releasing hormone
LHRHa	luteinizing hormone releasing hormone analogue
m	metre
m^3	cubic metre
M	male
mg	milligram
ml	millilitre
mm	millimetre
min	minute
mt	metric ton
n	number
NACA	*Network of Aquaculture Centres in Asia-Pacific*
pH	potantra of hydogeni (power of hydrogen)
ppm	part per million
PUFA	polyunsaturated fatty acid
R	Regression (correlation coefficient)
sGnRHa	salmon gonadotropin releasing hormone analogue
SD	standard deviation
SL	standard length
spp.	species
TL	total length
USA	United States of America
US$	United States Dollar
µg	microgram
WHO	*World Health Organization*

KEY WORDS

Pangasius hypophthalmus, *Clarias gariepinus*, Oocyte final maturation, Egg development, Egg quality.

CHAPTER 1

INTRODUCTION

1.1 Background of Global Catfish Culture

The governments of many countries in the tropical region emphasize development and expansion of aquaculture in their planning. Their main objectives are to provide sufficient protein to the people, increase income of fishermen and farmers, and extend the export market for foreign exchange earning (Ang, 1990; Chua & Tech, 1990; Pillay, 1990). In this region, most countries are developing and some are low-income food-deficit (LIFD) countries (Tacon, 2001). Therefore, the technology for the development of aquaculture should be acceptable and compatible with their financial resources, level and state of experience. The government of Malaysia has made concerted efforts to get the corporate sector to spearhead aquaculture in order to increase fish production in the future (Department of Fisheries Malaysia, 2000). Department of Fisheries Malaysia (2000) has also estimated aquacultural production to increase by as much as four times its present level (167,894 mt) by the year 2010 with a vast potential of inland and coastal areas.

Globally, human population is increasing every year and more people are undernourished due to the lack of sufficient protein in their diets. The world population doubled in size from 3 to 6 billion people from 1960 to 1999 and currently growing at 1.33% per year, and expected to reach 7.3 to 10.7 billion by 2050 (Tacon, 2001). Hunger and malnutrition remain amongst the most devastating problems facing the majority of the world's poor and needy, and continue to dominate the health of the world's poorest nations (WHO, 2000). Approximately 790 million people in developing countries and 34 million in developed countries are not eating sufficient food to meet their basic nutritional needs (FAO, 1999). Nowhere is this more critical than within many of the world's developing countries, within those LIFD countries (currently representing over 62% of the world's population) that are net importers of food and lack sufficient earnings to purchase food to cover basic dietary needs (Tacon, 2001; World Bank, 2000). In many developing and LIFD countries, there is a severe shortage of proteins. There is an urgent need to increase the supply of proteins in order to improve the situation. The long-term sustainability of many traditional agricultural systems required to meet increasing global demand for food (Tacon, 2001).

According to Tacon (2001), aquaculture is widely perceived as an important weapon in the global fight against hunger and malnutrition among the different global food production systems, particularly within developing countries. Aquaculture is regarded as an important domestic provider of much needed high-quality animal protein and other essential nutrients at affordable prices to the poorer segments of the community; and aquaculture is also an important provider of employment opportunities, cash income and valuable foreign exchange, with developing countries producing over 90% of total aquaculture production by weight in 1998 (Tacon, 2001). Aquaculture has been the world's fastest growing food production sector for nearly two decades with an overall growth rate of over 11.0% per year since 1984; it has expanded, diversified, intensified and technologically advanced (Jia *et al*., 2001; Tacon, 2001). Its potential contribution to local food security and livelihoods can be very significant, especially in remote and resource poor areas (Jia *et al*., 2001).

The total global aquaculture production was 39.43 million mt in 1998 (FAO, 2000). By economic country grouping, 90.0% and 82.2% of total global aquaculture production was produced within developing (35.49 million mt) and LIFD countries (32.41 million mt), respectively in 1998 (FAO, 2000; Tacon, 2001). At a species group level, finfish contributed 50.8% of total global aquaculture production (20 million mt) in 1998 (FAO, 2000; Tacon, 2001).

By region, Asia dominates global aquaculture production and produced 90.8% of total global aquaculture production (35.81 million mt), with China reporting 68.6% of total global aquaculture production (27.1 million mt) in 1998 (FAO, 2000; Tacon, 2001). The past 20 years have seen Asian aquaculture evolve from a traditional practice to a science-based activity and grow into a significant food production sector, contributing more to national economies and providing better livelihoods for rural and farming families (Kongkeo, 2001). According to Kongkeo (2001) aquaculture production in Asia has been growing at a rate nearly five times faster than landings from capture fisheries.

