

UNIVERSITI PUTRA MALAYSIA

EARLY DEVELOPMENT OF Rutilus frisii kutum KAMENSKII LARVAE WITH EMPHASIS ON THE ONTOGENY OF DIGESTIVE TRACT

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Thesis submitted to the School of Graduates Studies, Universiti Putra Malaysia, in Fulfilment of the Requirements for the Degree of Doctor of Philosophy

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DEDICATION

I would like to dedicate this thesis with love to

The memory of my father "MOHAMMAD TAGHI JAFARI"

To keep his spirit alive

My dear mother Shokat Farah Avar

My adorable brother,

Mohammad

and

my darling sister,

Erfaneh

for their love, constant truth and assistance during my difficulties

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

EARLY DEVELOPMENT OF *Rutilus frisii kutum* KAMENSKII LARVAE WITH EMPHASIS ON THE ONTOGENY OF DIGESTIVE TRACT

By

MAHDIEH JAFARI December 2011

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A series of experiments on the early development of Caspian kutum (*Rutilus frisii kutum*) larvae including the morphological and histological development of mouth and gut were conducted to determine the suitable feed type and size for the kutum larviculture and fry production on the basis of their morphological features. The embryonic and larval development of kutum were studied under hatchery conditions at 14-16° C using eggs were obtained through induced spawning of broodstock. Samples were taken every hour during the fertilization for two days and then six times per day till hatching (9 days). After hatching, daily observations were made until the fingerling stage. Nine main stages (zygote, cleavage, morula, blastula, gastrula, neurula, segmentation, pharyngula and hatching) and 30 sub-stages were observed during the embryonic development of kutum while three main stages and eighteen sub-stages were noted after the hatching until the juvenile stage. The mouth

and gut development were monitored using light microscopy after haematoxylineosin (H&E) staining. The larvae were first fed with egg yolk for 5 days and followed with *Artemia* nauplii and egg yolk until the end of 30-days. Ten to twenty larvae were daily sampled from hatching to 3 days after hatch (DAH) and thereafter at every 3 days until 30 days. The larval mouth opened at 3 DAH and the mouth size was $145 \pm 15.01 \,\mu\text{m}$ at 90° opening. A strong linear relationship between mouth size and total length was established. The digestive system was made of an undifferentiated straight tube at hatch and the system became sectioned into buccopharynx, oesophagus and intestine as the larva grew. Goblet cells in oesophagus were observed at 5 DAH and increased in number between 7 to 15 DAH. The first goblet cells appeared in the intestine at 7 DAH and increased in number and became abundant with the differentiation of intestinal mucosa from 10 to 20 DAH. A swimbladder was connected to the oesophagus via a pneumatic duct which can be seen from 1 DAH. Liver and pancreas were observed at 2 DAH and their ontogenetic changes were observed during the larval growth.

The effects of diets on histological changes in digestive tract, growth, survival, body composition and digestive enzyme activities of kutum larvae were also studied. Larvae were fed for 30 days on three diets (egg yolk, *Artemia* nauplii, *Artemia* plus egg yolk) and starved in triplicates. No significant histological differences were observed in the intestine development of larvae at 5, 10 and 15 days among the feeding larvae. Starved larvae had smaller size and shape intestine. Larvae fed with *Artemia* plus egg yolk had significantly higher (P<0.05) final mean total length and body weight (28.6 \pm 0.18 mm and 74 \pm 3.64 mg, respectively) and the highest survival rate (70.9 \pm 2.1%). The activities of digestive enzymes were detected at the

start of exogenous feeding. Protease activity increased with growth after 21 DAH. Specific pepsin content peaked between 5-7 DAH and then decreased in all treatments. No significant differences in total chymotrypsin activity were observed between larvae fed on *Artemia* and egg. The total lipase activity generally increased with the larval development. These findings suggested that Caspian kutum larvae should be able to ingest, digest and absorb food particles within 50- 100 μ m Ø from 3 DAH onwards. The functional alimentary tract of kutum larvae was completed by the 30 DAH. A combination diet of *Artemia* and egg yolk was the best for the culture of kutum during early life stages. Further studies on the larval feed development should be conducted to improve the production and quality of kutum fry.

