



UNIVERSITI PUTRA MALAYSIA

***BREEDING AND NURSERY CULTURE OF SEBARAU, *Hampala
macrolepidota****
(VAN HASSELT AND KUHL, 1823)

INTAN KHAIRULNISA BINTI ZAHARIN

FP 2017 22



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By

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

August 2015

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DEDICATION

To my deceased parents, Allahyarham Mej Zaharin Bin Dato' Md Zamani and Allahyarhamah Puan Khairani Binti Abdullahi who passed away last year and this year, may their gentle souls rest in peace, Amin.



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

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August 2015

Chairman: Annie Christianus, PhD
Faculty: Agriculture

Hampala macrolepidota or locally known as sebarau, is a native carp species of Malaysia, yet not a popular fish for aquaculture. This may be due to the lack of seed supply and proper nursery culture technique. This study was aimed to breed *H. macrolepidota* using Ovaprim, to observe its embryonic stages and to determine the effects of different salinity, pH, dietary protein and stocking density on the growth and survival of its fry.

For the first objective, induced breeding of *H. macrolepidota* was carried out with injection of 0.6 and 0.3 ml Ovaprim kg⁻¹ female and male, respectively. Results from the induced breeding shows that, *H. macrolepidota* with weight ranged from 180 - 280 g was able to produce 243,418 eggs/kg female. Mean of egg diameter for *H. macrolepidota* was 0.80 mm, while percentage of eggs fertilization was 22 % with majority of the eggs hatched within 24 h after fertilization. Embryonic development of *H. macrolepidota* lasted for about 24 h.

For the second objective, *H. macrolepidota* fry with initial body weight ranged from 0.35 – 3.00 g were used for experiments on salinities and pH for a period of 6 weeks. These experiments were conducted with 4 treatments (0, 5, 10 and 15 ppt) and pH also with 4 treatments (6.0, 6.5, 7.0 and 7.5). In both experiments, growth performance (weight and length increments) was determined through weekly sampling. Meanwhile survival and water quality were monitored throughout the study period. The highest percentages of survival for fry at salinity 0 -10 ppt and pH 6.0 -7.5 were 96.67% and 51.67%, respectively. Statistical analysis showed no significant differences ($p>0.05$) for survival, weight and length increments between all the treatments. The highest body weight and total length value were from salinity 0 ppt with the value of 2.50 g and 6.02 cm. As for pH experiment, there were slight differences for weight and length increments during the first three weeks of culture, however no significant differences

($p > 0.05$) were observed toward the end of the experimental period. The highest body weight value, 1.24 g was from pH 7.0 and highest total length of 5.00 cm from pH 6.0. The findings of this study showed that *H. macrolepidota* fry were unable to tolerate salinity above 15 ppt, while able to grow well in water with pH ranged from 6.5 to 7.5.

Finally, the third objective, experiments were conducted for 6 weeks to examine the effects of different dietary protein and stocking densities levels on the growth of *H. macrolepidota* fry with initial body weight ranged from 0.2 – 2.0 g. Dietary protein experiment was conducted with 3 treatments (23, 32 and 42% protein level) and stocking density also with 3 treatments (3, 4 and 5 fry/L water). In both experiments, survival and water quality were monitored throughout the study period and growth performance (weight and length increments) was determined through weekly sampling. For the dietary protein experiment, pellet with 32% protein produced the best growth performance for *H. macrolepidota* fry as compared to pellets containing 23 and 42% protein. Fry fed with pellet containing 23% protein showed the highest percentage of survival (76.67%). While fry fed with 23 and 32% of protein pellet showed significantly higher ($p < 0.05$) specific growth rate (SGR) than those fed with 42% of protein. In term of feed conversion ratio (FCR), fry fed with 23% protein pellet showed significantly higher ($p < 0.05$) FCR than those fed with 32 and 42% protein content. Fry fed with 32 and 42% showed significantly higher ($p < 0.05$) protein efficiency ratio (PER) than those fed with 23% protein. As for proximate composition of the feed, no significant difference ($p > 0.05$) were observed for crude fibre, moisture and energy between treatments except for the crude protein, ash and NFE. For lipid, pellet with 23 and 32% of protein showed significantly higher ($p < 0.05$) lipid content than pellet with 42% protein content. As for stocking density experiment, 3 fry/L showed the highest percentage of survival (100%). However, there was no significant different ($p > 0.05$) between all the treatments. The highest body weight and total length was observed in fry stocked at 5 fry/L. Slight differences were observed in body weight during the second week of culture and in length increments during the first week of culture. However, no significant differences ($p > 0.05$) were observed between treatment for the first week (body weight) and towards the end of the experimental period (body weight and total length).