Major freshwater species cultured in this region are carps, tilapias and catfishes. These include common carp, *Cyprinus carpio*; grass carp, *Ctenopharyngodon idella*; silver carp, *Hypophthalmichthys molitrix*; bighead carp, *Aristichthys nobilis*; Javanese carp, *Puntius gonionotus*, red tilapia, *Oreochromis niloticus* and black tilapia, *O. mossambicus*. Major cultured catfishes are *Clarias batrachus*, *C. macrocephalus*, *C. gariepinus* and *Pangasius hypophthalmus*.

According to FAO (2003), the freshwater catfish contributed 0.9% of the total global aquaculture production in 2001. Due to the leading role of the United States of America in catfish culture, global production is dominated by ictalurids, followed by clariids and pangasiids. In 2001, out of the 427,728 mt of catfish production, ictalurids contributed 63.6%, clariids 33.6%, pangasiids 2.4% and other species (*Silurus spp.*, *Mystus spp.* etc.) 0.4%, respectively (FAO, 2003).

Different catfish species are cultured in many parts of the world: channel catfish, *Ictalurus punctatus* in USA (Beleau, 1985); sheat fish, *Siluris glanis* in Europe (Bardach *et al.*, 1972), Asian catfish, *C. batrachus* and *C. macrocephalus* in Thailand (Arerat, 1987) Philippines (Tan-Fermin, 1992) and Malaysia (Thalathiah, 1998); Asian catfish, *C. batrachus* and *Heteropneustes fossilis* in India (Sinha, 1988); African catfish, *C. gariepinus* in Africa (Viveen *et al.*, 1985) and river catfish, *P. hypophthalmus* in many countries of Asian region (Csavas, 1994).

In Asia, clariids and pangasiids catfishes are of particular significance for catfish culture. In this region, clariids dominated in catfish production, representing nearly 92.3% of the total 144,641 mt of freshwater catfish production in 2001 (FAO, 2003). Although ranking beyond carps and tilapias, the total volume of cultured catfish in Asia has shown fast increase during the last 20 years. In 2001, Thailand, Indonesia and Malaysia were leading in catfish culture with an annual production of about 87,170; 36,979 and 15,124 mt, respectively (FAO, 2003).

Among pangasiids, the major cultured species have been *P. hypophthalmus*, *P. larnaudii* and *P. miconema* in Thailand, Cambodia, Vietnam and Laos for a long time (Thiemmedh, 1957; Hora & Pillay, 1962; Bardach *et al.*, 1972; Huet, 1972). *Pangasius pangasius* is also cultured in India, Pakistan, Bangladesh, and Myanmar (Mohsin & Ambak, 1983; Roberts & Vidthayanon, 1991). Four species are apparently endemic to the island of Borneo namely *P. humeralis*, *P. kinabatanganensis*, *P. lithostoma* and *P. nieuwenhuisii* (Inger & Chin, 1962; Roberts & Vidthayanon, 1991). In Sabah, wild *Pangasius spp.* is harvested in its natural habitat mostly in Beluran and Kinabatangan districts. Among clariids, the major cultured species are *C. batrachus*, *C. macrocephalus* and *C. gariepinus*.

1.1.1. Catfish Culture in Malaysia

The fisheries sector in Malaysia plays an important role in providing fish as a source of food and protein (Department of Fisheries Malaysia, 2000). In 2000, it contributed about 1.6% to the national Gross Domestic Product (GDP) and provided direct employment to 81,994 fishermen and 21,774 fish farmers. In 2000, the total production from the fisheries sector amounted to 1.45 million mt valued at RM5.37 billion.

In Malaysia, the aquaculture industry constituted 10.0% to the total fish production over the last decade. In 2000, the aquaculture sector recorded a production of 167,894 mt, which constituted about 11.6% of the total fish production. It is expected that the aquaculture industry would be the main potential sector playing a major role in fish and food production to meet the increasing demand of fish. However, even though tremendous efforts and planning have been put into developing aquaculture into a major and formidable industry, it will take some time to deliver the targeted production.

Among the catfishes, *Clarias spp.*, *P. hypophthalmus* and *Mystus nemurus* are cultured in Malaysia. Catfish culture began in the early 1960s with the culture of *C. batrachus* (Thalathiah, 1988). *Clarias macrocephalus* is cultured following the success of seed production in the mid 1980s (Thalathiah, 1988, 1990; Thalathiah *et al.*, 1990). At the same time, *C. gariepinus* became popular after introduced into this country (Thalathiah, 1998). The hybrid catfish (*C. macrocephalus* × *C. gariepinus*) is also cultured following its successful production in Thailand (Thalathiah & Ibrahim, 1991). The culture of *P. hypophthalmus* began in the early 1980s following the success of seed production in Thailand and the culture of *M. nemurus* is an even more recent development beginning only in the mid 1980s (Thalathiah, 1998).

