



UNIVERSITI PUTRA MALAYSIA

**BIOCHEMICAL COMPOSITION OF THE OVARY OF MYSTUS
NEMURUS (CUVIER AND VALENCIENNES) AND INFLUENCE OF
ASCORBIC ACID SUPPLEMENTATION ON THE EGG AND LARVAL
QUALITY**

SONIA G. SEBASTIAN SOMGA

FH 1996 1

BIOCHEMICAL COMPOSITION OF THE OVARY OF *Mystus nemurus* (CUVIER
AND VALENCIENNES) AND INFLUENCE OF ASCORBIC ACID SUPPLEMENTATION
ON THE EGG AND LARVAL QUALITY

BY
SONIA G. SEBASTIAN SOMGA

Thesis Submitted in Fulfillment of the Requirement for the
Degree of Master of Science in the Faculty of Agriculture
Universiti Pertanian Malaysia

November 1996



ACKNOWLEDGEMENTS

I am grateful to my chairman Dr.Sharr Azni Harmin, for all the support, guidance and motivation. My sincere thanks and appreciation to my committee members, Dr. Sharifah Kharidah Syed Mohammad for all the kind help and valuable advise, and Dr. Che Roos Saad for the encouragement and helpful comments throughout this study.

My appreciation goes to the Ministry of Science, Technology and Environment, Government of Malaysia for the research fund, under the Intensification of Research in Priority Areas (IRPA).

To Dr. Japar Sidek Bujang, I express my profound gratitude, for sharing his expertise in Statistics for the data analysis. I am thankful to Prof. Ang Kok Jee who kindly lend transport during the conduct of this study. My heartfelt thanks to Dr. Siti Shapor Siraj for all the help she extended. Thanks are also due to Mr. Chan tian Wan from the Faculty of Food Science and Biotechnology, and Zakaria Md. Shah for their kind assistance in the biochemical analysis. My appreciation to the Hatchery unit staff particularly Kimon, Jasni, Asmi, Ujang, Zaidi, Nordin and Krisnan for all the help while doing the study. Also, to Naharia Mat Lia and Mahamud Yusoh for their generous help, and the staff of the former Faculty of Fisheries and Marine Science for their contribution to make this study a success.



I am thankful to Dr. John Albaladejo for "introducing" me to the UPM post graduate education. Wishes of thanks are also due to Ate Dina, Gingging and Bebbot for the companionship, and for all those cheerful days. Special word of thanks to my friend, Annie Christianus and her family for their kindness and concern.

To my husband, Jojo and to my family whose love, support and help serve as inspiration, I wish to share this achievement. Above all. I thank the Almighty God for all his blessings.



TABLE OF CONTENTS

	Page
ACKNOWLEDGEMENTS	ii
LIST OF TABLES	vii
LIST OF FIGURES	xii
LIST OF PLATES	xiv
LIST OF ABBREVIATIONS	xvi
ABSTRACT	xviii
ABSTRAK	xx
CHAPTER	
I GENERAL INTRODUCTION	1
II LITERATURE REVIEW	7
Oocyte Development and Maturation	7
Concept of Egg Quality	9
Factors Affecting Egg and Larval Quality	10
Environmental Conditions	10
Nutrition	13
Induced Spawning	19
Assessment of Egg Quality	21
III GENERAL MATERIALS AND METHODS	28
Maintenance of Broodstock	28
Biochemical Analyses	28
Proximate Analyses	28



	Amino Acid Analysis	30
	Fatty Acid Analysis	32
	Ascorbic Acid Analysis	33
	Induced Spawning	34
	Hormone Preparation and Administration	34
	Staging of Oocyte	34
	Egg Collection and Artificial Fertilization	35
	Statistical Analysis	37
IV	OVARIAN DEVELOPMENT OF <i>MYSTUS NEMURUS</i> (C & V)	38
	Introduction	38
	Materials and Method	39
	Results	43
	Ovary of <i>M. nemurus</i>	43
	Histology of the Ovary	43
	Discussion	53
V	BIOCHEMICAL COMPOSITION OF <i>MYSTUS</i> <i>NEMURUS</i> (C & V) OVARIES	60
	Introduction	60
	Materials and Method	61
	Results	62
	Discussion	71



