N° d'ordre : 366/21

ABSTRACT

In 1983, the Chinese carps were imported from Hungary in order to control the eutrophication phenomenon observed in the Moroccan dams. Then the silver carp, Hypophthalmichthys molitrix began to have an important effect on the improvement of water resources of the kingdom. This specie is able to reduce the unwanted algal biomass and transform it into a highly appreciated fish flesh as consequence of its phytoplanctonophage diet. Indeed, the fry that weight just 0.0013 g can reach up to 6 kg in their second year. Moreover, their growth performances are really significant and they can weight more than 25 kg in the first five years (Droussi, 1998).

The present study seeks to determine the effect of the spawning period on eggs quality. During the breeding season of the silver carp, the quality of the eggs was assessed by monitoring the development of fertilized eggs and evaluating their quality by the determination of three viability rates: fertilization rate, embryos survival rate, and fry survival rate. This part will allow determining the period in the breeding season when the eggs record a satisfactory viability rates. The results showed that the fertilization rate (FR) varied from 60% to 72%, the embryos survival rate changed from 43% to 67% while fry survival rate recorded a variation of 80% to 99%.

On the other hand, our study treats the effect of eggs retention in the females' ovary of silver carp (in vivo aging). The purpose of this investigation is to determine how long eggs, ready to be ovulated, can remain in the ovarian cavity without losing their viability. The adopted method is based on inducing the ovulation of each female by pituitary injection. Then the extracted ova are fertilized immediately by the milt of males (at least three males).

Four egg extractions were performed at increasing times after ovulation. The time interval is 30 minutes between two successive extractions. The assessment of egg quality was performed by the evaluation of the variability of three rates indicated above. The results showed that fertilization rate and embryos survival rate decreased from 62% (at the ovulation time) to 30% (for fertilized eggs at 90 minutes post-ovulation). In addition, fry survival rate varied unfavorably from 87% to 49%.

Besides, the research was supplemented by biochemical analysis in order to find if there is a correlation between egg quality and biochemical composition of the ova.

The results showed that no effect of aging and periods on lipids, proteins and fatty acids composition. However, the antioxidants: vitamin C and Vitamin E in eggs do not show any relationship with the weight of silver carp breeders. The vitamin E is impacted by the time of the post-ovulatory aging. Moreover, both antioxidants balance the oxidative events throughout the breeding season by their changes.

Key words: *Hypophthalmichthys molitrix*, ova, aging, breeding, egg quality, lipids, proteins, fatty acids, antioxidants.

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Study of the aging of silver carp eggs (Hypophthalmichthys molitrix, Valenciennes 1844) and its impact on fish seeds production (DEROUA fish farming station, Fkih)

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For obtaining the grade of

DOCTOR

Disciplin: **Biology**

Speciality: ***Biochemistry and Animal Biology.***

Study of the aging of silver carp eggs (Hypophthalmichthys molitrix, Valenciennes 1844) and its impact on fish production (DEROUA fish farming station, Fkih Ben Salah, Morocco)

Defended on 17th July 2021, in front of the committee members:

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Pr. Abdelali BOULLI	Professor, University of Sultan Moulay Slimane, F.S.T. Béni-Mellal, Morocco.	Reporter
Pr Hicham CHAIRI	Professor, University of Sidi Mohamed Ben Abdellah, F.P, Larache, Morocco.	Reporter
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Pr.Mohammed DROUSSI	Aquaculture expert.	Guest and co-supervisor

FOREWORD

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This work started in January 2015

The results of this work were the source of the following publications and communications:

PUBLICATIONS

- F.Z. MAJDOUBI, R. BENHIMA, A. OUIZGANE, S. FARID, M. DROUSSI, G. GUERRIERO & M. HASNAOUI (2020). Ova Fatty Acids Composition and Spawning Performances of Silver Carp, *Hypophthalmichthys molitrix* (Morocco). Turkish Journal of Fisheries and Aquatic Sciences, 20, 879-888. http://doi.org/10.4194/1303-2712-v20_12_04
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- A. OUIZGANE, F.Z. MAJDOUBI, B. HARZANI, S. ELGHIZI, A. ELMOUJTAHID, M. DROUSSI & M. HASNAOUI (2017). Effect of Temperature and Density on Diet Training of the Largemouth Bass

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- F.Z. MAJDOUBI, A. OUIZGANE, M. DROUSSI, G. GUERRIERO & M. HASNAOUI (2019). Relationship between energetic components in eggs and the reproductive success of silver carp: fish specie used to control eutrophication. The 6th International symposium "Environment and Sustainable Development (6th ISESD 2019). (2-4 October 2019) Rabat, Morocco.
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Largemouth Bass, Chinese Carps) at Deroua Fisheries Station (Fkih Ben Saleh, Morocco). 1st Edition of “Euro-Mediterranean conference for environmental integration” EMCEI 2017, (21 - 25 November 2017) Sousse, Tunisia.

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ORGANIZATION OF SCIENTIFIC EVENTS

- Participation in the Organization of the side event in COP 22 November 8, 2016-green zone-in Marrakech: "climate change adaptation through valorization of water resources in arid and semi-arid areas".

INTERNSHIP

- Internship at the Laboratory of Endocrinology, Department of Biology, University of Federico II, Napoli, Italy (06 May to 06 June 2019).

THESIS PROJECT

This research project is carried out within the collaboration between the Laboratory of environmental engineering team, Faculty of Sciences and Techniques of Beni-Mellal and the High Commission for Water and Forests and the fight against desertification and the Comparative Endocrinology Laboratory, Department of Biology of University Federico II, Naples (Italy). It will complete many works interested in the Chinese carps farmed in the Deroua fish farm (artificial propagation, growth, diet, phytoplankton effect on the growth of silver carp, multitrophic farming, etc.).

ABSTRACT

In 1983, the Chinese carps were imported from Hungary in order to control the eutrophication phenomenon observed in the Moroccan dams. Then the silver carp, *Hypophthalmichthys molitrix* began to have an important effect on the improvement of water resources of the kingdom. This specie is able to reduce the unwanted algal biomass and transform it into a highly appreciated fish flesh as consequence of its phytoplanctonophage diet. Indeed, the fry that weight just 0.0013 g can reach up to 6 kg in their second year. Moreover, their growth performances are really significant and they can weight more than 25 kg in the first five years (Droussi, 1998).

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Besides, the research was supplemented by biochemical analysis in order to find if there is a correlation between egg quality and biochemical composition of the ova.

The results showed that no effect of aging and periods on lipids, proteins and fatty acids composition. However, the antioxidants: vitamin C and Vitamin E in eggs do not show any relationship with the weight of silver carp breeders. The vitamin E is impacted by the time of the post-ovulatory aging. Moreover, both antioxidants balance the oxidative events throughout the breeding season by their changes.

Key words: *Hypophthalmichthys molitrix*, ova, aging, breeding, egg quality, lipids, proteins, fatty acids, antioxidants.

ملخص

عام 1983، تم استيراد الشبوط الصيني من المجر من أجل السيطرة على ظاهرة التخثث التي لوحظت في مياه السدود المغربية. بعد استزراعه، لوحظ أن سمك الشبوط الفضي (*Hypophthalmichthys molitrix*) قد أثر بشكل كبير على تحسين جودة الموارد المائية في المملكة. نتيجة لنظامها الغذائي النباتي تتميز هذه الفصيلة بقدرتها على تقليل الطحالب غير المرغوب فيها وتحويلها إلى لحم سمكي ذي قيمة غذائية عالية. لقد أوضحت بعض الدراسات أنه يمكن أن يصل وزن الفرخ الذي يبلغ وزنه 0,0013 جرامًا في البداية إلى 6 كيلوجرامات في عامه الثاني. علاوة على ذلك، فإن قدرة نموه العالية تمكنه من أن يبلغ وزنا قياسيا يقدر ب 25 كيلوجراما في سنواته الخمس الأولى (الدروسي,1998).

تسعى الدراسة الحالية إلى تحديد تأثير فترة تبويض الشبوط الفضي على جودة البيض خلال موسم تكاثره؛ وذلك بتقييم جودة البيض عن طريق مراقبة نمو البويضات المخصبة. لذلك تم تتبع معدلات الجودة الثلاث: معدل الإخصاب، معدل بقاء الأجنة ومعدل بقاء اليرقات. ولقد أظهرت النتائج أن معدل الإخصاب يتراوح من 60٪ إلى 72٪ فيما تغير معدل بقاء الأجنة من 43٪ إلى 67٪، بينما سجل معدل بقاء اليرقات تفاوتًا من 80٪ إلى 99٪.

تطرقت نفس الدراسة أيضا إلى تقييم أثر شيخوخة البيض في مبيض إناث الشبوط الفضي. والغرض من هذه التجربة هو تحديد المدة التي يمكن أن تبقى فيها البويضات الجاهزة للإباضة في تجويف المبيض دون أن تفقد قابليتها للإخصاب والنمو. فيما تعتمد الطريقة على تحفيز التبويض لكل أنثى عن طريق الحقن الهرموني ثم استخراج البويضات في أربع أوقات متزايدة وقد تم تحديد 30 دقيقة كفترة زمنية تفصل بين عمليتي استخراج متتاليتين. ولقد أظهرت نتائج هذه الدراسة أن معدل الإخصاب ومعدل بقاء الأجنة على قيد الحياة انخفض من 62٪ (وقت الإباضة) إلى 30٪ (للبويضات الملقحة بعد 90 دقيقة من وقت الإباضة). بالإضافة إلى ذلك، تناقص معدل بقاء اليرقات على قيد الحياة من 87٪ إلى 49٪.

إلى جانب ذلك، تطرقت نفس الدراسة إلى التحليل البيو كيميائي للبويضات وذلك في سبيل البحث عما إذا كان هناك ارتباط بين المكونات البيو كيميائية للبويضة وجودتها؛ فقد أظهرت النتائج عدم وجود تأثير للشيخوخة وفترات التبويض على تكوين الدهون والبروتينات والأحماض الدهنية للبويضة. كما أن تركيز مضادات الأكسدة (فيتامين CوفيتامينE) في البويضة ليس مرتبطا بوزن إناث الشبوط الفضي؛ وبالنسبة لتغير هذين المضادين للأكسدة خلال ظاهرة شيخوخة البويضات، فقد أثبتت الدراسة الحالية أن تركيز فيتامينE في البويضات يتأثر بهذه الظاهرة.

الكلمات المفاتيح: الشبوط الفضي، البويضات، الشيخوخة، تربية الأسماك، جودة البيض، الدهون، البروتينات، الأحماض الدهنية، مضادات الأكسدة .

RESUME

En 1983, les carpes chinoises ont été importées de Hongrie afin de contrôler le phénomène d'eutrophisation observé dans les barrages Marocains. Après, la carpe argentée (*Hypophthalmichthys molitrix)* a commencé d'avoir un effet important sur l'amélioration de la qualité des eaux du Royaume. Grace à son régime phytoplanctonophage, cette espèce est capable de réduire la biomasse d'algues indésirables et de la transformer en chair de poissons appréciée. En effet, les alevins ayant un poids moyen de 0,0013 g peuvent atteindre jusqu'à 6 kg dans leur deuxième année. Il a été démontré que cette espèce se dote d'une performance de croissance impressionnante puisqu'elle atteint un poids dépassant 25 kg au cours des cinq premières années (Droussi, 1998).

L'objectif de la présente étude est de déterminer l'effet de la période de ponte sur la qualité des œufs durant la saison de reproduction de la carpe argentée. Pour cet effet, ladite qualité a été évaluée en surveillant le développement des œufs fécondés et en évaluant l'évolution de trois taux de viabilité : le taux de fécondation, le taux de survie des embryons et le taux de survie des alevins. Cette partie a permis de déterminer la période durant la saison de reproduction où les œufs enregistrent des taux de viabilité satisfaisants. Les résultats obtenus varient de 60% à 72% pour le taux de fécondation et de 43% à 67% pour le taux de survie des embryons alors que le taux de survie des alevins a enregistré une variation de 80% à 99%.

D'autre part, notre étude a traité l'effet de la rétention des ovules dans la cavité ovarienne des femelles de la carpe argentée sur la viabilité des œufs. Le but de cette expérimentation est de déterminer combien de temps les ovules, prêts à être ovulés, peuvent rester dans la cavité ovarienne sans perdre leur qualité. La méthode adoptée est basée sur l'induction de l'ovulation, chez les femelles, par injection hormonale. Ensuite, les ovules extraits sont fécondés immédiatement par la laitance des mâles. Quatre extractions d'œufs ont été exécutées à des moments croissants après ovulation. L'intervalle de temps est de 30 minutes entre deux extractions successives. L'évaluation de la qualité des œufs a été effectuée par l'évaluation de la variabilité des trois taux de viabilité. Les résultats ont montré que le taux de fécondation et le taux de survie des embryons varient de 62% (au moment de l'ovulation) à 30% (pour les ovules fécondés à 90 minutes après l'ovulation) alors que le taux de survie des alevins variait défavorablement de 87 % à 49 %.

En outre, la présente recherche a été complétée par un ensemble d'analyses biochimiques afin de déterminer s'il existe une corrélation entre la qualité des œufs et la composition biochimique des ovules. Les résultats n'ont montré qu'aucun effet du vieillissement et de la période de ponte sur la composition des lipides, des protéines et des acides gras. Ainsi, l'évaluation des antioxydants (vitamine C et vitamine E) dans les ovules a révélé que leurs concentrations ne dépendent pas du poids des géniteurs de l'espèce étudiée et la concentration de la vitamine E dans les ovules est affectée par le temps de la rétention post-ovulatoire.

Mots-clés : *Hypophthalmichthys molitrix*, ovule, vieillissement, élevage, qualité des œufs, lipides, protéines, acides gras, antioxydants.

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AA Arachidonic Acid (C20:4)
ABTS 2,2'-azino-bis (3-ethylbenzothiazoline-6-
ALA α-linolenic acid (C18:3)
ALT Alanine aminotransferase
ANOVA: One-way analysis of variance
AST: Aspartate aminotransferase
C14:0 Myristic acid; Tetradecanoic acid
C15:0 Pentadecylic acid; Pentadecanoic acid
C16:0 Palmitic acid; Hexadecanoic acid
C17:0 Margaric acid; Heptadecanoic acid
C18:0 Stearic acid; Octadecanoic acid
C20:0 Arachidic acid; Eicosanoic acid
C24:0 Lignoceric acid;Tetracosanoic acid
C16:1 Palmitoleic acid; Hexadecenoic acid
C17:1 heptadecenoic acid
C18:1 Oleic acid; 9-Octadecenoic acid
C19:1 cis-10-nonadecenoic acid
C20:1 eicosenoic acid
C17:3 heptadeca-5,8,11-trien acid
DHA docosahexaenoic acid (C22:6)
DPA docosapentaenoic acid (C22:5)
E2 Estradiol
EPA Eicosapentaenoic- acid (C20:5)
ESR embryos survival rate
FAME: fatty acids methyl esters
FA fatty acids
FR fertilization rate
FSH Follicle-Stimulating Hormone
FSR Fry survival rate
g: gram(s)
g (italicised): relative centrifugal force
GOT Glutamic-oxaloacetic transaminase
GPT Glutamic-pyruvic transaminase
GGT γ-glutamyltransferase
HCl hydrogen chloride
pH potential of hydrogen
MS mass spectrometry
GC gas chromatography
IBM International Business Machines Corporation
ID Identity
Kg kilogram(s)
LH Luteinizing Hormone
L litre(s)
M Molar mass
mg milligram(s)
MIS maturation-inducing steroids,
mL milliliter (s)
MS mass spectrometry
MUFAs: Monounsaturated Fatty Acids
mRNA messenger RNA
n-3 omega-3 fatty acids
n-6 omega-6 fatty acids
NADH nicotinamide adenine dinucleotide
NaOH Sodium hydroxide
nm: nanometer
ng/l nanograms per liter
m/s meter per second
Mm millimeter
p: probability value
P1-i Period
PUFAs Polyunsaturated Fatty Acids
r coefficient of determination
RNA ribonucleic acid
ROS reactive oxygen specie
SD standard deviation
SFA Saturated Fatty Acids
SPSS Statistical Product and Service Solutions.
TEAC Trolox equivalent antioxidant capacity
Tris: tris(hydroxymethyl)aminomethane
VTG: vitellogenin,
v/v volume/ volume
w/w weight /weight
Vis visible
µm micrometer

CONTENT

To my heroes, my father Lahcen Majdoubi and my mother Fatima Elhassani.

To my best friend and big brother, my husband Hamid Masaoudi.

To my two angels Hajar and Omar, hope you get a better life.

To my sister Amina and her beautiful family Hamid and little superboy Mohammed.

To my Brother Mohammed.

To all my family Majdoubi, ElHassani, Bahtat and Masaoudi.

To all my friends.

To all my Professors since the primary school.

To those, we have lost on the way.

To the beautiful souls, we had met and made us believe how amazing is life!

To those who hurted us and made us stronger.

To humanity.

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GENERAL INTRODUCTION

Recently, in 2018, an overall of 22.6 million fry and fingerlings, of freshwater fish, were produced **(HCEFLCD,2018)**. Moreover, in the same period, artificial dam lakes were stocked with 18 million fry of freshwater fish species, mainly Chinese carps. These statistics highlight the gorgeous efforts of the Moroccan government to ensure a sustainable supply of inland waters by numerous fish species, especially Chinese carp which play a major role in inland capture fisheries and also as an ecological tool to control water eutrophication.

To reach the established goals and enhance fry production, it is needed to improve our knowledge about factors that affect egg quality and fry growth and survival. Bromage and al. **(1992)** have defined an egg of good quality as one that would show high fertilization rate and low mortalities during embryonic development, hatching and first feeding. In addition, a good quality egg produces fry and adults with efficient and healthy growth. Furthermore, Brooks and al. **(1997)** reported that factors affecting egg quality may be linked to the conditions in which females are reared during the vitellogenesis, the broodfish diet, the physico-chemical quality of water used for eggs incubation, stress and over-ripening. The determination of these factors and the evaluation of their effect were identified by assessing fertilization rate, hatching rate and survival of embryos and fry **(Crandell and al., 1995; Kjorsvik, 2003; Yang and Chen, 2006; Johnsons and al., 2012)**. On the other hand, the determination of biomarkers used as indicators of egg quality was performed based on morphological, physiological and biochemical characterization of the egg **(Kjorsvik and al., 1990; Berkeley and al., 2004; Treasure and Ford, 2010)**.

Marshall and al. **(2008)** has reported that, in most fish species, females typically determine many aspects of the offspring phenotype while males' contribution is usually only genetic. The relationship between maternal traits and egg quality and quantity was also investigated in numerous studies **(Fleming and Gross, 1990; Linhart and al., 1995)**. It was showed that according to environmental factors females could adopt a trade-off strategy between egg size and number **(Fischer and al., 2011)**. In other studies, it was also demonstrated that females size (especially length and weight) and age are also factors that control eggs production **(Marteinsdottir and Begg, 2002; Macchi and al., 2004; Marshall and al., 2010).**

Stress induced by climate changes, manipulation, rearing conditions and interaction between fish individuals (e.g. competition, predation) influences significantly the reproductive

performances and output of fish (**Morgan and al., 1999; Schreck and al., 2001; Pankhurst and Munday, 2011**). Furthermore, stress effects are transuded through a hormonal cascade initiated by perception of stressor and it's an energetically costly process during which living organisms attempt to reset homeostatic norms (**Schreck, 2010**).

As other fish species, fry production of silver carp depends on the egg quality. This specie was a subject of numerous studies to investigate about the main factors that contribute to its reproductive success and high growth performances (**Deters and al., 2013; Lenaerts and al., 2015; Tucker and al., 2020).** Despite of being considered as an invasive specie that impacts negatively aquatic ecosystems in many countries (e.g. USA), in others (e.g. Morocco), silver carp is considered as a potential food resource and enhancer of water quality. Consequently, determining its spawning behavior and factors impacting its eggs quality will help in improving the breeding practices of silver carp taking in consideration labor costs.

This research project is carried out within the collaboration between EET Faculty of Sciences and Techniques of Beni-Mellal and the High Commission for Water and Forests and the Fight Against Desertification desertification and the Comparative Endocrinology Laboratory, Department of Biology of University Federico II, Naples (Italy). It will complete many works interested in seed production of the Chinese carps farmed in the Deroua fish farm (artificial reproduction, growth, diet, phytoplankton effect on the growth of silver carp, etc.). This study is carried out to determine factors that impact silver carp' fry production. It will help producers to improve artificial reproduction techniques by taking into account the environmental conditions of reproduction, prediction of egg quality, maternal effects on reproductive output and time management to enhance hatcheries' productivity.

This thesis is compiled from a comprehensive literature review where silver carp specie, its reproductive cycle and its relationship with the factors affecting egg quality were discussed and six experimental chapters with specific scientific goals:

- ✓ Chapter I: Variability of the reproductive performances of silver carp during the breeding season
- ✓ Chapter II: Relationship between maternal traits and fecundity of silver carp eggs.
- ✓ Chapter III: evaluation of the viability changes of silver carp eggs during the post-ovulation stage

- ✓ Chapter IV: Determination of levels of lipids, fatty acids and proteins in silver carp eggs and their relationship with egg viability.
- ✓ Chapter V: Interaction between lipids, fatty acids and proteins levels in silver ova and the in vivo aging phenomenon.
- ✓ Chapter VI: Relationship between stress markers, especially antioxidants, enzymes and steroids and the viability of silver carp eggs.

LITERATURE REVIEW

1. Presentation of silver carp

1.1. Overview

1.1.1. Taxonomy and Morphology

The taxonomic classification of silver carp is cited as follow:

Kingdom: Metazoa; **Phylum:** Chordata; **Subphylum:** Vertebrata;

Class: Actinopterygii; **Order:** Cypriniformes; **Family:** Cyprinidae;

Genus: *Hypophthalmichthys*; **Species:** *molitrix*

For morphologic characteristics (Figure 1), Silver carp has an elongated body recovered with small cycloid and silver scales. It was reported that only the lateral line contains between 95 and 103 scales. The lateral line curves downwards very markedly in the abdominal region, more or less following the profile of the belly. Its large head with short and blunt snout is scales free. This fish is characterized by small eyes setting below mouth level. The mouth, with no barbels, is relatively large, upturned and toothless. The pharyngeal teeth are well developed and compressed with a striated grinding surface. Concerning the fins of this species, small specimens do not have spines on their fins, whereas large specimens have a hard, stiff spine with fine serrations on the posterior margin, at the front end of the pectoral, and moderately strong spines on the dorsal and anal fins. The dorsal fin origin is behind the pelvic fin insertion.

Figure 1 *Silver Carp, Hypophthalmichthys molitrix. at Deroua fish farm, Morocco.*

1.1.2. Ecological requirements

a) Temperature

Temperature range for optimal growth of cyprinids was defined between 20°C to 30°C (**Arrignon, 1998; Stickney, 1979)**. The optimum of silver carp larval growth is between 26-30°C **(Panov and Khromov, 1970)** and it can tolerate temperature variation between 16 and 40°C **(Tripathi, 1989).** However, silver carp tolerates temperatures close to 0°C. In the Missouri River, silver carp are active in winter, with decreasing activity when the water temperature is under 4°C (**Kolar and al., 2005**). Moreover, it was reported that when water temperature is below 18°C or higher than 31°C, the rates of ovulation and hatching depleted and the rates of abnormal embryonic development are high **(FAO, 1980).** For feeding behavior, when the water temperature dropped below 15°C, the appetite of Silver Carp was reduced, and below 8-10°C, feeding almost ceased (**FAO, 1980; Tripathi, 1989**)

b) Dissolved oxygen

Silver carp is not very demanding in terms of dissolved oxygen (**Bruslé and Quignard, 2001**), so it can support oxygen levels close to zero for short periods. Although, oxygen concentration to ensure good growth conditions is estimated at 4 mg/l (**Domaizon and Devaux, 1999**).

c) Salinity

Silver carp can live in slightly brackish water, 5-12 ‰ depending on the stage of development **(Kolar and al., 2005).** Fingerlings have a tolerance of 1.5 ‰ **(Zang and al., 1989).** Zabka **(1983)** reported that silver carp bred in water salinity of 2.5‰. Moreover, it was demonstrated in another study that Silver Carp reared in water at 5.1‰ salinity increased in weight from 1.3 to 8.8 g/individual in 32 days **(Falk, 1986).**

d) Other chemical components

Table 1 *Water tolerances to chemical components according to life stage of silver carp (www.cabi.org)*

Life stage	**Egg**		**Larva**		**Adult**	
	Typical value	*status*	*Typical value*	*status*	*Typical value*	*status*
Ammonium (mg/l)	1.5	Optimum	-	-	>3	Harmful
Dissolved oxygen (mg/l)	>5	Optimum	>4	Optimum	4	Optimum
Iron (mg/l)	>0.02	Harmful	Below 0.02	Optimum	Below 0.02	optimum
Manganese (mg/l)	Below 0.02	Optimum	Below 0.02	Optimum	Below 0.02	Optimum
Nitrate (mg/l)	<1	Optimum	<1	optimum	Below 15	Optimum
Nitrite (mg/l)	< 0.5	Optimum	Below 0.5	Optimum	Below 0.5	Optimum

1.2. Silver carp industry

The worldwide capture fisheries production is estimated at 54.091 million tons **(FAO, 2018)**. Freshwater capture fisheries were estimated at 46.38 million tons representing more than 85% of the overall production. According to the FAO **(2018)**, the global fish production in aquaculture and capture fisheries increased by 8.12% and 2.54% respectively from 1950 to 2016. Besides, the Moroccan freshwater fish production was estimated at 16 000 tons between 2016 and 2017. Silver carp represents a high percentage of this production due to the massive stocking of this specie fingerlings in the Moroccan reservoirs (Figure 2).

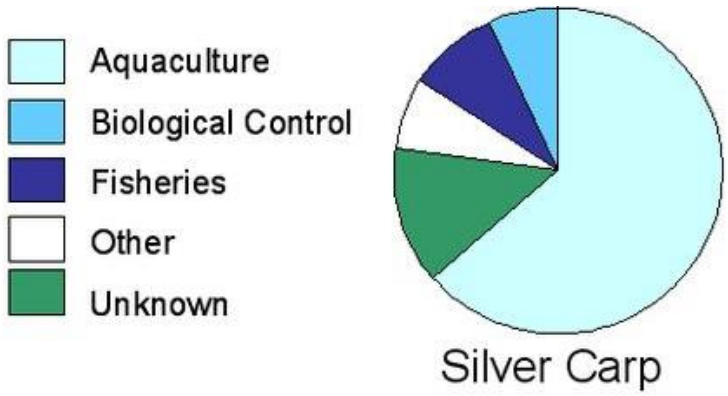


Figure 2 *The proportion of countries introducing Silver carps (H. molitrix) by various vectors. The 'other' category includes accidental introductions, diffusion from neighbouring countries, and introduction for research (by Chapman in: Kolar and al., 2005)*

Silver carp was introduced in numerous countries in the world for ecologic and socio-economic reasons. This specie is an efficient tool to control eutrophication phenomena in reservoirs thanks to its high capacity to ingest microscopic algae responsible of bad water quality. It has a good capacity to control the algal bloom in reservoirs and then enhance water quality. Further, it was demonstrated that at 23°C the consumption increased up to six times per day (**Omarov, 1970**). In many studies it was proved that silver carp has a considerable ability to control algae, zooplankton and also suspended organic matter (**Pillay, 1993; Robert and Uwe, 2002; Leventer and Teltsch, 1990; Farid et al., 2017; Starling, 1993; Dabbadie, 2005**). It was largely used for water quality management, especially for removal of the excessive algae from wastewater **(Henderson, 1977).**

On the other hand, the silver carp is considered as a food supply, it is reared and produced for domestic markets for local consumption. Moreover, many processed food products are made

from this specie (e.g vacuum packed sliced fillets, canned fish with oil, sauces) but it is mainly commercialized as live fish (**Kolar and al., 2005**). Furthermore, silver carp is also cultured in polyculture with other fish species to enhance their growth and production. The presence of this specie in polyculture improves growth of Common Carp and tilapias (**Yashouv, 1971**).

Surprisingly, this specie is not welcomed in some countries where it is considered as an invasive specie because of its negative impact on others fish. Due to its fast growth, the silver carp represents a potential hazard because it competes with the native fish for food and space which may damage the ecosystem. In the USA, silver carp, along with other Asian carps, have ended up in the Mississippi where they compete with the native species in their habitat.

2. Female's Reproductive cycle in fish

The reproductive cycle was defined by Mañanós and al. **(2008)** as a set of successive processes from immature germ cells to the production of mature gametes (Figure 3), with the final purpose of obtaining a fertilized egg after the insemination by spermatozoa. It can be divided into two main periods (or phases): the period of oocyte growth (gametogenesis or vitellogenesis) and the period of oocyte maturation.

2.1. Oogenesis

2.1.1. Vitellogenesis

The immature ovary contains nests of oogonia along the ovigerous lamellae, which proliferate through mitotic divisions. At a certain moment, part of the oogonia population enters meiosis and become primary oocytes, these later become inactive immediately at prophase I. The first step of gametogenesis is previtellogenic phase, which is hormone–independent phase. During previtellogenesis, the primary oocytes increase in size and the pale material in the cytoplasm, granulosa and theca cellular layers, become visible. Before the progression of vitellogenesis the oocyte is small and its cytoplasm is not clearly deprived of inclusions.