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

PEMBIAKAN DAN KULTUR NURSERI IKAN SEBARAU, *Hampala macrolepidota* (VAN HASSELT DAN KUHL, 1823)

Oleh

INTAN KHAIRULNISA BINTI ZAHARIN

Ogos 2015

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Hampala macrolepidota atau dikenali sebagai sebarau adalah spesis peribumi di Malaysia yang masih tidak begitu popular untuk akuakultur. Ini mungkin kerana kekurangan bekalan benih ikan dan teknik kultur nurserinya. Justeru itu, kajian ini dijalankan dengan tujuan untuk membiak *H. macrolepidota* dengan menggunakan hormon Ovaprim, memerhatikan peringkat embrionya dan untuk menentukan kesan saliniti, pH, protein pemakanan dan kepadatan stok ikan terhadap tumbesaran dan kemandirian fri ikan tersebut.

Untuk objektif pertama, pembiakan aruhan *H. macrolepidota* telah dijalankan dengan suntikan 0.6 ml Ovaprim kg⁻¹ bagi betina dan 0.3 ml Ovaprim kg⁻¹ bagi jantan. Hasil daripada eksperimen ini menunjukkan, *H. macrolepidota* dengan berat berjulat 180 - 280 g mampu mengeluarkan 243,418 telur/kg daripada seekor induk betina. Purata diameter telur *H. macrolepidota* adalah 0.80 mm, manakala kadar persenyawaan telur adalah 22% dengan kebanyakan telur menetas dalam tempoh 24 jam selepas disenyawakan. Perkembangan embrio *H. macrolepidota* mengambil masa lebih kurang 24 jam.

Untuk objektif kedua, fri *H. macrolepidota* dengan berat badan berjulat 0.35 - 3.00 g telah digunakan untuk eksperimen saliniti dan pH bagi tempoh 6 minggu. Eksperimen saliniti dijalankan dalam 4 rawatan (0, 5, 10 dan 15 ppt) dan eksperimen pH juga dalam empat rawatan (6.0, 6.5, 7.0 dan 7.5). Untuk kedua-dua eksperimen ini, prestasi pertumbuhan (berat dan pertambahan panjang) telah ditentukan melalui pensampelan mingguan. Manakala, kemandirian dan kualiti air dipantau sepanjang tempoh kajian. Peratusan kemandirian fri yang paling tinggi pada saliniti 0 -10 ppt, dan pH 6.0 -7.5, adalah 96.67% dan 51.67% masing-masingnya. Analisis statistik tidak menunjukkan perbezaan ketara ($p > 0.05$) untuk kemandirian, berat dan panjang fri antara semua rawatan. Berat badan dan panjang tertinggi yang dicatatkan untuk saliniti adalah pada 0

ppt dengan nilai 2.50 g dan 6.02 cm. Bagi eksperimen pH, terdapat sedikit perbezaan untuk pertambahan berat dan panjang untuk tiga minggu pertama pengkulturan, bagaimanapun tiada perbezaan ketara ($p>0.05$) yang diperolehi di akhir tempoh eksperimen. Berat badan tertinggi adalah pada pH 7.0 dengan nilai 1.24 g dan panjang tertinggi pada pH 6.0 dengan nilai 5.00 cm. Hasil kajian menunjukkan bahawa fri *H. macrolepidota* tidak dapat bertahan pada tahap kemasinan lebih dari 15 ppt, manakala ianya mampu membesar dengan baik dalam julat pH 6.5 hingga 7.5.