VI	CHANGES IN THE BIOCHEMICAL COMPOSITION DURING EMBRYONIC AND EARLY LARVAL DEVELOPMENT IN MYSTUS NEMURUS (C & V)	78
	Introduction	78
	Materials and Method	80
	Results	81
	Discussion	89
VII	THE EFFECT OF ASCORBIC ACID ON OOCYTE MATURATION, EGG FERTILITY, HATCHABILITY AND SURVIVABILITY IN MYSTUS NEMURUS (C & V)	101
	Introduction	101
	Materials and Method	103
	Results	106
	Discussion	117
VIII	GENERAL DISCUSSION, CONCLUSIONS AND RECOMMENDATIONS FOR FUTURE STUDIES	125
	REFERENCES	133
	APPENDICES	151
	BIOGRAPHICAL SKETCH	163



LIST OF TABLES

Table		Page
1	Modification of the Stages of Oocyte Maturation in <i>M. nemurus</i>	41
2	The Average Weight and the Gonad Somatic Index, Hepatosomatic Index and Fat Somatic Index of <i>M. nemurus</i> Collected from May to October 1995	44
3	Frequency Distribution (%) of Different Stages of Oocyte Development in <i>M. nemurus</i> from May to October 1995	47
4	Amino Acid Content of the Ovaries at Different Gonad Somatic Index Levels in <i>M. nemurus</i>	65
5	Fatty Acid Content of the Ovaries at Different Gonad Somatic Index Levels in <i>M. nemurus</i>	68
6	The Weight of the Broodstock and the Range of Fecundity, Fertilization Rate, Hatching Rate, Survival Rate and Percentage of Deformed Larvae	82
7	The Individual Broodstock and Their Fecundity, Fertilization Rate, Hatching Rate, Survival Rate and Percentage of Deformed Larvae	83
8	Changes in the Amino Acid Content During Embryonic and Early Larval Development in <i>M. nemurus</i>	87
9	Changes in the Fatty Acid Content During Embryonic and Early Larval Development in <i>M. nemurus</i>	90
10	The Diet Formulation Prepared with the Addition of Different Levels of Ascorbyl-2-Polyphosphate	105



11	The Average Gain in Weight of <i>M. nemurus</i> Broodstocks Supplemented with Different Levels of Ascorbyl-2-Polyphosphate from April to September 1995	107
12	The Average Fecundity and Egg Diameter of <i>M. nemurus</i> Broodstocks Supplemented with Different Levels of Ascorbyl-2-Polyphosphate from April to September 1995	109
13	Egg Quality Based on Fertilization Rate, Hatching Rate, Larval Deformity and Survival Rate of Eggs from Broodstocks Supplemented with Different Levels of Ascorbyl-2-Polyphosphate	115
14	Multiple Range Test of the Ascorbic Acid Content of Developing Eggs and Larvae from Broodstocks Supplemented with Different Levels of Ascorbyl-2-Polyphosphate	151
15	The Proximate Composition of the Developing Eggs and Larvae from Broodstocks Supplemented with Different Levels of Ascorbyl-2-Polyphosphate for Three Months	152
16	The Proximate Composition of the Developing Eggs and Larvae from Broodstocks Supplemented with Different Levels of Ascorbyl-2-Polyphosphate for Six Months	153
17	Summary of Water Quality Parameters Taken During the Experiment	154
18	ANOVA for Gonad Somatic Index of <i>M. nemurus</i> Broodstocks from May to October 1995	154
19	ANOVA for Hepatosomatic Index of <i>M. nemurus</i> Broodstocks from May to October 1995	154
20	ANOVA for Fat Somatic Index of <i>M. nemurus</i> Broodstocks from May to October 1995	154



21	ANOVA for Oocyte Diameter at Different Gonad Somatic Index Levels in <i>M. nemurus</i> Broodstocks	155
22	ANOVA for the Moisture Content of the Ovaries at Different Gonad Somatic Index Levels in <i>M. nemurus</i>	155
23	ANOVA for the Ash Content of the Ovaries at Different Gonad Somatic Index Levels in <i>M. nemurus</i>	155
24	ANOVA for the Protein Content of the Ovaries at Different Gonad Somatic Index Levels in <i>M. nemurus</i>	155
25	ANOVA for the Lipid Content of the Ovaries at Different Gonad Somatic Index Levels in <i>M. nemurus</i>	156
26	ANOVA for the Ascorbic Acid Content of the Ovaries at Different Gonad Somatic Index Levels in <i>M. nemurus</i>	156
27	ANOVA for Essential Amino Acid Content of the Ovaries at Different Gonad Somatic Index Levels in <i>M. nemurus</i>	156
28	ANOVA for Non Essential Amino Acid Content of the Ovaries at Different Gonad Somatic Index Levels in <i>M. nemurus</i>	156
29	ANOVA for Saturated Fatty Acid Content of the Ovaries at Different Gonad Somatic Index Levels in <i>M. nemurus</i>	157
30	ANOVA for Monosaturated Fatty Acid Content of the Ovaries at Different Gonad Somatic Index Levels in <i>M. nemurus</i>	157
31	ANOVA for Polyunsaturated Fatty Acid Content of the Ovaries at Different Gonad Somatic Index Levels in <i>M. nemurus</i>	157
32	ANOVA for the Moisture Content During Embryonic and Early Larval Development in <i>M. nemurus</i>	157



