Jitendra Kumar Sundaray
Mohd Ashraf Rather
Sujit Kumar
Deepak Agarwal *Editors*

Recent updates in molecular Endocrinology and Reproductive Physiology of Fish

An Imperative step in Aquaculture

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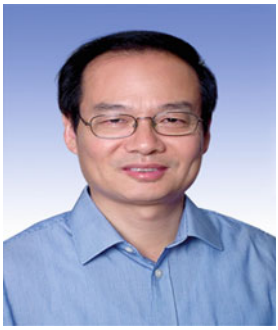
Dedicated to
Prof. Hiralal Chaudhuri (1921–2014)

Kolliyil Hameed Alkunhi (1918–2010)

Foreword



I took my first trip to India in December 2005 to attend the meeting of Frontiers in Molecular Endocrinology held in Hyderabad. It was in that meeting that I met Dr Jitendra Kumar Sundaray, a young and devoted scientist with strong passion for fish reproductive endocrinology and aquaculture. After 15 years, I am very glad to see that Jitendra has developed a successful career and leadership in the field of aquaculture, which, to some extent, reflects the fast development of Indian aquaculture.

Aquaculture has witnessed significant growth around the globe during the last decade. India is the second largest fish producer in the world with 13.7 million metric tonnes (MMT) of fish production, registering an average annual growth rate of more than 7% in recent years and stands at about 5% of total exports from India, and about 20% of agricultural exports (2018–2019). Aquaculture productivity gains can be achieved through optimal use of resources and quality inputs. The factor cost for production could be rationalized through effective use of better technologies, management process and reaping the economies of scale through an optimal scale of production. Current aquaculture practices are limited to very few species and require urgent species diversification to harness the existing potential. Quality fish seed is the prime requirement for aquaculture and it is the major area of concern for species diversification. Understanding reproductive physiology of fishes is essential for developing breeding and seed production technology. High-end technologies are now available which may facilitate more comprehensive exploration of reproductive

physiology and molecular endocrinology. Omics technology is an umbrella term for modern technologies like genomics, transcriptomics, proteomics and metabolomics. These techniques offer immense potential to unravel novel mechanisms underlying the complex neuro-endocrine regulation of breeding and reproductive behaviour. This book presents an extensive collection of articles on different key aspects associated with reproductive biology and endocrinology of fish such as sex determination and differentiation, gonadal development and maturation, vitellogenesis, steroidogenesis, etc. The book has all recent compilation and it will offer immense benefits to readers of all categories, viz. students and faculties for understanding the underlying mechanisms of reproductive physiology and their potential application in aquaculture.

I congratulate the team of book editors and all contributors for putting together such exhaustive compilation of excellent chapters on "Recent Updates in Molecular Endocrinology and Reproductive Physiology of Fish". I would like to encourage all readers of this book, especially those who may be stimulated by its insights and contents, to join the editors in this fascinating, challenging and rewarding endeavour.

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31 July 2020

Foreword

Aquaculture sector is considered as the fastest growing food producing sector and can meet the protein needs of an increasing world population. The current fish production data of FAO clearly shows the increasing trend of aquaculture growth over the years and it constitutes around 46% (82.1 million tonnes) of the total fish production in 2018. India is the second largest producer of fish in the world. The prospects for eradicating malnutrition and fulfilling the Sustainable Development Goal of UN partly lie with our efforts to increase aquaculture production. To achieve this, we must adopt species diversification and developing seed production technology is a prime requirement. Understanding of reproductive physiology through exploring the neuro-endocrine system by means of different tools such as transcriptomics, proteomics, bioinformatics, etc., would help in understanding key mechanisms in reproductive physiology. This new information can be used in refining the hatchery technology and will usher in technological innovations. This book brings together an informative compilation on different aspects of reproductive physiology and molecular endocrinology. It will help the readers to explore opportunities in reproductive physiology to realize blue revolution.