Akhir sekali, objektif ketiga, eksperimen dijalankan selama 6 minggu untuk menguji kesan protein pemakanan dan kepadatan stok ke atas tumbesaran fri *H. macrolepidota* dengan berat awal 0.2 – 2.0 g. Protein pemakanan dijalankan untuk 3 rawatan (23, 32 dan 42% paras protein) dan kepadatan stok, juga 3 rawatan (3, 4 dan 5 fri/L air). Dalam kedua-dua eksperimen, kemandirian dan kualiti air dipantau sepanjang tempoh kajian dan prestasi pertumbuhan (berat dan pertambahan panjang) telah ditentukan melalui persampelan mingguan. Untuk eksperimen protein pemakanan, pelet dengan 32% protein menghasilkan tumbesaran yang terbaik untuk fri *H. macrolepidota* berbanding dengan pelet 23 dan 42% protein. Fri yang diberi makan pelet yang mengandungi 23% protein memberikan kemandirian tertinggi (76.67%). Manakala fri yang diberi makan pellet dengan 23 dan 32% protein menunjukkan perbezaan yang ketara ($p<0.05$) untuk kadar pertumbuhan tertentu (SGR) berbanding dengan pelet 42% protein. Untuk kadar pertukaran makanan (FCR), fri yang diberi makan pelet mengandungi protein 23% menunjukkan FCR yang lebih tinggi ($p<0.05$) berbanding dengan pelet yang mengandungi 32 dan 42% protein. Manakala, untuk fri yang diberi makan pelet yang mengandungi 32 dan 42% protein menunjukkan nisbah kecekapan protein (PER) yang ketara lebih tinggi ($p<0.05$) berbanding dengan pelet mengandungi 23% protein. Komposisi proksimat untuk makanan, tidak menunjukkan perbezaan ketara ($p>0.05$) untuk serat mentah, lembapan dan tenaga antara rawatan kecuali untuk protein, abu dan NFE. Untuk lipid pula, pelet yang mengandungi 23 dan 32% protein menunjukkan kandungan lipid yang ketara lebih tinggi ($p<0.05$) berbanding dengan pelet yang mengandungi 42% protein. Untuk eksperimen kepadatan stok, 3 fri/L memberikan peratusan kemandirian tertinggi (100%). Walaubagaimanapun, tiada perbezaan ketara ($p>0.05$) untuk kadar kemandirian untuk semua rawatan. Berat badan dan panjang yang tertinggi adalah pada kepadatan 5 fri/L. Terdapat sedikit perbezaan dalam pertambahan berat badan bagi fri yang dikultur pada minggu kedua dan dalam pertambahan panjang badan fri pada minggu pertama pengkulturan. Walaubagaimanapun, tidak terdapat perbezaan ketara ($p>0.05$) di antara rawatan untuk minggu pertama (berat badan) sehingga ke akhir tempoh eksperimen (berat badan dan panjang badan).

ACKNOWLEDGEMENTS

Foremost, I would like to express my gratitude to ALLAH S.W.T. and great blessing for giving me the strength and patience in completing this research. I also like to thank my family especially my late father, Allahyarham Mej Zaharin Bin Dato' Md Zamani and my late mother, Allahyarhamah Puan Khairani Binti Abdullah for their unconditional support and love during the research time. A special thanks to my siblings, Intan Zuraazni, Intan Khairulazrin, Intan Zukhaireen and Muhammad Zayyani for their understanding and unconditional love. Without my family, I would never have the strength to finish this research.

In addition, I would also like to take this opportunity to thank all staffs of Aquaculture Extension Center Perlok, Department of Fisheries in Jerantut, Pahang, Aquaculture Department (UPM), Malaysian Technology Development Corporation (MTDC) laboratory, Putra Science Park, UPM and Fish Hatcheries Centre of UPM in Puchong: Mr. Muhamad Hatta Hj. Mahmud, Mr. Mohamad Nazri Puasa, Mr. Hairul Effendy Adzmi, Mr. Jasni Mohd Yusoff, Mr. Azmi Yaacob, Mr. Roszainal Yusop, Mrs. Shafika Maulad Abd. Jalil and Mr. Muhammad Farhan Nazarudin, who encouraged me to complete this independent study, who were always able to put a smile, guide and teach me and also kindness in lending their hand during the research.