The next phase is vitellogenesis during which the vitellogenin (VTG), lipophosphoglycoprotein, is synthesized in the liver and stimulated by the release of 17β-estradiol (E2). The vitellogenin is the precursor of the vitellin reserves of the egg **(Wallace, 1985)**. During the advancement of this process, vitellogenesis, the cortical alveoli, lipid globules and yolk granules and other inclusions appear in the cytoplasm. Moreover, the layers of zona radiata, granulosa and theca become more and more thick to support oocyte growth. At the end of vitellogenesis, the oocyte has a transparent cytoplasm entirely rich with yolk granules and lipid globules. The germinal vesicle (GV) is located

in the center of the oocyte. The thick layers of zona radiata become clearly striated and enveloped by the granulosa and theca follicular layers. The accumulation of the vitellogenin in the oocytes is of vital importance for the quality of the egg and, also, for the survival of the larvae before the exogenous feeding.

2.1.2. Oocytes Maturation and ovulation

The maturation of the oocyte started by the resumption of meiosis and its advance to metaphase II during which the oocyte becomes a secondary oocyte **(Nagahama and al., 1994)**. During maturation, the germinal vesicles migrate to the peripheral position, under the micropyle, and the yolk become translucent **(Billard, 1979)**. At advanced maturation, the nuclear membrane disappeared, which is called Germinal Vesicles Break Down (GVBD) **(Mañanós and al., 2008)**. During the breakdown process, a part of yolk protein, stored in the oocyte, break and the releasing of free amino acids and small peptides occurs which act as osmotic effectors of water influx **(Le Menn and al., 2007)**. As a consequence of this modification, the ionic composition of the cytoplasm changes, due to changes on lipids and yolk inclusions, and leads to the water incorporation inside the oocyte **(Mañanós and al., 2008)**. This hydration causes a rupture of the follicular wall and causes the release of the oocyte into the ovarian cavity, which is the ovulation phase. The ovulated egg contains the genetic code for the new individual, nutrients, maternal RNA and maternally deposited hormones which all may affect the egg quality **(Brooks and al., 1997; Lubzens and al., 2010)**.

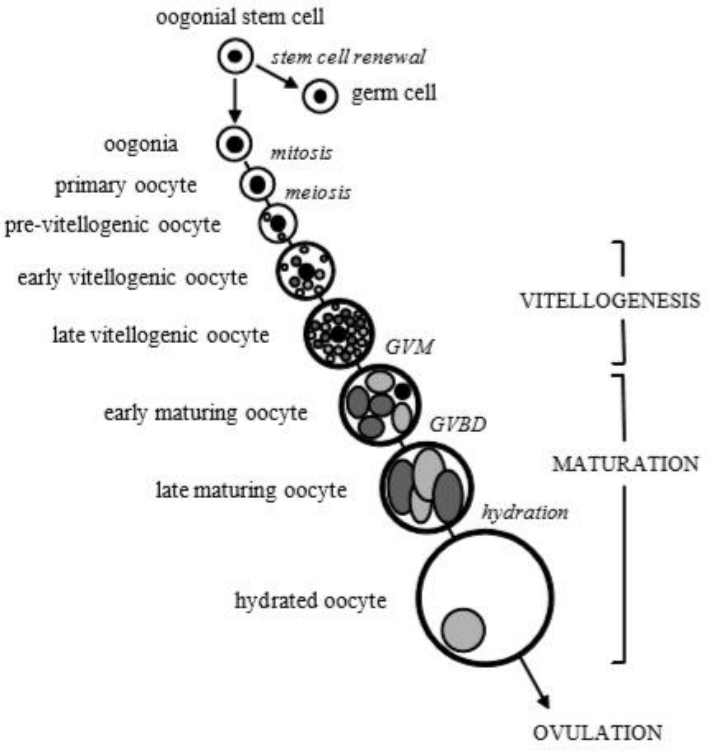


Figure 3 *The process of oocyte development and maturation in female fish. vitellogenin (VTG), as the cortical alveoli (white circles), lipid globules (light grey circles) and yolk granules (dark grey circles), germinal vesicle, GV (black circle) animal pole (GV) (Le Menn and al., 2007).*

3. Main Factors affecting the reproductive cycle in fish

Ova growth and production are controlled by environmental factors such as temperature and photoperiod. They impact the progress of gametogenesis by affecting hormones and neurohormones functions (Figure 4) (**Pankhurst, 2016; Billard, 1979**). As it was demonstrated by numerous studies, temperature and photoperiod are the main factors that have direct effects on the gametogenesis process in fish (**Le Menn and al., 2007; Pankhurst and Porter, 2003; Pankhurst and Munday, 2011).** For this reason, we will present a short review on the relationship between photoperiod, temperature and the reproductive process of fish in general and for cyprinids, silver carp in particular.

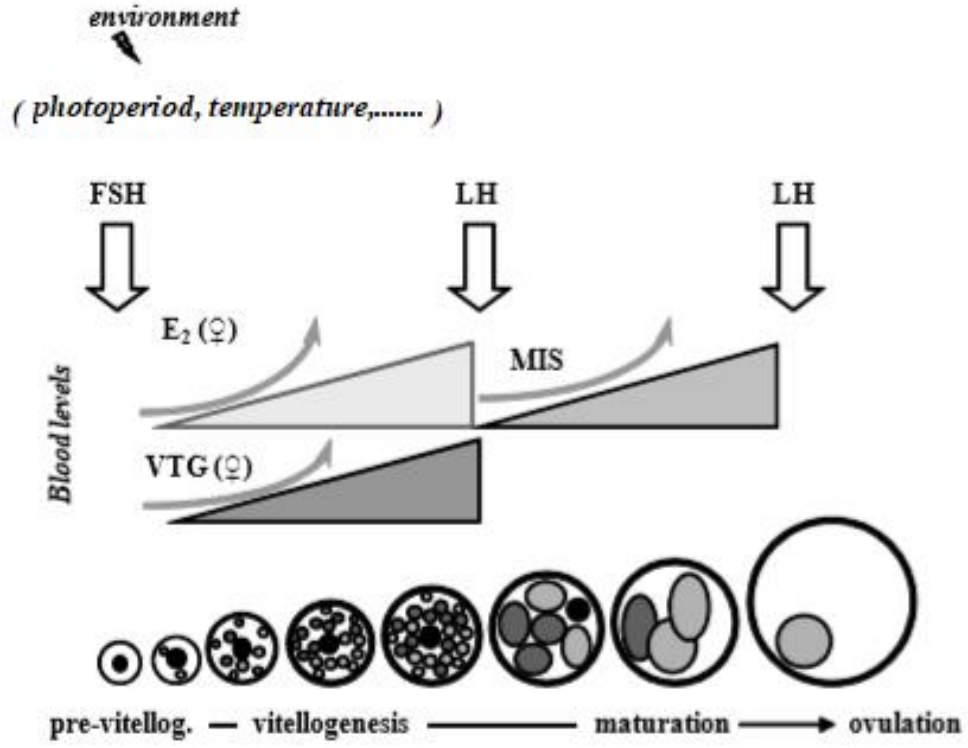


Figure 4 *The process of the effect of the environmental factors on the reproductive process in fish females the top half of the diagram shows the pituitary hormone secretions and plasma hormone concentrations, the bottom half dhows the correlated stages of oocyte development. The period of vitellogenesis is characterized by high blood levels of FSH and increasing levels of E_2 and VTG. At the end of oogenesis pituitary LH secretion induces the synthesis of maturation inducing steroids (MIS) which regulate the process of gonadal maturation. This is characterized by decreasing blood levels of FSH and androgens/ estrogens and increasing blood levels of LH and MIS. LH: Luteinizing Hormone, MIS: maturation-inducing steroids, E2:17β-estradiol, VTG: vitellogenin, FSH: Follicle-Stimulating Hormone (Mañanós and al., 2008; modified).*

3.1. Photoperiod

Several studies demonstrated that photoperiod has a significant effect on the all aspects of maturational development of fish (**Bromage and al., 1993; Bromage and al., 2001**). In captivity, controlling photoperiod (or day-length DL) can allow spawning fish species all year round. Manannos and al. **(2008)** reviewed that for freshwater species, especially tilapia and carps, controlled temperature and photoperiod conditions entrain spawning for an entire year. In 1995, Thomas and al. have succeeded on inducing spawning of red drum during 7 years only by maintaining photoperiod at 12:12 DL. Under natural conditions, salmonids' gametogenesis occurs in a decreasing photoperiod, from 16 hours to 8 hours during 6 months (**Billard, 1979**). However, the reproductive process is entrained not only by photoperiod but by its interaction with other factors. Furthermore, it also depends on the reproductive strategy of every fish species; for example**,**

for fish that spawn in spring or in early summer the gametogenesis is stimulated by long photoperiod combined with warm temperatures (**Htun-Han, 1977**). In cyprinids, vitellogenesis takes place under a natural photoperiod (LD 10:14 - 14:10) and when temperature vary between 18°C and 30°C. This combination between temperature and photoperiod was also demonstrated in other fish species such as the Japanese medaka, *Oryzias latipes* (**Yoshioka, 1962, 1963**), the sunfish, *Lepomis cyanellus* (**Kaya and Hasler, 1972**) and *L. megulotis* (**Smith, 1970**), the mosquitofish, *Gambusia affinis* (**Sawara, 1974**), the golden shiner, *Notemigonus cysoleucas* (**de Vlaming, 1975**), and the goldfish, *Carussius auratus* (**Ka- wamura and Otsuka, 1950; Fenwick, 1970; Gillet et al., 1978).**

3.2. Temperature

For the good process of vitellogenesis and maturation, most fish species have an optimum temperature range. If temperature is under this range, maturation will be delayed. While higher temperature accelerates oocyte maturation which would causes the overripeness of the ovum if it is not stripped and fertilized at the appropriate time **(Bye, 1990)**. For spring spawning species, increased temperature cues the reproductive development while low temperature levels stimulate reproduction for autumn-spawners (**Pankhurst and Munday, 2011**). In general, like other environmental factors which impact the reproductive cycle of the fish, temperature effect is related to the period during which temperature changes were occurred and it duration and severity depending on the optimum range of the species. Donelson and al. **(2010)** reported that nutritional status of damsel fish modulates the effects of temperature on it reproductive process. Moreover, the geographic distribution has a sort of effect on the acclimation and the adaptation to temperature changes (**Angilletta, 2009; Gardiner and al., 2010**)

Concerning temperate species, temperature has a strong effect on cyprinids' gametogenesis, it is the main factor that controls the reproductive cycle of this specie (**Billard, 1979**). Depending on the female's age of cyprinids, the spawning occurs only after reaching 1000 to 2000 degree days. For other freshwater species vitellogenesis can proceed in warm water such as *P. ambiguus*, which ovulation occurred at 23.6°C (**Lake, 1967)**, 21-24°C for channel catfish, *Zctalurus punctatus*, (**Huet, 1975**), 20°C for tench, *Tinca tinca*, (**Breton et al., 1980**).

On the other side, other fish species spawn in cold waters. For instance, the ovulation in rainbow trout starts at low temperatures (**Breton and Billard, 1977**). In general, in cold water (12.1°C), rainbow trout's vitellogenesis can proceed to the tertiary yolk-granule stage (**Yamazaki, 1965**,) but ovulation will not occur unless vegetation is present (**Pandey and Hoar, 1972; Pandey and al.,**

1973, 1977; Stacey and Pandey, 1975; Lam and al., 1975, 1976; Peter and al., 1978). In contrast, it was reported by Wiebe **(1968**) that in *C. aggregata*, the primary growth of the oocytes occurred in warm water while the late phase is stimulated by low temperature.

1.1. Reproduction of silver carp

1.1.1. Maturity and Fecundity

As all cyprinids, the reproductive cycle of silver carp is temperature dependent. It has been reported that this specie reaches its sexual maturity at the first year; but in general between 2 and 6 years (Table 2). Usually, males reach maturity one year before females (**Abdusamadov, 1986; Kolar and al., 2005**). According to Kolar and al. **(2005)**, the absolute fecundity of silver carp is high and can reach more than 5 million eggs for some females. Same author, revealed that the fecundity of silver carp varies greatly according to the geographical areas and especially according to the size of the fish, for example 597.000 - 4.329.600 eggs per female for fish of 6.4 - 12.1 kg.

Table 2 *growth, age and size of silver carp at maturity*

Table 2.1 Growth of silver carp.[a]

Age (years)	Body length (cm)	Weight (g)
2	50.0	1.803
3	57.6	4.650
4	60.3	5.340
5	63.0	6.400

[a] Adapted from Chang et al. (1983)

Table 2.2 Age and size at sexual maturity of silver carp

	Sexual maturity		
Country	Age (years)	Weight (kg)	Authority
South China	2-3	2-55-6	Kuronuma (1968)
Central China	4-5	2-5	Kuronuma (1968)
North China	5-6	2-5	Kuronuma (1968)
Rumania	6-9	6-8	Woynarovich (1968)

1.1.2. Reproductive cycle

The timing of spawning can be estimated by counting 1000-1200 (2 to 3-year-old fish) or 2000 (>3-year-old fish) degree days from when spring temperatures rise above 15°C. Therefore, vitellogenesis takes place under a natural photoperiod (LD 10:14 - 14:10) and thermal cycle (13-30°C). In general, gametogenesis of silver carp begins in summer immediately after spawning and it ends in autumn. Then, the oocytes stay blocked on the metaphase II until the spring or the following summer when spawning will occur again as soon as the oocytes maturation and ovulation will be stimulated by thermal conditions **(Billard, 1979**).

In its native area, Silver carp spawns in water bodies (specially in Asia). This fish breeds, often, after a sudden increase in water level (**Krykhtin and Gorbach, 1982; Kolar and al., 2005**). Eggs are scattered onto river beds (Chinese carps) and they drift with the water flow, sometimes up to 500 km downstream of the spawning grounds (**Kolar and al., 2005**). It appears that a minimum

length of 100 km is necessary for the development of eggs and larvae. Large lakes connected to rivers serve as nursery areas (**Kolar and al., 2005**). Silver carp is a batch spawner, it can spawn at least twice during the year 3-4 months apart, in tropical and subtropical regions. But in the northern latitudes this specie spawns only once a year.

2. Factors affecting eggs quality in fish

It was reported that good quality eggs are those which exhibit low levels of mortality at fertilization, hatch and first-feeding and those which produce the fastest growing and healthiest fry and older fish (**Bromage and al., 1992**). In the last decades, the enhancement of fish egg quality attracted scientists and aquaculturists interest. The knowledge of egg quality and mechanisms that impacts its viability was largely investigated through the determination of biochemical (**Zarski and al., 2012; Zakeri and al., 2011**), physiological (**Kjørsvik and al., 2003),** molecular **(Sohn and al., 1998; Cerda and al., 2008**) and endocrinological (**Srivastava and Brown, 1992; Thorsen and al., 2003**) analysis.

Also, to enhance seed production of several fish species, that have commercial and ecological importance, many investigations were carried out to decode the relationship between egg quality and broodstock diet, stress, maternal traits and the timing of eggs stripping (**Palacios and al., 2011).**

2.1. Nutrition and feeding

In many fish species it has been showed that the nutritional components of the diet and the feeding period can affect the spawning, egg and larval quality (**Izquierdo and al., 2001; Quintero, 2007; Palacios and al., 2011**). Limited feeding of breeders causes dysfunction of gonadal development, fish maturity and reduced fecundity in brown trout, *Salmo trutta*, (**Kjorsvik and al., 1990**). The richness of the diet by essential nutrients have also an important role in the enhancement of reproductive potential of fish and egg quality.

2.1.1. Lipids and Fatty acids

Lipids and their components, Fatty acids, are largely studied in fish nutrition and their effect on gametogenic process and egg quality. This is due to the fact that fatty acids composition of the eggs is directly related to the fatty acids content in the mothers' diets (**Fernández-Palacios and al., 2011)**. Furthermore, it was proved that these components play an important and significant role in preserving egg quality and improving larval viability (**Watanabe and al., 1984; Mourente and al., 1989; Dhert and al., 1991; Bruce and al., 1993; Fernández-Palacios and al., 1995; Navas and al., 1997; Rodriguez and al., 1998; Lavens and al., 1999; Furuita and al., 2002, 2003; Mazorra**

and al., 2003; Aijun and al., 2005; Izquierdo, 2005; Valdebenito and al., 2015). Their levels vary with species and the environmental conditions in which gametogenesis occurred **(Dantagnan and al., 2007)**. Also, fatty acids are the main energetic source used during the embryonic development (**Tocher and al, 1985a; Sargent, 1995**). They are the main components of the phospholipids in the bio-membrane, they regulate the fluidity of the cell and they have a vital function in the development of the neural tissue **(Bell and al., 1986, 1997; Takeuchi, 1997; Sargent, 1995; Sargent and al., 1999b, 1993; Benitez and al., 2007).** Also, it was showed that lipids are mobilized from other fish organs, especially liver and muscles, to the gonads in order to ensure their normal development during the reproduction period (**Castell and al.,1972).** Besides, Brooks and al. **(1997**) reported that a deficiency in polyunsaturated fatty acids (PUFA), docosahexaenoic acid (DHA) and eicosapentanoic acid (EPA) affect negatively egg quality and survival in teleost fish. Moreover, n-3 series of fatty acids improve females' fecundity and egg and larval quality (**Aby-Ayad and al., 1997; Fernández-Palacios and al., 2005).**

Besides, it was demonstrated that n-3 PUFAs have a significant effect on the reproductive performances in many fish species such as *Sparus aurata* (**Almansa and al., 1999**), *Plectorynchus cinctus* (**Li and al., 2005**), *Paralichthys olivaceus* (**Furuita and al., 2003**), *Acanthopagrus latus* (**Zakeri and al., 2011**), *Astacus leptodactylus* (**Harlioğlu and al., 2012**) and *Lateolabrax japonicas* (**Xu et al, 2010**). However, high levels of n-3 PUFAs reduced egg and larval viability (**Furuita and al., 2000; Lavens and al., 1999; Fernandez-Palacios and al., 1995**). High levels of n-3 PUFAs in diet leads to low number of produced ova with reduced quality in *Sparus aurata L.* **(Fernández-Palacios and al., 1995),** *Paralichthys olivaceus* **(Furuita and al., 2002)** and *Xiphophorus helleri* **(Ling and al., 2006).** On the other hand, Cavalli and al. **(1999**) revealed that feeding *Macrobrachium rosenbergii* broodstock with high levels of C18:2n-6 and n-3 PUFAs improved fecundity, fertilization rate, egg hatchability and larval quality. Diets containing optimal amounts of arachidonic acid (ARA) enhance fecundity, egg viability, hatching rate and larval survival (**Bruce and al., 1999; Fernández-Palacios and al., 1995, 2005; Navas and al., 2001)**. In *Trichopodus trichopterus*, the inclusion of 2% of arachidonic acid (ARA) in broodstock diet enhance fecundity and hatching rate (**Asil and al., 2017**). Nevertheless, data about the effect of fatty acids on reproductive performances in numerous fish species are limited.

2.1.2. Proteins

Proteins are the main energy source during embryonic development of the majority of teleost fish whose vitellogenin are the main protein precursor of yolk (**Watanabe and Kiron, 1994**

Sivaloganathan and al., 1998). They have an important role in fertilization and embryo development and they are involved in the normal development of tissues and organs (**Hart, 1990; Srivastava and Brown, 1992; Srivastava and al., 1995; Da-Silva and Anderson, 1995; Rønnestad and al., 1999; Lanes and al., 2012**). Moreover, many studies determined amino acids that have functional role in regulating osmotic pressure for oocyte hydration and usable supply of nutrient for embryo, and present positive correlations with egg buoyancy (**Matsubara and al., 1999; Lahnsteiner, 2005; Ohkubo and al., 2008; Wu, 2009; Tong and al., 2017**).

Proteins and their constituents, amino acids, serve as regulators of gene expression, key precursors for hormones synthesis and they take a part in cell signaling **(Wu, 2009).** In sea bass, broodstock receiving diets with low proteins alter the secretion of GnRHs (**Kah and al., 1994**) and LH (**Navas and al., 1996**) which are important hormones for the regulation of oocyte maturation and ovulation. Another amino acid that is important for fish reproduction is tryptophan. It's affects the gonadal maturation of fish. For example, ayu *(Plecoglossus altivelis)* fed with diets supplemented with 0.1% of tryptophan induce testosterone secretion which induces female maturation (**Akiyama and al., 1996**). On the other hand, Al-Hafedh and al. **(1999**) showed that a high levels of proteins in the Nile tilapia (*Oreochromis niloticus*) diet enhance it fecundity and increase the number of eggs. Moreover, optimal proteins' content in diet of grass carp (*Ctenopharyngodon idella*) and common carp (*Cyprinus carpio*) have positive effect on increasing egg quality and larval performance (**Manissery and al., 2001; Khan and al., 2004).** A positive impact was found on fecundity and larval deformities for sea bass (*Dicentrarchus labrax*, **Cerdá and al., 1994**) and Japanese sea bream (*Pagrus major*; **Watanabe and al., 1984**) if they received sufficient amount of dietary protein with balanced amino acids.

However, high levels of proteins in diet could negatively impact reproductive performances in fish. For instance, in Labeo roho, *Labeo rohita*, fecundity was high when dietary protein levels varied from 20 to 25 and 30%, while an increase over 35 or 40% reduced fecundity **(Khan and al., 2005**). Similarly, maximum fecundity of catfish, *Mystus nemurus*, was obtained when the diet contains around 35% of proteins (**Abidin and al., 2006**).

1.1.1. Vitamins E and C

Vitamins are vital nutrients which are used, in small amounts, by fish for the normal growth, health and reproduction. They exist in two forms: hydro-soluble vitamins (e.g. vitamins B and C) and fat-soluble vitamins (e.g. vitamins A, D and E). The demand for vitamins differs from one species to another, it also depends on the age, size and physiological state of the fish. The relationship between

vitamin C, E and A and fish growth and reproduction was extensively studied by Fernandez-Palacios and al. **(2011)** and Volkoff and London **(2018)**.

Vitamin E inhibit lipids peroxidation in animal cells (**Huber, 1988; Atkinson and al., 2010**). It is the most important molecules that protect eggs during the early development **(Packer, 1991)**. It was reported that this vitamin promotes gonadal maturation and enhances hatching rate and survival rate of larvae in common carp (*Cyprinus carpio*), ayu (*Plecoglossus altivelis*), Japanese sea bream (*Pagrus major*) (**Watanabe, 1990, Watanabe and al.,1991).** Watanabe and al. **(1991**) studied the effects of increasing vitamin E in the diet (above 200 mg/kg) in *P. major*, and found that it improved the percentage of floating eggs, the incubation rate and the percentage of normal larvae. In Pindani (*Pseudotropheus socolofi*), females fed with diets supplemented by vitamin E (α-tocopherol) had increased size of eggs, fertilization and hatching rates, number of spawns, batch fecundity and relative fecundities and pre and post larval survival rates **(Erdogan and Arsalan, 2019).** Likewise, others studies revealed the existence of vitamin E from females' tissue to ovary during vitellogenesis which leads to high levels of vitamin E in eggs and low in tissue (**Lie and al., 1994; Hemre and al., 1994; Mukhopadhyay and al., 2003b**).

On the other hand, high level of vitamin C in feed enhances eggs viability **(Izquierdo and al., 2001)**. Vitamin C have significant effect on steroidogenesis and vitellogenesis in salmonids **(Eskelinen, 1989; Sandnes, 1991; Blom and Dabrowski, 1995)**. It has a significant impact on fish by promoting growth and survival, reducing malformations, enhancing immune and optimizing stress responses **(Darias and al., 2011)**. Furthermore, supplementing broodstock diet by high vitamin concentrations improves egg quality and increases hatching in rainbow trout (**Ridelman, 1981; Sandnes and al., 1984)** and hatching rate and larval viability in Nile tilapia (*Oreochromis niloticus*) (**Soliman and al., 1986**). In milkfish (*Chanos chanos*), supplementing females's diet with 1% of vitamin C enhances eggs' viability, hatching rates and larval survival **(Emata and al., 2000)**.

Finally, it was demonstrated that not only vitamin C and E levels in diets affect Fecundity and egg quality separately, but the interaction between them have a significant impact on the enhancement of the reproductive performance of fish (**Silveira and al., 1996; Emata and al., 2000**). It was showed, by Lebold and al. **(2013),** that the interaction between vitamins C and E increases the antioxidant protection of organic molecules.

1.2. Stress

Many definitions have been established to give a better understanding of stress **(Selye, 1950, 1973; Pickering, 1981; Emlen and al., 1998)**. For instance, Schreck **(2000)** defined stress as a physiological cascade of events that occurs when the organism is attempting to resist death or reestablish homeostatic norms in the face of a threat. For this reason, stress is considered as one of the key factors that should be controlled in aquaculture in order to have a successful stripping and good quality eggs. Furthermore, stress effect is related to the timing when was occurred in fish life cycle and also its severity (Figure 5). Westernhagen **(1988)** stated that nutritional deficiency is one of factors causing stress and affects fecundity and gamete quality. He reported that this effect is related to different tolerance thresholds that eggs have against stress factors at different stages of development. Additionally, based on fish density, as a factor leading to stress, many authors outlined that a prolonged exposure to stress leads to a dysfunction in reproductive process and oocytes atresia which gives low eggs viability **(Bromage, 1992; Brooks, 1997; Valdebenito and al., 2011)**. On the other hand, it was demonstrated that fish exposed to a chronic stress affects the secretions of some hormones (cortisol, sexual hormones) and leads to delaying gonadal development **(Campbell and al., 1994; Sumpter, 1997)**. In numerous fish species, it was demonstrated that stress causes a decline of sex hormones such as testosterone, 11-ketotestosterone, and 17ß-estradiol in C. *nebulosus* **(Safford and Thomas, 1987)**, rainbow and brown trout **(Pickering and al., 1987; Pankhurst and Dedualj, 1994)**, snapper, *Pagrus auratus* **(Carragher and Pankhurst, 1991**), red gurnard, *Chelidonichthys kumu* **(Clearwater, 1992)**, and white sucker **(Van Der Kraak and al., 1992; McMaster and al., 1994)**. Additionally, it was proved that females subjected to stress can have negative impact on the cortisol levels in the eggs depending on the severity and longevity of stressor. This negative impact was investigated in coho salmon (*Oncorhynchus kisutch*) **(Stratholt and al., 1997)**, tropical damsel fish *(Pomacentrus amboinensis)* **(McCormick, 1998, 2009)**, Sockeye salmon (*Oncorthynchus nerka*) **(Taylor and al., 2016)**. Many studies showed that Stress induced cortisol release affects the overall metabolic system, including the immune response system, and may increase the susceptibility of the fish to diseases **(Harris and Bird, 2000; Davis and al., 2002)**. It was showed that injecting cortisol into adult tilapia, *Oreochromis mossambicus*, causes a decrease in oocytes size and circulating testosterone and 17ß-estradiol concentrations **(Foo and Lam, 1993)**.

However, Schrek and al. **(2001)**, suggested that the females have a mechanism which allow them to protect or buffer their eggs from the deleterious effects of stress (Figure 5). He proposed two

mechanisms that stressed females adopt to maintain the quality of eggs: (1) the quantity and nature of substances transferred from the female to the egg, and (2) the timing of maturation and ovulation.