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

PERKEMBANGAN AWAL LARVA Rutilus frisii kutum KAMENSKI, DENGAN PENEKANAN KEPADA ONTOGENI SALURAN PENGHADAMAN

Oleh

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Satu siri eksperimen terhadap perkembangan awal larva ikan kutum Kaspian (*Rutilus frisii kutum*) termasuk perkembangan morfologi dan histologi mulut dan saluran pencernaan telah dilakukan untuk menentukan jenis dan saiz makanan yang sesuai bagi larvikultur dan pengeluaran fri ikan kutum berdasarkan ciri morfologi. Perkembangan embrio dan larva ikan kutum dalam persekitaran hatceri pada 14- 16° C dikaji menggunakan telur yang diperolehi secara pembiakan aruhan. Persampelan dilakukan setiap satu jam sewaktu peringkat persenyawaan selama dua hari diikuti sebanyak enam kali sehari sehingga peringkat penetasan (9 hari). Selepas penetasan, pemerhatian harian dilakukan sehingga ke peringkat jejari. Sembilan peringkat utama (zigot, belahan, morula, blastula, gastrula, neurula, segmentasi, pharyngula dan penetasan) dan 30 sub peringkat utama dan 18 sub-peringkat didapati selepas penetasan sehingga peringkat juvenil. Perkembangan mulut dan saluran pencernaan dipantau menggunakan mikroskopi cahaya setelah diwarnakan menggunakan

hematoksilin-eosin (H&E). Larva diberi makan kuning telur selama 5 hari dan diikuti dengan naupli Artemia - kuning telur sehingga hari ke 30. Sepuluh hingga dua puluh larva dari peringkat penetasan hingga 3 hari selepas penetasan (DAH) telah disampel setiap hari dan seterusnya disampel pada setiap 3 hari sehingga 30 hari. Mulut larva membuka pada 3 DAH dengan saiz mulut 145 ± 15.01 µm pada pembukaan 90°. Terdapat hubungan linear yang kukuh antara saiz mulut dan panjang keseluruhan. Sistem pencernaan larva berbentuk tiub lurus semasa peringkat penetasan dan sistem itu menjadi bahagian bukofarink, esofagus dan usus apabila larva membesar. Sel goblet kelihatan dalam esofagus pada 5 DAH dan bertambah bilangannya di antara 7 hingga 15 DAH. Sel pertama muncul di dalam usus pada 7 DAH dan bilangan sel bertambah dan menjadi semakin banyak dengan pembezaan mukosa usus dari 10 hingga 20 DAH. Pundi renang bersambung ke esofagus melalui saluran pneumatik yang boleh dilihat dari 1 DAH. Hati dan pancreas kelihatan pada 2 DAH dan perubahan ontogenetik diperhatikan sepanjang pertumbuhan larva.

Kesan diet ke atas perubahan histologi saluran pencernaan, pertumbuhan, kemandirian, komposisi badan dan aktiviti enzim pencernaan larva ikan kutum telah juga dikaji. Larva diberi makan tiga diet (kuning telur, naupli Artemia, dan *Artemia* - kuning telur) dan dilaparkan selama 30 hari dalam triplikat. Tiada perbedaan histologi yang ketara dilihat dalam perkembangan usus larva yang diberi makanan pada hari 5, 10 dan 15. Larva yang dilaparkan mempunyai saiz dan bentuk usus yang lebih kecil. Larva yang diberi *Artemia* - kuning telur mempunyai panjang keseluruhan dan berat badan akhir (28,6 \pm 0,18 mm dan 74 \pm 3,64 mg, masing-masing) yang ketara lebih tinggi (P<0.05) serta kadar kemandirian yang tertinggi (70.9 \pm 2.1%). Aktiviti enzim pencernaan dikesan pada permulaan pemakanan

eksogenus. Aktiviti protease meningkat dengan pertumbuhan terutamanya selepas 21 DAH. Kandungan spesifik pepsin memuncak pada 5-7 DAH dan kemudian menurun pada semua rawatan. Tiada perbezaan ketara dalam aktiviti keseluruhan kimotripsin diperhatikan antara larva yang diberi *Artemia* dan kuning telur. Aktiviti keseluruhan lipase secara amnya meningkat dengan perkembangan larva. Penemuan kajian ini menunjukkan bahawa larva ikan kutum Kaspian berupaya untuk memakan, mencerna serta menyerap partikel makanan dalam julat 50 - 100 μm Ø bermula dari 3 DAH. Saluran pencernaan larva ikan kutum yang berfungsi menjadi lengkap pada 30 DAH. Campuran *Artemia* dan kuning telur merupakan makanan terbaik untuk pengkulturan ikan kutum di peringkat awal hidupnya. Kajian lanjut mengenai pembangunan makanan larva perlu dilakukan untuk mempertingkatkan pengeluaran dan kualiti fri ikan kutum.

