



**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**

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**FH 1996 1**

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AND VALENCIENNES) AND INFLUENCE OF ASCORBIC ACID SUPPLEMENTATION  
ON THE EGG AND LARVAL QUALITY

BY  
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## TABLE OF CONTENTS

	Page
<b>ACKNOWLEDGEMENTS</b> . . . . .	ii
<b>LIST OF TABLES</b> . . . . .	vii
<b>LIST OF FIGURES</b> . . . . .	xii
<b>LIST OF PLATES</b> . . . . .	xiv
<b>LIST OF ABBREVIATIONS</b> . . . . .	xvi
<b>ABSTRACT</b> . . . . .	xviii
<b>ABSTRAK</b> . . . . .	xx
 <b>CHAPTER</b>	
<b>I GENERAL INTRODUCTION</b> . . . . .	1
<b>II LITERATURE REVIEW</b> . . . . .	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
<b>III GENERAL MATERIALS AND METHODS</b> . . . . .	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
<b>IV</b>	<b>OVARIAN DEVELOPMENT OF <i>MYSTUS NEMURUS</i></b> <b>(C &amp; V) . . . . .</b>	<b>38</b>
	Introduction . . . . .	38
	Materials and Method . . . . .	39
	Results . . . . .	43
	Ovary of <i>M. nemurus</i> . . . . .	43
	Histology of the Ovary . . . . .	43
	Discussion . . . . .	53
<b>V</b>	<b>BIOCHEMICAL COMPOSITION OF <i>MYSTUS</i></b> <b><i>NEMURUS</i> (C &amp; V) OVARIES . . . . .</b>	<b>60</b>
	Introduction . . . . .	60
	Materials and Method . . . . .	61
	Results . . . . .	62
	Discussion . . . . .	71



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



## 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 <sub>3</sub>	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  
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**BIOCHEMICAL COMPOSITION OF THE OVARY OF *MYSTUS NEMURUS*  
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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  
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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

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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.