33	ANOVA for the Ash Content During Embryonic and Early Larval Development in <i>M. nemurus</i>	158
34	ANOVA for the Protein Content During Embryonic and Early Larval Development in <i>M. nemurus</i>	158
35	ANOVA for the Lipid Content During Embryonic and Early Larval Development in <i>M. nemurus</i>	158
36	ANOVA for the Ascorbic Acid Content During Embryonic and Early Larval Development in <i>M. nemurus</i>	158
37	ANOVA for Essential Amino Acid Content During Embryonic and Early Larval Development in <i>M. nemurus</i>	159
38	ANOVA for Non Essential Amino Acid Content During Embryonic and Early Larval Development in <i>M. nemurus</i>	159
39	ANOVA for Saturated Fatty Acid Content During Embryonic and Early Larval Development in <i>M. nemurus</i>	159
40	ANOVA for Monounsaturated Fatty Acid Content During Embryonic and Early Larval Development in <i>M. nemurus</i>	159
41	ANOVA for Polyunsaturated Fatty Acid Content During Embryonic and Early Larval Development in <i>M. nemurus</i>	160
42	ANOVA for the Initial Weight of Broodstocks Before Supplementation with Ascorbyl-2-Polyphosphate in <i>M. nemurus</i>	160
43	ANOVA for the Weight Gained of Broodstocks After Three Months of Supplementation with Ascorbyl-2-Polyphosphate in <i>M. nemurus</i>	160
44	ANOVA for the Weight Gained of Broodstocks After Six Months of Supplementation with Ascorbyl-2-Polyphosphate in <i>M. nemurus</i>	160



45	ANOVA for the Ascorbic Acid Content of Eggs from Broodstocks Supplemented with Different Levels of Ascorbyl-2-Polyphosphate	161
46	ANOVA for the Fertilization Rate of Eggs from Broodstocks Supplemented with Different Levels of Ascorbyl-2-Polyphosphate	161
47	ANOVA for the Hatching Rate of Eggs from Broodstocks Supplemented with Different Levels of Ascorbyl-2-Polyphosphate	161
48	ANOVA for the Larval Survival from Broodstocks Supplemented with Different Levels of Ascorbyl-2-Polyphosphate . . .	161
49	ANOVA for Percentage of Deformed Larvae from Broodstocks Supplemented with Different Levels of Ascorbyl-2-Polyphosphate	162



LIST OF FIGURES

Figure		Page
1	The Average Gonad Somatic Index and Oocyte Diameter of <i>M. nemurus</i> Broodstocks Collected from May to October 1995	45
2	The Proximate Composition of the Ovaries of <i>M. nemurus</i> Broodstocks from May to October 1995	63
3	The Total Essential and Non Essential Amino Acid Contents of the Ovaries at Different Gonad Somatic Index Levels in <i>M. nemurus</i>	64
4	The Total Saturated, Monounsaturated and Polyunsaturated Fatty Acid (PUFA) Contents of the Ovaries at Different Gonad Somatic Index Levels in <i>M. nemurus</i>	70
5	The Ascorbic Acid Content of the Ovaries at Different Gonad Somatic Index Levels in <i>M. nemurus</i>	72
6	Changes in the Proximate Composition During Embryonic and Early Larval Development in <i>M. nemurus</i>	84
7	Changes in the Total Essential and Non Essential Amino Acid Contents During Embryonic and Early Larval Development in <i>M. nemurus</i>	85
8	Changes in the Total Saturated, Monounsaturated and Polyunsaturated Fatty Acid (PUFA) Contents During Embryonic and Early Larval Development in <i>M. nemurus</i>	92
9	Changes in the Ascorbic Acid Content During Embryonic and Early Larval Development in <i>M. nemurus</i>	94