I congratulate the team of editors and all the authors for bringing out a comprehensive compilation in the form of this book entitled "Recent Updates in Molecular Endocrinology and Reproductive Physiology of Fish". I compliment the entire team

of young researchers for their contributions. The book will be of great use to all fishery students, faculty members and researchers to enhance their knowledge in reproductive physiology and molecular endocrinology of fishes.

ICAR-CMFRI, Cochin, India A. G. Ponniah
ICAR-CIBA, Chennai, India
ICAR-NBFGR, Lucknow, India
WorldFish Centre, Bayan Lepas, Malaysia
30 July 2020

Foreword

This book is a compilation of topics on different aspects of reproductive physiology and molecular endocrinology of commercially important and aquaculture-promising fish species with a focus on species diversification to maximize efficient utilization of available diverse water resources. The knowledge of reproductive physiology of fish is essential in developing breeding strategy for use in commercial aquaculture. Reproduction is a highly coordinated physiological process controlled by the interplay of environmental–nervous–endocrine activities. Molecular biology methods and techniques have revolutionized biological research in all fields including reproduction biology and there is inevitable need for regular updating of the vast data and reviewing the progress. With this in mind, topics were selected to address key aspects of reproductive endocrine and neuro-endocrine systems at the cellular and molecular levels. There are twenty-one chapters under six categories. The subject areas covered are gonadal biology and reproductive cycle, regulation of gonadotropins, gonadotropin inhibitory hormone, melatonin, steroid hormones and receptors, oestrogen control of reproduction, endocrine control of vitellogenesis, ghrelin and reproduction, sex determination, role of small RNA in reproduction, gene regulation of catfish reproduction, modulation of Hypothalamus–Pituitary–Gonadal Axis by phytochemicals, role of dietary supplements on reproduction, bioinformatics tools and their importance in reproduction. A chapter is devoted to zebrafish as a model species for reproductive biology and toxicology. Topics also cover anthropogenic activities and their impacts on fish reproduction, hormonally

active environmental agents and their effects on oogenesis and fertility, and endocrine disrupting chemicals of emerging concerns. Lastly, the book deals with climate change and its impact on reproduction.

The chapters are contributed by experts who have established themselves in their fields of specialization. This book offers an attractive compilation of highly relevant topics in current and future aquaculture curriculum and research. In view of the growing awareness of aquaculture across the globe, the book will be a useful guide and reference text for scientists and students working on commercial fish culture, animal biology, fish genetics, comparative endocrinology and so on in aquaculture and academic institutions. The contents would be relevant to policymakers working towards blue revolution and blue economy. The publication of the book is very timely and relevant to boost the sustainable growth and development goals in aquaculture research. The editors who have vast experience and expertise in aquaculture research have taken the bold initiative and responsibility to compile the book and get it published by an international leading publishing house. I congratulate them for this timely academic venture.

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31 July 2020

Preface

The entire globe is looking for sustainable source of nutritional security and aquaculture is standing firmly with the expectations. Today's food production industry scenario clearly indicates the dominancy of fish and fishery products in terms of growth rate and future potential of providing nutritional security. According to "The State of World Fisheries and Aquaculture", total fish production in 2018 crossed the mark of 178 million tonnes, of which around 46% was contributed by aquaculture. Major portion of total fish production, i.e. around 88%, was used for direct human consumption (SOFIA 2018). India has witnessed tremendous growth in aquaculture during the last ten years and also geared up to move up through adopting advance aquaculture technologies along with species diversification to utilize the diverse potential water resources. The biggest obstacle in species diversification is unavailability of breeding and hatchery technology. Standard hatchery technology is a primer for aquaculture of any species. Understanding the reproductive behaviour and its underline regulation by means of exploring the complex neuro-endocrine system along with external factors control are the prime requirement for development of hatchery technology. Such knowledge of reproductive physiology of fish will help in development of breeding strategy for use in commercial aquaculture.