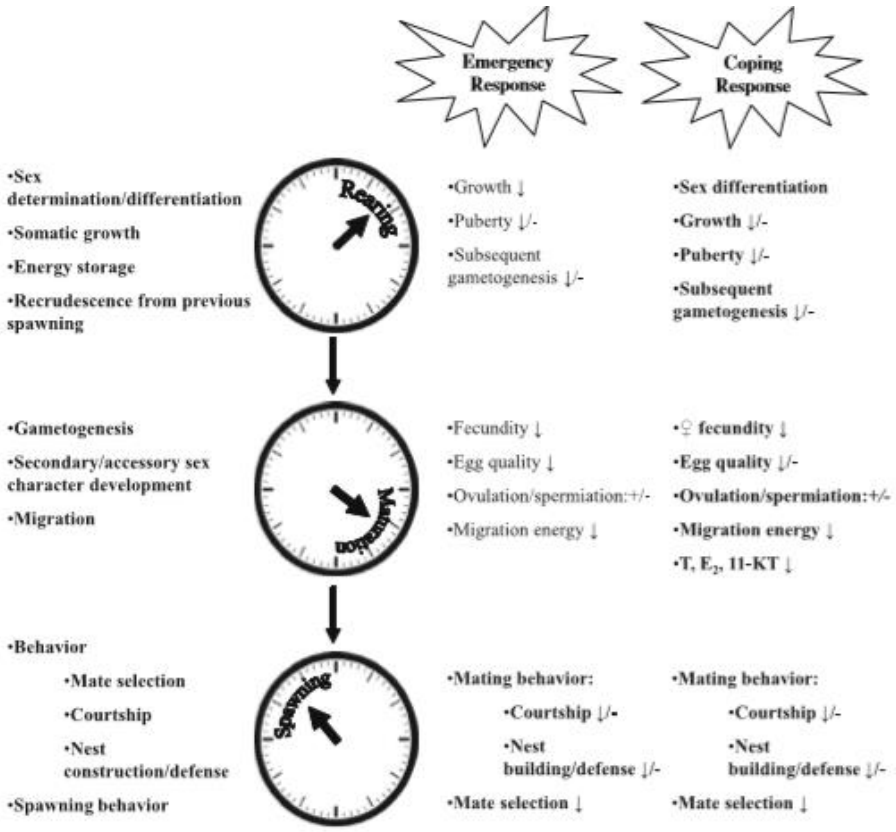


Figure 5 *The organism level response to stressors causingemergency responses and coping responses as part of allostatic load are shown to the right of the clocks. Responses in bold lettering are based on solid information; responses not in bold lettering are based on strong inference. A down arrow indicates a decrease. A minus sign (-) indicates inhibition (Schreck, 2010)*

1.3. Overripening (Post-ovulatory aging)

The overripening (or post-ovulatory aging) is a phenomenon during which fertilizable oocytes capacity progressively decrease **(Aegerter and Jalabert, 2004)**. This phenomenon is observed in farmed fish that are kept to be spawned artificially in hatcheries such as salmonids, carps, pike perch. During overripening, eggs are exposed to morphological, biochemical and molecular changes that cause a decrease in their quality and viability **(Springate and al., 1984; Barnes and al., 2000, 2003; Barnes and Durben, 2004; Aegerter and Jalabert, 2004; Rime and al., 2004; Mansour and al., 2008; Bobe and Labbé, 2010)**. Furthermore, salmonids are the most used species to characterize and decode the mechanism and the effect of aging on egg quality **(Escaffre and Billard, 1979; Bry, 1981; Springate, 1984; Bromage and al., 1992; Samarin and al., 2008; Milla and al., 2011)**. Thus, the evaluation of egg quality was based on the assessment of fertilization success (**Hay, 1986;**

Yang and Chen, 2006), survival of embryo **(Flett and al., 1996)**, hatching rate **(Baidya and Senoo, 2004)**, larval survival **(Crandell and al., 2004)** and biochemical composition **(Craik and Harvey, 1984; Bahre kazemi and al., 2010; Lord and Aitken, 2013)**. Also, due to the strong relationship between temperature and oocytes maturation, experiments were carried out to determine the effect of temperature on the aging process **(Gillet, 1991; Mansour and al., 2008)**. For instance, it was proved that, according to temperature, the ova retention in rainbow trout could last 9 days after ovulation at 13°C **(Bry, 1981)** and 14 days at 10°C without affecting the success of the fertilization **(Azuma and al., 2003)**. For silver salmon, this period varies between 4 and 10 days after ovulation at 10°C **(Gordon and al., 1987; Bromage and al., 1994)**. Based on the assessment of the embryos survival, Bonnet and al. **(2007)** showed that, rainbow trout ova retained in the abdominal cavity for 16 days after ovulation presented an embryo survivability of 37%, compared with 93% in a control group. Similarly, Barnes and al. **(2000)** proved that over-ripened eggs gave a low survival of embryos (30%) compared to ova stripped immediately at ovulation (50%).

Numerous studies have investigated the biochemical changes in the egg during the overripening process **(Craik, 1984; Bahre kazemi and al., 2010; Samarin and al., 2015b)**. Kjorsvik and al. **(1990)** reported that overripe turbot (*Scophthalmus maximus*) eggs contained more lipids of all groups, especially phospholipids, than viable eggs. In salmonids, specially rainbow trout, it was proved that during post-ovulatory aging, egg quality can be predicted by determining pH and osmolarity in the ovarian fluid **(Aegerter and Jalabert, 2004)**. On the other side, it has been proved that aged eggs have lower mRNA expression **(Aegerter and al., 2005; Ma and al., 2015)**. Moreover, hepcidin-1 can be a potential egg quality marker for the post-ovulatory aging of Atlantic salmon eggs, **(Bizuayehu and al., 2019)**.

CHAPTER I: VARIABILITY OF THE REPRODUCTIVE PERFORMANCES OF SILVER CARP DURING THE BREEDING SEASON

1. Introduction

Fish, as known, is a special food not only recognized by its value in macronutrients like healthy fats and proteins, providing energy and structural molecules, but also by being an excellent source of micronutrients. Vitamins and minerals are micronutrients with a very important role in the development and health. Fish are very rich in many essential minerals such as iodine, selenium, zinc, iron, calcium, phosphorus, potassium, and vitamins such as A, B and D **(FAO, 2016)**. Carp production represents over 50% of all animal biomass produced by aquaculture all over the world **(Stickney and al., 2000)**. The silver carp, *Hypophthalmichthys molitrix*, represents an important source for guaranteeing food supply and developing commercial fishing in Morocco and in Deroua fish farm at Fkih Ben Saleh since 1990. Silver carp is one of the most cultured fish species in the world due to its zootechnical performances (high growth) and feed efficiency. It provides high quality proteins with a lower cost than other farmed fish species. Carp meat contains 78.33% of moisture, 15.80% of proteins and 2.12% of lipids and nutritional composition may be different between farmed and wild fish **(Ashraf and al., 2011).** Also, the amino acids were higher in farmed silver carp which can be due to the diet afforded to the cultivated specie **(Kindong and al., 2017)**.

Besides, silver carp meat presents an important source of essential fatty acids for the human nutrition. It contains high proportions of Palmitic acid, Oleic acid, Alpha-linolenic acid, docosahexaenoic (DHA) and eicosapentanoic (EPA) **(Kindong and al., 2017)**. As a consequence, silver carp present a potential source of the n-3 fatty acids which are important for human health, early development, and the prevention of some diseases **(Taşbozan and Gökçe, 2017)**.

Normally, the greater knowledge of the factors influencing reproduction, eggs quality and fry survival contribute to improving the yield of fish production on farms and guarantee a sustainable food resource for the growing population. For this reason, numerous studies have been conducted to decode the determinants of eggs quality and their relationship with the survival and growth performance of the larvae. Nutrition, physiological antioxidative defense, temperature, photoperiod, salinity, pollutants are key aspects that influence the oocyte quality and its development **(Guerriero and al., 2004; Guerriero and Ciarcia, 2006; Parisi and Guerriero, 2019)**. Intrinsic factors such as maternal age and genetics also have a direct impact on egg quality **(Carillo and al., 2000;**

Heinimaa and Heinimaa, 2004; Venturelli and al., 2010; Macchi and al. 2013). For many farmed fish species, the identification of these factors and the degree of their impact will serve as tools for fish farmers to produce a large number of viable eggs with high survival and optimal fry growth **(Lubzens and al., 2010).** Much research is aimed at identifying factors that influence the success of reproductive production and being used as direct morphological, physiological and biochemical biomarkers of egg quality **(Berkeley and al. 2004; Guerriero and al., 2004; Guerriero, 2007; Treasurer and Ford, 2010, Majdoubi et al., 2017)**. The success of fertilization or "fertilization rate" is widely used to determine the quality of eggs in the early stages of embryo development **(Papadaki and al., 2008; Bobe and Labbé, 2010)**. Evaluation of embryos development during incubation and their survival are also used to evaluate the quality of eggs in commercial hatcheries **(Kjorsvik, 2003; Mylonas and al., 2003)**. Knowledge of the changes on egg quality during fish breeding season is important for improving productivity in farms. Determining seasonal and inter-annual changes in egg quality is an effective tool for optimizing egg collection and larval production **(Mylonas and al., 2004; Castet, 2011)**.

In this study, we present an effective method for highlighting the viability changes applied in silver carp eggs. Our goal is to increase the productivity of the carp culture and ensure a sustainable food resource without additional costs based on the biostatistical management of breeding operations during the breeding season. For this reason, we rely on the analysis of data and statistical analysis of the fry survival rate as a parameter to predict the yield of the fry production of this specie. Also, the relationship between maternal age and weight and different viability rates (fertilization rate, survival of embryos and fry) was investigated. As a case study, we chose broodfish bred in Deroua fish farm in Morocco.

2. Material and methods

2.1. Presentation of the study area

The Deroua Fish Farm Station is located in the Fkih Ben Saleh Province, 25 km southwest of the city of Beni-Mellal. Its coordinates, presented as decimal degrees, are: latitude = 32.500 and longitude= -6.700. Based on its climate conditions it is considered as semi-arid zone. The temperature oscillates between 18.5°C and 37.5°C in august and between 4.4°C and 17.8°C in January (Table 3). Furthermore, the region is known by moderate precipitations in winter that can reach 83 mm in December.

Table 3 *Temperature and precipitations profile in the province of Fkih Ben Saleh (Source: Climate data.org)*

	Janvier	Février	Mars	Avril	Mai	Juin	Juillet	Août	Septembre	Octobre	Novembre	Décembre
Température moyenne (°C)	11.1	12.2	14.5	16.9	19.9	23.9	27.8	28	24.9	20.6	15.1	11.8
Température minimale moyenne (°C)	4.4	5.2	7.2	9.1	11.6	15.2	18.1	18.5	16.5	12.9	8.5	5.2
Température maximale (°C)	17.8	19.3	21.9	24.8	28.3	32.7	37.5	37.5	33.3	28.3	21.7	18.5
Précipitations (mm)	55	64	68	53	26	9	1	2	8	42	72	83

The main missions of Deroua fish farm are:

1. Seed production of Chinese carp and common carp by carrying out the artificial breeding. Since its establishment in 1990 the main purpose of Deroua fish farm is to ensure a continuous supply of fingerlings of these species to be stocked in reservoirs and irrigation canals to control the eutrophication and anarchic vegetal development in irrigation canals and enhance fish production.
2. Production of Tilapia seeds to promote cage culture in reservoirs. This specie was introduced in Morocco since 2004.
3. Production of black bass fingerlings since 1998 to promote sport and commercial fishing.

2.2. Period of the experiment

Silver carp is a batch spawner that ovulates once a year. In Morocco, its breeding season starts from April and lasts until June. In the present study we have assessed the artificial breeding operations performed during three reproductive seasons (2016, 2017 and 2018). The number of the breeding operations depends on the total number of fingerling (especially fry) that Deroua fish farm has to produce every year. This quantity is fixed by the National Center of Hydrobiology and Fish culture (CNHP). The Table 4, below, shows the total number of breeding operations that we assessed and the number of females manipulated during this study.

Table 4 *Number of breeding operation and number of reproduced females and their weight in each breeding season.*

Reproductive season	Starting date of the breeding season	Number of breeding operations	Number of females	Average weight(kg)
2016	07th-April	4	40	3.98
2017	14th-April	7	39	3.53
2018	24th-April	4	35	3.25

2.3. Artificial breeding of silver carp

2.3.1. Fish rearing condition and feed

For the first time, silver carp breeders were imported in 1983 from Hungary. The fish are reared in captivity in 2000 m^2 earthen ponds with 1.5 m depth (Figure 6). The water is originated from groundwater and from the Bin El Ouidane' Dam. Water temperature in ponds varies between 10°C in winter and 33°C in summer.

Silver carp breeders are reared in polyculture with grass carp and they are feed on phytoplankton that are present naturally in ponds **(Farid et al., 2017)**. Also, they receive a fish meal, containing 65% of proteins, spread on water surface.

Figure 6 *Rearing pond in Deroua fish farm*

2.3.2. Brood stock selection and harvesting

Mature females are known by their inflated and soft abdomen, inflated anus and red pectoral fins. The seining of fish is performed smoothly in order to avoid fish stress (Figure 7). Mature males are detected by a gentle pressure on their flanks which induces milt release.

Selected, mature and healthy, fish (Figure 8) are transferred to the hatchery and kept in tanks where water temperature is thermo-regulated and maintained at 24°C.

Figure 7 *Fish harvesting in Deroua Fish farm*

Figure 8 *Mature fish of silver carp*

2.3.3. Hormonal injection

After weighting and color marking in the dorsal fin, they are gently injected by pituitary solution to induce ovulation for females and spermiation for males (Figure 9). Females receive 0.3 mg/kg of their weight as a first injection. 12 hours later, they receive 3 mg/kg of their weight as a second injection. Males receive a single injection of 3 mg/kg of their weight at the moment of the females' first injection.

Figure 9 *Hormonal injection of breeder near to its dorsal fin*

2.3.4. Gamete collection and in vitro fertilization

At ovulation, breeders were anesthetized, in clove solution (1g/10 liters), in order to make their manipulation easier and to avoid their stress. Gametes were stripped by a gentle pressure on the posterior part of the belly, of each breeder. The fertilization process was carried out in dry conditions. For each female, the extracted ova were collected in a container and immediately fertilized by the milt of, at least, three males (Figure 10). To improve the fertilization rate, the fertilized eggs were washed with saline solution (20% NaCl) three times. Afterwards, the eggs are rinsed with fresh water and left in water for 15-20 min to allow the chorion hardening.

After the swelling process of the fertilized eggs, 1 liter of dry eggs yield 6–9 liters of swollen eggs as reported by **(Woynárovich and Horváth, 1980**). After hydration of eggs, every single batch was incubated in conical hatching jars of 40 liters (Figure 11). These incubators are powered by an inlet water flow of 0.6 to 0.8 l/min. During the incubation, water temperature was maintained between 23°C and 24°C.

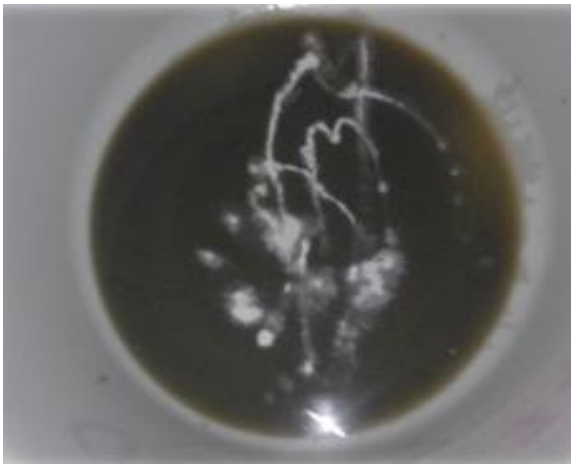


Figure 10 *Eggs and milt of silver carp*

Figure 11 *Incubation system in hatchery*

2.3.5. Eggs hatching and larval rearing

After 36 hours of incubation, the eggs begin to hatch. Then, the larvae are placed in the aquariums (Figure 12) of the first nursery where they feed on their yolk sac for 60 hours. From the moment of hatching to the resorption of the yolk sac, we determine the fry survival rate. Fry remain in aquariums for 7 days before being transferred to nursing ponds. During this period the fry are fed on chicken eggs diluted in hot water.

Figure 12 *Aquariums used for larval rearing*

2.4. Assessment of fertilization, embryos and fry survival

The eggs quality was determined based on three viability rates (fertilization, embryos survival rate and fry survival rate) assessed during the incubation process (Figure 13). These rates are calculated as cited below:

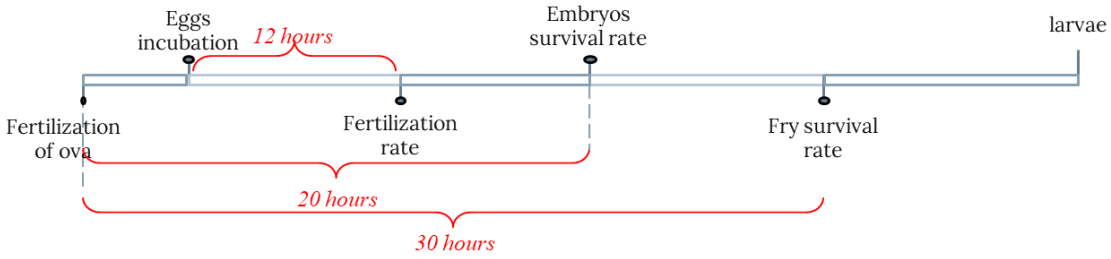


Figure 13 *Assessment of viability rates during the incubation of silver carp eggs*

- 12 hours after incubation, the fertilization rate was estimated as the ratio of the number of fertilized eggs and total number of eggs in the sample.

$$\text{Fertilization rate} = \frac{\text{number of developing eggs}}{\text{total number of eggs in the sample}} \times 100$$

- 20 hours after fertilization, the survival rate of embryos was detected as the ratio of the number of viable embryos and total number of embryos in the sample.

$$\text{Embryos survival rate} = \frac{\text{number of viable embryos}}{\text{total number of embryos in the sample}} \times 100$$

- The survival rate of fry is determined after 30 hours after fertilization.

$$\text{Fry survival rate } = \frac{\text{number of viable pre} - \text{hatchnig embryos}}{\text{total number of pre} - \text{hatchnig embryos in the sample}} \times 100$$

2.5. Data analysis and statistics

A fifteen days' period was adopted to evaluate the changes that occur in the eggs viability during the breeding season. The periods are determined as: first period (1^{st} – 15^{th} April), second period (16^{th} – 30^{th} April), third period (1^{st} – 15^{th} May) and fourth period (16^{th} – 31^{st} May). This consideration is used to determine the existence of changes in reproductive performances during the same month. Data were analyzed using the one-way analysis of variance ANOVA, followed by Tukey test at a significant level of 0.05. Then, Pearson's test was carried out to determine the correlation between the three viability rates (Fertilization rate (FR), embryos survival rate (ESR) and fry survival rate (FSR)) and spawning time. All presented data were expressed as mean ± SEM (standard error of mean).

3. Results

3.1. Fertilization rate (FR), embryos survival rate (ESR) and fry survival rate (FSR)

Data, in Table 5, present results of the overall viability rates for every 15 days of the reproductive season. During 2016 reproductive season, which started from 07^{th} April 2016, the reproductive performances of silver carp were fairly good in terms of eggs' viability rates. The best fertilization rate was obtained in the middle of the season (16^{th} – 30^{th} April) with an overall mean of 68.39 ± 4.44 % followed by the starting from 1^{st} to 15^{th} May by 65.50 ± 5.5%. The lowest fertilization rate was obtained at the beginning of this season with an overall mean of 53.83 ± 7.9%.

The highest mean of embryos survival rate was obtained in the second period (16-30 April), the mean was 55.83 ± 5.29%, while the lowest rate was obtained in the third period (1^{st}-15^{th}May) with a mean of 42.5 ±8.7%. Concerning the survival of the fry, results show that highest survival was obtained in the beginning of the season (92.69%) and the lowest in the middle of the season with a mean of 53.50%. Furthermore, the one-way ANOVA test revealed the absence of the effect of periods on the changes of fertilization rate and the survival of the embryos rate. However, it was found that the survival of the fry is a period dependent. This means that viable fry is obtained at the beginning of the reproductive season.

During 2017 reproductive season, which started from 14th April, the highest fertilization success was obtained at the end of the season with an overall mean of 58.72 ±6.09%, while the lowest rate was obtained in the second period with a mean of 49.53 ±5.2%. However, the lowest mean survival rate of the embryos was observed in the third period (1st-15th May) (40.60%±6.4) while the highest mean of this rate was obtained at the end of the season (16th-31st May) with an overall average of 51.37±4.02%. Additionally, it is important to highlight that survival of embryo wasn't significantly different between the first, second and the third period during this season (2017). The mean survival rate of fry decreased drastically from the first period to the third period from 81% to 43%, respectively. Based on the data's statistical analysis (ANOVA 1), there is no significant changes on viability rates within the reproductive season.

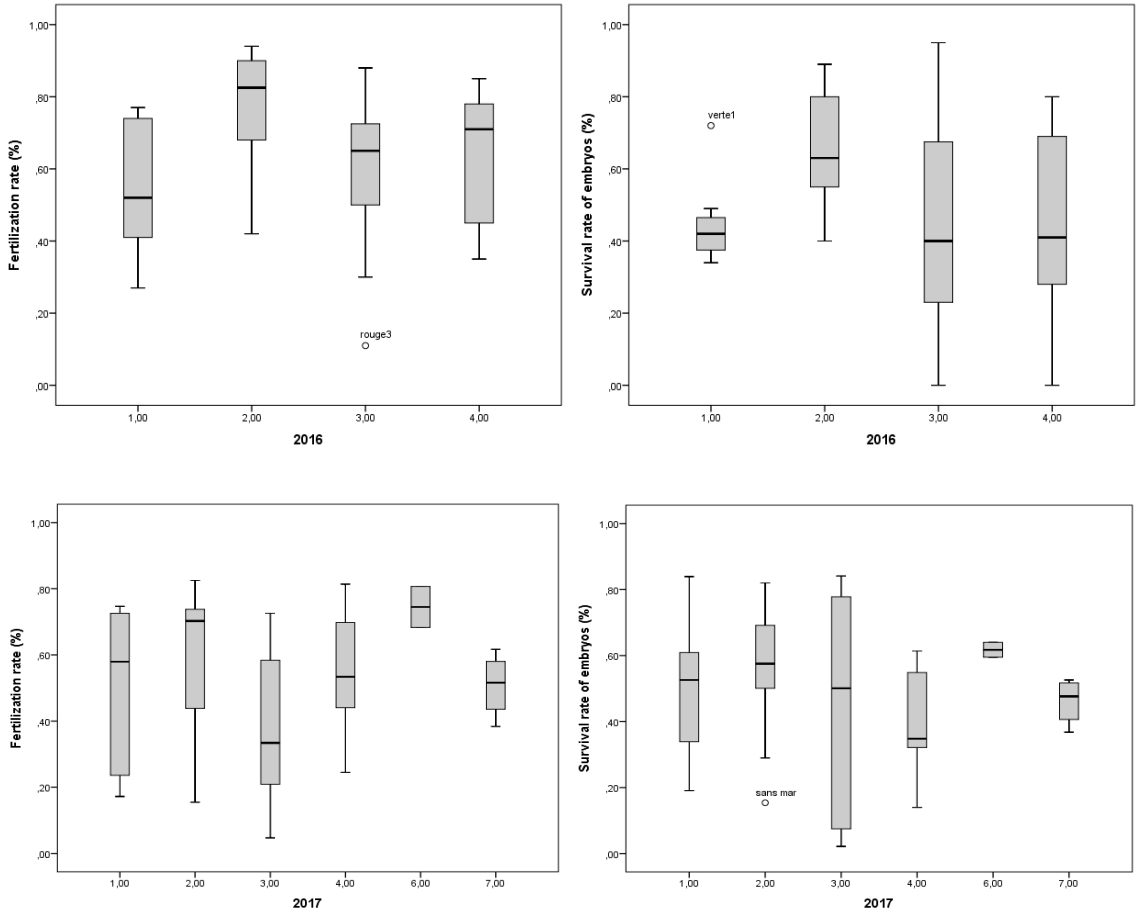
In the third breeding season (2018), the artificial reproduction operations of silver carp started in 24th-April and lasted to June. Further, the hatchery reached its desired goal, expressed by the number of the produced fry, only after four breeding operations. 2 breeding operations were conducted during the second period and the 2 others were conducted during the third period. Consequently, we had only the possibility to collect data between second and the third period. During this season, between the two periods (16th-30th April and 1st -15th May) the fertilization rate increased from 47.94% to 52.48%, while the embryos survival rate increased from 41.98% to 49.26%. However, the survival of the fry hadn't the same trend as the fertilization and the embryos survival rate. This rate decreased from 59.89% to 33.21%.

Table 5 *Viability rates (Fertilization rate, FR; embryos survival rate, ESR and Fry survival rate, FSR) of ova extracted within the same reproductive season of the silver carp (Data are presented as mean± standard error of mean).*

	SEASON 2016			**SEASON 2017**			**SEASON 2018**		
PERIOD	**FR (%)**	**ESR (%)**	**FSR (%)**	**FR (%)**	**ESR (%)**	**FSR (%)**	**FR (%)**	**ESR (%)**	**FSR (%)**
1st-15th APRIL	53.83 ±7.9	45.14 ±4.8	92.69	50.67 ±10	50.50 ± 9.11	81	-	-	-
16th-30th APRIL	68.39 ±4.44	55.83 ±5.29	53.50	49.53 ± 5.2	50.54 ±5.71	66	47.94 ±4.77	41.98 ± 4.09	59.89
1st-15th MAY	65.50 ±5.5	42.5 ±8.7	79.60	55.29 ±7.4	40.60 ± 6.4	43	52.48 ± 4.4	49.26 ± 4.43	33.21
16th-31st MAY	-	-	-	58.72 ±6.09	51.37 ±4.02	51	-	-	-

Box plots (Figure 14) show that fertilization rate and the embryos survival varied between females manipulated in every breeding operation. It highlights, also, the variability of these rates between

the breeding operations. Moreover, Pearson's test revealed that fertilization rate correlates significantly with the embryos survival rate. The correlation factor was 0.724 (p=0.000), 0.483 (p=0.002) and 0.640 (p=0.000) for the first, second and third reproductive seasons, respectively. On the other hand, maternal weight and age didn't correlate with the reproductive performances during the first and the second season. However, in the third season it has found, surprisingly, that female weight correlates negatively with the embryos survival rate (r = -0.459; p= 0.032).



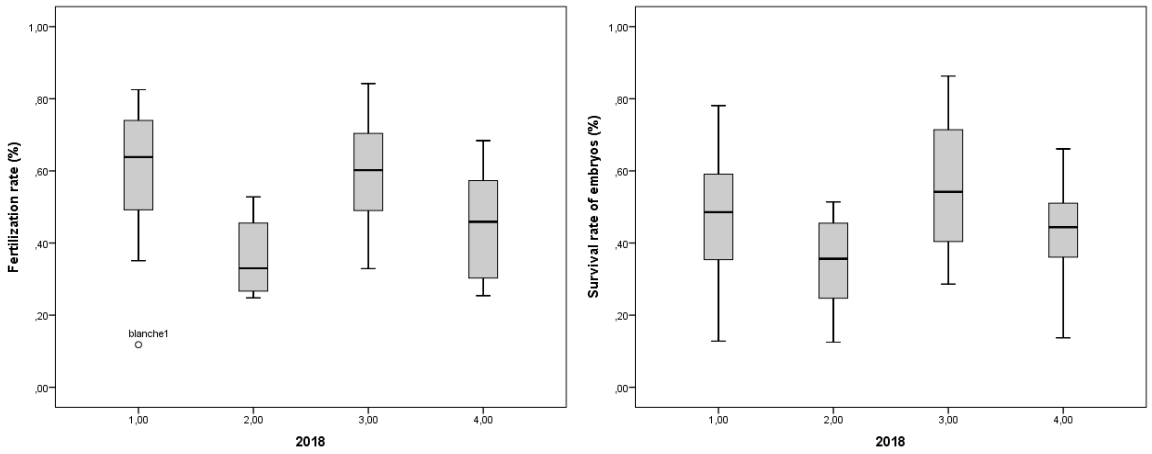


Figure 14 *Boxplots presenting the variation of fertilization rate and embryos survival rate for all the performed breeding operations during the reproductive seasons of silver carp. (The centre horizontal line in the box is the median, the top and the bottom of the box are the 25% and 75% percentiles (quartiles), and the end of the whiskers are the 5% and 95% percentiles. The notch is the 95% confidence interval of the medians).*

3.2. Interannual variability of reproductive performances

During the present study, the collected data proved that reproductive success of silver carp varies significantly between the reproductive seasons (Figure 15). The overall average of fertilization rate decreased between the seasons. The highest fertilization success was observed in 2016 (64.71%) and was only 50.15% in 2018. The same trend was observed for the survival of the embryos. It was higher in the first season by 12.6% than the third season. Finally, fry survival decreased also between seasons from 83.21% in 2016 to 51.75% in 2018 (Table 6).

Table 6 *The overall average of females' weight, ova weight and reproductive performances during the three reproductive seasons (Data are presented as mean± Standard Error)*

	SEASON 2016	**SEASON 2017**	**SEASON 2018**
Ova weight (grams)	525.87 (±25.5)	512.78 (±38)	560.02 (±34.29)
Fertilization rate (%)	64.71 (±7)	51.79 (±3.93)	50.15 (±3.23)
Embryos Survival rate (%)	58.10 (±2.7)	51.24 (±4.06)	45.5 (±3.03)
Fry Survival rate (%)	83.21 (±5)	67.64 (±3.6)	51.75 (±5.81)

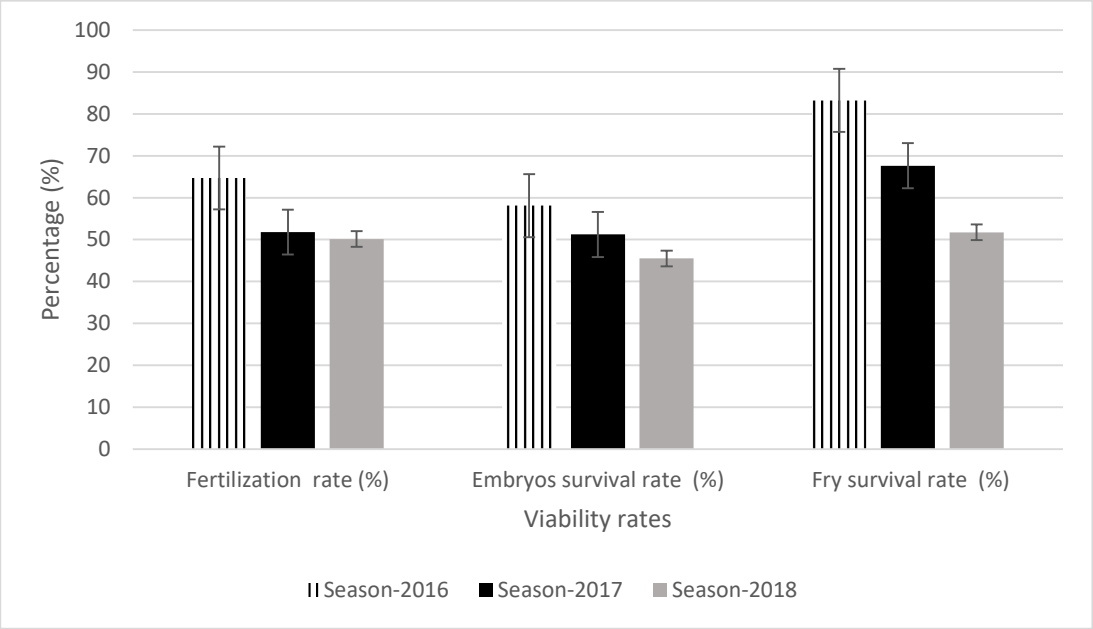


Figure 15 *Variation of viability rates (fertilization rate, embryos survival and fry survival rates) between the three successive breeding seasons of silver carp.*

4. Discussion

Analysis of data using one-way ANOVA revealed that there is no significant difference between the means of the fertilization rate and the survival rate of the embryos ($p > 0.05$) during the three reproductive seasons. This revealed that fertilization success and the normal development of the embryos are not related to the spawning period. So, they can't be used to determine the changes of the females' reproductive outcome in silver carp during the same reproductive season. However, these viability rates are useful tools that give predictive informations about the success and the yield of the performed breeding operation. That is to say that determining the fertilization success ''fertilization rate'' could give aquaculturists an idea about the quality of the stripped eggs and by this knowing the tendency of the breeding operation's success. Similarly, Skaalsvik and al. **(2015)** found that in Atlantic halibut (*Hippoglossus hippoglossus L.*) the fertilization rate and survival rate of larvae didn't change throughout the spawning season. The authors explained this invariability by the absence of parental effect on the eggs quality during the experiment. However, the same study highlighted that the fertilization rate can be a predictive tool for the hatching success only for batches with low fertilization success. Same results were found for the Ballan wrasse (*Labrus bergylta*) whose fertilization rate remain high during its reproductive season (up to 87.5%) **(Grant and al., 2016).** However, other studies revealed that fertilization rate is a good parameter to determine egg quality in many fish species **(Brown and al., 2006; Policar and al., 2010; Jerez and al., 2012).** In

gilthead seabream (*Sparus aurata*), fertilization success changed during the spawning season. It was showed that higher rates were obtained in early and mid-season then it depleted at the end of the reproductive season **(Jerez and al., 2012).** The assessment of the reproductive performances of common barbel (*Barbus barbus)* revealed that lowest fertilization rates were obtained at the beginning and the end of the reproductive season **(Policar and al., 2010)**.