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I certify that a Thesis Examination Committee has met on 23rd December 2011 to conduct the final examination of Mahdieh Jafari on her thesis entitled "Early development of Caspian kutum (*Rutilus frisii kutum* kamenskii, 1901) larvae with an emphasis on the development of larval digestive tract" in accordance with Universities and University Colleges Act 1971 and Constitution of the Universiti Putra Malaysia [P.U. (A) 106] 15 March 1998. The Committee recommends that the student be awarded the Doctor of Philosophy.

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DECLARATION

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

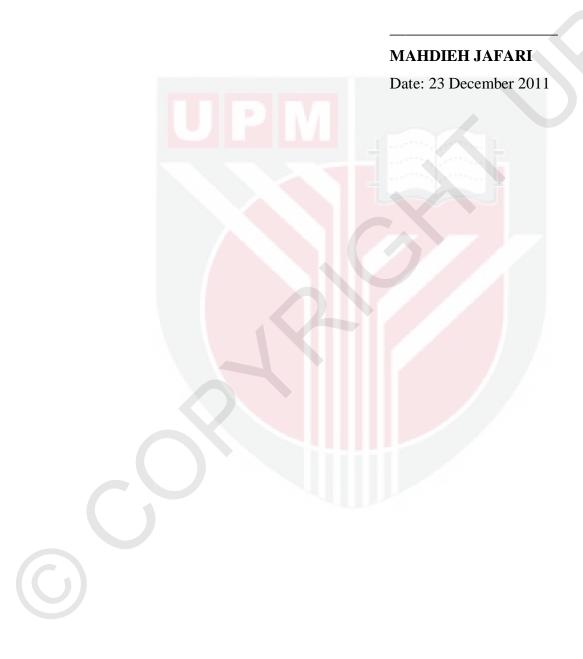


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LIST OF ABBREVIATIONS

	μm	Micro meter			
	А	Anal			
	Af	Anal fin			
	ai	anterior intestine			
	b	blastomers			
	b	buccopharynx			
	bb	basibrancial			
	Bb	Brush border			
	Bc	Buccal cavity			
	bl	blastula			
	BV	blood vessel			
	С	Cranium			
	cb	ceratobranchial			
	Ce	cuboidal epithelium			
	cm	centimeter			
	Cr	cephalic region			
	CRD	completely randomized design			
	DAF	Days after fertilization			
	DAH	Days after hatching			
	Df	Dorsal fin			
	Е	embryo			
	e	eye			
	g	gram			
	G	gut tube			
	G	glycogen			
	Gb	Gas bladder			
	Gc	Goblet cell			
	GL	Gut length			
	Gr	germ ring			
	Н	head			
	h	heart			

 \bigcirc

H & E	Hematoxylin & Eosin
hb	hypobranchial
He	hepatocytes
Ι	intestine
Il	islet of Langerhance
IS	intracellular
IV	intestinal valve
kg	Kilogram
km	kilometer
km ²	Square kilometer
km ³	Cubic kilometer
L	liver
Li	lipid
LJ	Lower jaw
Ll	Lateral line,
lp	lamina propria
LV	lipid vacuoles
m	myomere
m	maxilla
М	mouth
Ma	Mandible
mc	meckel's cartilage
mc	mucus cell
MF	myomere fiber
mm	millimeter
MU. C	mucus cell
Mv	Microvilli
Ν	notochord
n	Nostril
oc	otic capsul
°C	Degree centigrade
Oe	oesophagus
op	optic vesicle

