EMBRYONIC DEVELOPMENT AND NUTRITIONAL REQUIREMENTS OF KUTUM FRY, *RUTILUS FRISII KUTUM*

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BY

DAVOUD TALEBI HAGHIGHI

Thesis Submitted to the School of Gaduate Studies, Universiti Putra Malaysia, in Fulfilment of the Requirement for the Degree of Doctor of Philosophy

August 2006

DEDICATION

To my parents

For their true love, constant trust and helps

To my wife

For her kindness, devotion and support during all difficulties

To my children

For their undetstanding, dignities and tolerance

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

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Chairman: Associate Professor Che Roos Saad, PhD

Faculty: Agriculture

This study consisted of a series of experiments that were conducted to determine some biological characteristics, artificial reproduction, embryonic development, optimal level of protein and lipid in artificial diets and food regime spectrum in the stage of secondary nursing of kutum fish larvae (*Rutilus frisii kutum*).

The above study was carried out with artificial reproduction to determine some biological characteristics such as: age, weight, length, percentages of ovary weight to broodstock weight, and also estimation of absolute and relative fecundity and evaluated their relationships in kutum fish broodstock. The results of this study showed that the optimal ratio of female to male was 1:2 (♀ :♂), the first sexual maturity occurred at 2 year old with well developed sexual products with length ranging from 25 cm to 48cm and weight ranging from 213.5 g to 1588

g. Most age, length and weight–frequencies belonged to age groups III^+ (23.20%) to IV^+ (20%). The results of this study indicated that the absolute fecundity increased as the fish grows, ranging from 12022 to 78856 eggs per fish; on the contrary, relative fecundity of fish decreased with the increasing of fish age and it ranged from 67 to 42 egg for per g of ovary for age groups II^+ to VII^+ respectively. Correlation coefficient for absolute fecundity against length, weight and age were observed to be r=0.76, r=0.68 and r=0.73 respectively, but the correlation coefficient between relative fecundity against length, weight and age were lower with values of r=0.0.37, r=0.30 and r=0.33 respectively.

The stripping of eggs was performed in semi-dry condition. Males and females were injected with carp pituitary gland extracts (GnRh) to induce ovulation at 2 mg of GnRh per kilogram of broodstock body weight. Seven period of embryogenesis were defined; **I)** the newly fertilized egg was zygote, occurred with 1 cell, with an average egg diameter of 2.21 mm, and mean weight of 9.99 mg and lasted for 15 min. **II)** Cleavage stage began from 2 to 64 divided cells, with egg diameter of 2.715 mm, weight at 12.99 mg, in 7 hours. **III)** Blastula stage was made up of early epiboly at the animal pole, with egg diameter 2.8 mm, with weight of 13.51 mg, and time of 26 hours. **IV)** Gastrula stage was formed when two layers of cells including epiblast which gave rise to ectoderm tissues and hypoblast which gave rise to both mesoderm and endoderm; consequently, the embryonic axis and epiboly were formed at the 90% of gastrulation period. It lasted for 33 h, with egg diameter of 2.96 mm and egg weight of 13.61 mg. **V)** Segmentation stage began with the formation of the

primary organs; somites, early organogenesis, neuromeres, elongation of tail and earliest movement appeared. It lasted for 29 h, with diameter of egg at 3.04 mm, weight egg of 13.24 mg. **VI)** Organogenesis occurred with rapid development towards functionality in most organs, particularly the senses, the heart and the gut. It lasted for 15 h with egg diameter of 3.16 mm and weight of 14.64 mg. **VII)** Hatching ended when the larvae were pelagic and avoids sinking. It lasted 20 h after organogenesis with diameter egg 3.009 mm and weight 14.39 mg. Total times for hatching was 130 hours and 15 minutes.