10	The Fertilization Rate, Hatching Rate, Survival Rate and Percentage of Deformed Larvae of Eggs Produced by <i>M. nemurus</i> Broodstocks Supplemented with Different Levels of Ascorbyl-2-Polyphosphate for Three Months	110
11	Ascorbic Acid Content of Developing Eggs and Larvae from <i>M. nemurus</i> Broodstocks Supplemented with Different Levels of Ascorbyl-2-Polyphosphate for Three Months	113
12	The Fertilization Rate, Hatching Rate, Survival Rate and Percentage of Deformed Larvae of Eggs Produced by <i>M. nemurus</i> Broodstocks Supplemented with Different Levels of Ascorbyl-2-Polyphosphate for Six Months	116
13	Ascorbic Acid Content of Developing Eggs and Larvae from <i>M. nemurus</i> Broodstocks Supplemented with Different Levels of Ascorbyl-2-Polyphosphate for Six Months	118



LIST OF PLATES

Plate		Page
1	Mature Ovary with Thick Ovarian Wall, Blood Vessels and Mature Oocyte	48
2	Mature Ovary Showing Different Oocyte Stages. Oogonia, Chromatin Nucleolus, Early Perinucleolus, Late Perinucleolus	48
3	Oogonia Occur in Cluster and Surrounded by Small Epithelial Cells	49
4	Oocytes at Chromatin Nucleolus Stage. Nucleoli, Chromatin	49
5	Oocyte at Early Perinucleolus Stage. Provitelline Nucleoli	50
6	Higher Magnification of Oocyte at Early Perinucleolus Stage. Note the Movement of Provitelline Nucleoli Toward the Nuclear Membrane, Nucleus	50
7	Oocyte at Late Perinucleolus Stage. Note the Formation of Nucleoli as a Clear Ring in the Nuclear Membrane. Nucleus, Zona Radiata, Follicular Epithelium	52
8	Oocyte at Cortical Alveoli Stage. Yolk Granules, Yolk Vacuoles, Zona Radiata, Follicular Epithelium, Nucleus	52
9	Higher Magnification of Oocyte at Cortical Alveoli Stage. Note the Follicular Cells, Yolk Vacuoles, Nucleoli, Zona Radiata	54
10	Oocyte in Advanced Vitellogenic Stage. Note the Large Bright Red Yolk Granules that Occupy the Cytoplasm. Yolk Vacuoles, Nucleus	54



11	Higher Magnification of Oocyte in Advanced Vitellogenic Stage. Note the Yolk Granules, Distinct Zona Radiata, Follicular Cell, Theca Layer.	55
12	Normal Physical Characteristic of <i>M. nemurus</i> Larvae with Proportionately Well-Defined Shape of the yolk	111
13	Abnormal Larva Showing Curved Body with Strange Size Compared to its Yolk	111
14	Larva with Twisted Body and Abnormal Swimming Behaviour	112
15	Developing Larva Demonstrated a Spinal Deformity at the Posterior Part of the Body	112



LIST OF ABBREVIATIONS

AA	Ascorbic Acid
AABA	Alpha Amino Butyric Acid
AAPP	Ascorbyl-2-Polyphosphate
Ala	Alanine
Arg	Arginine
Asp	Aspartic Acid
CHCl ₃	Chloroform
CP	Crude Protein
Cys	Cysteine
EAA	Essential Amino Acids
EtOH	Ethanol
FAME	Fatty Acid Methyl Ester
FSI	Fat Somatic Index
GC	Gas Chromatography
Glu	Glutamic Acid
Gly	Glycine
GnRH-A	Gonadotropin Releasing Hormone Analogue
GSI	Gonad Somatic Index
GV	Germinal Vesicle
GVB	Germinal Vesicle Breakdown
GVM	Germinal Vesicle Migration
His	Histidine
HPLC	High Performance Liquid Chromatography



HSI	Hepatosomatic Index
Ile	Isoleucine
KCl	Potassium Chloride
Leu	Leucine
Lys	Lysine
Meth	Methionine
MSA	4n-Methanesulfonic Acid
NaOH	Sodium Hydroxide
OI	Ovarian Index
Phe	Phenylalanine
Pro	Proline
PUFA	Polyunsaturated Fatty Acids
Ser	Serine
TCA	Trichloroacetic acid
Thr	Threonine
Tryp	Tryptophan
Tyr	Tyrosine
Val	Valine
YG	Yolk Globule