In 2000, Malaysia's freshwater aquaculture production was 50,688 mt, which contributed 30.2% of the total aquaculture production. In the same year, the catfish production amounted 12,115 mt, which contributed 23.9% of the total freshwater aquaculture production. The main species of catfishes cultivated are the *Clarias spp.* The production rose from 4,175 mt in 1997 to 9,904 mt in 2000. The production of *P. hypophthalmus*, the next most important catfish cultured, increased from 837 mt in 1997 to 1,625 mt in 2000 (Department of Fisheries Malaysia, 1999, 2000).

In Malaysia, Production of *Pangasius spp.* was 1,625 mt and this amount contributed 3.2% of the total freshwater fish production in 2000 (Figure 1.1). In Sabah, the aquacultual production of *P. hypophthalmus* was 31 mt and this amount

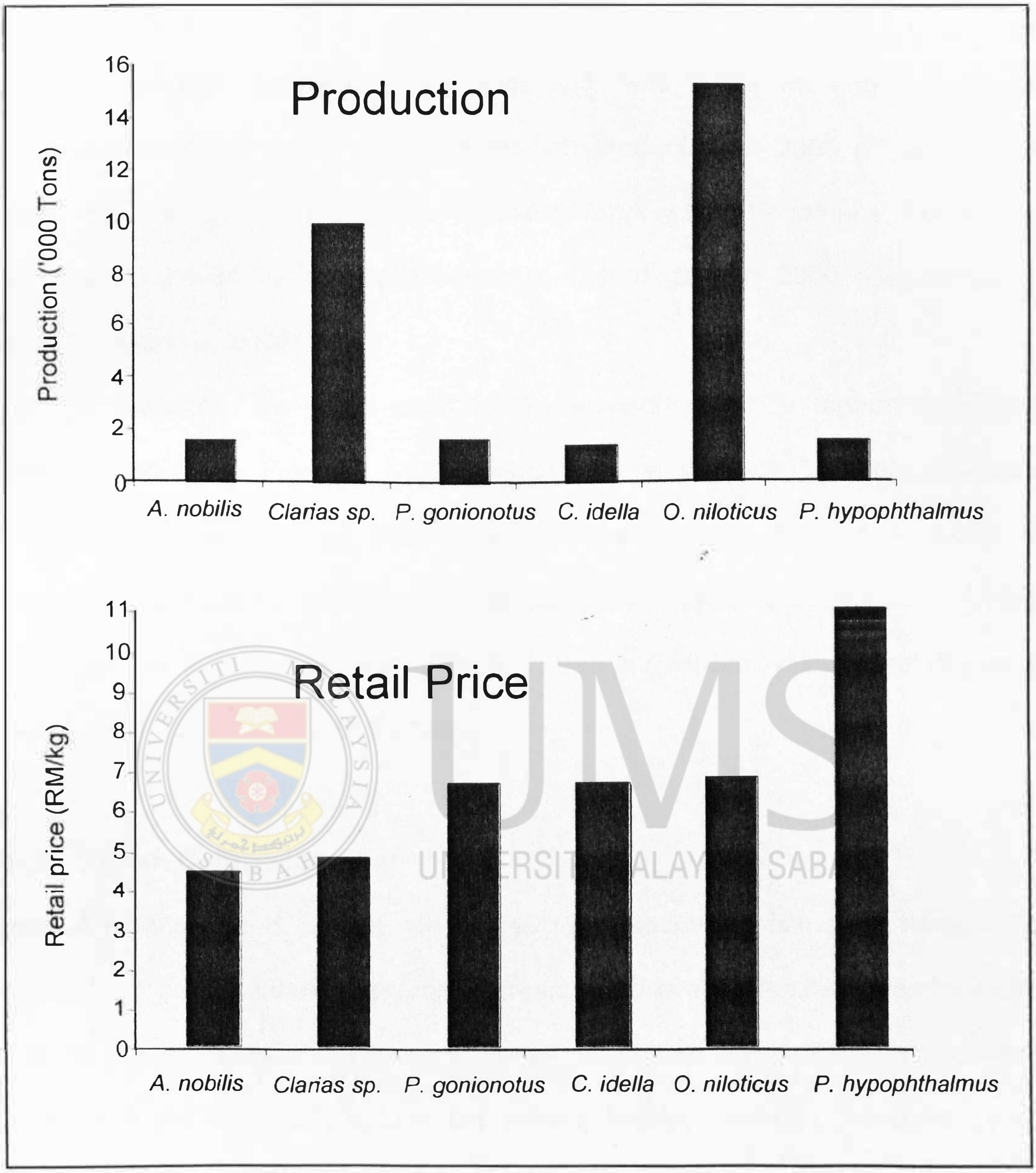

Figure 1.1: Comparison of production and retail price of some major freshwater fish in Malaysia in 2000 (Source: Annual Fisheries Statistics 2000, Volume 1, Department of Fisheries, Malaysia, 2000).