Food regime spectrum of kutum fry at the secondary nursing under pond culture condition was estimated based on their stomach contents analysis. During the rearing period water temperature varied from 16 °C to 28 °C. In general, the frequency and diversity of phytoplankton included 5 phylums, 10 orders, 21 families and 36 genuses and zooplankton community included 5 phylums, 10 orders, 12 families and 31 genuses were obtained from gut contents analysis of kutum fry. Observation was carried out

Observation was carried out periodically for 11 weeks, and it was found that kutum fry collected its food from below to surface of water. The results of this study indicated that kutum fry had low growth and high mortality at the early stage of rearing period. When the intestinal digestion system became more developed, the utilization of greater diversity of food items increased. The feeding regime spectrum of kutum fry consisted of phytoplankton, zooplankton, benthos, detritus and artificial food.

Determination of optimal level of crude protein in kutum fry diet was conducted with five levels of protein concentrations (35%, 40%, 45%, 50% and 55%). The results showed significant difference among 5 concentrations on growth of fish (p<0.05). The best final weight was 1095 mg, weight gain of 877.3%, specific growth rate of 3.21%, protein efficiency ratio of 1.00, food conversion ratio of 1.99, and at 28% mortality obtained at 50% protein level diet. Polynomial regression analysis between protein levels and weight gain of fish indicated that a weight gain peak was obtained at 46.8% protein level. Results of this experiment showed that the optimum protein level of kutum larvae for best growth was 46.8%.

Determination of optimal level of crude lipid in kutum fish fry diet was conducted with four levels of lipid concentrations (8%, 10%, 12% and 14%). The results showed significant difference among lipid levels on growth of fish (P< 0.05). The highest final weight was 1926 mg, weight gain 852%, specific growth rate 2.78%, protein efficiency ratio 1.07, food conversion ratio 2.05 and mortality 2.5% obtained when dietary lipid level was 8%. Polynomial

regression analysis between lipid levels and weight gain of fish indicated that a weight gain peak was at 7.67% lipid level. Result of this experiment showed that the optimum level lipid of kutum larvae for best growth was at 7.67% lipid level.

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

PERKEMBANGAN EMBRIO DAN KEPERLUAN NUTRIEN FRI KUTUM, *RUTILUS FRISII KUTUM*

Oleh

DAVOUD TALEBI HAGHIGHI

August 2006

Pengerusi: Profesor Madya Che Roos Saad, PhD

Fakulti: Pertanian

Satu siri eksperimen telah dijalankan untuk menentukan beberapa ciri biologi, pembiakan aruhan, perkembangan embrio, paras protein dan lipid yang optimum dalam diet buatan dan spektrum makanan pada peringkat penjagaan sekunder bagi fri ikan kutum (*Rutilus frisii kutum*).

Kajian di atas telah dijalankan menggunakan pembiakan aruhan untuk menentukan ciri-ciri biologikal seperti**:** umur, berat, panjang kelas, peratus berat ovari kepada berat ikan induk, anggaran fekunditi absolut dan fekunditi relatif serta menilai perhubungan tersebut terhadap induk ikan kutum. Hasil kajian menunjukkan bahawa nisbah optimum induk spesis jantan kepada betina adalah sama 1:2 **(**♀**:**♂**)**; kematangan seksual paling awal berlaku pada umur 2 tahun dengan pembentukan produk seksual yang matang sehingga umur 7 tahun, dengan panjang ikan dalam lingkungan 25cm ke 48cm dan berat

daripada 213.5 g ke 1588 g. Kebanyakan umur, panjang dan kekerapan berat tergolong dalam kumpulan umur III^{+} – IV^{+}. Hasil kajian ini juga menyatakan bahawa fekunditi keseluruhan semakin meningkat mengikut pembesaran ikan iaitu daripada 12022 kepada 78856 bilangan telur bagi setiap ikan atau ovari. Walau bagaimanapun, fekunditi relatif ikan semakin berkurang dengan peningkatan umur ikan dan termasuk dalam lingkungan 67 ke 42 telur sehari bagi setiap gram ovari bagi kumpulan umur tersebut, masing-masing dalam lingkungan II^{+} ke VII^{+} setiap satu. Pekali koefisien korelasi antara fekunditi keseluruhan terhadap panjang, berat dan umur adalah r=0.76, r=0.68 dan r=0.73 masing-masing, tetapi pekali korelasi antara fekunditi relatif terhadap panjang, berat dan umur adalah lebih rendah iaitu r=0.37, r=0.30 and r=0.33 masing-masing.