Op	Operculum
Ov	optic vesicle
Р	preblast
Р	pancreas
pd	pneumatic duct
PD	pancreatic duct
pe	posterior oesophagus
Pef	Pelvic fin
Pf	Pectoral fin
pg	pigmentation
pi	posterior intestine
Pm	Pre maxilla
R	Rectum
RGI	Relative gut index
S	Somite
Sm	submucosa
s	sinusoids
SB	swimblader
SV	supranuclear
Т	Tail
tb	taste bud
TL	Total length,
UJ	Upper jaw
Y	Yolk

G

CHAPTER 1

INTRODUCTION

1.1 Background of the Study

The Caspian Sea is the unique lake on the Earth with a surface area of 371,000 km², a volume of 78,200 km³ and covers a coast line of 1200 km long, and the width varies between 204 and 566 km (Froehlich *et al.*, 1999). It is a delimit basin (without outflows) and is bordered by southern Russia, western Kazakhstan and Turkmenistan, northern Iran and eastern Azerbaijan. The maximum depth of the Caspian Sea is about 1025 meters (Zaidi and Mustafaev, 2003). Its salinity is about 12.7 ppt, approximately a third of the salinity of most seawater (CEP, 2004).

The Caspian Sea has a high economic value in terms of the natural resources. About 130 fish species live in the Caspian Sea. Some of which are commercially important, including the five unique sturgeon species, namely the beluga sturgeon (*Huso huso*), the Persian sturgeon (*Acipenser persicus*), the Russian sturgeon (*Acipenser gueldenstaedtii*), the Ship sturgeon (*Acipenser nudiventris*) and the Stellate sturgeon (*Acipenser stellatus*). In addition bony fishes present are Caspian kutum (*Rutilus frisii kutum*), mullets (*Mugil auratus* and *M. saliens*), breams (*Abramis brama*), carps (*Cyprinus carpio*), salmons (*Salmo trutta caspius*), Pike-perch (*Lucioperca lucioperca*), Roach (*Rutilus rutilus*), and kilka fish (*Clupeonella delicatula, C. engrauliformis, C. grimmi*). After the sturgeon fish, the Caspian kutum is the second commercially important fish in the Caspian Sea (Coad, 1979).

This species is a migratory fish, similar to most of Caspian Sea fishes and it migrates to fresh water river for spawning (Derzhavin, 1934; Azari, 1979). The Caspian kutum is an anadromous fish with three populations (one autumn form and two spring forms). The spring forms enter the rivers in March to April and autumn form enters the rivers in November to December (Razavi, 1997; Abdoli, 1999). However, the spring population of kutum stock faced a decline since early 1950s resulting in a significant drop in the catch (CEP, 2002).

Factors contributing to the decline in kutum population and catch (1950-1980) include illegal fishing during spawning season, over-exploitation of kutum resources, hydro- electricity engineering buildings (dams) as well as redistribution of water for agriculture fields' irrigation that caused changes the normal hydrological cycle. The releases of agricultural pesticides, domestic sewage and industrial pollutions into the rivers also have led to the deterioration of the water quality of natural habitats while sand removal from river bottom for building construction destroyed the spawning substrates. All these factors plus the illegal fishing and use of nets with smaller than the permitted mesh size have threatened the Caspian kutum stocks.

The Iran Fisheries Organization (IFO) has decided to restore the depleted spring forms stocks kutum in the Caspian Sea. Caspian kutum artificial breeding for releasing fingerlings had actually been attempted in 1939 for three rivers in Iran. The number was small and accurate data was not recorded. Faridpak in (1961) conducted an experimental on restocking of fingerlings in several rivers in the south Caspian Sea basin. Emadi (1979) reported that 28-44 million fingerlings were released in year 1979. Following the drastic decline in kutum stocks in 1980-81 when kutum catches reached less than 500 tons in a year, the Guilan Fisheries Research Center has been tasked to develop a practical artificial breeding program for the spring form kutum.

The stock conservation program for kutum was started in 1979 when the fingerlings of the spring population of this species were released to rivers that has successfully increased to kutum stocks (Azari, 1991). Since then artificial breeding and the release of kutum fingerlings have been included in annual activities of the Iranian Fisheries Organization until today. Fingerlings are released to improve natural spawning in few areas and raising the water level which had contributed to increase stocks (IFRO, 2010).