The statistical treatment of data related to the fry survival showed that it didn't change during 2016 breeding season. However, this rate was significantly variable throughout the second and the third reproductive seasons (seasons 2017 and 2018). Low fry mortalities were obtained at the beginning of the seasons (1^{st}-15^{th} April and 16^{th}-30^{th} April) while at the end of the reproductive seasons, mortalities rates were higher. The same reproductive kinetics were observed in *Dentex Dentex* **(Giménez and al., 2006)**, *Merlangius merlangus* L (**Treasurer and Ford, 2010)** and *Perca fluviatilis* **(Castets, 2011)**. For the European perch (*Perca fluviatilis*), the eggs viability was evaluated by determining fertilization rate, hatching rate and larval survival and deformities. The obtained results showed that eggs viability of this specie decreased during the breeding season. Eggs of high quality were those stripped at the beginning of the period, while those obtained at the end of the same season are considered of poor quality (**Castets, 2011).** Treasurer and Ford **(2010)**, found that for the *Merlangius merlangus L*, the eggs quality correlate negatively with time. The fertilization rate, egg diameter, and dry and wet weight drop significantly with the progress of the spawning season.

During the reproductive season, the variability of the fertilization success and the embryos survival could be explained by dominance of the intraspecific factors, as showed in figure 14, which reveals the existence of heterogeneity between females of the same group. This can be supported by other studies that proved the existence of strong effect of parental fitness on eggs quality and embryos survival **(Young and al., 1990; Rubin, 1995; Johnson and al., 2012)**. However, in the present study, the female's age and weight have no effect on egg viability which means that other maternal factors may have a direct effect on egg quality. For instance, it was proved in numerous studies that the maternal nutritional status has a strong effect of on eggs and fry quality (**Bruce et al, 1993; Izquierdo and al., 2001; Mazorra and al., 2003; Watanabe and Vassallo-Agius, 2003; Sink and Loch-mann, 2008)**. It was also shown that maternal RNA has a strong effect of fry survival and quality in other fish **(Chapman and al., 2014; Lubzens and al., 2017)**. As a consequence, more studies should be carried out to investigate about the relationship between maternal diet and egg viability in silver carp in order to better understand this correlation. Knowing that their diet is strictly

phytoplanktonophagous and that the food preferences of this specie in the Deroua fish farm have been well studied **(Farid et al., 2014; Hasnaoui et al., 2017; Farid et al., 2020).**

The correlation between the chosen viability rates revealed that there is only significant correlation between fertilization rate and embryos survival rate. What is more, no significant correlation between fry survival rate, fertilization rate and embryos survival rate was observed during this study. So these findings highlight that fertilization success or embryos survival cannot be used as a predictive criterion to estimate fry viability in silver carp. Contrariwise, in other fish species fertilization success is used to predict fry survival **(Bromage and Cumaranatunga, 1988; Mylonas and al., 2004)**.

Finally, the inter-seasonal difference in fertilization success, survival of embryos and fry shed light on the significant effect of environmental changes on reproductive performances of silver carp. Further, temperature is the main environmental factor that affects, significantly, fish reproduction **(Billard, 1979; Pankhurst and Munday, 2011).** During this study the temperature profile was different between the three years. The significant increase of temperature during the second breeding season could be responsible of the perturbation of oogenesis process and as a consequence on the decrease of egg quality. A gradual increase of water temperature in rearing pond would allow a normal repining of fish eggs. It was reported by Lam **(1983)** that under temperature conditions extremely different from the optimal temperature range, gametogenesis is stopped and atresia is induced. Furthermore, Temperature changes impact the duration (length) of the reproductive season. It was observed that in 2016 first mature females were spawned on April 7th while in the second season (2017) first females spawned in 14th April. In the last season (2018), the reproduction was delayed and the first females were spawned in 24th April. These results prove the high effect of temperature on the length of the reproductive season and also on gametogenesis of silver carp. Moreover, these fluctuations in temperature caused perturbation in reproductive behavior of fish which was expressed by a low fecundity (see chapter II) and failure of ovulation for a high number of females. Also, deformities of embryos and fry were largely observed during the second season. Brown and al. **(2006)** showed that for Atlantic halibut (*Hippoglossus hippoglossus L.*), high temperatures during the vitellogenesis causes a decrease in quantity and quality of eggs. Also, it was proved that high temperatures (>12°C) inhibit and delay spawning activities in Atlantic salmon, *Salmo salar*, **(Beall and Marty, 1983)**. Besides, high temperatures accelerate oocytes maturation **(Gillet, 1991)** which may cause eggs overripening known by its negative effect on eggs quality **(Bry, 1981).** In Deroua Fish farm, it was observed over more than 25 years that at end of the

spawning season eggs quality of the silver carp decreases drastically due to the important increase of water temperature **(Droussi, Personal com)**.

5. Conclusion

During this study, it was found that reproductive success of this specie is related to the interaction of several factors such as environmental and maternal effects. The obtained results revealed that the determination of fertilization rate and the assessment of the embryos development during incubation can give informations about the quality of the obtained eggs but they didn't determine the tendency of the egg quality throughout the same reproductive season. However, Fry survival showed a high rate at the beginning of the season and low values at the end. Consequently, this can be used, in hatcheries, as a parameter to predict the yield of seed production of silver carp (*Hypophthalmichthys molitrix*). Along with other factors that should be investigated about, the fry survival could help to establish a map of reproductive performances of silver carp during the breeding season

CHAPTER II: RELATIONSHIP BETWEEN MATERNAL TRAITS AND FECUNDITY OF SILVER CARP

1. Introduction

Fish present an important and wide diversity of reproductive strategies, which make of them an excellent group to investigate about the life history variation **(Johnston and Leggett, 2002).** The assessment of the variation of fecundity is one of the tools used to achieve this goal. Its data are used by scientists to understand and describe the investment of an organism into reproduction **(Sibly and Hone, 2002)**. In many studies, it was demonstrated how fish species invest in gamete size, quality and quantity to produce viable offspring and to ensure their continuity **(Sargent et al., 1987; Beacham and Murray, 1993; Einum and Fleming, 2004)**. According to the interaction of numerous environmental conditions, fish females can adopt a trade-off strategy between eggs size and number (fecundity) **(Morrongiello and al., 2011, Einum and Fleming, 2004; Fischer and al., 2011)**. Furthermore, Fox and Czesak **(2000)** reported that mothers inhabiting harsh environments conditions will produce fewer, larger offspring. So, as a reaction toward environmental changes, fish females could produce fewer and larger offspring or high number of small eggs taking in consideration if there are good chances of their offspring to survive. Those environmental factors could be a variation in temperature **(Johnston and Leggett, 2002)**, photoperiod **(Campos-Mendoza and al., 2004)**, food availability, richness of essential nutrients **(Lukšiené and al., 2000; Nowosad and al., 2017)** hydrological variation and physiochemical stress **(Humphries, 1995; Morrongiello and al., 2011)**.

Additionally, maternal traits are also factors that have direct effect on the reproductive output in fish. Numerous studies were carried out to decode the relationship between maternal characteristics (e.g. age, length, weight) and fecundity. For some species, it was shown that reproductive output (e.g. fecundity) is positively correlated to female body size **(Fleming and Gross, 1990; Beacham and Murray, 1993; Humphries, 1995)**. Also, it was proved that in other fish species, female's weight correlates significantly with fecundity (**Ishrat and al., 2018; Saç and Gaygusuz, 2020).** Further**,** Bernardo **(1996)** demonstrated that fecundity can't be brought just through a trade-off with egg size but also through an increase of body size. He revealed that body size differences among populations are potentially a very real and important source of adaptive variation in reproductive investment.

The reproductive potential and strategies of silver carp were largely studied in order to determine its spawning behavior and factors leading to its reproductive success (**Chapman and George, 2011; Coulter and al., 2013; Deters and al., 2013; Lenaerts and al., 2015; Tucker and al., 2020)**. Its high fecundity and rapid growth are the main factors contributing to its high spread in water bodies **(Kolar and al., 2007)**. In general, fecundity of Chinese carp female is very high. It's ranging from 100 000 to 300 000 eggs per kg body weight per oogenesis cycle, with one cycle/year in the case of females reared in an outside natural pond environment **(Linhart and al., 1995)**. Moreover, Williamson and Garvey **(2005)** used fecundity as a metric tool, along with growth, age structure and diets, to evaluate the reproductive potential of silver carp and its relationship with the rapid spread and invasion of this specie.

The present study aims to evaluate the reproductive potential of silver carp females by quantifying the fecundity and determining its seasonal variation. The obtained data can provide insight into the trend of egg production within and between the reproductive seasons. Also, it seeks to determine the relationship between maternal traits (age, length and weight) and the reproductive output of silver carp. This allows for better evaluation of the consequences if the maternal age, length and weight have a direct effect on the seed production of silver carp. Moreover, the determination of the trends of ova production during the breeding season is useful for attempts to increase the yield of silver carp larvae without impacting labor costs.

2. Material and methods

2.1. Experimental Fish

The broodfish of this study were reared in Deroua Fisheries Station, located in the middle of Morocco. They were reared in earthen ponds and feed on phytoplankton **(Hasnaoui et al., 2001; Farid et al., 2014; Farid et al., 2017)** available naturally in these ponds. Over three years, 126 females were manipulated. the average of their weight was 3.99 kg, 3.51 kg and 3.25 kg obtained in the first, second and third reproductive season, respectively. Performed breeding operations (BO) were 14 in total (Table 7).

Healthy and Fully matured fish are captured and transported to the hatchery. Then they were, immediately, weighted using a dynamometer scale to the nearest g. After weighting, females were marked using colored threads to facilitate their identification at the moment of the injection with a solution of pituitary extract of common carp to induce ovulation. Female's length was measured to

the nearest cm to determine the effect of females' size (length) on the reproductive output. For age determination, we used scalimetry instead of otholimetry to avoid fish sacrifice.

2.2. Ova stripping and weighting

To induce ovulation, females received two hormonal injections of pituitary extract of common carp. At ovulation, the Ova are stripped by gentle pressure on the posterior part of the belly, of each female. The fully-stripped ova are weighted to the nearest g, for each female, to investigate the relationship between ova weight and females' profile (age, weight, length).

2.3. Determination of Fecundity

To determine the impact of the breeding season on fecundity we adopted two methods to calculate fecundity.

2.3.1. Relative fecundity

Relative fecundity expresses the number of grams of the ova per kg of female body weight. Even if there are objections to the use of relative fecundity, we adopted it because it is considered a useful working index used by all fish producers. This parameter helps to establish the relationships between ova production and several factors such as stocking density, feeding rates, water quality and broodstocks age.

2.3.2. Gravimetric fecundity

During the season 2018, we had attempted to determine gravimetric fecundity by calculating the proportion of the weight of 100 ova in the total weight of the produced ova **(Bromage and al., 1992)**. This method gives the same accuracy as the volumetric fecundity does. This method is widely used by scientists and it relies on measurements of mean egg diameter and the total volume of water-hardened eggs **(Von Bayer, 1950).**

2.4. Data analysis

The collected data were analyzed by the one-way ANOVA to investigate whether there is a significant difference between fecundity during the spawning season ($P < 0.05$ is considered significant). The linear analysis of regression was carried out to determine the correlation between age, weight, length of female, weight of stripped ova and fecundity. We used SPSS statistical software (IBM Corporation, Somers, NY, USA).

3. Results

3.1. Seasonal variability of relative and gravimetric fecundity

During the three breeding seasons, a total of 126 females were manipulated. The overall average weight was 3.99 kg, 3.51 kg and 3.25 kg respectively for the first, second and third reproductive season. The highest ova weight was obtained in the third year (551.44 grams) followed by the first year (522.16 grams) then the second year (427.79 grams) (Table 7).

Table 7 *Variation of weight, ova weight and fecundity during the three breeding seasons.*

	SEASON 1		**SEASON 2**		**SEASON 3**	
	April	May	April	May	April	May
NUMBER OF PERFORMED BREEDING OPERATIONS (BO)	3	1	5	1	2	2
NUMBER OF FEMALES	39	10	34	8	18	17
AVERAGE AGE (YEARS)	6	7	4	4	3	3
AVERAGE WEIGHT (KG)	4.097	3.7	3.576	3.18	3.15	3.355
AVERAGE OF RELATIVE FECUNDITY (GRAMS OF OVA/KG OF BODY WEIGHT)	114.967	146.06	122.292	139.42	178.385	162.43
AVERAGE OF OVA WEIGHT (GRAMS)	521.89	523	426.946	432.02	563.66	539.22

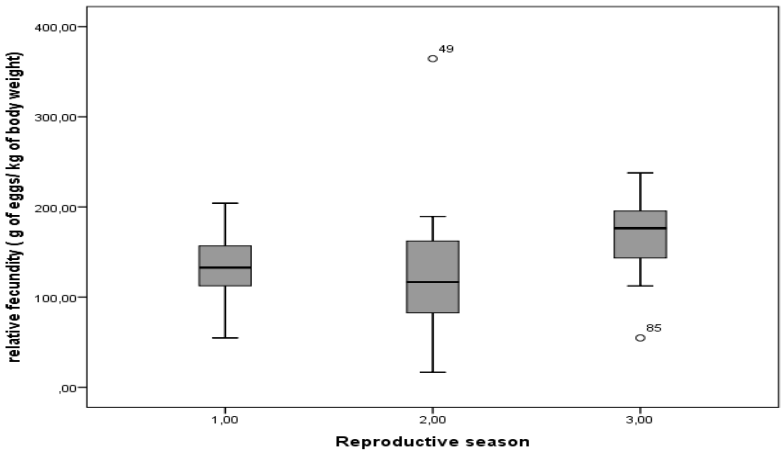


Figure 16 *Box plots presenting variation of relative fecundity (grams of ova/ kg of body weight) of silver carp within and between three reproductive seasons.*

Additionally, the relative fecundity didn't show a specific trend during the three breeding seasons (Figure 16). During the first year, relative fecundity ranged from 107.81 to 146.06 g of ova/kg of body weight obtained in the BO3 and BO4, respectively. At the beginning of the season (BO1 and BO2), relative fecundity was similar. It reached about 118 g of ova/kg of body weight. In the second year, the highest fecundity was obtained at BO1 with an overall average of 153 g of ova/kg of body weight. The lowest fecundity was 89.79 g of ova/kg of body weight at the BO5. During the third

reproductive season, relative fecundity was higher compared to the previous two seasons. The highest value was obtained at BO1 with an overall average of 185.42 g of ova/kg of body weight and the lowest fecundity was obtained at BO3 with an overall average of 151.74 g of ova/kg of body weight. Pearson's test revealed that, during the three years, there is no significant correlation between breeding season and relative fecundity.

During the third breeding season, gravimetric fecundity was measured and it showed an increase from 30.47% to 34.32% until the third breeding operation (BO3). Then it depleted at the last breeding operation (BO4) to reach 14.23% (weight of 100 ova in the total weight of the produced ova). Moreover, Pearson's test revealed a negative correlation between breeding season and gravimetric fecundity ($r=-0.352$, $p=0.038$). On the other hand, the test showed that gravimetric fecundity correlates significantly with relative fecundity ($r=-0.686$, $p=000$).

3.2. Relationship between maternal traits and fecundity

High gravimetric fecundity was obtained for females with total length between 45 cm and 50 cm, while the highest values of relative fecundity were obtained for females with a total length between 50 and 60 cm. In contrast, the female with the longest length (76 cm) and the highest weight (5.5 kg) had the lowest relative fecundity (54.73 g of ova/kg of body weight) (Figure 17). The One-way ANOVA test showed a significant effect of body length on gravimetric fecundity ($P<0.05$) and relative fecundity ($P<0.05$). Furthermore, the highest gravimetric fecundity corresponds to the lowest weight females while the highest relative fecundity values were obtained for females whose weight varied from 3.5 kg to 5 kg.

Moreover, a one-way ANOVA test was conducted to study the effect of maternal age and length on reproductive fitness. The test showed that age has no impact on the fecundity and the egg quantity (ova weight) of silver carp. On the other side, the length of the female significantly affects fecundity and ova weight.

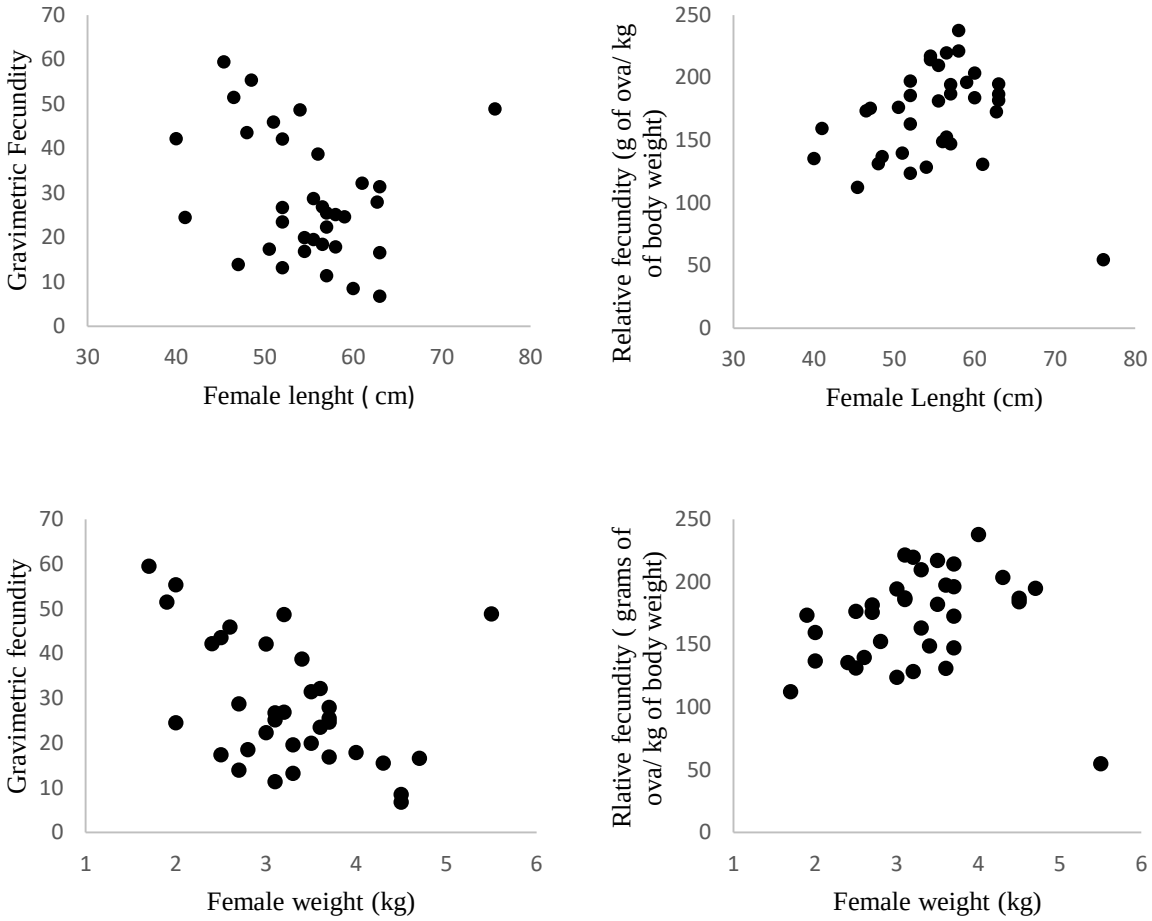


Figure 17 *Variation of gravimetric fecundity and relative fecundity according to female length (cm) and weight (kg).*

During the third breeding season, Pearson's test revealed a negative correlation between relative fecundity and gravimetric fecundity (r= -0.686, p= 0.000). A positive correlation was also found between female length and ova weight (r= 0.494, p=0.003). In addition, a negative correlation was found between female weight and gravimetric fecundity (r=-0.44, p=0.008) and ova weight (r=0.665, p=0.000). The same correlation test revealed the absence of correlation between female's age and gravimetric and relative fecundity (Table 8).

The results obtained at the third year confirm the results obtained during the two previous years. This similarity concerns the correlation between female's weight and ova weight (Table 8). Nevertheless, the correlation was significant between female's weight and relative fecundity in the first year (r=-0.328, p=0.030). Contrariwise to the second and the third year where the correlation between female's weight and relative fecundity wasn't significant (r=-0.140, p=0.452 for year 2; r=0.127, p=0.467 for year 3) (Table 8).

Table 8 *Summary statistics of correlation between maternal traits (age, weight and length) and ova weight, relative fecundity and gravimetric fecundity. The correlation between gravimetric and female's length was verified in the third reproductive season.*

	SEASON1		SEASON2		SEASON3		
Females traits	***Age***	***Weight***	***Age***	***weight***	***Age***	***Length***	***Weight***
Gravimetric fecundity	-	-	-	-	r=0.182; p=0.296	r= - 0.27; p=0.117	r=-0.440; p=0.008
Relative fecundity	r=-0.283; p=0.062	r=-0.328; p=0.030	r=-0.088; p=0.38	r=0.140; p=0.452	r=-0.109; p=0.535	r=0.038; p=0.829	r=0.127; p=0.467
Ova weight	r=-0.155; p=0.315	r=0.668; p=0.000	r= -0.015; p=0.937	r=0.494; p=0.005	r=-0.120; p=0.494	r=0.490; p=0.003	r=0.665; p=0.000

The box plots showed that the values of the gravimetric and relative fecundity were different during the breeding seasons (Figure 18 and 19). For each spawning period, the position of the median is asymmetric, which reveals the existence of heterogeneity between females of the same group. Otherwise, box plots indicate the existence of intra-specific factors that impact fecundity during the breeding season, revealing the existence of other factors involved in controlling the fecundity of silver carp' females.

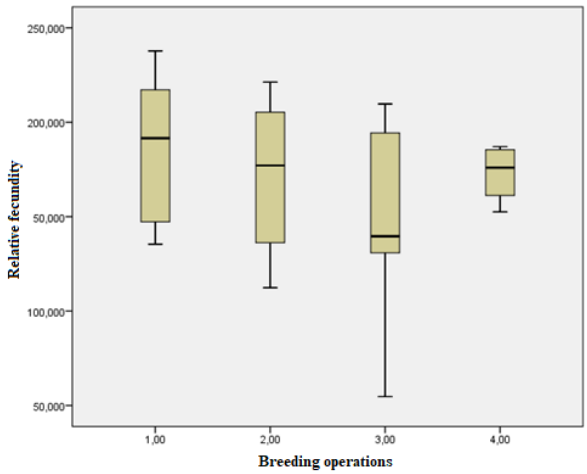


Figure 18 *Box plots of relative fecundity in year 3.*

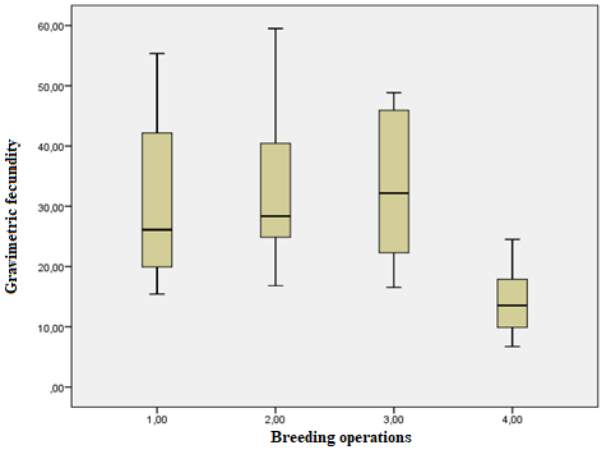


Figure 19 *Box plots of gravimetric fecundity in year 3.*

4. Discussion

Relative fecundity seems to be independent on temporal factor (time of reproduction). Statistical data analysis (ANOVA1) showed that during the reproductive season there is no significant variation in relative fecundity. However, the study revealed the existence of an interannual effect on relative fecundity. It increased between the three breeding seasons. Further, Tukey test demonstrated that the reproductive performance obtained in the first year was significantly lower than the reproductive performance in the third year; the obtained values were 130.75 and 170.73 g of ova /kg of body weight in 2016 and 2018 respectively. These results are in accordance with studies conducted on other fish species **(Kjesbu and al., 1998; Kraus and al., 2000; Kennedy and al., 2007; Gunnarsson, 2017)**. For example, relative fecundity of Atlantic wolfish was different among the reproductive seasons (from 2003 to 2005). Fertility in 2003 was shown to be 11% lower than in 2005 **(Gunnarsson, 2017).**

This inter-annual variability in fecundity could be linked to the nutritional status of the female, which determine the energy that can be devoted to egg production **(Lambert and al., 2003)**. Furthermore, many studies have highlighted the influence of food availability and its richness in essential nutrients on fecundity **(**Atlantic Salmon**, Rennie and al., 2005;** Sea bass**, Czesny and Dabrowski, 1998;** Striped jack**, Watanabe and Vassallo-Agius, 2003**). What is more, fecundity also depends on the interaction of numerous environmental factors **(Smith and Fretwell, 1974; Parker and Begon, 1986; Tanasichuk and al., 1987; Johnston and Leggett, 2002; Kamler, 2005)**. The study carried out on crayfish by Skurdal and al. **(2011),** revealed that the increase in temperatures during the breeding period studied was correlated with increased fecundity. On the other hand, low temperature inhibits vitellogenesis in virgin Atlantic cod **(Yoneda and Wright,**

2005). But in European hake, temperature does not seem to have a direct effect on its reproductive potential **(Recasens and al., 2008).** Additionally, it was proved that photoperiod (daylenght) have significant effect on the fecundity of Nile tilapia. Fish with a long daylenght had a high total fecundity **(Campos-Mendosa and al., 2004)**.

The present study also demonstrated the variability of reproductive performance in females which is related to the reaction of the female towards her living conditions (nutritional status and environmental factors cited below).

Many researches have focused on the relationship between female characteristics (age, length and weight) and their reproductive output (fecundity, number of eggs) **(Thrope and al., 1984; Kallio, 1986; Skurdal and al., 2011)**. This relationship between fish size (usually expressed as length or weight) and eggs production has been explained by the strong relationship between female body and size **(Wootton, 1992, 1999).** In our case of study, the female length has a positive and strong correlation with the ova weight. The correlation test proved that 24% of the variability of the ova weight can be explained by the female length. However, female length explains only 1% and 7.3% of the variability in relative fecundity and gravimetric fecundity, respectively. Similarly, according to our results, reproductive output is size independent for many fish species **(Kraus and al., 2000; Johnston and Leggett, 2002; Recasens, 2008)**. For instance, the relative fecundity of Atlantic salmon was not dependent on female size but was related to body weight **(Heinimaa and Heinimaa, 2004).** However, in other fish, female size is closely related to fecundity. Kamler **(2005)** have reported that female size explains 25 to 98% of the potential fecundity of many freshwater and marine fishes. This has been confirmed by other researchers **(Almatar and Bailey, 1989; Winters and al., 1993; Kraus and al., 2000; Marteinsdottir and Begg, 2002; Lambert and al., 2003**). Regarding relative fecundity, which was studied in this work, Marshall and al. **(1998)** reported that female size (expressed in length) has a significant effect on relative fecundity. It has also been shown that relative fecundity is higher in small females than in large ones **(kallio, 1986).**

This study also highlights the relationship between female age and fecundity. The obtained data demonstrated that age has no effect on relative fecundity or gravimetric fecundity. However, some authors have found that the relative fecundity of early 2-year- old haddock was significantly lower than that of older fish **(Hislop, 1988)**. However, it is important to point out in this study, the ova weight is closely related to the female weight. During the three years, female weight explains respectively 44.6%, 24.4% and 44.2% of the variability in the weight of the ova. Maternal age has

no effect on the ova weight unlike the female length. Large females have been shown to give high grams of ova than small ones, and length explains 24% of the variability in ova weight.