Proses melurut telur ikan telah dijalankan dalam keadaan separa kering. Ikan jantan dan betina telah disuntik dengan ekstrak daripada kelenjar pituitari sebanyak 2 mg GnRh setiap kilogram berat ikan untuk mempercepatkan kematangan. Sebanyak 7 peringkat pembentukan embrio yang terlibat telah didefinasikan sebagai: I) **Zigot**, telur baru yang telah disuburkan, terbentuk dengan 1 sel dan diameter purata telur adalah 2.21 mm, min berat sebanyak 9.99 mg dan mengambil masa sehingga 15 min. II) Peringkat ***Cleavage*** bermula daripada pembahagian sel daripada 2 hingga 64, dengan diameter telur sebanyak 2.715 mm, berat sebanyak 12.99 mg, dalam masa 6 jam. III)

Peringkat **Blastula** terdiri daripada *epiboli* awal pada kutub haiwan, dengan diameter telur sebanyak 2.8 mm, berat sebanyak 13.51 mg, selama 26 jam. IV) Peringkat **Gastrula** pula terbentuk apabila 2 lapisan sel termasuklah epiblast yang telah bertukar kepada tisu ektoderma dan *hipoblast* yang juga telah bertukar kepada tisu mesoderma dan endoderma; pada masa yang sama paksi embrionik telah dibentuk dan *epiboli* terbentuk pada 90% proses grastulasi. Ia berlangsung selama 33 jam, dengan diameter telur sebanyak 2.96 mm dan berat telur sebanyak 13.61 mg. V) Peringkat **Segmentasi** bermula dengan pembentukan organ-organ primer, *somit*, organogenesis awal, *neuromeres*, pemanjangan ekor dan berlakunya pergerakan awal. Ia berakhir dalam masa 29 jam, dengan diameter dan berat telur sebanyak 3.04 mm dan 13.24 mg masing-masing. VI) **Organogenesis** berlaku dengan perkembangan yang pantas ke arah kefungsian sebahagian besar organ, terutamanya organ sensori, jantung dan gastrousus. Ia berakhir selama 15 jam dengan diameter dan berat telur sebanyak 3.16 mm dan 14.64 mg. VII) **Penetasan** berakhir apabila larva bersifat *pelagik* dan dapat mengelak dari tenggelam. Proses ini berlaku dalam tempoh 20 jam selepas organogenesis dengan diameter telur sebanyak 3.009 mm dan berat sebanyak 14.39 mg. Tempoh penetasan keseluruhan adalah selama 129 jam 25 min. Spektrum makanan fri ikan kutum pada penjagaan sekunder di bawah keadaan kultur kolam telah dianggarkan berdasarkan analisis kandungan perut ikan. Semasa tempoh pemeliharaan, suhu air dan keadaan cuaca berbeza antara 16°C dan 28°C. Secara amnya, kekerapan dan kepelbagaian fitoplankton termasuklah 5 filum, 10 order, 21 famili dan 36 genus sementara komuniti zooplankton pula terdiri daripada 5 filum, 10 order, 12 famili dan 31 genus yang

diperolehi daripada analisis kandungan perut fri ikan kutum. Pemerhatian telah dijalankan secara berkala setiap 2 minggu selama 11 minggu, dan didapati bahawa fri ikan kutum mendapatkan makanan dari bahagian bawah sehinggalah ke permukaan air. Spektrum rejim pemakanan bagi fri ikan kutum terdiri daripada fitoplankton, zooplankton, benthos, detritus dan makanan tiruan. Hasil kajian mendapati bahawa fri ikan kutum mempunyai kadar tumbesaran yang rendah dan kadar kematian yang tinggi pada peringkat awal tempoh penternakan. Apabila sistem penghadaman usus terbentuk, penggunaan jenis-jenis makanan yang berbeza dan pelbagai dipertingkatkan.