This book presents an updated extensive collection of different key aspects associated with reproductive endocrine system of fish such as endocrine regulation of key events associated with reproduction such as vitellogenesis, sex hormones and gonad developments, sex determination, etc. Reproductive endocrinology with respect to gene regulation and small RNA is also incorporated to provide detailed insight on how omics regulates the endocrine system. How external factors such as dietary supplements, climate, contaminants, etc., impact the reproductive system is a burning issue in today's generation. This book has also covered the reproductive biology of some key species.

In the past few decades, scientist around the world had tried to explore the reproductive system of several species through molecular endocrinology study. It has helped them to develop the breeding and hatchery technology. The availability of different advanced tools such as transcriptomics, proteomics, bioinformatics, etc., has helped extensively in such type of exploration. Many potential indigenous species are available within India, which require such research to boost hatchery production and reduce pressure on natural system.

This book (*Recent Updates in Molecular Endocrinology and Reproductive Physiology of Fish*) is in current shape due to contribution made by several researchers working at different level with reputed institute across India and having research experience on reproductive endocrinology. This book will provide invaluable support to the scientists, teachers, researchers, students, etc., who are interested in the field of reproductive physiology and molecular endocrinology of fish.

Bhubaneswar, India — Jitendra Kumar Sundaray
Kashmir, Jammu and Kashmir, India — Mohd Ashraf Rather
Gandhinagar, Gujarat, India — Sujit Kumar
Chennai, India — Deepak Agarwal

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First and foremost, we are grateful to the Almighty for establishing us to complete this innovative book. We would like to express our heartfelt gratitude and respect to all contributors who have contributed to this book in the form of their chapters. Indeed, it is our utmost pleasure to acknowledge all the people behind the completion of this book. It was not possible to complete this work without their continuous encouragement and support.

We are very much thankful to Dr Wei GE, Chair Professor, Vice-Rector (Research), University of Macau, Avenida da Universidade, Taipa, Macau, China, Dr A. G. Ponniah, Former Director, ICAR-CIBA, Chennai and ICAR-NBFGR, Lucknow and Former Discipline Leader, World Fish Centre, Malaysia and Dr Keerikkattil Paily Joy, Retired Professor of Zoology, Banaras Hindu University and INSA Senior Scientist and Emeritus Scientist, Department of Biotechnology, Cochin University of Science and Technology for their compliments, praise and blessing to the entire team of young researchers for their contributions in this book.

We are thankful to Director, ICAR-CIFA, Bhubaneswar, Vice-Chancellors of Sher-e-Kashmir University of Agricultural Sciences and Technology—Kashmir, Tamil Nadu Dr. J. Jayalalithaa Fisheries University—Chennai and Kamdhenu University—Gandhinagar for their constant support and encouragement.

We will also take this opportunity to express our sincere gratitude to Springer Nature Publishers, Singapore for publishing this work.

Support of our students, colleagues and family members is highly appreciable.

Finally, we would like to put our head down in front of Almighty, who has given us courage, passion and strength for completing this work.

31 July 2020

Jitendra Kumar Sundaray
Mohd Ashraf Rather
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Jitendra Kumar Sundaray is the Head, Division of Fish Genetics and Biotechnology at ICAR-Central Institute of Freshwater Aquaculture, India. He has completed his undergraduate from Odisha University of Agriculture and Technology, Master in Science from Ravenshaw University and the Post Graduate Diploma in Fisheries Science from ICAR-Central Institute of Fisheries Education, Mumbai. He has completed Ph.D. from Kyushu University, Fukuoka, Japan. His major research interest is captive maturation of fish through hormonal manipulation. He is leading a Center of Excellence project on the genetic improvement of freshwater fish. He is a fellow of Odisha Environmental Society, Zoological Society of India, Applied Zoologist Research Association, India, and Fellow of Indian Society of Coastal Agricultural Research.