5. Conclusion

In the present study, it was proved that relative fecundity didn't change within the reproductive season. However, the existence of inter-annual variability sheds light on the impact of the environmental changes on the reproductive potential of silver carp. Besides, the study highlighted the impact of intraspecific factors on the reproductive performance of silver carp. It was demonstrated that the female weight could be used to estimate the yield of seed production of this specie. Big females produce more ova than smaller females. However, female reproductive output (ova weight) is length and age-independent.

As a consequence, more work should be done to determine the reaction of females toward changes in the main environmental factors such as temperature and photoperiod. On the other side, it is better to investigate the variation of ova size and its relationship with the reproductive potential and success.

CHAPTER III: THE EFFECT OF THE POST-OVULATORY AGING ON EGGS VIABILIY IN SILVER CARP.

1. Introduction

The over-ripening (or post-ovulatory aging) is a phenomenon in which the rate of fertilizable oocytes progressively decreases **(Aegerter and Jalabert, 2004)**. In fish farming, many efforts have been made to determine the safe time-lapse in which eggs remain viable even if stripping was performed after ovulation. This phenomenon was largely studied in salmonids due to their high commercial value **(Escaffre and Billard, 1979; Bry, 1981; Springate, 1984; Bromage and al., 1992; Samarin and al., 2008; Milla and al., 2011)**. For instance, experiment carried out on the Caspian brown trout showed that the ova retention in the female's abdominal cavity for 40 days' post-ovulation declines the eyeing and hatching rate of the eggs **(Bahre Kazemi and al., 2010).** Determining the effect of eggs aging becomes important to quantify the percentage of the loss of egg quality and to enhance hatcheries management **(Bry and al., 1978; Rizzo and al., 2003; Samarin and al., 2016).** Further, the evaluation of this effect was obtained through the assessment of fertilization success **(Hay, 1986; Yang and Chen, 2006)**, hatching rate (**Baidya and Senoo, 2004)**, survival of embryos **(Flett and al., 1996)**, survival of larvae **(Crandell and Smoker, 1995)** and biochemical composition **(Craik and Harvey, 1984; Bahre Kazemi and al., 2010; Lord and Aitken, 2013)**.

During the breeding practices of silver carp, the handling time of a single female lasts for 15 to 20 minutes, increasing the possibility of eggs over-ripening for females reaching ovulation at the same moment. The present study deals with the evaluation of the effect of ova aging of silver carp (*Hypophthalmichthys molitrix*) on the viability of their reproductive parameters. Ova quality was determined by assessing the variability of fertilization success, survival of embryos and larvae survival for ova fertilized between 0 and 90 minutes post-ovulation. This experiment will help determine how long the eggs, when ready to be stripped, can remain in the ovarian cavity without affecting reproductive success and fry production.

2. Material and methods

2.1. Experimental design

The experiment was carried out over three successive years (2016, 2017 and 2018) at Deroua fish farm (Table 9). Healthy, mature females and males were transferred to the hatchery and given a

hormonal injection to induce ovulation (females) and spermiation (males). At ovulation, fish are anesthetized in a clove solution to facilitate their handling and to avoid their stress. Then, the ova of the same female were stripped at ovulation as well as at 30, 60 and 90 minutes after ovulation. The stripping was performed by a slight pressure on the posterior part of the fish belly. The retention of ova in the female cavity post-ovulation was achieved by suturing the female's genital papilla (Figure 20).

For each female and at every retention period, ova were immediately fertilized by sperm obtained from at least 3 males. Then the eggs were washed with saline solution (NaCl solution) to stimulate the sperms and promote the fertilization process. Then, they were washed with clear water three times. Lastly, the swollen eggs were held in incubators with a volume of 40 liters at 23-24°C. These incubators are equipped with a system that ensures the continuous renewal of water and whose flow rate is adjustable according to the stage of development of the fertilized eggs.

Table 9 *Number of females used for the three aging experiments and their average weight.*

YEAR	**2016**	**2017**	**2018**
NUMBER OF FEMALES	9	6	7
AVERAGE WEIGHT (KG)	2.96	3.03	3.12

Figure 20 *Suturing female's genital papilla to avoid eggs release in tanks during aging process.*

2.2. Assessment of viability rates

As cited previously in Chapter I, we assessed the fertilization success and the development of the embryo into larvae during eggs incubation.

2.2.1. Fertilization success

12 hours after incubation, fertilization rate was evaluated. This rate was calculated as the number of fertilized ova in relation to the total number of ova.

2.2.2. Observations of living embryos

20 hours after fertilization, dead embryos were easily detected; they were characterized by a white mass lying on the yolk. The survival rate was defined as the ratio of living embryos to total embryos. The result is expressed as a percentage.

2.2.3. Observations of viable larvae

30 hours after fertilization, the survival rate of larvae was determined by calculating the ratio of the number of viable larvae to the total number of larvae in the sample. The result is also expressed as a percentage.

2.3. Statistical analysis

Data were analysed using SPSS version 23 (IBM Corporation, Somers, NY, USA). We used one-way ANOVA to evaluate the changes of fertilization rate, survival of embryos and survival of larvae between the moment of ovulation and 30,60 and 90 minutes after ovulation ($P < 0.05$ is considered significant). Graphs were generated using Microsoft Excel 2016.

3. Results

During the three experiments, the highest fertilization rates were recorded for eggs stripped at ovulation followed by those fertilized 30 minutes after ovulation. At ovulation, the mean fertilization value was 63.35%, 70.24% and 63.26% for 2016, 2017 and 2018, respectively (Table 10). The ova stripped 60 and 90 minutes after ovulation showed a significant decrease in fertilization success. During the experiment of 2016, this rate (fertilization success) dropped to 29.51% and 15.43% at 60 and 90 minutes' post-ovulation, respectively. A similar tendency of fertilization success was obtained in experiments performed in 2017 and 2018. The fertilization success decreased from 70.24% to 31.2% in 2017 and from 63.26% to 44.48% in 2018 for eggs obtained at ovulation and 90 minutes' post-ovulation.

Besides, embryos survival rate declined progressively during aging; it has the same trend as the fertilization rate (Table 10). During the three experiments, this rate was high for eggs obtained at ovulation with an overall average of 61.63%, 52.63% and 44.99% for 2016, 2017 and 2018,

respectively. After 90 minutes' post-ovulation, the embryos survivability declined to reach 29.76%, 16.35% and 27.60% in 2016, 2017 and 2018, respectively.

Concerning the fry survivability, it decreased progressively during aging experiment of 2016 from 87.32% at ovulation to 48.81% at 90 minutes' post-ovulation. Nevertheless, the results obtained in the next experiments (2017 and 2018) were surprisingly different (table 10). During the aging experiment performed in 2017, the highest fry survival was obtained for eggs stripped at ovulation (93.75%) followed by those stripped at 30 minutes' post-ovulation (64.84%) then it depleted to reach the lowest survival rate for eggs stripped at 60 minutes' post-ovulation (47.20%). Further, eggs stripped at 90 minutes' post-ovulation have high fry survival rate (63.93%).

During the aging experiment of 2018, the fry survival rate reached its highest values for the eggs stripped at ovulation (81.59%), 60 minutes (92.36%) and 90 minutes' post ovulation (96.67%). The lowest fry survival was obtained for eggs stripped at 30 minutes' post-ovulation with an overall average of 25.91%.

Table 10 *Variability of Fertilization rate (FR), Embryos survival rate (ESR) and Fry survival rate (FSR). Data are presented as percentage (mean ± standard error).*

Reproductive season	Viability rates	0 minutes Post-ovulation	30 minutes post-ovulation	60 minutes post—ovulation	90 minutes post-ovulation
2016	FR	63.35 ± 2.7^{a}	61.24 ± 1.58^{a}	29.51 ± 3.47^{b}	15.43 ± 6.33^{b}
	ESR	61.63 ± 5.75^{c}	56.44 ± 7.19^{c}	$38.27 \pm 5.76^{c,d}$	29.76 ± 8.8^{d}
	FSR	87.32^{e}	86.71^{e}	79.74^{e}	48.81^{f}
2017	FR	70.24 ± 2.81^{g}	$61.18 \pm 6.01^{g,h}$	$44.14 \pm 4.01^{h,i}$	31.2 ± 5.04^{i}
	ESR	52.36 ± 6.37^{j}	$35.21 \pm 2.81^{j,k}$	31.01 ± 4.33^{k}	16.35 ± 6.01^{k}
	FSR	93.75	64.84	47.20	63.93
2018	FR	63.26 ± 4.17^{l}	$61.04 \pm 1.70^{l,m}$	52.93 ± 4.55^{m}	44.48 ± 6.67^{m}
	ESR	44.99 ± 3.95^{n}	$41.81 \pm 4.39^{n,o}$	31.12 ± 3.61^{o}	27.60 ± 3.14^{o}
	FSR	81.59	25.91	92.36	96.67

The means followed by the same letters are statistically not different

Additionally, Pearson's test showed that post-ovulatory aging correlates significantly with the fertilization rate (r=-0.840, p=0.000) and the embryos survival rate (r=-0.537 p=0.001), in 2016. These findings were confirmed during the two following years (2017 and 2018). The performed experiment in 2017 revealed the existence of a high and significant correlation between fertilization time and the post-ovulatory aging and the fertilization rate (r= -0.819, p=0.000) and the embryos survival rate (r=-0.732, p=0.000). The last experiment, in 2018, highlighted this strong correlation

between post-ovulatory aging and the fertilization rate (r= -0.532, p=0.004) and the embryos survival rate (r=-0.596, p=0.001).

On the other hand, the box plots below (Figure 21) highlight the variability of the fertilization rate and the embryos survival within females manipulated during the aging experiments. It shows that the dispersion of fertilization rate and embryos survival rate is impacted by aging. Moreover, the asymmetric position of the media reveals the existence of heterogeneity between females of the same group.

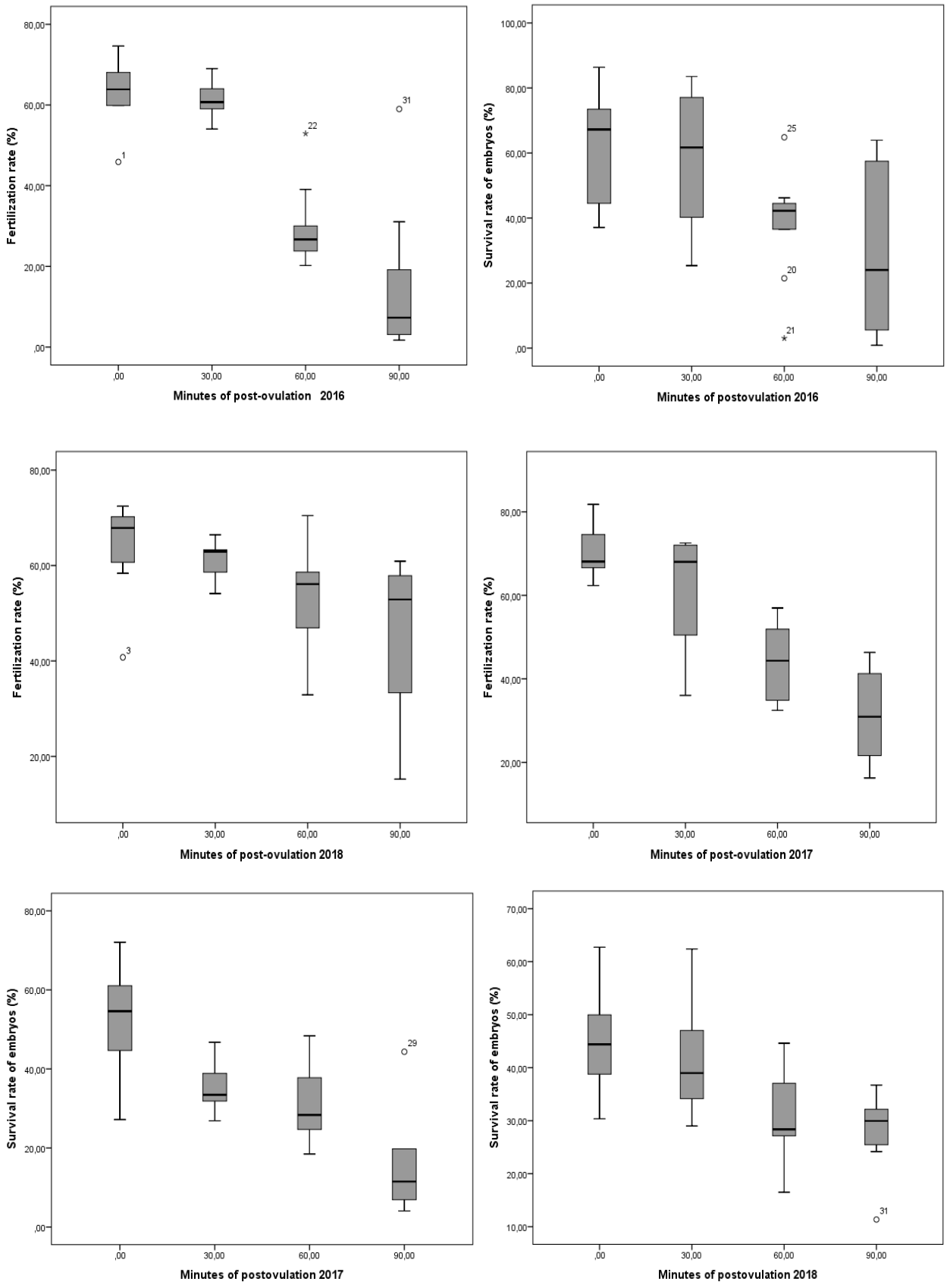


Figure 21 *Variation of FR and ESR during post-ovulatory aging performed in 2016, 2017, 2018.*

4. Discussion

Data statistical analysis by the one-way ANOVA highlighted the strong effect of the post-ovulatory aging on the fertilization success and the survival of the embryos in the three performed experiments. These results shed light on the significant effect of the ova retention on egg quality of silver carp and therefore on the yield of its seed production. In general, the egg fertilization ability and survival of the embryos decreased, during aging, by 35.24% and 28.42%, respectively. The obtained results are in accordance with other studies that highlighted the impact of aging on eggs quality (**Barnes and al., 2000; Yang and Chen, 2006; Bonnet and al., 2007; Bahre Kazemi, 2010; Moran and al., 2018**). After ova retention for 90 minutes, the fertilization success decreased from 63.35% to 15.43% in 2016, 70.24% to 31.2% in 2017 and from 63.26% to 44.48% in 2018. Identically, Bayless **(1972**) demonstrated that retained striped bass *(Morone saxatilis)* eggs lose their viability and become completely infertile after 15 to 30 minutes' post-ovulation. On the other hand, the survival of embryos was impacted by the ova retention in silver carp. The survival was higher at ovulation (61.63% in 2016, 52.63% in 2017 and 44.99% in 2018) then, at 90 minutes' post-ovulation, it decreased progressively to reach 29.76%, 16.35% and 27.60% in 2016, 2017 and 2018, respectively. Similarly, Bonnet and al. **(2007**) showed that embryo survivability was higher for ova obtained at ovulation (93%) compared to those retained in the abdominal cavity for 16 days after ovulation (37%). Barnes and al. **(2000**) proved that in rainbow trout, aged eggs showed a decrease of 20% of embryos survival compared to those stripped immediately at ovulation. For the obscure puffer fish (*Takifugu obscurus*), it was demonstrated that after 18 hours of eggs retention in females' body cavity, all eggs lost their viability **(Yang and Chen, 2006)**. For the Caspian brown trout females, retained eggs for 40 days' post-ovulation causes a decrease of eyeing rate from 90.6% to 1.34%, and hatching rates from 86.33% to 0.98% (**Bahre Kazemi, 2010)**. However, a study done by Kharroubi et al. **(2000)** in Deroua fish farm showed that there is no impact of aging on survival of embryos and hatching rate in grass carp.

Concerning the fry survival, in the current study it was found that fry survival didn't have the same tendency during the three experiments. This difference can be related to the interaction of many factors like environmental changes and handling conditions. Maternal profile (maturity, life experience, stress, nutritional status…) could be responsible of these variation, as well.

The present study showed that silver carp ova can be retained only for 30 minutes without impacting, significantly, eggs viability, while in other carp species ova can be retained more longer without having any loss in eggs viability **(Kharroubi et al., 2000; Samarin and al., 2015a)**. The study

performed on grass carp by Kharroubi et al. **(2000)** demonstrated that ova can remain in female's body cavity for 2 hours without impacting the eggs quality and embryos development. Moreover, Samarin and al. **(2015a)** proved that for the common carp, the complete loss of the eggs' viability occurs after 12 to 14 hours' post-ovulation. This difference between these carp species can be explained by their nature and the adopted strategies to face aging phenomenon when it is occurred. It can be related to the specie's zootechnical performances that makes common and grass carps have more ability and resistance to keep ova in the abdominal cavity, more longer, without affecting their quality; this can be ensured by sustainable supply of essential nutrients to the ova until the moment of ovulation. On the other hand, the present study showed that in silver carp, ova lose their fertility after 60 minutes' post-ovulation which make this fish less resistant toward aging. What is more, this resistance could be impacted by the climate changes, especially temperature variation, which have recently occurred. Further, the genetic profile of every specie is different and maybe the low resistance to aging of silver carp could be related to the changes occurred in their genes. Because, this specie was introduced in 1983 and never been introduced again since that time. So it will have several genetic modifications in this fish which can impact the resistance of the ova to aging process. For other fish species such as turbot (*Scophthalmus maximus L.*), eggs can be retained 10 hours in the body cavity without losing their viability **(McEvoy, 1983).** Moreover, in rainbow darter (*Etheostoma caeruleum*), eggs lost their viability only after 6 hours of post-ovulation **(Moran and al., 2018).** On the other hand, postovulatory aging can have positive effect in other fish species. Eggs remaining for 12 hours in female's body cavity don't influence their viability in Pike perch (*Sander lucioperca L.*). It seems that the ability to keep ova in a female's body cavity after the time of ovulation provides synchronous artificial egg insemination and thereby easing the hatchery management **(Samarin and al., 2015b).**

Morocco is a semi-arid zone where temperature increases in March. This leads to the start of Chinese carps breeding season in the period between April and June. Because of the strong relationship between temperature and ova maturation, many studies were interested in the evaluation of the impact of temperature on the aging process **(Piper and al., 1982; Espinach and al., 1984; Harvey and Kelly, 1984; Formacion and al., 1993; Legendre and al., 2000; Bahre Kazemi and al., 2010).** For instance, it was demonstrated that, according to temperature, maximum fertilization potential in Arctic charr (*Salvelinus alpinus)* eggs is limited to approximately 5 days after ovulation at 5°C **(Gillet, 1991)**, and even shorter at higher temperatures (**Gillet and Breton, 2009).** For salmonids, which are widely used to study the aging of the eggs, it was found that rainbow trout eggs can maintain their viability for at least 2 weeks of post-ovulation at 8°C **(Samarin and al.,**

2008) and 10°C **(Azuma and al., 2003)**. Also, when temperature varied between 10°C and 12°C, the eggs of this specie can be retained for at least 1 week without any decrease in egg quality **(Springate and al., 1984; Aegerter and Jalabert, 2004).** These studies give insights into the importance of the environmental factors, especially temperature, on the degree of eggs damages caused by the postovulatory aging. So, studying the relationship between temperature and post-ovulatory aging's effect on silver carp eggs will give more details that can be used in hatcheries to control the egg quality during the breeding operations.

Additionally, determining the biochemical, hormonal and molecular changes that occur during the post-ovulatory aging process is important to better understand the effect of this phenomenon on egg viability and to decode its biochemical pathway in the fish. These research aspects were studied in numerous fish species such as turbot (**Kjorsvik and al., 1990**), rainbow trout **(Aegerter and Jalabert, 2004)** and Atlantic salmon (**Bizuayehu and al., 2019**). For the silver carp, the present study determined some biochemical (Chapter V and VI), hormonal and enzymatic (Chapter VI) changes caused by post-ovulatory aging.

5. Conclusion

Based on fertilization success and embryos survival, the current study reveals that the post-ovulatory aging has a significant effect on silver carp's fry production. The ova aging causes a significant decrease in egg fertility. Also, retained ova for 90 minutes' post-ovulation causes a decrease of the embryos survival by 36% which leads to a drop in fry production. This fact proves that the stripping time of silver carp eggs after ovulation is a critical step during its breeding manipulation. To avoid silver carp ova aging, it is recommended to establish planning of injection of females so that ovulation would not occur at the same time for all the injected females. Further, a detailed study on the relationship between temperature and the impact of post-ovulatory aging could bring up some practical solutions to better manipulate a high number of females without decreasing the viability of eggs.

CHAPTER IV: DETERMINATION OF LIPIDS, FATTY ACIDS AND PROTEINS IN SILVER CARP EGGS AND THEIR RELATIONSHIP WITH EGG VIABILITY.

1. Introduction

Determining the role, function and the mechanism of chemical components in egg and their effect on the progeny will lead to optimize resources and to better understand the mechanisms that determine high eggs and larvae quality **(Tocher, 2003)**. For this reason, Scientists considered that the determination of biomarkers as indicators of egg viability is a useful tool in aquaculture for improving the production of eggs and larvae of good quality **(Kjorsvik, 1983; De-Silva and al., 2018)**.

Proteins and their constituent amino acids affect fish reproduction and eggs development. They involve in the normal development of tissues and organs and they are mainly important for normal fertilization and embryonic development **(De-Silva and Anderson, 1995; Rønnestad and al., 1999; Lanes and al., 2012)**. Moreover, it was demonstrated that amino acids regulate osmotic pressure for oocyte hydration and usable supply of nutrient for embryo, and influence egg buoyancy **(Matsubara and al., 1999; Ohkubo and al., 2008; Wu, 2009; Tong and al., 2017)**. Besides, it was proved that changes of protein levels in eggs could be related to genetic factors (maternal mRNAs) **(Bobe and Labbé, 2010)**, photoperiod **(Bonnet and al., 2007)** and broodstock feed **(Khan and al., 2004)**.

On the other hand, Lipids and their constituent fatty acids are, along with proteins, the major organic constituents of fish **(Taşbozan and Gökçe, 2017; González-Félix and al., 2019)**. They are used for ova production, embryos and first stage of larval development. In general, lipid utilization in freshwater fish eggs can occur during the whole developmental stages including embryogenesis **(Palacios and al., 2007)**. Moreover, catabolism of lipids results in the release of free fatty acids which can be used in several purposes. They have significant function in the success of the reproduction outcome of various animal species like, ruminants **(Mattos and al., 2000)**, pink shrimp **(Emernciano and al., 2014)**, oysters **(Bayne, 2017)** and Sprague Dawley rats **(Ye and al., 2019)**. Concerning fish, many studies showed that there is some relationship between reproductive success and fatty acids composition. It was demonstrated that they are the major source of metabolic energy for reproduction and they are used for growth from the egg to the adult fish regulating the biophysical properties of cellular membranes **(Bacle and al., 2020)**.

The eggs of most freshwater fish contain higher levels of n-6 PUFA, particularly arachidonic acid (20:4n-6) and Linoleic acid (18:2n-6) **(Wiegand, 1996)**. As mentioned before, they are essential for the reproductive success of fish. To illustrate, PUFAs in common carp 'eggs (*Cyprinus carpio*) are very essential in terms of fertilization success and larval development **(Mukhopadhyay and Ghosh, 2003)**. Also, a previous study performed on common snook (*Centropomus undecimalis*) proclaims that the success of fertilization and larval survival rate are related to high concentration of DHA in eggs **(Yanes-Roca and al., 2009)**. Moreover, various studies proved that many egg quality criteria, including hatching, fertilization rates and survival of early life stages (embryos and larvae), were positively correlated with increased levels of n-3 HUFA (Highly Unsaturated Fatty Acids) **(Bruce and al., 1999; González-Félix and al., 2019)**. On the other hand, balanced ratio of n-3 and n-6 fatty acids enhances the reproductive performances of zebrafish, *Danio rerio*, females and increases the hatching rate **(Jaya-Ram and al., 2008)**. Furuita and al. **(2007)** showed that n-3 and n-6 fatty acids are necessary for Japanese eel, *Anguilla japonica*, reproduction but a high ratio affects negatively the embryos development. In addition, fatty acids impact significantly the eggs and larval quality in Eurasian perch, *Perca fluviatilis*, and common carp, *Cyprinus carpio*, (**Mukhopadhyay and al., 2003; Henrotte and al., 2010)**.

Fatty acids composition in the eggs is related to many factors. The most important causes of the variation in the fatty acid profiles are the differences among species and the broodstock nutrition too, since all nutrients needed during embryogenesis until the first exogenous feeding are provided maternally and transferred to the oocyte during vitellogenesis **(Izquierdo and al., 2001; Grote and al., 2012; Asil and al., 2017; Taşbozan and Gökçe, 2017)**. For the European eel (*Anguilla anguilla*, L.), it was proved that fatty acids levels in fish body and tissues decreased during gametes maturation **(**sperm, **Baeza and al., 2015 and Butts and al., 2015**; oocytes, **Nowosad and al., 2015)**. It was demonstrated that during the process of maturation of the European eel female's, docosahexaenoic acid (DHA) eicosapentanoic acid (EPA) decreased in muscles and increased in ovaries **(Nowosad and al., 2015)**. Further, Clevestam and al. **(2011)** reported that deficient fat reserves in European eel body could prevent oocytes maturation and the production of good quality eggs. It was also found that fatty acids profile in eggs differ between captive and wild fish **(Salze and al., 2005; Palacios and al., 2007; Seaborn and al., 2009; Lanes and al., 2012)**. Therefore, it has been reported that environmental conditions have significant impact on the biochemical composition of fish eggs **(Krautz and al., 2010)**. Castro and al. **(2010)** revealed that eggs collected from cold water contained higher PUFA while eggs collected from warm waters contained higher

MUFA. Moreover, it was showed in other studies that species and stock differences influence the variability of fatty acids in fish eggs **(Pickova and al., 1997)**.

The aim of the present study is to investigate about the temporal changes in eggs lipids and proteins, as a major energetic content, and how they are impacted by the maternal age and weight. On the other hand, we are looking for the relationship between the energetic nutrients and the fertilization success and survival of embryos and larvae.

On the other side, the present study seeks to determine the variability intra spawning of fatty acids profile in ova collected from silver carp (*Hypophthalmichthys molitrix*), reared in Morocco at Deroua fish farm. It investigates the relationship between these fatty acids and the fertilization success and embryos survival. In particular, it examines the effects of maternal age and weight on the fatty acids composition in the unfertilized eggs.

2. Material and methods

2.1. Eggs sampling

During this study, eggs were collected from 20 females used in all performed operations (P1, P2, P3 and P4) of artificial breeding of silver carp (Figure 22). P1 and P2 were performed in April while P3 and P4 were performed in May. The samples were stored at -20°C until use. The biochemical analysis was performed in June.

Figure 22 *Eggs sampling for biochemical analysis.*

2.2. Lipids

Total lipids extraction was performed using Folch and al. method **(1957)**. Extraction is performed through the use of a solvent mixture (chloroform, methanol, 2: 1 v / v). About 2 grams of silver carp

eggs were weighed and crushed. the sample was mixed with 8 ml of chloroform and 4 ml of methanol and homogenized for 2 minutes. After decantation, the lower phase, which contains lipids, is recovered. A second extraction was carried out, for the residue, by the addition of 5 ml of chloroform: methanol (2:1, v / v). After recuperation of lipids, the solvent was evaporated at 50°C then lipids mass was weighted.

2.3. Proteins

For every collected sample, 0.5g of silver carp eggs were grounded cold in 2ml of Tris-HCl buffer (pH 7.5) and 5% glycerol (v / v). The grounded matter thus obtained was centrifuged at 4500 *g* for 30 min and at 4°C. 0.1 ml of supernatant was taken and its absorbance was measured at 540 nm after adding Gornall reagents. Total proteins concentration was calculated using the calibration curve.

2.4. Fatty acids

To determine the ova fatty acids profile, every sample of extracted lipids, about 2 grams, was subjected to trans-esterification. The reaction was catalyzed by 1.5% NaOH (w/w) in methanol 1:20 at 80 °C during 5hours. The resulting fatty acids methyl esters (FAME) were analyzed by capillary gas chromatography (Agilent 7890 A Series GC) Tandem to mass spectrometry (MS) equipped with multimode injector and 5 MS column (30 m × 250 µm × 0.25 m) and electron impact ionization. The detection of fatty acid methyl esters profile was done using full scan mode between 35 and 500 m/s with gain factor 5 and identification was performed using NIST 2011 MS Library and known standards. The fatty acids methyl esters composition was calculated as percentage of the total FAME presents in the sample, determined from the peak areas.