Penentuan paras optimum kandungan protein kasar dalam diet fri ikan kutum telah dijalankan mengikut lima kepekatan protein (35%, 40%, 45%, 50%, 55%) yang menunjukkan perbezaan yang signifikan terhadap pertumbuhan ikan ($P< 0.05$). Nilai terbaik bagi berat akhir, pertambahan berat, kadar pertumbuhan spesifik, nisbah kecekapan protein, nisbah pertukaran makanan dan kematian adalah 1095 mg, 877.3 %, 3.21 %, 1.00 %, 1.99 dan 18 % masing-masing apabila diberi diet yang mengandungi kandungan protein sebanyak 50 %. Analisis regresi polinomial di antara paras protein dan pertambahan berat badan ikan menunjukkan bahawa puncak pertambahan berat ikan adalah pada paras protein 46.8%. Keputusan eksperimen ini menunjukkan bahawa paras protein diet yang optimum untuk pertumbuhan larva ikan kutum yang terbaik adalah pada 46.8%.

Penentuan paras optimum kandungan lemak kasar dalam diet fri ikan kutum telah dijalankan mengikut empat kumpulan dengan kepekatan (8%, 10%, 12%, 14%) yang menunjukkan perbezaan yang signifikan terhadap pertumbuhan ikan ($P< 0.05$). Nilai tertinggi bagi berat akhir, pertambahan berat, kadar pertumbuhan spesifik, nisbah keberkesanan protein, nisbah pertukaran makanan dan kematian adalah 1926 mg, 852%, 2.78%, 1.07, 2.05 dan 2.5% masing, yang diperolehi pada paras lipid diet adalah 8 %. Analisis Regresi Polinomial di antara paras lipid dan pertambahan berat badan ikan menunjukkan bahawa puncak pertambahan berat ikan adalah pada paras lipid 7.67%. Keputusan eksperimen ini menunjukkan bahawa paras lipid diet yang optimum untuk pertumbuhan larva ikan kutum yang terbaik adalah pada 7.67%.

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I certify that an Examination Committee has met on 9th August 2006 to conduct the final examination of Davoud Talebi Haghighi on his Doctor of Philosophy thesis entitled "Embryonic Development and Nutritional Requirements of Kutum Fry, *Rutilus frisii kutum*" 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 and has been accepted as fulfilment of the requirement for the degree of Doctor of Philosophy. The members of the Supervisory Committee are as follows:

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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 UPM or other institutions.

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DAVOUD TALEBI HAGHIGHI

Date:

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A	Anal
AF	Absolute Fecundity
ANOVA	Analysis of Variance
AP	Anterior- Posterior
Cm	centimeter
CF	Condition Factor
CL	Crude Lipid
CP	Crude Protein
CRD	Complete Randomized Design
D	Dorsal
DEL	Deep Cell Layer
DMRT	Duncan Multiple Range Test
DMSO	Dimethyl Sulfoxide
DO	Dissolved Oxygen
DV	Dorsal- Ventral
DW	Dry Weight
Epine	Epinephalus
ECE	Energy Conversion Efficiency
EPL	Early Postlarvae
EFA	Essential Fatty Acid
FCR	Feed Conversion Ratio
G	gram

GnRh	Gonadotropin hormone
H	Height
H	hours
HUFA	Highly Unsaturated Fatty Acids
Kg	Kilogram
K cal	Kilocalorie
L. L	Late larvae
L	Liter
L	Length
L^3	Cubic Length
LINDO	Linear Interactive and Discrete Optimizer
LP	Linear Programming
M	meter
m^2	Square meter
mm^2	Square millimeter
m^3	cubic meter
MBW	Mean Body Weight
MD	Mean Development
Mg	milligram
Min	minutes
Mm	millimeter
Ml	milliliter
Mm	micronmeter
MUFA	Monounsaturated fatty acids

NaOH	Sodium hydroxide
NRC	National Research Council
PER	Protein Efficiency Ratio
PCE	Protein Conversion Efficiency
PL	Postlarval
P/L	Protein/ Lipid ratio
PS	Protein Sparing
PUFA	Polyunsaturated Fatty Acids
RF	Relative Fecundity
R	regression
SD	Standard Deviation
SE	Standard Error
SFA	Saturated Fatty Acids
SGR	Specific Growth Rate
SR	Survival Rate
W	Weight
WG	Weight Gain
YS	Yolk Sac
YSL	Yolk Syncytial Layer