Abstract of the thesis submitted to the Senate of the
Universiti Pertanian Malaysia in fulfilment of the
requirement for the degree of Master of Science

**BIOCHEMICAL COMPOSITION OF THE OVARY OF *MYSTUS NEMURUS*
(Cuvier & Valenciennes) AND INFLUENCE OF ASCORBIC ACID
SUPPLEMENTATION ON EGG AND LARVAL QUALITY**

BY

Sonia G. Sebastian-Somga

November 1996

Chairman : Dr. Sharr Azni Harmin

Faculty : Fisheries and Marine Science

Selected biochemical compositions of the ovaries such as proximate composition, amino acid, fatty acid and ascorbic acid were determined and their role in developing eggs and larvae were evaluated. The ovaries contained substantial amounts of protein, lipid, and ascorbic acid. The protein comprised of high levels of both essential and non essential amino acids, while, the lipid consisted of higher proportions of saturated and monounsaturated fatty acids, and lower proportion of polyunsaturated fatty acid (PUFA), respectively. These biochemical components did not show any significant ($p>0.05$) changes at different gonad somatic index levels. Although histology revealed that ovaries demonstrated asynchronous oocyte development, it appeared that there is continuous accumulation of nutrients into the oocyte until final maturation stage. In developing



eggs and larvae, marked reduction in protein, lipid, essential amino acid, saturated fatty acid and ascorbic acid contents indicated endogenous utilization. Quantitative decrease of these constituents suggested that more nutrients were utilized during embryonic development.

Supplementation of ascorbyl-2-polyphosphate in the broodstock diet demonstrated that the dosage and duration of feeding both affected the accumulated ascorbic acid in the eggs and their quality. Egg quality increased significantly ($p < 0.05$) from broodstocks fed higher dosage of ascorbyl-2-polyphosphate based on fertilization rate, hatching rate, survival rate and percentage of abnormal larvae.

The results of this study indicated that the egg composition and its quality were affected by the biochemical composition of the ovary. While it is directly regulated by the broodstock nutrition it can nevertheless be considered as an important element for optimum reproductive efficiency that may reduce factors limiting aquaculture production.



Abstrak tesis yang dikemukakan kepada Senat Universiti
Pertanian Malaysia sebagai memenuhi syarat
keperluan untuk ijazah Master Sains

**KOMPOSISI BOKIMIA OVARI *MYSTUS NEMURUS* (Cuvier &
Valenciennes) DAN KESAN PEMBERIAN ASID ASKORBİK
KE ATAS KUALITI TELUR DAN LARVA**

Oleh

Sonia Sebastian-Somga

November 1996

Pengerusi : Dr. Sharr Azni Harmin

Fakulti : Perikanan dan Sains Samudra

Komposisi biokimia ovari seperti komposisi proksimat, asid amino, asid lemak dan asid askorbik telah ditentukan dan peranannya di dalam perkembangan telur dan larva telah dinilai. Ovari ini mengandungi banyak protein, lipid, dan asid askorbik. Protein tersebut mengandungi kedua-dua asid amino perlu dan tidak perlu pada paras yang tinggi, sementara lipid mengandungi lebih banyak bahagian asid lemak tepu dan asid lemak mono tak tepu dan kurang asid lemak poli tak tepu (PUFA). Komponen biokimia ini tidak menunjukkan sebarang perbezaan bererti ($p > 0.05$) pada paras indeks gonad somatik yang berlainan. Walaupun kajian histologi menunjukkan bahawa ovari mengalami perkembangan oosit tidak serentak, namun jelas kelihatan terdapatnya pengumpulan berterusan nutrien di dalam oosit sehingga ke peringkat akhir kematangan. Di dalam telur dan larva yang

sedang berkembang terdapat pengurangan yang ketara pada kandungan protein, lipid, asid amino perlu, asid lemak tepu dan asid askorbik yang menunjukkan terdapatnya penggunaan endogenus. Pengurangan kuantitatif komponen-komponen ini, mencadangkan bahawa lebih banyak nutrien telah digunakan semasa perkembangan embrionik.