2.5. Statistical analysis

The statistical determination of the fertilization success classes was based on the equation below. The range between the highest and lowest rates of the fertilization was approximately classified into three equal classes. Same equation was used to determine weight class of females and age class in order to investigate about the relationship between maternal characteristics and fatty acids composition in eggs.

$$\text{Class limit} = \frac{\text{The highest value} - \text{ the lowest value}}{3}$$

Data were statistically analyzed by SPSS Statistics software version 23 (IBM Corporation, Somers, NY, USA). One-way analysis of variance (ANOVA 1) was used to study the variability of lipids, fatty acids and proteins in eggs throughout the breeding season. Pearson's correlation was used to determine the relationship between lipids, fatty acids and proteins and fertilization rate, embryos survival rate.

2. Results

2.1. Variability of lipids and proteins in eggs during the reproductive season

2.1.1. lipids and proteins in silver carp's unfertilized eggs and their variation during the breeding season

During the breeding season, the mean of total lipids content varied from 3.90 ± 0.59% to 5.40 ± 0.57 %. The proteins amounts varied between 2.26 ± 0.33% and 2.14 ± 0.37% (Table 11). High level of lipids was obtained at P2 (second operation in April) with an overall average of 5.40%. The lowest level of lipids was obtained at P3 (first operation in May) with an average of 3.90%. Concerning proteins in silver carp eggs, no significant difference was observed between the artificial breeding operations performed during the same month. But these proteins levels varied between April and May. The high amounts were obtained in April (2.26% for P1 and 2.21% for P2) and the lowest in May (2.14% for P3 and 2.16% for P4). The one-way analysis of variance revealed that lipids and proteins content, of the ovulated eggs, do not vary significantly throughout the breeding season (P>>>0.05). Moreover, the correlation between proteins and lipids in eggs was extremely weak and non-significant. Data about the assessment of viability rates were presented and discussed in Chapter I.

Table 11 *Relative biochemical composition and viability rates of stripped ova from silver carp females, during the breeding season.*

	April		**May**	
Spawning period	**P1**	**P2**	**P3**	**P4**
Lipids (% wet weight)	4.45 ± 0.75	5.40±0.57	3.90±0.59	4.67± 0.89
Proteins (% wet weight)	2.26±0.33	2.21±0.62	2.14±0.37	2.16± 0.72
Fertilization rate (%)	56.37±9.75	34.88±4.58	52.80±6.32	51.54±6.97
Embryos survival rate (%)	42.72±7.079	35.87±4.72	48.51±6.35	48.08±6.072

Means given with ±standard deviation

2.1.2. Relationship between chemical composition of eggs and viability rates (FR and SRE)

No significant impact of lipids and proteins content was found on the fertilization rate (FR) and the embryos survival rate (ESR) (P>>>0.05). The correlation values between the lipids content, fertilization rate and embryos survival rate were r = 0.103 (sig=0.665) and r= 0.074 (sig=0.845), respectively. while the correlation between proteins level, fertilization rate and embryos survival rate were r= -0.435 (sig=0.055) and r= -0.513 (sig=0.021).

2.1.3. Relationship between maternal characteristics and richness of eggs by lipids, proteins.

The lipids and proteins levels varied within females manipulated during the breeding season. The Pearson's test revealed the existence of negative correlation between lipids and females age (r=-0.42, sig=0.032). This means that old females gave eggs with low levels of lipids contrariwise of the youngest females which gave eggs containing more lipids. Negative correlation was found, also, between proteins amount in eggs and the females weight (r=-0.418, sig=0.053). Females with high weight gave eggs with low proteins and female with low weight their eggs contain high levels of proteins. The same correlation was observed between female's length and proteins in eggs (r= -0.32) but it wasn't significant.

2.2. Variability of fatty acids in eggs during the reproductive season

2.2.1. Fatty Acids profile of the silver carp' eggs

In silver carp' eggs, the proportions of polyunsaturated (PUFAs), monounsaturated (MUFAs) and saturated fatty acids (SFAs) were not highly different. The overall average was 33.22%, 29.60% and 32.44%, respectively for PUFAs, MUFAs and SFAs (Table 12). Furthermore, the fatty acids composition, of silver carp eggs, is dominated by docosahexaenoic acid, DHA (C22:6n-3), oleic acid (C18:1n-9), palmitic acid (C16:0) and Eicosapentaenoic acid, EPA (C20:5n-3) (Table 12).

Within the PUFA the n-3 series were more abundant than the n-6 series, the total mean was 31.57±1.01% for the former and 5.33±0.32% for the later. The overall ratio (n-3)/ (n-6) was 6.50 % (±0.50).

Table 12 *Proximate composition of fatty-acids in total lipids extracted from silver carp' eggs. Data expressed as weight % of total identified fatty acids and represent means ± SD (standard deviation) of 23 sample.*

Fatty acids	Eggs fatty acids %
C14:0	1.63±0.12
C15:0	0.94±0.09
C16:0	17.70±0.53
C17:0	4.28±0.22
C18:0	8.32±0.20
C20:0	0.11±0.01
C24:0	0.25±0.03
SFA	**33.22±1.21**
C16:1	5.37±0.16
C17:1	0.20±0.04
C18:1	21.19±1.03
C19:1	0.24±0.05
C20:1	2.78±0.09
MUFA	**29.79±1.37**
C17:3	0.03±0.02
C18:3ALA	0.08±0.03
C20:5 EPA	10.29±0.84
C20:4 AA	2.57±0.24
C22:6 DHA	21.21±1.19
C22:5 DPA	2.76±0.29
PUFA	**36.94±2.61**
n-3	31.57± 1.01
n-6	5.33±0.33
n-3/n-6	6.50 ± 0.50

2.2.2. Fatty acids effect on fertilization success, embryos mortality and survival of larvae

The One-way ANOVA test revealed the existence of the relationship between fertilization success and fatty acids profile of eggs, especially C14:0 and C15:0. Moreover, the experiment showed that high levels of the two fatty acids, cited bellow, belongs to eggs that had a medium fertilization success. The values were 2.14% for C14:0 and 1.34 % for C15:0. However, levels of fatty acid were

not related to ova with high fertilization success and those of low fertilization success (Figure 23).

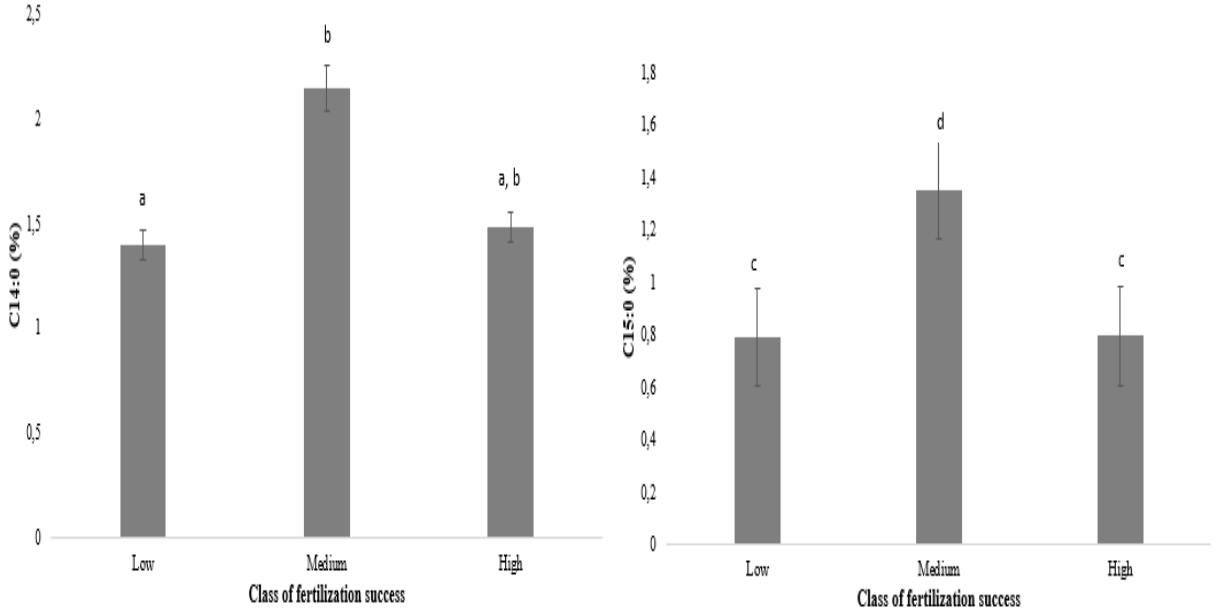


Figure 23 *Variation of C14:0 and C15:0 in eggs according to the fertilization success. Low< 41.53%; Medium<58.28%; High>58.28%. The means followed by the same letter do not show a significant difference between them.*

2.2.3. Changes of fatty acids composition of silver carp eggs throughout the breeding season

The overall amount of PUFAs, MUFAs and SFA vary respectively between 37.98% and 35.31%, 27.73% and 31.23%, 34.46% and 31.89% (Table 13). On the other hand, the n-3 series didn't change during the breeding season. The overall amount varied between 29.34% and 33.24% (Table 13). Likewise, the C16:1 increased during the breeding season. Except the C16:1, ANOVA 1 test showed that fatty acids' composition doesn't vary during the breeding season of the studied fish. But, it has revealed the variation of n-6 series and n-3/n-6 ratio throughout the breeding season ($F(3, 19) = 3.987$, $p=0.023$) and ($F(3, 19) = 5.166$, $p=0.009$) respectively. A Tukey post hoc test showed that n-6 series were statistically lower at the P3 (4.38 ± 0.45, $p=0.041$) compared to the end (6.72 ± 0.71) of the breeding season (P4) (Table 13). The n-3/n-6 ratio was lower at the P2 (4.93 ± 0.19, $p=0.041$) and the P4 (4.70 ± 0.28, $p=0.037$) compared to the beginning (P1) (8.21 ± 1.15) of the breeding season (Table 13). The same test showed the absence of the effect of fatty acids content on the fertilization ratio.

Table 13 *Variation of fatty acids composition of eggs, during the breeding season. No statistical difference was detected between the means of the fatty acids during the reproductive season.*

Fatty acids	Spawning detection			
	April		May	
	P1	P2	P3	P4
C14:0	1.64 ± 0.18	1.41±0.2	1.49±0.15	2.01±0.41
C15:0	0.90 ± 0.21	0.89 ±0.14	0.91±0.12	0.90 ± 0.26
C16:0	17.40±1.55	18.69±.93	16.60±.68	18.15±.80
C17:0	3.58±0.48	4.78±0.28	4.19±0.52	4.61±0.29
C18:0	8.47±0.53	8.4±0.36	8.23±0.28	8.15±0.51
C20:0	0.14±0.03	0.08±0.028	0.11±0.026	0.10±0.028
C24:0	0.31±0.073	0.19±0.066	0.28±0.065	0.16±0.070
SFA	32.51 ± 2	34.46 ± 1.71	31.89 ± 0.76	34.26 ± 1.51
C16:1	4.86±0.26	5.23 ±0.20	5.62 ±0.4	5.83 ±0.21
C17:1	0.24±0.109	0.14±0.050	0.26±0.062	0.12±0.082
C18:1	21.4±0.91	22.24±0.736	22.10±0.551	18.57±4.699
C19:1	0.165±0.106	0.161±0.102	0.358±0.114	0.298±0.123
C20:1	2.94±0.169	2.43±0.149	2.86±0.145	2.89±0.223
MUFA	29.70 ± 0.92	30.22 ± 0.73	31.23 ± 0.46	27.73 ± 4.36
C17:3	n.d.	0.04 ± 0.043	0.06±0.067	n.d.
C18:3	0 ± 0	0.11 ± 0.060	0.08±0.041	0.12±0.124
C20:5 EPA	9.85 ± 0.566	8.81 ± 0.710	12.71±2.969	9.65±0.820
C20:4 AA	2.51 ± 0.663	2.55 ± 0.123	2.19±0.525	3.10±0.489
C22:6 DHA	23.38 ± 1.658	20.40 ± 1.332	19.59±3.910	21.48±1.987
C22:5 DPA	2.01 ± 0.768	3.36 ± 0.163	2.19±0.619	3.61±0.272
PUFA	37.77 ± 2.75	35.31 ± 2.31	36.85 ± 1.12	37.98 ± 7.63
n-6	4.53 ± 0.69	5.92 ± 0.27	4.39 ± 0.45	6.72 ± 0.71
n-3	33.24 ± 2.18	29.34 ± 2.089	32.39 ± 1.03	31.26 ±2.81
(n-3)/(n-6)	8.21 ± 1.15	4.93 ± 0.19	7.83 ± 0.97	4.71 ± 0.34

Abbr. n.d. not detectable. Data expressed as weight % of total identified fatty acids and represent means ± SD.

2.3. Maternal effect on fatty acids profile in eggs

2.3.1. Effect of weight and length

High levels of C18:1 were detected on eggs originated from females of high weight (>4 kg) and it reached 23.28% in total lipids. Then followed by eggs of females with medium weight (2.7 kg<medium<4 kg) with an average of 22.66% in total lipids. The females with low weight (low<2.7 kg) gave eggs that contain low levels of C18:1 (16.62% in total lipids). On the other hand, arachidonic acid (C20:4) decreased with the increase of female weight. It declines from 3.70% to 2.27%, then in eggs of females with high weight this fatty acid decreased to reach 1.87%. The same

variation was observed for the docosahexaenoic acid (C22:6) decreased with the increase of the weight class. It reached 25.16%, 21.23% and 15.21% for low, medium and high weight, respectively (Table 14). Additionally, Pearson's test demonstrated that female's weight and length correlate with EPA, DHA and AA (Table 15).

Table 14 *Variationof fatty acids according to the females' weight class*

	Females'weight class		
	Low	**Medium**	**High**
	≤2.7	**2.7<**	**4 kg <**
C18:1	16.62 ± 8.2[a]	22.66 ± 1.42[b]	23.28 ± 1.09[a,b]
C20:4	3.701 ± 1,05[c]	2.27 ± 0.81[d]	1.87 ± 1.25[d]
C22:6	25.16 ± 2.71[e]	21.23 ± 3.08[e,f]	15.21 ± 10.5[f]

Data expressed as weight % of total identified fatty acids and represent means ± SD. The means followed by the same letter do not show a significant difference between them.

Table 15 *Correlation between females'weight and length and EPA, DHA and AA in eggs. Pearson's coefficients (r) and corresponding significance level (P) are presented for each correlation.*

	EPA	**DHA**	**AA**
Female's weight	NS	P = 0.003; r = -0.588	P = 0.009; r = -0.530
Female's lenght	P = 0.013; r = 0.508	P = 0.002; r = -0.602	P = 0.01; r = -0.525
Statistical significance was set at P < 0.05.			

2.3.2. Effect of age

High values of C16:1 were obtained in eggs of medium class then eggs of low age the lowest value was reached in eggs of high class of age. The values were 5.5%, 5.7% and 4.35% respectively for low, medium and high class of age. C20:5 was high in eggs of females with medium age (18.7%) followed by eggs of older females (10.74%) then the younger females which contains the lowest value (9.27%).

High values of C22:6 were reached for eggs stripped from older females (25.22%) followed by younger females (21.54%) then eggs of females with medium age (12.17%). Furthermore, female's age correlates significantly with C16:1 (p=0.025; r=-0.458), C16:0 (p=0.045; r=-0.422) and C17:1 (p=0.007; r=0.544) (Figure 24).

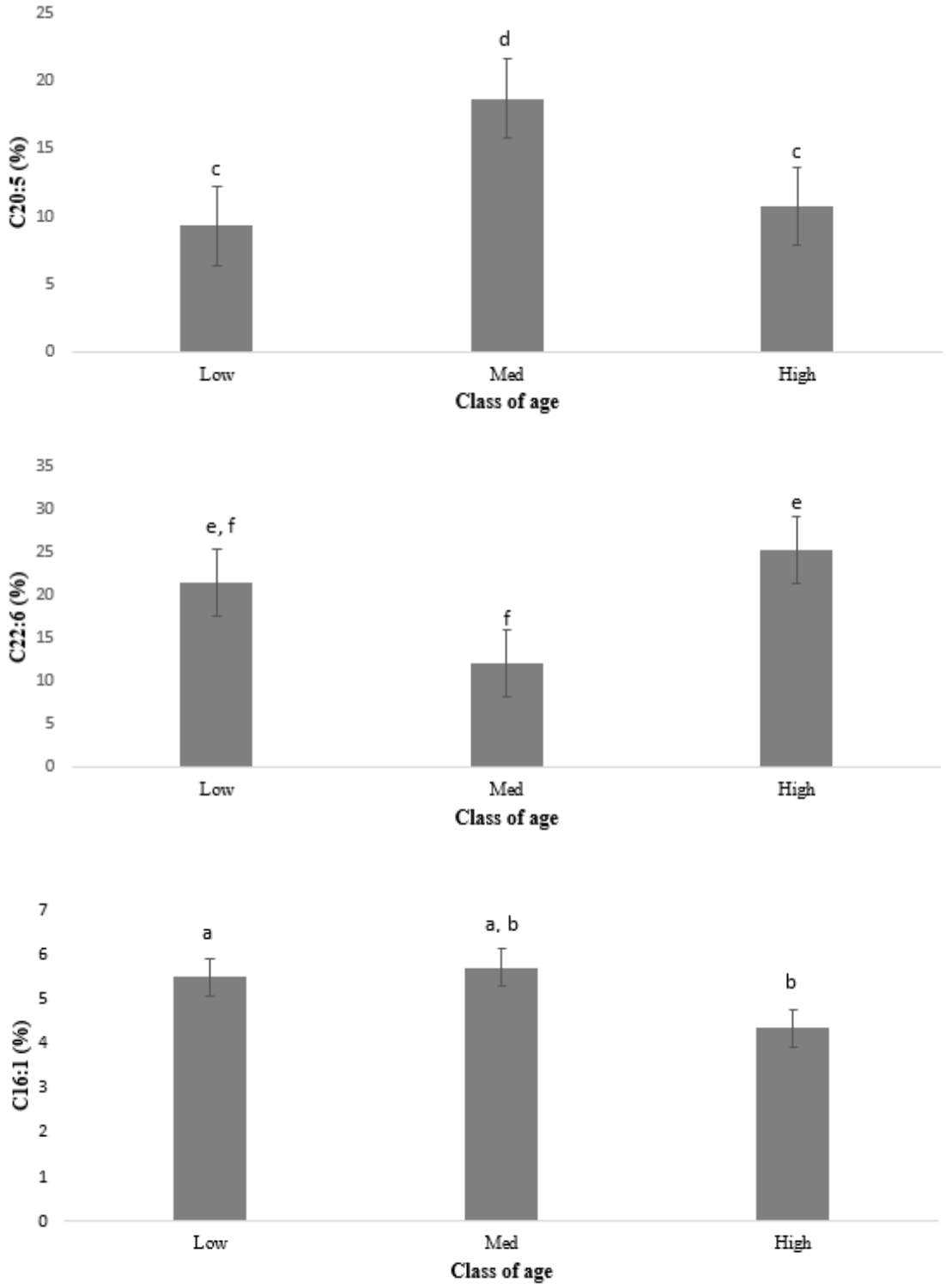


Figure 24 *Variation ofC16:1, C20:5 and C22:6 according to the class of females' age. Low ≤2.7 ; 2.7<Med; 4 <High. The means followed by the same letter do not show a significant difference between them.*

3. Discussion

lipids and proteins are the major source of energy in fish. They are used for ova production, embryos and first stage of larval development **(Tocher and al., 1985)**. The present study revealed that silver carp unfertilized eggs contain less than 6% of fat and 2.26% of proteins proportion. Based on the one-way ANOVA test, lipids in silver carp' eggs didn't vary during the breeding season. These findings are in concordance with previous studies performed on silver carp's eggs **(Lahnsteiner, 2001)** and also tissues **(Richard and al., 2017)**. The performed study on three cyprinid species, by Lahnsteiner **(2001)** revealed that no relationship was found between the lipids and proteins levels and the fertilization rate in silver carp eggs which is similar to our findings. Same results were revealed by studies done on eggs of other fish species which showed the invariability of egg lipids during the reproductive season **(**Atlantic cod**, Lanes and al., 2012;** White trevally**, Nogueira and al., 2018)**. White trevally *(Pseudocaranx dentex)* Egg's total lipids (16% Dry Weight) remained constant throughout the spawning season and were not correlated with any egg viability parameters **(Nogueira and al., 2018)**. However, eggs of many fish species are impacted by the temporal factor. The performed study by Bransden and al. **(2007)** showed that total lipids in striped trumpeter decreased throughout its reproductive season. On the other hand, Turbot (*Scophthalmus maximus*) which its eggs are composed by 13.8% of lipids **(Silversand and al., 1996).** In a study performed in turbot, it was found that the weight of total lipids per egg fell as the spawning season progressed **(McEvoy and al., 1993)**.

Concerning the Proteins variation, the study revealed that they remain stable during the breeding season. Similar findings were obtained for the farmed Atlantic cod while for the eggs of wild fish increased during the reproduction season. No significant relationship was found between the content of proteins and fertilization or hatching rates **(Lanes and al., 2012)**. In contrast to other fish species which the proteins correlate with eggs viability **(***Scophthalmus maximus***, Fauvel and al., 1993;** *Salmo trutta f. lacustris.***, Lahnsteiner and al., 1999).**

Our findings, revealed that energetic component, lipids and proteins, in silver carp eggs do not change during the breeding season. Otherwise, the abundance of lipids and proteins in eggs depends on the intraspecific factors and also diet factors. Moreover, the invariability of lipids and proteins may be due to the resistance of silver carp eggs to changes and conditions that the female lived during the oogenesis and the reproductive season. In addition, nonspecific relationship was detected between the energetic components and the egg viability in silver carp. The absence of changes in

lipids and proteins levels in eggs could be due to food availability. In this study, the females are reared in captivity so the food is available as it was demonstrated by Farid et al. **(2017)**. This performed research was interested in the determination of the diet of the broodstocks of silver carp reared in ponds. The study revealed that this fish feed on phytoplankton (*Diatomophyceae* and *Chlorococcales*) available naturally in ponds.

lipids content in eggs depend on several factors like broodstock diet which is linked in many studies with the eggs quality and its richness by biochemical nutrients that are needed for embryonic development and growth **(Sargent and al., 1999; Pavlov and al., 2004; Lubzens and al, 2010; Tocher, 2003; Quintero, 2007)** and if it is a captive broodfish or a wild fish **(Czesny and Dabrowski, 1998; Ashton and al., 1993; Salze and al., 2005; Hauville and al., 2015)**. Bransden and al. **(2007)** found that for striped trumpeter, the total lipids in eggs of wild fish are higher than those of fish in captivity; the values were 18.9% and 17.6% respectively. Proteins' concentration in eggs is also related to genetic factors which are represented by the maternal contribution by the mRNAs **(Bobe and Labbeé, 2010)** photoperiod **(Bonnet and al., 2007)** and broodstock feed **(Khan and al., 2004)**.

Saturated and monounsaturated fatty acids are usually catabolized for energy, long-chain polyunsaturated fatty acids accumulate in sufficient quantities in the oocytes to ensure adequate hatching and early growth and survival of the larvae **(Izquierdo and al., 2001; González-Félix and al., 2019)**. Our study highlights the richness of silver carp 'eggs by PUFAs which is barely distinguishable from the general freshwater fish fatty acids profile **(Tocher, 2003)**. The DHA (C22:6) was the major fatty acid in this group followed by EPA (C20:5), palmitic acid C16:0 (SFA) and oleic acid C18:1 (MUFA). These findings are in concordance with previous studies performed on silver carp's tissues. Kindong and al. **(2017)** found that the tissues of this specie contains palmitic acid (C16:0), with high proportion, oleic acid (C18:1), α-linolenic acid (C18:3), DHA and EPA. These results are different from our findings except the α-linolenic acid (C18:3) which was found with a very small amounts in silver carp eggs than tissues. This difference could be related to the habitat changes and diet of the broodfish used for the studies in fact oleic acid (C18:1n-9) is produced by the desaturation of saturated fatty acids synthesized in the fish organism from energy-rich supplemented feed **(Buchtová, 2011)**.

For instance, a study performed on egg of the chinook salmon, *Oncorhynchus tshawytscha* showed that n-3 FA in total lipids for wild and cultured fish was 40.2% and 29.4%, respectively. The study

showed that wild fish contained higher concentration of C20:5n3 than cultured fish **(Ashton and al., 1993; Palacios and al., 2007)**. The eggs of walleye, *Stizostedion vitreum* contain high level of DHA for both wild and domesticated fish. But the fatty acids profile differed significantly among the wild and the domesticated fish **(Czesny and Dabrowski, 1998; Palacios and al., 2007)**.

During the current study, it was found that silver carp eggs contain high percentage of n-3 fatty acids. The diet of this specie may be responsible for this result. As it was demonstrated previously by other studies, silver carp feeds on phytoplankton which is well known by high concentration of n-3 fatty acids **(Steffens and Wirth, 2005; Farid et al., 2017)**. For other fish species, it was demonstrated that n-3 fatty acids are essential for the fertilization success **(Mukhopadhyay and Ghosh, 2003; Ovissipour and Rasco, 2011)**. The fatty acids profile of Beluga's eggs (*Huso huso*) are predominated by linolenic and linoleic acids and a high amount of n-3 fatty acids. It was found that low levels of PUFAs in the cultivated specie affects negatively the fertilization and the hatching ratios **(Ovissipour and Rasco, 2011)**. The richness of silver carp feed by n-3 series is probably responsible of the absence of fertilization variability of the fertilization success during the breeding season. In other fish species, some fatty acids varied within the breeding season, for instance in the eggs of the common snook, *Centropomus undecimalis*, the PUFAs changed during the spawning season and the fertilization success and larval survival rate are related to high concentration of DHA **(Yanes-Roca and al., 2009)**.

The association between fertilization and fatty acids in general has been well reviewed previously **(McKeegan and Sturmey, 2011; Dunning and al., 2014)**. The current study revealed that there are no changes of fatty acids profile in silver carp eggs during the breeding season. This can be due to the food availability and that silver carp is not a multi-spawner fish. In addition, no direct relationship was found between fatty acids profile of the egg and the fertilization success. This result was also found in other fish species which fatty acids remain stable during the reproductive season **(**captive walleye**, Mejri and al., 2014;** ballan wrasse**, Grant and al., 2016;** white trevally**, Nogueira and al., 2018)**. However, studies performed on turbot eggs, showed that they are impacted by the progress of the spawning season; the weight of total lipid per egg fell as the spawning season progressed and there were significant increases in the levels of 18:3n-3,22:4n-6 and 22:5n-3 in the neutral lipid fraction, but neutral 20:5n-3 and 22:6n-3 levels remained fairly constant throughout the season **(McEvoy and al., 1993)**. Moreover, the present study revealed the presence of an effect of female's age and weight on the concentration of specific fatty acids in the egg. It revealed that there is an effect of female weight on the concentration of C18:1, C20:4 and C22:6 in eggs. In

addition, it was showed that female's weight and length correlate with EPA, DHA and AA (Table 15). On the other hand, female's age correlates significantly with C16:1, C16:0 and C17:1. Many studies were interested in maternal contribution on fatty acid profile in fish eggs **(Bachan and al., 2012; Ulvund and Grahl-Nielsen, 1988)**. It was demonstrated that eggs stripped from small females, low weight and length, contained lower amounts of fatty acids than the bigger females **(Ulvund and Grahl-Nielsen, 1988)**. Further, Brekely and al. **(2004)** demonstrated that older females of the black rockfish, *Sebastes melanops,* gave eggs rich in lipids than younger females. Furthermore, many studies revealed that inter-females' differences of fatty acids in eggs is related to rearing conditions, especially the feeding of the broodstock **(Mazorra and al., 2003; Krautz and al., 2010; Asil and al., 2017)**.

4. Conclusion

The present experiment reveals that total lipids and proteins are not impacted by the temporal factor during the same season. Moreover, it leads to conclude that fertilization success and survival of the embryos are not related to the total energetic component in the egg. Additionally, the fertilization success in silver carp eggs is related to the presence of C14:0 and C15:0. These findings could be used as a reference for the elaboration of diet that enhances the eggs quality and by this increasing the yield of the fry production of silver carp. Further, the determination of fatty acids profile and egg quality of wild silver carp could be a useful tool to determine the broodstocks origin which can give egg with high viability and as a consequence, the best way to ensure an optimal and high seed production of this specie.

On the other hand, the study presented the fatty acids profile of eggs of silver carp reared in Moroccan inland waters and showed that this profile remains stable during the same reproductive season. Since nutrient in eggs are transferred from the mother, the high amount of n-3 fatty acids in eggs reflect the richness of the fish by these high quality fatty acids. So silver carp present a potential source of n-3 fatty acids for the human alimentation.

Concerning the contribution of maternal traits in the fatty acids amounts in eggs, the study revealed that maternal age and weight have an important effect on their levels.

CHAPTER V: RELATIONSHIP BETWEEN LIPIDS, FATTY ACIDS, PROTEINS AND THE IN VIVO AGING PHENOMENA OF SILVER CARP OOCYTES

1. Introduction

In order to understand the mechanism of aging and the relationship between fertilization success, embryo's development and changes of nutrient reserve in eggs, numerous researches were carried out to determine the egg's biochemical changes during the aging (over-ripening) **(Craik, 1984; Bahre kazemi, 2009; Samarin and al., 2015)**. Nevertheless, the knowledge of the aging mechanisms still not fully understood. In salmonids, especially rainbow trout, it was proved that during post-ovulatory aging, egg quality can be predicted by determining pH (potential of hydrogen) and osmolarity in the ovarian fluid **(Aegerter and Jalabert, 2004)**. For the same objective, which is determining biomarkers of egg quality, it has been demonstrated that fatty acids, proteins and aspartate aminotransferase are suitable biomarkers for predicting egg quality in rainbow trout **(Lahnsteiner, 2000)**. Bahre Kazemi and al. **(2009)** demonstrated that during post-ovulation Caspian trout's pH decreased significantly while the levels of glucose, proteins increased in the ovarian fluid.