There are a few friends in particular that I would like to say a special thank for always helping me to look on the bright side. I honestly don't think I could have made it through this without their helping, advice, support and made me sitting through the research. This is dedicated to Afzan Muntaziana Mohd Pazai, Mohamad Faizul Mat Isa, Eng Hueh Theng, Zaaim Zahari, Farhana Affandi, Mursyida Ayob, Sairatul Dahlianis Ishak, Syahida Ahmad, Saufinas Ismail, Zamri Zainudin, Dayana Dimiyati and Zubir Zainudin.

Most importantly I would like to thank my supervisor, Dr. Annie Christianus, who had help me accomplish effort that at first seemed impossible and specifically, I would like to thank her for instilling a sense of confidence within me. Without this self-assurance I would not have been able to persevere and ultimately accomplish such an incredible task. I owe my deepest gratitude to my co-supervisor, Dr. S.M. Nurul Amin for generating ideas of the whole research, provide comments and help me in interpretation of results. Last but not least, a special acknowledgement to those who involve directly and indirectly in producing this research.

I certify that a Thesis Examination Committee has met on 24 August 2015 to conduct the final examination of Intan Khairulnisa Binti Zaharin on her thesis entitled “Breeding And Nursery Culture Of Sebarau, *Hampala macrolepidota* (Van Hasselt And Kuhl, 1823)” in accordance with the Universities and University Colleges Act 1971 and the Constitution of the Universiti Putra Malaysia [P.U.(A) 106] 15 March 1998. The Committee recommends that the student be awarded the Master of Science.

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LIST OF ABBREVIATIONS

ANOVA	Analysis of variance
cm	Centimetre
DMRT	Duncan's Multiple Range Test
DOF	Department of Fisheries
FAO	Food and Agricultural Organization
FCR	Feed conversion ratio
g	gram
hr	hour
IM	Intramuscular injection
L	Litre
mL	Millilitre
mm	Millimetre
MTDC	Malaysian Technology Development Corporation
PER	Protein efficiency ratio
pH	potential Hydrogen
ppt	part per thousand
SGR	Specific growth rate
TL	Total length
Wt	Weight
°C	Degree Celsius
%	Percentage

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CHAPTER 1

INTRODUCTION

1.1 Background of the Study

1.1.1 Economic Status of Aquaculture

Since 1920's, aquaculture sector has developed rapidly in Malaysia. It started with freshwater then continues with brackish water culture in the late of 1930 (FAO, 2011). However, this sector is small when compared to neighboring countries such as Indonesia and Thailand (Tan, 1998). Limited supplies of suitable fish seed is the main reason why aquaculture potential has not been fully realized in Malaysia.

Fishery sector supplies one of the protein source and at the same time assists in the rural development through creation of employment in Malaysia (Safa, 2004). Fisheries statistic showed that this sector has contributed RM11, 440.31 million to the nation's economy in 2012 compared to 2011 by showing increases of 13.61%. Moreover, production of food fish from the aquaculture sector was 302, 886.32 tonnes with valued at RM2, 559.17 million in 2012. This showed an increase of 5.76 and 7.27% respectively compared to 2011. As for freshwater fish culture, it has contributed of 163,756.81 tonnes valued at RM992.39 million in 2012. The production and value showed an increase of 33.99 and 45.05 % respectively when compared to 122,218.73 tonnes valued at RM684.15 million in 2011. This information indicates that aquaculture sector has potential to become the main industry to contribute to the economy. Furthermore, fishing was listed as a secondary occupation (Keskinen, 2003; Hap *et al.*, 2006).

1.1.2 Hormone Usage in Aquaculture

Some species of fish show an extended breeding season and it is desirable to control the timing to synchronize of the breeding process. The most effective and reliable solution for ensuring the availability of good quality fish seed all the year around and sustainable for the aquaculture industry is through induced breeding. This technique includes the use of synthetic hormones to induce ovulation and spawning of farmed fishes (Viveen *et al.*, 1985). Hence, there are few hormone used in aquaculture. For example, culturists utilized carp pituitary (CP) gland, human chorionic gonadotropin (HCG), luteinizing hormone-releasing hormone analogue des-Gly10 [D-Ala6] LHRH Ethylamide (LHRHa), Ovotide and Ovaprim.