Penambahan askorbil-2-polifosfat dalam diet induk menunjukkan bahawa dos dan jangkamasa pemberian makanan kedua-duanya memberi kesan ke atas pengumpulan asid askorbik di dalam telur dan terhadap kualitinya. Kualiti telur meningkat dengan ketara ($p < 0.05$) dari induk yang telah diberi makanan yang mengandungi dos askorbil-2-polifosfat yang tinggi dan ini adalah berdasarkan pada kadar persenyawaan, kadar penetasan, kadar kemandirian dan peratus larva yang abnormal.

Keputusan-keputusan daripada kajian ini menunjukkan bahawa komposisi telur dan kualitinya adalah dipengaruhi oleh komposisi biokimia ovari. Sehubungan dengan itu, sementara ia dikawal secara langsung oleh nilai pemakanan induk, ia juga boleh disifatkan sebagai elemen yang penting untuk kecekapan pembiakan yang optimum dan mungkin mengurangkan faktor-faktor penghad dalam pengeluaran akuakultur.



CHAPTER I

GENERAL INTRODUCTION

Egg quality in general reflects the egg production capability of the broodstock together with the survival of the egg and growth rates of fry. Egg quality is basically influence by the conditions under which the broodstock are maintained, their husbandry, types of diet and genetic make-up (Bromage *et al.*, 1992). Thus, the parent condition apparently regulates the physical and chemical dimensions of the egg as well as the subsequent progeny survival (Springate *et al.*, 1984).

In fish, reproduction is triggered by external cues such as photoperiod, temperature, feeding and social factors. These stimulate the multifaceted reproductive hormonal centres that induce the maternal production of vitellogenin and deposition of yolk into the oocyte (Peter, 1983). Vitellogenin constitutes the carrier molecule for various classes of compounds and ample amounts of nutrition being accumulated by the developing oocyte for proper assembly (Wallace, 1985). Incorrect composition of the circulating vitellogenin due to unbalanced diet can cause a negative effect on vitellogenic and oocyte maturation process (Cerdeira *et al.*, 1994).



It is well known that nutrition influences reproduction by affecting fecundity and egg size, egg hatchability and fry viability as well as its biochemical composition (Eskelinen, 1989). The quality and quantity of broodstock diet is important for spawning and egg quality (Rainuzzo, 1993; Watanabe et al., 1984). Positive correlation of broodstock nutrition on reproduction and egg quality have been found in red seabram (Watanabe et al., 1991), Atlantic salmon (Eskelinen, 1989) and rainbow trout and carp (Sato et al., 1987). Likewise, food restriction generally reduces total fecundity and may delay oocyte maturation process and decrease the proportion of maturing fish (Horwood et al., 1989; Kjesbu, 1988; Springate and Bromage, 1985).

Mobilization of nutrients during gonadal development was reviewed by Lie and Mangor-Jensen (1993). Nutrient distribution seems to become intense in the ovaries during oocyte maturation process. For instance, changes in the plasma constituents of the maturing turbot has been related to the transport of nutrients from various tissues to the gonads (Lie and Mangor-Jensen, 1993). There was a shift in metabolism and redistribution of nutrient reservoir during gonad maturation.

Fecundity represents the true reproductive capability of the broodstock (Bromage et al., 1992). Fecundity is expressed in terms of the number of eggs released at spawning and the total volume of water-hardened eggs

(Bromage *et al.*, 1992). It is deemed to be related to the size of the broodstock as well as the size of the egg. Changes in fecundity can be achieved through modification of the rate of recruitment of pre-vitellogenic oocytes and cortical alveoli into vitellogenesis (Bromage *et al.*, 1992), but knowledge regarding the dynamics of these processes is limited.

There are a number of egg characteristics that are considered essential in measurement of egg quality. Physiological processes that occur from fertilization until hatching is a complex mechanism and regarded as universal criteria of egg quality. After fertilization, activation process by enzyme reaction cause hardening of the egg chorion that serves as the egg's ability to sustain mechanical resistance (Kjorsvick *et al.*, 1990). Deviation in chorion integrity and morphological malformation precede failure in hatching.

Egg size is known to be correlated to the larval size. Larger larvae tend to survive longer without food than those hatched from smaller eggs (Springate *et al.*, 1984). This may however does not give long term advantage as far as growth and survival of the larvae are concerned (Blaxter, 1988). Springate and Bromage (1985) suggest that size-dependent survival rates might be a reflection of differences in stage of ripeness of the egg and it is not a basis of a lesser quality than the larger eggs.