Besides, studies were interested to investigate about the relationship between maternal genetic expression and the state of egg quality during post-ovulation **(Aegerter and al., 2005; Ma and al., 2015; Bizuayehu and al., 2019)**. For the rainbow trout, which is used as a good sample for studying post-ovulation mechanisms, it has been proven that aged eggs have lower mRNA expression **(Aegerter and al., 2005; Ma and al., 2015)**. Moreover, hepcidin-1 can be a potential egg quality marker during the post-ovulatory aging in Atlantic salmon **(Bizuayehu and al., 2019)**.

The current study seeks to determine some biochemical changes that may occur during the in vivo aging process of the silver carp ova. Moreover, it investigates about the relationship between eggs quality and its biochemical composition. For this purpose, we have investigated the possible variation of total lipids, fatty acids composition and total proteins during post-ovulation in vivo retention of eggs.

2. Material and methods

2.1. Eggs sampling

Ovulated eggs originated from females used for the aging experiment. Based on results obtained in Chapter III, which revealed that there is no significant difference on viability rates between eggs

stripped at ovulation and those obtained at 30 minutes' post-ovulation, we performed the biochemical analysis only for eggs collected at ovulation as well as those collected at 60 and 90 minutes after ovulation. The determination of lipids and proteins of aged eggs was carried out for number of 9 females while fatty acids were determined for eggs of 5 females.

2.2. Determination of proteins contents in unfertilized eggs

As presented in Chapter IV, for every collected sample, 0.5g of silver carp eggs were grounded cold in 2ml of Tris-HCl buffer (pH 7.5) and 5% glycerol (v / v). The grounded matter thus obtained was centrifuged at 4500 *g* for 30 min at 4°C. 0.1 ml of supernatant was taken and its absorbance was measured at 540nm after adding Gornall reagents. Proteins concentration was calculated using the calibration curve.

2.3. Determination of total lipids in unfertilized eggs

Total lipids are extracted from eggs stripped in different retention times (at ovulation, 60 and 90 minutes after ovulation). Lipids extraction was performed using Folch and al. **(1957)** method (Chapter IV).

2.4. Determination of fatty acids profile

The determination of fatty acids profile was carried out in official Laboratory of Chemical Science and Analysis (LOARC). About 1.5 g of lipids were extracted and dissolved in 2ml of KOH (2N) and 2ml of iso-octane. Then the samples were homogenized and a quantity of Sodium bisulfite was added to the preparation. The fatty acids were analyzed by gas-liquid chromatography (HP6890 series; GC system HP). The dissolved Fatty-acid methyl esters, in iso-octane, were injected automatically in a capillary column (Blx70). Individual fatty-acid methyl esters in the samples were identified by comparison of their retention time with the retention time of a known standard sample (fish oil; BIPEA).

2.5. Statistical analysis

Data were analysed using SPSS version 23 (IBM Corporation, Somers, NY, USA). We used one-way ANOVA to evaluate the biochemical changes (total lipids, total proteins and fatty acids) between moment of ovulation and 60 and 90 minutes after ovulation ($P < 0.05$ is considered significant). We excluded the analysis of the ova stripped at 30 minutes after ovulation due to the absence of significant difference between them and those stripped at ovulation. This was in order to avoid the lake of reagent in the laboratory.

3. Results

3.1. Variability of total lipids and total proteins in eggs during post-ovulatory aging

High proteins concentration was obtained in eggs stripped at ovulation 2.21% then the concentration of proteins decreased after 60 minutes of post-ovulation to reach 2.10%. After 90 minutes, the concentration of proteins was lower and reached 2.05%. Moreover, total lipids levels increased from 3.76% to 4.86% then it dropped to 4.07% in eggs stripped at ovulation, 60 and 90 minutes after ovulation, respectively (Table 16). Further, the one-way ANOVA demonstrated that no statistical difference between the means of proteins and lipids during the aging phenomenon.

Table 16 *Variation of proteins and lipids levels in unfertilized eggs during post-ovulatory aging (Data are presented as mean± Standard Error).*

	At ovulation	**60 minutes post-ovulation**	**90 minutes post-ovulation**
Proteins (g /dl)	2.21± 0.182	2.109 ± 0.165	2.049 ± 0.195
Lipids (% wet weight)	3.76±0.327	4.86 ± 0.883	4.07 ± 0.837
no statistical difference between the means			

On the other side, linear regression revealed that only 2.3% of the variability of total proteins levels in eggs could be explained by aging. On the other hand, aging could explain only 1.7% of the variability of total lipids levels in eggs. This weak relationship between lipids, proteins and aging is confirmed by the one-way ANOVA which proved that eggs retained in body cavity of females for 90 minutes didn't show any significant changes in their total lipids and proteins content ($P>0.05$). These results were confirmed by the results obtained in 2018. The total proteins and lipids determination in aged eggs of three females has shown that there are no significant effects of aging on lipids and protein content in eggs post-ovulation.

3.2. Variability of fatty acids in eggs during post-ovulatory aging

No significant difference was detected in fatty acids composition in eggs during aging. But, some fatty acids declined with the post-ovulatory aging. C16:0, C20:5 (EPA) and C22:6 (DHA) have varied from 30.65%, 1.68% and 17.31% in eggs stripped at ovulation, to 29.22%, 1.33 % and 15.83%, respectively in eggs stripped after 90 minutes after ovulation (Table 17). On the other hand, the levels of C18:1 in eggs increased with post-ovulation, its level was 19.98% in eggs stripped at ovulation and reached 25.11% in eggs stripped 90 minutes after ovulation. The correlation test has

revealed no relationship between aging and fatty acids except C17:1. The correlation was negative and significant between aging and C17:1 (r=-0.453; p=0.037) whose concentration depleted with post-ovulation from 1.39% at ovulation to 1.07% 90 minutes after ovulation. Moreover, Pearson's test showed the existence of a correlation between C20:0 and aging (r=-0.488) but this correlation was barely non-significant (p=0.065).

Table 17 *Changes of proximate composition of fatty acids in eggs stripped at different times of post-ovulation.*

Fatty acids	At ovulation	60 minutes post-ovulation	90 minutes post-ovulation
C14:0	1.475	1.466	1.245
C16:0	30.657	29.292	29.221
C16:1	5.986	5.901	5.329
C17:0	1.924	1.501	1.864
C17:1	1.396[a]	1.304[a,b]	1.077[b]
C18:0	12.496	11.792	12.727
C18:1	19.984	23.764	25.119
C18:2	3.506	3.467	2.878
C18:3	1.954	1.935	1.553
C20:0	0.009	0.031	0.006
C20:1	1.617	1.501	1.811
C20:5 EPA	1.686	1.601	1.333
C22:6 DHA	17.311	16.444	15.836

The means followed by the same letters are statistically not different for the means corresponding to C17:1. For the other fatty acids. no statistical difference was detected.

Additionally, during aging, significant correlation was found between some fatty acids in retained eggs. High and significant correlation was found between C14:0 and C18:0 (r=-0.755, p=0.001), C18:1 (r=-0.863, p=0.000), C18:2 (r=0.844, p=0.000), C18:3 (r=0.916, p=0.000), C20:1 (r=-0.738, p=0.002), C20:5 (r=0.775, p=0.001). The correlation was also high and significant between C17:1 and C18:1 (r=-0.586, p=0.022) and C20:5 (r=0.682 p=0.005). On the other hand, DHA correlates with C18:0 (r=-0.681, p=0.005) and C18:1 (r=-0.658, p=0.008).

4. Discussion

During this study it was proved, using the one-way ANOVA, that total lipids and proteins concentration in silver carp eggs were not impacted by the post-ovulatory aging. It has been shown that the concentration of the cited components didn't vary significantly from ovulation to 90 minutes of post-ovulation. The values were 2.21% for proteins and 3.76% for lipids at ovulation and 2.05 % for proteins and 4.07% for lipids 90 minutes' post-ovulation. Same results were found for the

majority of fatty acids except C17:1 which its concentration decreased with post-ovulation. Consequently, C17:1 could be used as a predictive marker of post-ovulatory aging in silver carp. Our findings are in accordance with experiment performed by Clarkson **(2018)** who has found that there are no significant changes in lipids and fatty acids during the post ovulatory aging of Atlantic salmon eggs. On the other hand, our results have a point of similarity with those reported by Lahnsteiner **(2000)**. This author showed that in aged eggs of rainbow trout there was a decrease in esterified fatty acids during over-ripening. In our study, this decrease of fatty acids was only obtained for C17:1 levels in aged eggs of silver carp. However, other authors have found significant changes in lipid classes related to the post-ovulatory aging of rainbow trout eggs **(Craik and Harvey, 1984).**

Moreover, proteins levels in eggs of silver carp didn't show any significant difference during post-ovulatory aging even if there is a decrease. However, in Atlantic salmon it was found that proteins content was significantly reduced at 10 days of post-ovulation compared to 5 days of post-ovulation **(Clarkson, 2018)**. Nevertheless, eggs of Caspian brown trout showed a significant decrease in proteins levels during the post-ovulatory aging **(Bahre Kazemi et al., 2010)** as well as those of herring **(Schreck and Moyle, 1990)**.

5. Conclusion

During the aging process of silver carp ova, their lipids and proteins contents did not vary significantly. Furthermore, the assessment of the variation of fatty acids profile revealed that C17:1 level decreased during aging. As a results this component could be considered as a biomarker for retained eggs in silver carp. However, more research should be done on the molecular and cellular approach to determine precisely the mechanism of aging in silver carp ova and to decode the biochemical pathways that are related to this phenomenon and eggs viability.

CHAPTER VI: EFFECTS OF STRESS ON SILVER CARP EGGS

1. Introduction

Many factors are thought to affect the quality and survival of eggs, including nutrition, genetics, stress, health status, water temperature and time after ovulation **(Billard, 1979; Lam, 1983; Izquiero and al., 2001; Schreck and al., 2001; Abidin and al., 2006; Guerriero and Ciarcia, 2006)**. Describing the effect of these factors and their biochemical pathway leads to the determination of quality markers in fish eggs that can be used to enhance fish production in aquaculture. So this knowledge will help to increase the fry production of numerous species which have commercial value. Stress is one of the factors that have a significant and direct effect on fish's reproductive cycle and potential. This stress results in the activation of a cascade of biochemical reactions which can alter the good progress of the vitellogenesis, the quality and quantity of the ova (fertilization success, fecundity, egg size, hatching success) and the survival and the growth of the fry **(Clearwater and Pankhurst, 1997; Contreras-Sanchez and al., 1998; Coward and al., 1998; Schreck and al., 2001; Okumura and al., 2002; king and al., 2003; Berg and al., 2004)**.

Cortisol is an important component for metabolic functions in fish **(Mommsen and al., 1999)**. Along with other nutrients and hormones, cortisol is stocked in the egg and used for the embryos development in fish **(Hwang and al., 1992; Tanaka and al., 1995)**. During vitellogenesis, cortisol is taken up by the oocyte from material liver **(Brooks and al., 1997; Tylor and al., 1991).** Besides, this component is mainly released in response to stress which affects the overall metabolic system, including the immune response system, and may increase the susceptibility of the fish to diseases **(Harris and Bird., 2000; Davis and al., 2002)**. Moreover, it has direct effect on the reproductive performances of fish such as decrease in body size, gonado-somatic index, egg size and gamete quality **(Foo and Lam, 1993; Kimeand Nash, 1999)**. In many studies it was showed that the cortisol in eggs is transferred from female during oogenesis **(Leatherland and al., 2010; Eriksen and al., 2006)**. Stressors experienced by the mother can have varying effects on the levels of cortisol in eggs depending on severity and longevity of stressor, and when in the reproductive process the stressor is experienced **(Schreck and al., 2001; Stratholt and al., 1997)**. Berg and al**. (2004)** showed that in Arctic charr *(Salvelinus alpinus*) cortisol reduces the vitellogenin proteins which are used as nutritional source for the embryos. For Masu salmon (*Oncorhynchus masou*), Mingist and al. **(2007)** investigated about the relationship between cortisol levels in serum and the percentage of eyed eggs. They found that higher cortisol levels may have caused the lowering of the eyed-egg

percentage. Moreover, high levels of cortisol in Atlantic salmon (*Salmo salar*) eggs led to reduced embryo size and yolk sac use **(Eriksen and al., 2006).**

Estrogens are responsible for growth and maturation of oocytes in teleost **(Peter and Yu, 1997)**. Together with testosterone and progesterone they regulate final oocyte maturation and ovulation **(Peter and Yu, 1997; Kime, 1993)**. Further, 17β-estradiol is the most potent estrogen that is involved for the vitellogenin production **(Berg and al., 2004)**. For instance, Zebrafish (*Brachydanio rerio*) females were exposed to a level higher than 1.67 ng/l of 17-ethinylestradiol to induce vitellogenin production, reduced the egg number produced per female, the fertilization success and the growth rate of fish **(Segner and al., 2003)**. On the other hand, a study performed on female grayling showed that females exposed to 1.0 ng/l of 17β-estradiol, during the prespawning time, speeds up ovulation and all females ovulated in a shorter time span than in the control group **(Lahnsteiner and al., 2006)**. The same study showed that a female of rainbow trout exposed to 0.5 ng/l of 17β- estradiol the egg quality was poor and variable throughout the experiment. Significant amounts of testosterone, 17β- estradiol (E2), cortisol, thyroxine and triiodothyronine are consistently found in vitellogenic eggs prior to oocyte maturation, as well as in freshly ovulated eggs and fertilized mature eggs of teleost **(Tagawa and al., 1990; Deane and Woo, 2003; Raine and Leatherland, 2003).**

Additionally, stress is also related to enzymatic activity. Many studies investigated about the evaluation of stress on cells, especially human cells. These studies were based on the determination of enzymatic biomarkers such as Glutamic-oxaloacetic transaminase (GOT) – also known as aspartate aminotransferase (AST), glutamic-pyruvic transaminase (GPT)-synonym alanine aminotransferase (ALT) and γ-glutamyltransferase (GGT) **(Ahluwalia, 2001; Rao and al., 1989; Ryder and Beckingham, 2001; Stone and Harrison, 2001)**. These enzymes are largely used as diagnostic tool in human medicine to detect cellular damages **(Adolph and Lorenz, 1978; Moss and al., 1986)**. GOT is an enzyme found in the mitochondrion and cytoplasm of all cells. Its increase levels in the cell reflect the extent and severity of cellular damage **(Li and al., 2010)**. Moreover, it catalyses the formation of glutamate and oxaloacetate while GPT catalyses the production of glutamate and pyruvate **(Moss and al., 1986)**. Additionally, the changes of those enzymes in fish serum are related to water pollution effect (**Bucher and Hofer, 1990; El-Khayat and al., 2016)**. The toxicity and the damage caused by certain pollutants were investigated in many species such as bluegill sunfish (*Lepomismacrochirus*)**, (Oikari and Jimenez, 1992**), carp (*Cyprinus carpio*, **Balint and al., 1995)** flounder (*Platichthysflesus*, **Beyer and al., 1997**) rockfish *(Sebastes schlegeli*, **Kim**

and Kang, 2004), major carp (*labeorohita*, **Vutukuru and al., 2007**). As the GPT and GOT, the γ-glutamyltransferase (GGT) is present in plasma membrane of all tissues **(Goldberg, 1980)**. It's metabolises the extracellular glutathione (GSH), which is the main intracellular antioxidant, through a process known as γ-glutamyl cycle **(Meister, 1974; Whitfield, 2001)**. It's widely used to evaluate human health and diagnosis several diseases **(Lee and al., 2004; Meisinger and al., 2005; Lim and al., 2007)**. Although, this antioxidant enzyme is barely used in fish to assess the damages caused by pollution. It was determined in tilapia, *Oreochromis niloticus*, by Elkhayat and al., **(2016)** to evaluate water pollution in Lake Manzala fish.

On the other side, antioxidants help reducing the stress effect. They are important molecules in all living organisms because they protect from the harmful effects of molecule oxidation. This protection is executed through a series of enzymatic and non-enzymatic reactions that together constitute a complex antioxidant defense-system **(Halliwell and Gutteridge, 2007)**. For example, the primary function of some vitamins, mainly vitamin E and vitamin C, is serving as antioxidants **(Packer, 1991; Hernandez and al., 2018)**. Valenzuela and al. **(2002)** revealed that the vitamin C levels decreased when, rainbow trout, *Oncorhynchus mykiss*, is subjected to stress. It was proved that diet containing sufficient vitamin C amount increases the growth and survival, as well as reduces the skeletal malformation and enhances the stress response in fish **(Darias and al., 2011)**. On the other hand, vitamin E (fat-soluble antioxidant) is considered as a powerful antioxidant that protects highly relevant biomolecules in aquatic organisms such as HUFAs which are essential nutrients in early-stage development and growth for all marine organisms **(Gao and al., 2014)**. Additionally, **Liebler (1993)** revealed that one tocopherol molecule can protect up to 1000 lipid molecules in the membrane. Moreover, high levels of HUFAs and insufficient vitamin E content leads to yolk sac hypertrophy and decreased survival **(Fernández-Palacios and al., 1998; Baker and Davies, 1996)**.

This study aims to investigate about stress levels in unfertilized eggs of silver carp, during the reproductive season and during the post-ovulatory aging phenomenon. This objective is achieved by evaluating the levels of cortisol and 17β-Estradiol. The cell damage was obtained by determining the enzymatic activity (GGT, GPT, GOT). On the other hand, antioxidants capacity was identified by quantifying the fat-soluble and hydro-soluble antioxidants to evaluate their use during the spawning period and also during the aging phenomenon. Furthermore, the study seeks to determine the relationship between these components and the reproductive performance of the fish, especially fertilization success and the survival of the embryos.

2. Material and methods

The determination of the components cited below were performed in Comparative endocrinology laboratory (EC lab), Department of Biology, Federico II University, Naples, Italy.

2.1. Preparation of the samples for steroids and enzymes determination

One gram of eggs, belonging to the females, was weighted and well crushed. 1ml of bi-distillated water was added to the eggs. Then the samples were centrifuged at 3000 *g* for 1 min. the supernatant of each sample was subdivided in two Eppendorf tubes of 0.5 ml each. The supernatant that shows the presences of pellet were crushed again and centrifuged at 3000*g* for 5 min.

2.2. Determination of Cortisol and 17β-estradiol

The determination of cortisol and 17β-estradiol was carried out using a commercial method: AIA-2000 (Tosoh Bioscience) using the prepared samples as described previously. The used samples belong to females used during breeding season (ID F1-6) and females used in aging experiment.

2.3. Determination of the enzymes activity

Enzymes activity was determined in eggs belonging to females F1-9 used during breeding season and females used in aging experiment.

2.3.1. Alanine Aminotransferase (GPT)

Alanine aminotransferase (ALT) or Glutamate pyruvate transaminase (GPT) catalysis the reversible transfer of an amino group from alanine to α-ketoglutarate forming glutamate and pyruvate. The pyruvate produced is reduced to lactate by lactate dehydrogenase (LDH) and NADH. The rate of decrease in concentration of NADH, measured photometrically at wavelength 340 nm, is proportional to the catalytic concentration of ALT present in the sample (**Murray, 1984**).

2.3.2. Aspartate aminotransferase (GOT)

Aspartate aminotransferase (AST) formerly called glutamate oxaloacetate (GOT) catalysis the reversible transfer of an amino group from aspartate to α-ketoglutarate forming glutamate and oxaloacetate. The oxaloacetate produced is reduced to malate dehydrogenase (MDH) and NADH. The rate decrease in concentration of NADH, measured photometrically at wavelength 340 nm, is proportional to the catalytic concentration of AST present in the sample (**Murray, 1984**).

2.3.3. Gamma-Glutamyl transferase (GGT)

Gamma-Glutamyl transferase (γ-GT) catalysis the transfer of γ-glutamyl group from γ-glutamyl-p-nitroanilide to acceptor glycylglycine. The rate of 2-nitro-5-aminobenzoic acid formation, measured photometrically at wavelength 405 nm, is proportional to the catalytic concentration of γ-GT present in the sample **(Gendler, 1984; Persjin and al, 1976).**

2.4. Antioxidants

Antioxidants levels were determined in eggs stripped from females whose ID are F1-10 during breeding season and females used in aging experiment. The analysis of antioxidants was performed according to the method of Prieto and al. **(1999)**. For each situation, 500 mg of eggs were weighted for hydro-soluble antioxidants. A volume of distillated water (1 ml/g) was added, and the suspension was homogenized and shaken for 1 h at room temperature in the dark. The samples were centrifuged at 10000 *g* for 15 min. The pellets were suspended in another volume of solvent and centrifuged once more. The two supernatants were combined and kept at 4°C. For fat-soluble antioxidants we used acetone instead of water.

For determining antioxidants, we used a spectrophotometric method. Antioxidants in the samples were complexed by ABTS solution at 0.7 M. For each sample we read the antioxidants concentration by UV-Vis spectrophotometer (LLG-uniSPEC 2) at 732 nm.

2.5. Statistical analysis

Data were statistically analyzed by SPSS Statistics software version 23 (IBM Corporation, Somers, NY, USA). One-way analysis of variance (ANOVA 1) was used to study the variability of cortisol, 17β-estradiol, enzymes (GOT, GGT, GPT) and antioxidants in eggs throughout the breeding season and during aging. Pearson's test and linear regression were used to determine the relationship between the studied components and fertilization rate, embryos survival rate.

3. Results

3.1. Stress during the reproductive season of silver carp

3.1.1. Cortisol and 17β-estradiol

During the operations of the artificial breeding of silver carp, the overall amount of cortisol was higher in ova stripped in April while in May this amount was lower. On the other side, 17β-estradiol levels were extremely higher in ova stripped in May than those obtained in April (Table 18).

In April the stripped ova from Female F3 had the highest amount of cortisol (8.65 U/I) and the lowest amount of 17β-estradiol (100 U/I) (Table 18). Also, it was observed that for the same female (F3) the FR and SRE were the highest with a rate of 82.47% and 63.48%, respectively (Table 18). The lowest amount of cortisol was obtained in ova stripped from female F1 (5.26 U/I) which had the highest amount of 17β-estradiol (1358 U/I). for the F1 the FR was 47.80% While the SRE was the lowest with an average of 12.50%. For female F2, 17β-estradiol was also higher (1096U/I) and the FR was the lowest within the three manipulated females with an average of 26.92%.

In May, Cortisol levels in stripped ova were lower than those obtained in April. The highest amount of cortisol was found in ova originated from female F4 (4.95U/I) whose FR and SRE were higher (70.39% for FR and 54.19% for SRE). Although, Contrary to what we obtained in April for F3, ova of female F4 had the highest 17β-estradiol amount (4940 U/I) and, surprisingly, ova from female F5 the 17β-estradiol level was out of range. The same batch of ova, from F5, had the lowest FR (34.66%) and SRE (40.37%). For female F6, ova had the lowest amount of cortisol (3U/I) and 17β-estradiol (535U/I) but those lowest amounts didn't impact significantly the FR and SRE.

On the other hand, Pearson's test revealed the existence of negative correlation between female's weight (r=-0.61, p=0.076) and length (r=-0.728, p=0.026) and cortisol levels in eggs. Also, it was found that 17β-estradiol correlates negatively with female's weight (r=-0.668, p=0.049) and length (r=-0.634, p=0.067). The one-way ANOVA didn't prove any variation of 17β-estradiol and cortisol levels in the ova according to the spawning period. In addition, linear regression revealed that 14.7% of fertilization success and 11.7% of survival of the embryos variability, during the reproductive season, could be explained by cortisol changes. Nevertheless, 17β-estradiol can explain only 3.7% and 1.8% of the variability of fertilization success and survival of the embryos, respectively.

Table 18 *Proximate composition of cortisol and 17β-estradiol in eggs stripped during May and April of the same reproductive season. The data present also FR: fertilization rate, SRE: Survival of the embryos, length of females and also their weight*

	Female ID	**Cortisol (U/l)**	**17β-estradiol (U/l)**	**Weight (Kg)**	**Lenght (cm)**	**FR (%)**	**SRE (%)**
April	F1	5.26	1358	2.7	55.5	47.80	12.50
	F2	6.98	1096	3.7	62.7	26.92	34.77
	F3	8.65	100	3.7	57	82.47	63.48
May	F4	4.95	4940	3.3	55.5	70.39	54.19
	F5	3.98	out range	3.6	61	34.66	40.37
	F6	3	535	3.3	52	49.27	41.30

3.1.2. Enzymatic activity

No specific trend was observed in GOT and GGT variation in eggs stripped during the reproductive season. In April, highest amounts of GOT and GPT were recorded in ova from female F5; with an average of 622.7 (U/I) and 62.7 (U/I), respectively. For GGT, the level in ova of female F5 was low (4.7 IU/L). Moreover, the lowest amount of GOT was recorded in ova of F1 (275.8U/I) followed by F2 (314.7U/I). This last female had the highest amount of GGT (7.04UI/L) and the lowest amount of GPT (10.5U/I) (Table 19).

In May, the highest levels of GOT and GPT were recorded in ova of female F9 which had lowest amount of GGT. The levels of GOT, GPT and GGT were 630.5 (U/I), 45.3 (U/I) and 0.82 (IU/L), respectively. The lowest levels of GOT and GPT were obtained in ova from female F8 with an average of 222.4 (U/I) and 15.2 (U/I), respectively. Same female, F8, had the highest level of GGT (6.46 IU/L) (Table 19).

Pearson's test revealed that no significant correlation was found between the studied enzymes and the fertilization success and the survival of the embryos. No correlation was found between GGT and GOT and the breeding period. However, GPT correlates significantly with the period ($r=0.774$, $p=0.024$). Also, GPT in eggs correlates with the 17β-estradiol levels ($r=0.767$, $p=0.026$). Moreover, no correlation was observed between female's weight and length and the enzymes amount in stripped eggs.

Table 19 *Proximate composition of GOT, GGT, GPT in silver carp eggs stripped during April and May during the reproductive season*

	Female ID	**Weight (kg)**	**GOT (U/I)**	**GPT (U/I)**	**GGT (IU/L)**
April	F1	3.7	275.8	13.8	3.29
	F2	3.7	314.7	10.5	7.04
	F3	2.7	420	15.4	5.52
	F4	2.9	346.2	18.2	5.75
	F5	3.26	622.7	62.7	4.7
May	F6	3.3	243	46	2.93
	F7	3.3	592.7	40.4	5.92
	F8	3.2	222.4	15.2	6.46
	F9	3.98	630.5	45.3	0.82

On the other hand, the overall levels of GPT and GOT were higher in eggs stripped in May than April (Figure 25). The values varied from 395.88 to 422.15 (U/l) for GOT and from 24.12 to 3.72 (U/l) for GPT. GGT levels were higher in April than May (Figure 25). It depleted from 5.26 for

April to 4.03 (IU/l) for May. The one-way ANOVA revealed that there are no changes of GOT and GGT levels in eggs between April and May. However, GPT showed a significant difference between April and May ($p<0.05$).

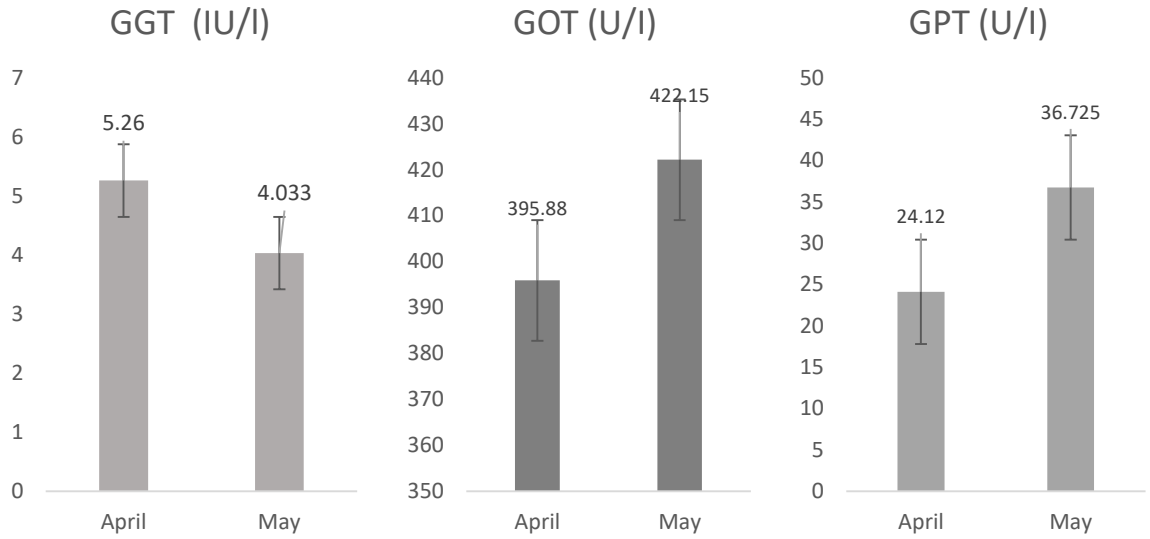


Figure 25. *Variation of GOT, GPT and GGT between April and May of the same reproductive season.*

3.1.3. Antioxidants activity

The investigations about the amount of antioxidants in stripped ova revealed that those obtained in May had highest levels of antioxidants than those obtained in April (Table 20). The ova stripped in April recorded high amounts of HSA and low amounts FSA originated from female F4. The values were 1.48 (Teac) for HAS and 1.50 (Teac) for FSA. On the other side, the lowest value of HSA was recorded in ova of female F3 (0.39 Teac) and the lowest level of FSA was recorded in ova of female F4 (0.80 Teac). In May, high levels of HSA were obtained in ova from female F9 (1.52 Teac) while highest amount of FSA was recorded in ova originated from female F6 (1.48 Teac). Moreover, the lowest amounts of HSA were recorded in female F8 (1.33 Teac) and the lowest amount of FSA was recorded in female F9 (0.91 Teac).