Primarily, farmer used carp pituitary (CP) to induce breed fish and it is still widely used especially for the Chinese carps, Indian carps and the common carp *Cyprinus*

carpio (Lam, 1983; Park *et al.*, 1994). HCG has been found to be useful in inducing final maturation of oocytes, and widely used in commercial aquaculture (Mylonas *et al.*, 2009; Park *et al.*, 1994; Donaldson and Hunter, 1983; Park *et al.*, 1997; Kelly and Kohler, 1994). As for LHRHa, the function of this hormone is to induce final maturation and synchronize ovulation of many commercial fish species (Donaldson and Hunter, 1983; Park *et al.*, 1997).

Ovaprim and Ovotide are products which contained salmon gonadotropin releasing hormone analogue (sGnRH_a) with a dopamine blocker (Syndel International Inc., 2003). Salmon gonadotropin releasing hormone analogue resulted in successful ovulation of some of cyprinids (Drori *et al.*, 1994; Glasser, 2004; Rutaisire and Booth, 2004; Hill *et al.*, 2005) and catfishes (Sahoo *et al.*, 2007). These hormones are usually administered through intramuscular or intraperitoneal injection. Ovaprim has been used on a various type of fishes for aquaculture production (Mohd-Zaini *et al.*, 1994; Ingram *et al.*, 2005), research (Pinillos *et al.*, 2002; Viveiros *et al.*, 2002; Hill *et al.*, 2005), veterinary purposes (Yanong *et al.*, 2003) and conservation (Van Eenennaam *et al.*, 2001; Sarkar *et al.*, 2006). Overtime, Ovaprim has proven to be very effective, thus became widely accepted synthetic hormones (Nwokoye *et al.*, 2007; Olubiyi *et al.*, 2005). Ovaprim also is effective at room temperature and it is more eminent compared to carp pituitary extract in inducing fish to breed (Osman *et al.*, 2012). By using Ovaprim, fertilization and hatching rates are higher and larger size of eggs after hardening process. It's also more conserved than pituitary extract (Surnar *et al.*, 2015).

1.2 Problem Statement

Artificial propagation is an alternative way to increase fry production. It was first elaborated in 1765 and has been distributed to many parts of Europe, America, China, Japan and Israel (Omoniyi *et al.*, 2013). During the rainy season in the tropical river system, majority of the fishes breed (Alkins - Koo, 2000; Ballesteros *et al.*, 2009), but there are few fish breed during the dry season (Pusey *et al.*, 2002; Torres-Mejia & Ramírez-Pinilla, 2008) or throughout the year (Alkins-Koo, 2000). For *Hampala macrolepidota*, spawning may not occur due to several factors, such as physicochemical and environmental conditions (Abidin, 1986). Besides sunlight and temperature, changing of water level cause by the monsoonal rains can damage the spawning time on some species (Schields, 1957; Siddique *et al.*, 1976).

Until now, *H. macrolepidota* is the most popular among fish consumer and aquaculturist as ornamental and recreational fishes and also as food fish in Malaysia. However, the presence of its culture is very much limited due to the lack fry supply, which dependant on hatchery productions and wild seed. The production of freshwater fish fry and hatchlings from government hatcheries in Malaysia for 2014 were 12, 797, 586 pieces for fry and 4, 729, 349 pieces for hatchling. While for *H. macrolepidota*, the production of its fry from Aquaculture Extension Centre in Perlok, Jerantut, Pahang has declined from 57, 000 pieces in 2013 (DOF, 2013) to 50, 300 pieces in 2014 (DOF, 2014).

Since this fish is a seasonal species that breed naturally, the production in the hatchery is insufficient and inconsistent. Presently, little information is available on its artificial reproduction (Ambak *et al.*, 1982; Rosli, 1987) and culture (Aizam and Ang, 1984). To date, with no documented report on the embryonic development and culture requirements. Therefore, further study should be carried out for the successful breeding, larval rearing and culture of *H. macrolepidota*.

1.3 Objectives

Thus, the objectives of this study were:

1. to induced breed *Hampala macrolepidota* using Ovaprim, observe and describe its embryonic stages;
2. to determine the growth performances of the resulted *H. macrolepidota* fry culture at different salinity and pH;
3. to determine the growth performance of the *H. macrolepidota* fry fed with different dietary protein and cultured at different stocking density

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