



UNIVERSITI PUTRA MALAYSIA

***EMBRYONIC AND EARLY LARVAL DEVELOPMENT OF SEVEN-LINE
BARB (*Probarbus jullieni* Sauvage 1880) AND LEMON FIN BARB HYBRID
(♂ *Hypsibarbus wetmorei* Smith 1931 X ♀ *Barbonymus gonionotus*
Bleeker 1849)***

MOHD HAZMADI BIN ZAKARIA

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By

MOHD HAZMADI BIN ZAKARIA

**Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia,
in Fulfillment of the Requirements for the Degree of Master of Science**

July 2015

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DEDICATION

To my lovely mom who always keep praying for me to achieve my goal

To my brothers and sisters

To all my colleagues

and

To all my friends who supported me all those past years



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Abstract of thesis was presented to the Senate of Universiti Putra Malaysia in fulfillment of the requirement for the degree of Master of Science.

EMBRYONIC AND EARLY LARVAL DEVELOPMENT OF SEVEN-LINE BARB (*Probarbus jullieni* Sauvage 1880) AND LEMON FIN BARB HYBRID (♂ *Hypsibarbus wetmorei* Smith 1931 X ♀ *Barbonymus gonionotus* Bleeker 1849)

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Embryonic and larval development of Temoleh, *Probarbus jullieni* and hybrid of Kerai ♂ (*Hypsibarbus wetmorei*) X Lampam Jawa ♀ (*Barbonymus gonionotus*) were investigated under laboratory condition. The matured eggs and sperms were collected by stripping each of the brood stocks species. Temoleh eggs were fertilized with a diluted concentration of normal milt of the same species while eggs of Lampam Jawa were fertilized with sperm of Kerai in two different experiments. The embryonic developing stages were observed in stages, beginning every 10 min intervals for the first hour, 20 min intervals at the second hour, 30 min for the next hour and then hourly intervals up to hatching. When hatching was completed, the observations were continued at 2-hour interval for the first day and at least every 6-hour interval for the following days. In *P. jullieni*, hatching started at 22-hour post-fertilization and fully completed within 25-hour at temperature ranged from 26.0 to 28.0 °C. The hatching of Kerai hybrid occurred at 14-hour after fertilization at temperature ranged from 23.0 to 25.0 °C. The yolk sac of the early larval development of *P. jullieni* was completely absorbed after 61-hour of hatching and the larvae started to swim actively and fed exogenously. The larvae of Kerai hybrid swam actively when the yolk sac became absent at 46-hour after hatching, and completed its early larval development. This study represents the first time description of the early development stages for *P. jullieni* and the hybrid of *H. wetmorei* ♂ X *B. gonionotus* ♀ in captive breeding condition. The valuable knowledge and information emerged from this designated studies will ultimately be useful towards understanding the reproductive biology of the two freshwater fishes. It will specifically enhance knowledge on seed production and larval rearing of the endangered *P. jullieni* and Kerai hybrid for aquaculture purpose.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Master Sains.

**PERKEMBANGAN EMBRIO DAN LARVAL AWAL BAGI TEMOLEH
(*Probarbus jullieni* Sauvage 1880) DAN HIBRID KERAI (♂ *Hypsibarbus
wetmorei* Smith 1931 X ♀ *Barbonymus gonionotus* Bleeker 1849)**