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Sujit Kumar is an Assistant Professor at the Department of Fish Biotechnology, Postgraduate Institute of Fisheries Education and Research, Kamdhenu University, Gandhinagar. He graduated from Fisheries College and Research Institute, Thoothukudi, TNJFU, Tamil Nadu and obtained Master's degree from Department

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Abbreviations

AP	Anterior Pituitary
AHA	Anterior Hypothalamic Area
ALT	Alanine Aminotransferase
ANH	Atrial Natriuretic Hormone
AMH	Anti-Mullerian Hormone
AP	Anterior Pituitary
AR	Androgen Receptor
α-AR	Alpha Type Adrenergic Receptor
β-AR	Beta Type Adrenergic Receptor
ARE	Androgen Response Element
ASH	Aldosterone Stimulating Hormone
ASIF	Aldosterone Secretion Inhibition Factor
AVP	Arginine Vasopressin
AVT	Arginine Vasotocin
CAH	Congenital Adrenal Hyperplasia
CAP	cAMP Activated Protein
CBG	Corticosteroid Binding Globulin
CBP	Cortisol Binding Protein
CCK	Cholecystokinin
cGMP	Cyclic Guanosine 3′5′-Monophosphate
CG	Chorionic Gonadotropin
CGRP	Calcitonin Gene Related Peptide
CIF	Cytosolic Inhibitory Factor
CLIP	Corticotropin-Like Intermediate Peptide
cLRF	Chorionic Luteinizing Hormone Release Factor
hCLRF	Human Chorionic Luteinizing Hormone Release Factor
CoA	Coenzyme A
CRE	Cyclic AMP Regulatory Element
CREB	Cyclic AMP Response Element Binding Protein
CRF	Corticotropin Release Factor
CRH	Corticotropin Releasing Hormone
cAMP	Cyclic Adenosine 3′5′-Monophosphate
DDT	Dichlorodiphenyldichloroethane

DM	Diabetes Mellitus
DOCA	11-Deoxycorticosterone Acetate
DOC	11-Deoxycorticosterone
EPO	Erythropoietin
ER	Estrogen Receptor
ERE	Estrogen Response Element
FRF	Follicle Stimulating Hormone Release Factor
FRP	Follicle Stimulating Hormone Releasing Peptide
FSH	Follicle Stimulating Hormone
FSH-BI	Follicle Stimulating Hormone Binding Inhibitor
FSH-RF	Follicle Stimulating Hormone Release Factor
FSH-RH	Follicle Stimulating Hormone Releasing Hormone
GAP	Gonadotropin Releasing Hormone Associated Protein
GH	Growth Hormone
GHBP	Growth Hormone Binding Protein
GHIH	Growth Hormone Inhibiting Hormone
GHRH	Growth Hormone Releasing Hormone
GIS	Gonadotropin Inhibitory Substance
GLP-1, GLP-2	Glucagon Like Peptides
GnRH	Gonadotropin Releasing Hormone
GR	Glucocorticoid Receptor
GRP	Gastrin Releasing Peptide
GAPs	GTPase Activating Proteins
hCS	Human Chorionic Somatomammotropin
hCT	Human Chorionic Thyrotropin
HGF	Hepatocyte Growth Factor
hGH	Human Growth Hormone
HHPS	Hypothalamo-Hypophyseal Portal System
HMG	Human Menopausal Gonadotropin
hTG	Human Type Thyroglobulin
ICSH	Interstitial Cell Stimulating Hormone
IGF	Insulin Like Growth Factor
IGF-BPs	Insulin Like Growth Factor Binding Proteins
IGFRcs	Insulin Like Growth Factor Receptors
IP_3	Inositol Triphosphate
JAK Kinase	Janus Kinase
JH	Juvenile Hormone
JOD	Juvenile Onset Diabetes Mellitus
LATS	Long Acting Thyroid Stimulator
LATS-P	Long Acting Thyroid Stimulator Protector
LH	Luteinizing Hormone
LH-RF/LRF	Luteinizing Hormone Release Factor
LH-RH/LRH	Luteinizing Hormone Releasing Hormone
LTH	Luteotropic Hormone