No Specific trend was observed on the overall antioxidants according to females' weight. Pearson's test showed that there is no significant correlation between antioxidants amount in the egg and its fertilization success, the survival of embryos and maternal weight and length. However, the test showed that there is a high and significant correlation between fat-soluble and hydro-soluble antioxidants ($r=0.981$, $p=0.00$).

Table 20 *Antioxidants' proximate compositionin silver carp eggs through the same reproductive season (2018). HSA= hydro-soluble antioxidants, FSA= fat-soluble antioxidants*

Period	Female ID	Lenght (cm)	Weight (kg)	HSA (Teac)	FSA (Teac)	FR (%)	SRE (%)
April	F1	60	4.3	1.48	1.50	60.15	57.40
	F2	55.5	2.7	1.31	1.32	47.88	12.5
	F3	40	2.4	0.94	0.94	35.1	78.06
	F4	52	3.1	1.47	0.80	11.75	12.81
	F5	45.4	1.7	1.37	1.13	26.37	36.51
May	F6	55.5	3.3	1.41	1.48	70.39	54.19
	F7	61	3.6	1.42	1.43	34.66	40.37
	F8	48	2.5	1.33	1.40	49.03	42.20
	F9	51	2.6	1.52	0.91	60.24	71.36
	F10	57	3.1	1.39	1.38	25.37	13.67

The overall levels of hydro-soluble and fat-soluble antioxidants were higher in eggs stripped in May while they were lower in eggs stripped in April (Figure 26). The hydro-soluble antioxidants varied between 1.30 for April and 1.41 for May. The fat-soluble antioxidants varied between 1.14 for April and 1.32 for May. However, the one-way ANOVA showed that there is no significant change of antioxidants concentration between April and May.

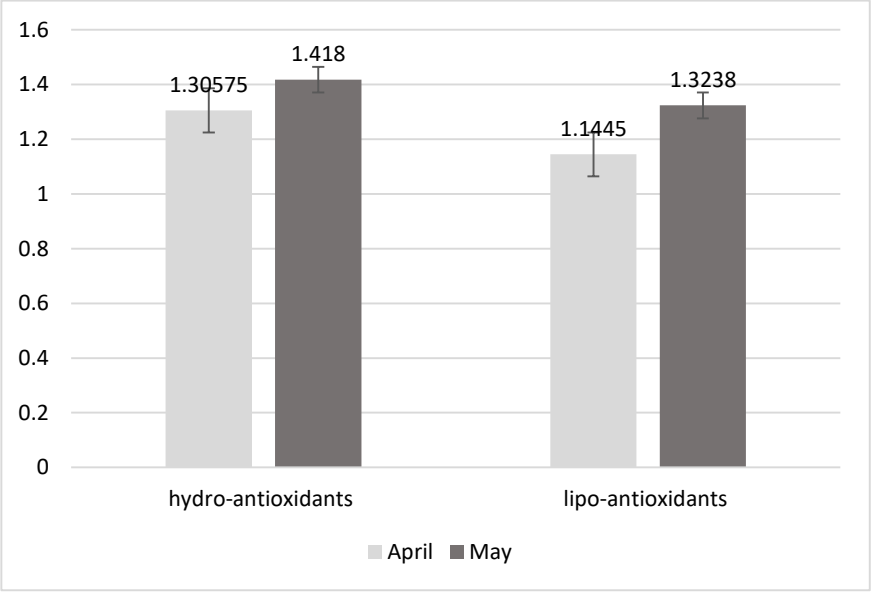


Figure 26 *Variation of antioxidants between April and May of the same reproductive season*

3.2. Stress during post-ovulatory aging in silver carp

Due to the aging process, cortisol amount in stripped ova decreased from 11.16 (U/I) at ovulation to 9.15 (U/I) in ova remained in ovarian lumen for 60 minutes after ovulation moment (Table 21). Then the amount suddenly increased in ova stripped after 90 minutes of pot-ovulation to reach 11.5 (U/I). On the other hand, 17β-estradiol was higher in ova stripped at ovulation (2652.33 U/l) then its concentration decreased to reach 667.33 (U/I) then 644 (U/I) in ova stripped after 60 minutes and 90 minutes of post-ovulation, respectively (Table 21). The linear regression revealed that variability of cortisol during the aging phenomenon could explain only 28.9% of the variability in the fertilization success and only 5% for the variability of the embryos survival. Moreover, Pearson's test revealed the existence of high and significant correlation between Cortisol levels in the ova and the GOT ($r=0.758$, $p=0.018$).

Additionally, 17β-estradiol didn't show any significant correlation with other chemical components assessed during the aging experiment. However, Pearson's test revealed the existence of positive correlation between 17β-estradiol and GPT but it wasn't statistically significant ($r=0.599$, $p=0.089$). The linear regression showed that 17β-estradiol levels in aged eggs could explain only 4.5% of the variability of fertilization success and didn't explain the variability of the survival of embryos ($r^2= 0.001$).

Concerning enzymatic activity, GOT decreased from 423.03(U/I) at ovulation to 400.56 (U/I) 60 minutes' post-ovulation . Then it increased to reach 413.066 after 90 minutes of post-ovulation (Table 21). Linear regression showed that GOT could explain 10.5% of variability of the fertilization success and 33% of the variability of embryos survival. However, GPT decreased in ova during the post-ovulatory aging. It depleted from 32.2 (U/I) at ovulation to 27.86 (U/I) 60 minutes' post-ovulation then it reached 24.99 (U/I) 90 minutes' post-ovulation. Statistically, the variation of GPT levels in ova could explain only 8.9% of embryos survival but it didn't explain the fertilization success variability. The GGT amount in aged ova depleted from 4.26 (IU/L) at ovulation to 3.95 (IU/L) in ova stripped after 60 minutes of post-ovulation. Then the level of GGT increased in ova retained for 90 minutes to reach an overall average of 4.15 (IU/L) (Table 21). For the GGT, it can explain only 3.7% of the variability of fertilization success and 10% of embryos survival variability.

Hydro-soluble antioxidants decreased from 0.86 (Teac) in ovulation to 0.82 (Teac) in ova stripped at 60 minutes after ovulation. Then, it increased to 1.39 (Teac) in ova stripped at 90 minutes after ovulation. Same variation was observed for fat-soluble antioxidants which the value decreased from 1.07 (Teac) at ovulation to 0.74 (Teac) after 60 minutes of post-ovulation then it increased to reach

1.24 (Teac) after 90 minutes of post-ovulation (Table 21). Pearson's test showed that there is significant correlation between hydro-soluble antioxidants and the time of ova retention in body cavity of females (r=0.71, p=0.032) while no significant correlation was found between aging and fat-soluble antioxidants (r=0.151, p=0.698), GOT (r=-0.052, p=0.895), GPT (r=-0.234, p=0.544) and GGT (r=-0.025, p=0.950). The one-way ANOVA showed that aging has only effect on the levels of hydro-soluble antioxidants.

Table 21 *Overall proximate composition of antioxidants, GOT, GPT and GGT in eggs stripped from three females used in post-ovulatory aging experiment.*

Post-ovulation time (minutes)	**0**	**60**	**90**
Hydro-soluble antioxidants (Teac)	0.86	0.82	1.39
Fat-soluble antioxidants (Teac)	1.07	0.743	1.24
GOT (U/I)	423.03	400.56	413.06
GGT (IU/L)	4.26	3.95	4.15
GPT (U/I)	32.2	27.86	24.96
Cortisol	11.16	9.15	11.5
17β-estradiol	2652.33	667.33	644

4. Discussion

4.1. Stress during the reproductive season of silver carp

Cortisol levels in fish plasma reflect the stress response toward several factors that the individuals faced during their evolutionary history **(Foo and Lam, 1993; Kime and Nash, 1999)**. Further, in numerous experiments it was proved that cortisol, along with other hormones are transferred from the mother to the eggs at spawning **(Ayson and Lam, 1993; Brown and Kim, 1995; kausar and al., 2013; Nesan and Vijayan, 2013; Andersson and al., 2011)**. These facts pushed the curiosity of many researchers to investigate about the relationship between cortisol (maternal and egg) and the reproductive success, the embryonic and larval growth, behavior and survival **(Ayson and Lam, 1993; Brooks and al., 1995; Leatherland, 1999; McCormick; 1998, 1999; Suter, 2002; Bruce, 2002; Auperin and Geslin, 2008)**. In the performed study, based on the one-way ANOVA test, no changes in ova's cortisol was detected through the same reproductive season. This could be due to the fact that silver carp is not a multiple spawner which, probably, explains the absence of the allocation of the maternal transferred content to the ova. Further in salmonids, Suter **(2002)** showed that steroid content of eggs from a single female might arise during the spawning period but no specific trend of this variation was detected. The high levels of cortisol content in silver carp ova could be due to the fact that this corticosteroid increases during final stages of ovarian development

as it was revealed in many salmonids **(Leatherland, 1999; Pickering and Christie, 1981)**. However, our study revealed that there is a difference in the overall amount of cortisol between ova stripped in April and those stripped in May. The amount of cortisol in ova obtained in April was higher than those obtained in May. This difference highlights the effect of temperature changes during the reproductive season on the reproductive cycle of the females. This could be explained by the fact that in April younger females still are not acquainted and used to the stress which affects the cortisol levels in the plasma which is transferred to the ova.

Moreover, our study demonstrated that egg's cortisol levels are unrelated to the fertilization success and the survival of the embryos of silver carp. These findings confirm the results of the experiment performed on the same specie by Kausar and al. **(2013)** and in other fish species such as coho salmon **(Sopinka and al., 2015)**, white sturgeon **(Simontacchi and al., 2009)**, and zebrafish **(Nesan and Vijayan, 2012)**. Leatherland **(1999)** reviewed that for salmonids, there is no correlation between egg cortisol content and embryonic development or mortality. Furthermore, Brooks and al. **(1995)** and Stratholt and al. **(1997)** reported that the absence of the impact of high cortisol levels in the egg on rainbow trout or Coho salmon survival could be explained by the ability of the egg to clear the maternal cortisol. This conclusion was proved by Li and al. **(2012, 2014)** who demonstrated that rainbow trout's ovarian follicles metabolize cortisol to cortisone, which is the biologically inactive Glucocorticoid in fish **(Bury and Strum, 2007)**. Similarly, Tagawa and al. **(2000)** detected metabolism of cortisol to cortisone in the thecal/granulosa layer of tilapia (*Oreochromis mossambicus*) follicles. In general, the elevation of circulating glucocorticoids in response to an environmental stressor is considered to be adaptive, initiating physiological and behavioral changes that function to promote survival **(Wingfield and al., 1998; Sapolsky and al., 2000; McEwen and Wingfield, 2003)**. In our case of study, further work is required to determine if there is a long term effect of the high levels of cortisol, detected in ova, on the growth, survival and behavior of silver carp larva. Also, testing the hypothesis that silver carp egg clears the cortisol is required. Further, our findings highlight the existence of the intraspecific effect on the variation of cortisol and 17β-estradiol amount among eggs of different females within the same specie. This was also reported in early studies which demonstrated that the concentration of cortisol tends to vary between eggs of different females **(Pottinger and Moran, 1993; Stratholt and al., 1997; Pottinger and Carrick, 1999; Leatherland, 1999; Suter, 2002)**.

17β-estradiol is the main estrogen that controls the vitellogenin production in the oocyte, the egg yolk precursor **(Norris, 1996)**. The effect of this hormone on the reproductive performances was

studied in many fish species **(Ortiz Tirado and al.,2017; Paolucci and al., 2020)**. However, the variation of 17β-estradiol in fish eggs is rarely studied. There were investigations about the hormonal treatment for sex differentiation in commercial fish species **(Piferrer, 2001; Carvalho and al., 2014)** and the relationship between this estrogen and the stress which causes disruptions in endocrine cascade of fish **(Jobling and Tyler, 2003; Park and al., 2010)**. The present study revealed that there is no specific trend of the 17β-estradiol during the reproductive season of silver carp. Lahensteiner and al. **(2006)** demonstrated that no significant difference was observed in fertilization rate and fecundity between fish receiving 17β-estradiol treatment and the control. Moreover, the interaction between cortisol and 17β-estradiol and its effect on the reproductive success was highlighted by Lethimonier and al. **(2000)** and Berg and al. **(2004)**. It was demonstrated that Cortisol interferes with the 17β-estradiol and decreases the vitellogenin production **(Berg and al., 2004)**. In this study we found that there is no correlation between cortisol and 17β-estradiol in stripped ova. However, the levels of 17β-estradiol in ova is related to fertilization success and embryos survival. Except Female F4, we noticed that eggs with low fertilization rate and low embryos survival have high concentration of 17β-estradiol in ova. So, the variation of this estrogen is related to other factors which need to be studied. Moreover, we can hypothesize that the effect of the high levels of cortisol and 17β-estradiol, in the stripped ova, could be related to the changes of other chemical components in the eggs which may alter egg, embryo and larval development. This hypothesis needs to be studied as well.

Determining the enzymes levels in the cell provides insights into cellular damages. Further, this method is largely used in human medicine and still limited in fish. The determinations of GPT and GOT levels in fish plasma was, often, used to indicate intoxications and water pollution, bacterial, viral and parasitic infections **(Van der Oost and al., 2003; Barcellos and al., 2007)**. However, there is no data related to the relationship between the activity of the studied enzymes (GOT, GPT and GGT) and egg viability of fish. The studies related to the determination of the enzymatic activity were mainly interested in the evaluation of the effect of environmental pollution on fish growth **(Bucher and Hofer, 1990; El-Khayat and al., 2016)**, especially the exposure to a xenobiotic **(Kim and Kang, 2004; Vutukuru and al., 2007)**. For instance, Kim and Kang **(2004)** reported that the exposure of the rockfish to Cu (>50 mg/kg) induced a reduction of its growth rate. Also, they highlighted that Serum GOT and GPT activities in rockfish were increased at relatively low doses (50 mg/kg). So, our study could be considered as a first step to determine the levels of the enzymes (GOT, GPT and GGT) in unfertilized eggs of silver carp and investigate about their relationship with eggs quality. As a result, fertilization success and the survival of the embryos are not affected

by the high levels of the studied enzymes. Moreover, the levels of GGT, antioxidant enzyme, decreased while GOT and GPT increased, which means that GGT is consumed by the cell when a stress is occurred. This stress is manifested by the increasing levels of GOT and GPT in ova. So, based on the obtained results and the fact that GGT have a protective action against oxidative stress across a number of cell types, tissues, or organs **(Whitfield, 2001)**, we can hypothesize that thanks to GGT action, the silver carp ova still protected from stress that the mother is subjected to during its reproductive cycle. More studies should be performed in this approach to well understand this protective action on silver carp eggs and its relationship with the reproductive performances of this fish specie. On the other hand, a significant difference in these enzymes levels was observed between ova stripped in April and those stripped in May. This provides insights on the fact that at the beginning of the reproductive season of silver carp, females seem to be more stressful. This stress could be caused by temperature changes, manipulation and female conditions.

As mentioned before, antioxidants have an important role for reducing and preventing the damages caused by stress. In the present study, Hydro-soluble antioxidants and Fat-soluble antioxidants are higher in May. While in April, the obtained ova contained lower levels of these antioxidants. This provides insights on the extensive use of the antioxidants during April while the high levels of antioxidants in ova stripped in May reflect their reduced use by the ova or the female. On the other hand, the study revealed that there is no relationship between the levels of the antioxidants and the maternal traits (age, weight and length). These findings could be explained by the large availability of feed in ponds during the rearing of the broodfish.

On the other hand, the invariability of eggs viability (fertilization rate, survival of the embryos, see chapter I) during the reproductive season of silver carp could be caused by the availability of the essential antioxidants in the egg. Emata and al. **(2000)** showed that supplementing fish diet by vitamin C improves eggs viability and increases the hatching rates, and larval survival. similarly, other studies had proved that supplementing broodstock diet with optimal amounts of vitamin C (hydro-soluble antioxidant) improves reproductive performances of fish, such as eggs weight, diameter and hatching rate **(Furuita and al., 2009; Sarmento and al., 2018)**. What is more, the larvae from females, of Nile tilapia, receiving high levels of vitamin C showed resistance to stress caused by increased salinity **(Sarmento and al., 2018)** since vitamins can be transferred to the eggs, and thus absorbed by the larvae **(Ortuño and al., 2003)**.

On the other side, vitamin E helps PUFAs to maintain membrane fluidity by protecting them against peroxidation **(Atkinson and al., 2010)**. Meanwhile, it was proved that high levels of vitamin E

reduce the percentage of abnormal eggs and increase overall fecundity in fish **(Izquierdo and al., 2001)**. The use of these vitamins as antioxidants is largely used in aquaculture to enhance gametes quality, embryos survival and larval growth and survival **(Mollah and Sarowar, 2009; Miller and al., 2012)**. In our case of study, the determination of antioxidants in ova showed that the levels of antioxidants have no effect on the reproductive success of females which could lead to hypothesis that the low fertilization and survival of embryos rates can be related to other factors such as the quality of male gametes (spermatozoa) and conditions in which eggs were incubated and embryos were developed. Moreover, Vitamin E requirements is also provided by vitamin C through the regeneration of vitamin E from the vitamin E radical **(Tappel, 1962; Olsen and al., 1999)**. Based on this fact, we can hypothesis that this balance between the two antioxidants (vitamin C and vitamin E) maintains the ova quality of silver carp during the stress to ensure the normal development of the embryos and growth of larvae. Further, we can hypothesis that antioxidants levels in the ova are maintained at high level to protect the eggs from stress damage and afford the required amount for normal development of the embryos. Especially for embryos since they do not have an active enzymatic antioxidant defense mechanism **(Zengin and al., 2015; Arslan and al., 2016).** So, the presence of sufficient antioxidant defenses in their lipid rich yolk reserves, deposited by females during vitellogenesis, is necessary **(Erdogan and Arslan, 2019)**. Also, the present study revealed that stress, evaluated by the measurement of the antioxidants capacity, have not a significant effect on the unfertilized eggs of silver carp. But more investigations should be carried out to determine the damage and the repair of the ova during stress as Metcalfe and Monaghan **(2013)** in their review. On the other hand, the effect of stress during reproduction could be significant in other tissues in the fish **(Selman and al., 2012)** which, also, opens up new perspectives and aspect to investigate about the stress effect during silver carp reproduction.

4.2. Effects of stress during post-ovulatory aging on silver carp ova

In the current study we investigated the effect of aging, as one of the main stress source in captive fish, on eggs quality of silver carp based on the hormonal, enzymatic and antioxidants activity. ANOVA I test revealed the absence of the effect of aging on the variation of cortisol and 17β-estradiol levels in ova. The obtained data revealed that there is no specific trend in variation of cortisol in aged eggs while the overall amount of 17β-estradiol decreased through aging process. However, the significant correlation between Cortisol levels in the ova and the GOT ($r=0.758$, $p=0.018$) revealed that the cellular damage increased in eggs during aging. However, the present study proved the existence of difference between the females' response to the stress caused by the

aging process. As Metcalfe and Monaghan **(2013)** reported in their review that when studies are performed under laboratory conditions, the number of offspring produced over a fixed time can show huge inter-individual variation which reflects the phenotypic differences between parents. Based on this, we can hypothesize that the observed difference in stress response between females could be due to the specific conditions under which the experiment was carried out. Or, this difference could be a result of maternal investment during reproduction which is explained by an energy trade-off between reproduction and lifespan **(Monaghan and al., 2009)**. Concerning the relationship between egg's viability and cortisol and 17β-estradiol levels, the study showed that 17β-estradiol levels in aged eggs could explain only 4.5% of the variability of fertilization success and didn't explain the variability of the survival of embryos (r^2= 0.001).

Numerous studies revealed that aging causes an increase in levels of endogenous reactive oxygen species (ROS) and decreases in antioxidant defenses which lead to oxidative damage in cell structures, including lipid peroxidation of membranes, enzyme inactivation, protein oxidation **(Dean and al., 1993; Orrenius and al., 2007)**. The present study revealed that enzymes activity in ova (GOT, GPT and GGT) have not a direct and significant relationship with aging process. However, the levels of these enzymes showed a correlation with fertilization success and the survival of the embryos. It was found that GOT could explain 10.5% of variability of the fertilization success and 33% of the variability of embryos survival. The GPT levels in ova could explain only 8.9% of embryos survival but it didn't explain the variability of the fertilization success. Further, GGT levels in aged eggs can explain only 3.7% of the variability of fertilization success and 10% of the variability of the survival of the embryos.

Moreover, the antioxidants measurement in aged ova revealed that there is a significant effect of aging on the variation of hydro-soluble antioxidants in ova. During stress, the production of reactive oxygen species (ROS) increases. These products are neutralized by antioxidant processes **(Finkel and Holbrook, 2000; Fletcher and al., 2012)**. Further, the study showed that they were lower in eggs stripped at ovulation and at 60 minutes of post-ovulation which reflect their high consumption by the ova to preserve their viability during aging which represent a high source of stress. However, the ova stripped after 90 minutes' post ovulation contained a high amount of antioxidants which reveal the reduction of the use of antioxidants and the start of ova apoptosis. The decrease of fat-soluble antioxidants in ova stripped at 60.

minutes' post-ovulation (Table 21) could be caused by the lack of their regeneration process by hydro-soluble antioxidants, we specify here the process of regeneration of the vitamin E by the Vitamin C **(Olsen and al., 1999)**.

5. Conclusion

In the present study it was demonstrated that cortisol, 17β-estradiol, enzymes (GGT, GOT, GPT) and antioxidants are not impacted by the temporal factor during the same season. Further, reproductive performances, especially fertilization success and embryos survival, are unrelated to the levels of cortisol, enzymes (GGT, GOT, GPT) and antioxidants in ova. Nevertheless, the evaluation of 17β-estradiol concentration in ova revealed that low fertilization rate and low embryos survival belong to those containing high amounts of this hormone.

Additionally, a difference between eggs stripped in April and those stripped in May was detected. This highlights the existence of other factors that impact the amounts of the studied components in ova and their effect on the reproductive success such as temperature changes, stress and females' conditions. During the reproductive season, our study demonstrated that GGT levels, antioxidant enzyme, decreased while GOT and GPT increased, which means that GGT is consumed by the cell when a stress is occurred. This stress is manifested by the increasing levels of GOT. Meanwhile, antioxidants were lower in ova stripped in April while in May their levels were higher. These findings provide insights on the extensive use of the antioxidants during April while the high levels of antioxidants in ova stripped in May reflect their reduced use by the ova or the female. Based on these data we can conclude that stress is more important at the beginning of the reproductive season (April).

On the other side, we have demonstrated that enzymes activity in ova (GOT, GPT and GGT), cortisol and 17β-estradiol have not a direct and significant relationship with aging process. However, the enzymes (GOT, GPT and GGT) levels in aged ova showed a correlation with fertilization success and the survival of the embryos. Further, the antioxidants measurement in aged ova revealed the existence of a significant effect of aging on the variation of hydro-soluble antioxidants. It demonstrated that hydro-soluble antioxidants increased during aging. Their amounts were lower in eggs stripped at ovulation and at 60 minutes of post-ovulation which reflect their high consumption by the ova to preserve their viability during aging which represent a high source of stress. However, the ova stripped after 90 minutes' post ovulation contained a high amount of antioxidants which reveal the reduction of the use of antioxidants and the start of ova apoptosis

GENERAL CONCLUSION

The carpiculture industry is one of the important sectors in Moroccan inland aquaculture due to its ecologic and socio-economic importance. The production of silver carp and Chinese carps in general is highly demanded in Morocco and for this reason the present study was performed to investigate the effect of the aging phenomenon on the egg quality and therefore on the hatcheries production of the seeds of this species.

The study revealed that no temporal effect was detected on the fertilization success and the embryos survival within the same reproductive season. However, it revealed that fry survival could vary within periods of the same reproductive seasons but more studies should be carried out to reveal the interaction between environmental factors and the survival of the fry. On the other hand, the study demonstrated the existence of the interannual variability of the fertilization success, the survival of the embryos and the survival of fry. These results highlight the sensibility of silver carp fish to the environmental changes in term of temperature and photoperiod variation during the vitellogenesis process. This sensibility was explained by the over-ripening of eggs and the delayed ovulation observed in many induced females during the experiments.

In order to determine the most efficient approach to enhance silver carp seed production, the evaluation of fecundity during the breeding season was the main criteria. The results showed that fecundity didn’t vary during the same reproductive season but it changes within the different seasons. Also, the investigations showed that there is no relationship between the fecundity and maternal traits (age, weight and size). However, relative fecundity (number of ova/kg of female) is directly related to the female weight.

Proteins, lipids and fatty acids are the major organic components used by fish for ova production, embryos and larval development. In our study, the determination of these components in eggs, stripped during the same reproductive season, showed that these constituents do not vary during the reproductive season. This can be explained by the abundant food availability in rearing ponds of silver carp breeders. Also, it can be explained by the fact that this species pawns once during the reproductive season which do not lead the females to adopt an energetic strategy as multiple spawners do. The high reproductive performances (fertilization success and the survival of embryos and fry) of silver carp can be explained by the nature of its feeding habits based on the phytoplankton, which is highly rich with n-3 fatty acids. These category of fatty acids are essential for fertilization success and hatching.

Cortisol and17β-estradiol are important component that have a direct effect on fish reproductive performances. Moreover, these two hormones are considered as stress biomarkers. The assessment of their levels in silver carp eggs proved that they are not affected by the temporal factor. However, changes in cortisol levels in the egg could explain 14.7% of the variability of fertilization success and 11.7% of the variability of the survival of the embryos. Moreover, the difference in stress biomarkers, especially cortisol and 17β-estradiol, could be due to females' conditions and environmental factors.

Additionally, antioxidants and enzymatic activity are stress dependent. The evaluation of antioxidants levels in silver carp eggs during the same reproductive season showed that they were highly consumed, by the ova, in April than May. Nevertheless, the statistical study showed that this difference is not significant. Concerning the fertilization success and the embryos survival, we have found that they are not related to the antioxidants levels in the eggs. Same results were obtained for the variation of GOT, GPT and GGT activity in the stripped ova throughout the reproductive season.

In addition, that it's a main source of stress; aging has a significant impact on eggs. The performed study proved that aging has a direct effect on the egg viability. It was demonstrated that fertilization rate, survival of embryos decreases significantly with the increasing ova retention time in the female ovaries. As an outcome, the aging phenomenon may cause a highly significant reduction in fry production of silver carp. Based on the obtained results, the safe retention time during which eggs can preserve their optimal viability was set to 30 minutes of post-ovulation. For this reason, it is recommended to dress a planning of the females' hormonal injection in such a way that ovulation will not occur simultaneously for all manipulated females. The biochemical approach revealed that the main energetic components, total lipids and proteins, do not vary during the ova retention. However, the determination of the fatty acids profile of the aged eggs detected a decrease of C17:1 level with the advancement of aging time.

To evaluate the degree of stress caused by aging, we evaluated the variation of the antioxidants, cortisol, 17β-estradiol, GPT, GOT and GGT levels in eggs stripped during the aging experiment. The study revealed that no variability was detected in cortisol and 17β-estradiol levels in eggs during aging phenomenon. This could be due to the fact that females were anesthetized to avoid their stress before manipulation which limited these steroids variation in the eggs. On the other side, the hydro-soluble antioxidants increased during aging, which revealed their low consumption by cells and the onset of apoptosis.

Overall, the results presented in this thesis contribute to increase the knowledge of silver carp reproduction and factors impacting its eggs quality. These findings will promote the breeding

practices in hatcheries and enhance silver carp seed production. They lead to better understand the impact and the interaction between many factors (environmental conditions, prediction of egg quality, maternal traits, time management) in order to have optimal fry production.

Recommendations and perspectives for seed production of silver carp:

- ✓ The best period to perform artificial breeding of silver carp with optimal reproductive performances is in the middle of the reproductive season.
- ✓ Ova should be stripped within 30 minutes after ovulation in order to reduce embryos mortality and the fail of the breeding operation.
- ✓ The determination of the survival rates in different embryonic stages (Morula, Blastula, Gastrula, etc..) as well as the biochemical components are required to determine the exact biomarker that have a direct effect on embryos mortality.
- ✓ A comparison between eggs of wild and reared broodstocks could be useful to determine in which case we have eggs of high viability.
- ✓ The effect of feed quality and quantity should be investigated to find out the most appropriate feeding techniques to be adopted in the brood stock rearing ponds which allow to get the highest reproductive performances.
- ✓ The effect of water quality parameters the brood stock rearing ponds should be investigated. A monitoring of these parameters should be carried out over the whole growing season in different rearing ponds managed in different manners.

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