1.2 Statement of Problems

At the present, three government hatcheries (Shahid Ansari in Guilan Province, Shahid Rajaee in Mazandaran Province and Sijoval in Golestan Province in the south of Caspian Sea) are responsible to propagate and release spring forms of kutum fingerlings (Abdolhay, 1997). As mentioned earlier Caspian kutum is the most popular fish with the highest economic value consumed by the Iranian people. Kutum population in the Caspian Sea is now dominated by the stocks produced from artificial breeding programs. Although restocking enhancement plays a significant role in revitalization the kutum population and fishery in the Caspian Sea, recent studies showed that the decrease in the average weight of released Caspian kutum from 1.56g in 1992 to 0.7g in 1998 has resulted in lowering survival coefficient and decreasing the annual kutum catch (Afraei *et al.*, 2010; IFRO, 2000, 2010).

In the present artificial breeding practices, no selection program is involved in choosing Caspian kutum male and female broodstocks which subsequently will cause the loss the gene bank of this species. Loss of variation in genetic stocks and the gradual depletion of the gene bank are parameters of great concern in the long run. The decrease in growth rate, mean length and fecundity and the increase in the number of abnormal larvae will become evident to the next 25 to 40 years (Pourkazemi, 2000).

Rearing of fish larvae is the most critical stage in the aquaculture in which the balance between rapid fish growth and optimum use of food should be considered. In kutum larval culture, feeding management is very important because suitable or correct feeding will increase the survival and growth of fish (Haghighi, 2006; Fallahi *et al.*, 2009; Gholami, 2010). Survival rate of fingerlings in the first years of life depends on their weight and quality at the time of release into the sea (Steiger and Schulz, 1989).

In Iran, two hundred million fingerlings of Caspian Kutum, with an average of one gram weight of fingerlings are released to open ecosystem annually, although, high percentages of larvae die before reaching the fingerling stage. Some of them needs a longer time to reach to this stage and some are remain underdeveloped (Fallahi *et al.*, 2009). Heavy mortality in fish larvae occurs in early life stage because lack of suitable foods which subsequently leads fish larvae to a point no return (PNR) even under good conditions (Fallahi *et al.*, 2009). Furthermore, feeding of fish larvae is strongly relevant to food size in relation to mouth size and gut structure of larvae

(Keast and Webb, 1966; Person *et al.*, 1993; Kohinoor *et al.*, 1995; Ghada Ahmed, 2000).

Studies on Caspian kutum larvae feeding (Effatpanah, 1992; Haghighi, 2006; Afraei *et al.*, 2009; Fallahi *et al.*, 2009) gave some useful information. However, a major gap remains on kutum larval nutritional requirements and digestive tract physiology. These lack of knowledge have caused high mortality and low quality larvae, which commonly observed in kutum hatchery in Iran as reported by Kazeroni (1996) and Fallahi *et al.* (2009).

1.3 Significance of Study

Knowledge of larviculture conditions is required for a successful rearing through the critical periods of fish larvae in early life stages such as fertilization, hatching, and nursery. Suitable behavioral development and the ability to rear preferred larval food sources consistently in large quantities are required for the growth and success of the commercial fish aquaculture industry.

Significant progression of normal embryogenesis allows an early screening of hatchery production runs, which can help establish the value of broodstock fish, and assist decision making on the distribution of resources such as rearing environment to different sets of embryo. Embryology can help to improve comparative studies with other species. To date, there is no published data about the early development of *R. frisii kutum* in hatchery conditions, histology and morphology of mouth and gut

development, relationship between the mouth size and gut, and suitable size of food during larval stages digestive enzyme.

As feeding of fish larvae is strongly affected by food particle size, mouth size and gut structure, study on mouth and gut system is necessary for Caspian kutum rearing in intensive culture. Understanding of nutritional physiology of fish larvae, the knowledge on the development of the alimentary tract and its function during ontogenetic development is vital and recommended for nursery and pond culture (Segner *et al.*, 1993). Information on the beginning time of the full function digestive tract also shows the best time for larvae releasing to the sea.

1.4 Objectives of Study

The objectives of this study were:

- i. To describe embryonic development in early life stage of Caspian kutum.
- ii. To examine development of mouth and gut morphology and histology in Caspian kutum larvae.
- iii. To determine the effects of diets on histological features of digestive system, growth, survival and body composition.
- To determine ontogenetic changes of some digestive enzymes of Caspian kutum larvae.

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