MAP Kinases	Mitogen Activated Kinases
MBH	Medial Basal Hypothalamic Region
MDI	Mullerian Duct Inhibitor
MEN	Multiple Endocrine Neoplasia
MITS	Monoiodotyrosines
MOD	Maturity Onset Type Diabetes Mellitus
MODY	Maturity Onset Type Diabetes Mellitus in the Young
MRH	Melanocyte Stimulating Hormone Releasing Hormone
MSH	Melanocyte Stimulating Hormone
NE	Norepinephrine
NOD	Non-Insulin Dependent Diabetes Mellitus
OT	Oxytocin
PAG	Pineal Antigonadotropin
PEP	Phosphoenolpyruvate
PHA	Posterior Hypothalamic Area
PKA	Protein Kinase A
PKC	Protein Kinase C
PP	Pancreatic Polypeptide
PRF	Prolactin Release Factor
PSP	Parathyroid Secretory Protein
PTH	Parathyroid Hormone
SCF	Sertoli Cell Factor (Inhibin)
SH Domain	Src Homology Domain
SIADH	Syndrome of Inappropriate ADH Secretion

Sex Hormones and Their Role in Gonad Development and Reproductive Cycle of Fishes

1

Prem Kumar, P. Behera, L. Christina, and M. Kailasam

Abstract

The development of captive broodstock and reproductive maturation is essential for induced breeding. The reproductive cycle of fish is divided into two phases, that is, growth and maturation phase, which is controlled by the cascade of hormones along the brain-pituitary-gonad (BPG) axis. In this axis, gonadotropin-releasing hormone (GnRH) and dopamine secreted from the brain regulate the gonadotropin secretion from the pituitary. The gonadotropins from the pituitary gland are released into the blood which act on the gonad to trigger the production of the sex steroid hormones. Estradiol-17β (E2) under the control of gonadotropin (follicle-stimulating hormone, FSH) regulates the vitellogenesis and oocyte growth. Maturation-inducing steroid (MIS) or maturation-inducing hormone (MIH) under the control of gonadotropin (luteinizing hormone, LH) regulates the maturation and spawning. In synchronous fish, the level of FSH increases during the vitellogenic phase and drops during follicular maturation and spawning, whereas the LH level is low at vitellogenesis and high before ovulation. The first peak of testosterone occurred during vitellogenesis, and the second peak is observed just prior to spawning. The E2 level remains high during late-vitellogenic and vitellogenic stages and low during post-vitellogenic and hydrated stages. In male, FSH increases gradually at the initial stage of spermatogenesis, reaches peak during testicular growth, and then declined after spawning, whereas LH is low at early spermatogenesis, increases during spermiation, and attained peak during the spawning. In male, the first peak of testosterone is noticed during pre-spawning and the second just prior to the spawning. In

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asynchronous fish, two peaks of gonadotropins (FSH and LH) are observed in the plasma of multiple spawner female fish (tilapia) during two consecutive spawning. The first peak is evident 2 to 3 days after spawning (vitellogenic phase), and the second peak is noticed just before the next spawning. The knowledge on hormonal cycle will help in the captive breeding of fishes.

Keywords

Broodstock · Maturation · Hormones · Reproduction

Introduction

Teleost represents more than half of all vertebrate species, which is distributed in freshwater and marine water environment (Nelson 2006). To develop the commercial aquaculture, the domestication of aquatic species is increasing (Duarte et al. 2007). Control breeding to produce quality seed is a prerequisite for the domestication and development of sustainable aquaculture. Similar to the vertebrates, the reproductive cycle of fish is divided into two major stages. The first stage includes proliferation, growth, and differentiation of the gametes (spermatogenesis and vitellogenesis/growth). The second stage includes maturation and release of oocytes and spermatozoa (spermiation and oocyte maturation). Gametogenesis (spermatogenesis and oogenesis) and final maturation (spermiation and oocyte maturation) are regulated by the series of hormones from the brain-pituitary-gonad (BPG) axis. The secretion of gonadotropins (FSH and LH) from the pituitary gland is controlled by the GnRHs of the brain (Peter and Yu 1997; Yu et al. 1997; Rather et al. 2017, 2020). GnRHs are the main neuropeptides regulating reproduction and act as integrators of external information from the environment (temperature, water fall, and social interactions). Dopamine (DA) has an inhibitory effect on the release of the pituitary gonadotrophs (Chang and Jobin 1994). The FSH and LH are released from the pituitary, which act on the gonad and stimulate the synthesis of sex steroids (androgen, estrogen, and progestogen). These steroids ultimately influence the gonadal development.