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Perkembangan embrio dan larva bagi ikan Temoleh, *Probarbus jullieni* dan hibrid Kerai ♂ (*Hypsibarbus wetmorei*) X Lampam jawa ♀ (*Barbonymus gonionotus*) telah dikaji dalam keadaan makmal. Telur dan sperma ikan yang matang diperolehi dengan cara melerek setiap spesies induk. Telur Temoleh disenyawakan dalam kepekatan yang sederhana cair dengan spesies yang sama manakala telur Lampam jawa disenyawakan dengan air mani Kerai dalam dua eksperimen yang berlainan. Peringkat perkembangan embrio diperhatikan secara berperingkat dengan selang 10 minit bagi jam yang pertama, selang 20 minit bagi jam yang kedua, selang 30 minit bagi jam yang seterusnya dan selang setiap jam sehingga menetas. Apabila penetasan selesai, pemerhatian diteruskan dengan selang 2 jam bagi hari pertama dan sekurang-kurangnya selang 6 jam untuk hari berikutnya. Bagi *P. jullieni*, penetasan bermula pada jam ke-22 selepas persenyawaan dan sepenuhnya selesai dalam masa 25 jam pada julat suhu dari 26.0 ke 28.0 °C. Penetasan bagi pembenihan hibrid Kerai berlaku pada 14 jam selepas persenyawaan pada julat suhu dari 23.0 ke 25.0 °C. Kantung telur bagi perkembangan awal larva *P. jullieni* diserap sepenuhnya selepas 61 jam penetasan di mana pada masa yang sama juga larva mula berenang secara aktif dan makan dengan cara menelan. Larva hibrid Kerai didapati berenang secara aktif bila kantung telur telah tidak kelihatan pada jam ke-46 selepas menetas, dan pada ketika ini proses perkembangan awal larva dianggap tamat. Kajian ini merupakan yang pertama kali menghurai peringkat perkembangan awal untuk *P. jullieni* dan hibrid *H. wetmorei* ♂ X *B. gonionotus* ♀ dalam pembiakan ternakan kurungan. Pengetahuan dan maklumat yang bernilai ini berguna dalam memahami biologi pembiakan kedua spesies ikan air tawar ini. Ia juga secara spesifiknya meningkatkan pengetahuan dalam pengeluaran benih dan pemeliharaan larva *P. jullieni* yang terancam dan hibrid Kerai untuk tujuan akuakultur.

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I certify that a Thesis Examination Committee has met on 02 July 2015 to conduct the final examination of Mohd Hazmadi Bin Zakaria on his thesis entitled “Embryonic and Early Larval Development of Seven-Line Barb (*Probarbus jullieni* Sauvage 1880) and Lemon Fin Barb Hybrid (♂ *Hypsibarbus wetmorei* Smith 1931 X ♀ *Barbonymus gonionotus* Bleeker 1849)” in accordance with Universities and University Colleges Act 1971 and the Constitution of the Universiti Putra Malaysia [P.U. (A) 106] 15th March 1998. The committee recommends that the candidate be awarded the relevant degree Master of Science.

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LIST OF ABBREVIATIONS

ANOVA	Analysis of variance
SD	Standard deviation
SE	Standard error
spp.	Species
DOF	Department of Fisheries
UPM	Universiti Putra Malaysia
DO	Dissolved oxygen
NaCl	Sodium Chloride
^o C	Degree Celsius
%	Percentage
MT	Matrix tone
kg	Kilograms
cm	Centimeters
mm	Millimeters
µm	Micrometer
h	Hour
min	Minutes
<	Less than
>	More than

CHAPTER 1

INTRODUCTION

The wild river carp, *Probarbus jullieni* (Sauvage, 1880) belonging to the family Cyprinidae, is one of the commercially important freshwater fish in Malaysia. It has been documented to be the largest species of cyprinids in Peninsular Malaysia (Mohsin and Ambak, 1983). The English names species are: Jullien's Golden Carp, Seven-stripped Barb, Seven-lined Barb, Price Carp and Isok Barb (Phuriphong and Ukkatawewat, 1992; Anonymous, 1994; Rainboth, 1996b; FishBase, 2012). In Malaysia, *P. jullieni* is commonly known as "Temoleh" or "Temelian". It has attracted considerable interest from fisheries scientists to conservationists due to its large size, high market price, excellent taste, endangered status and alleged migratory behavior (Suvatti, 1981; Roberts, 1993; Anonymous, 1994; Roberts and Baird, 1995; Mattson *et al.*, 2002; FishBase, 2012). In recent years, the stocks of *P. jullieni* have declined drastically from its natural habitats (such as lakes and rivers) due to unsustainable fishing pressure as well as habitat degradation, resulting from intensive development activities such as land clearing, deforestation and dam construction (Chew *et al.*, 2010). These factors have not only destroyed the breeding and feeding grounds but also caused destruction to the biodiversity of this important fish. Therefore, is a need for increasing concern on reproduction and conservation of the endangered fish, as the information on the early life history of a fish is very important for the optimization of its large-scale seed production, culture and management (Rahman *et al.*, 2004; Miah *et al.*, 2009).

Probarbus jullieni is currently listed as an endangered species in the Convention on the International Trade in Endangered Species (CITES), and also in the International Union for Conservation of Nature (IUCN) Red List of Threatened species (Appendix 1) (Baird, 2006). Considering its value, knowledge on breeding biology, reproduction and feeding are of prime importance for stock management of this species (McAllister *et al.*, 2000; Rahman *et al.*, 2011). Several studies on reproduction, especially on early life history of various endangered species have been reported (Mookerjee, 1945; Nakamura and Motonobu, 1971; Chakraborty and Murty, 1972; Rahman, 1975; Bruton, 1979; Boglione *et al.*, 1992; Tripathi, 1996; Haniffa *et al.*, 2003; Rahman *et al.*, 2009 and Mollah *et al.*, 2011). For *P. jullieni*, sperm cryopreservation study has been done by Chew *et al.* (2010) but till now, no published information on the early life history as well as described the embryonic and larval development of this species.

Silver barb (*Barbonymus gonionotus*) commonly known as 'Lampam jawa', is a highly popular freshwater food fish and constitutes an important aspect of inland fisheries in Malaysia. It is considered as an important tropical fish species due to its fast growth rate, palatability, easy and year-round reproduction, and adaptability to a wide range of culture conditions (Hussain *et al.*, 1989). It has been reported to be one of the most popularly cultured freshwater fish species in many parts of the world, especially in Southeast Asian countries (Alim *et al.*, 1998; Sarker *et al.*, 2002;

Bhuiyan *et al.*, 2006). In record, it was introduced to Bangladesh in 1977 (Rahman, 1989). The species is believed to be introduced to Peninsular Malaysia through movement of the Sumatran people migrating to Malaya during those pre-independence days. The production potentiality of *B. gonionotus* for culture in seasonal ponds, ditches and canals has already been proven and created a significant profitability in many countries of Southeast Asia including Bangladesh (Sarker *et al.*, 2002). In 2013, total landing of *B. gonionotus* from the fresh water resources in Malaysia was 114 MT while from the freshwater aquaculture production was 3,559 MT (DOF, 2013a and b).

Hypsibarbus wetmorei (Smith, 1931) locally known as ‘Kerai’ (Tan, 1980) or ‘Kerai Kunyit’ is one of the most important and high value carp fish in Malaysia (Chew and Zulkafli, 2012). The extensive aquaculture trials of *H. wetmorei* are widely observed in the region, but information on its present status is unclear (Vidthayanon, 2001). In addition, some biological data on the species related to its reproduction in the wild especially for spawning seasons and grounds have been reported (Poulsen *et al.*, 2002) and the investigation of the biology and the morphological development of the early life stages on the larvae and juveniles of this species has been reported by Ogata *et al.* (2010). Till date, no published information on the hybridization of *H. wetmorei* was reported while knowledge of recruitment success has thus far also been limited. Moreover, descriptions of embryonic and larval development are important in order to obtain a better understanding of the evolutionary ecology and phylogeny (Yamaguchi *et al.*, 2000). This information is essential for species identification and technical improvements in seed productivity. The current availability of this species in Malaysia mostly come from Pahang River resources and was seen to decrease along the year. The total landing of *H. wetmorei* species was recorded about 290 MT in 2012 and only 11 MT in 2013 (DOF, 2012 and 2013a).

Due to the biological, cultural and conservational higher values of *P. jullieni* and *H. wetmorei*, it is very important to develop the appropriate techniques for breeding and larval rearing in captive condition. Good quality juveniles are needed to establish a successful culture of the both species. The present study has therefore been undertaken with the following objectives:

- (i) to investigate the detailed embryonic and early larval development of Temoleh, *Probarbus jullieni* and,
- (ii) to examine the detailed embryonic and early larval development of hybrid Kerai (*Hypsibarbus wetmorei* ♂) and Lampam jawa (*Barbonymus gonionotus* ♀).

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