Ovarian Development

Three types of ovarian development, namely, synchronous, group-synchronous, and asynchronous, are reported in the fish (Wallace and Selman 1981). In the synchronous type, all oocytes develop and ovulate together. In the group-synchronous type, at least two populations of oocytes (vitellogenic and maturing) are seen in the ovary throughout the reproductive season. In the asynchronous type, oocytes of all stages of development are seen. Two essential steps to complete the final maturation of oocytes are growth and maturation. The synchronous type of ovarian

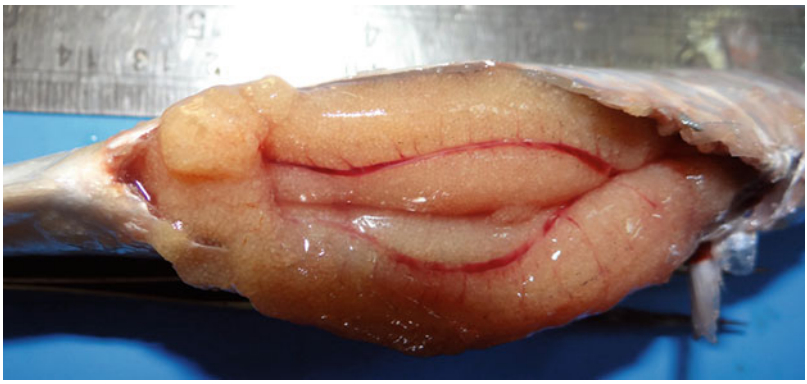

Fig. 1.1 Mature ovary of *Liza parsia*

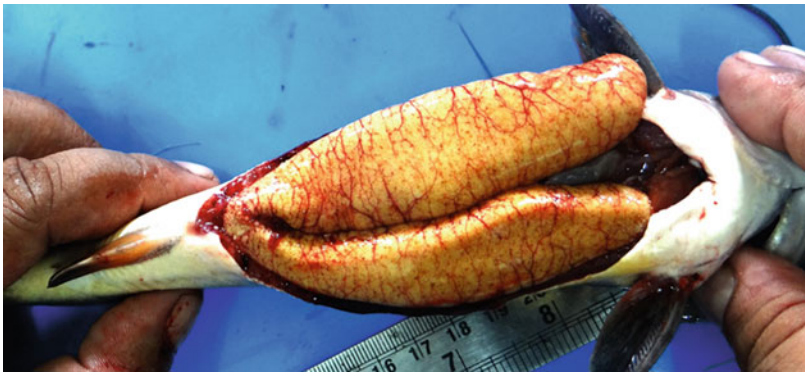

Fig. 1.2 Mature ovary of *Mystus gulio*

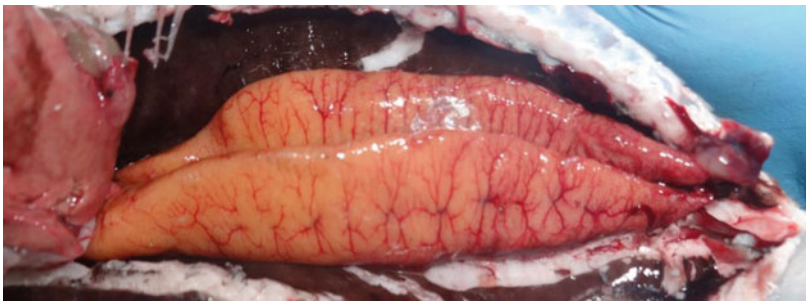

Fig. 1.3 Mature ovary of *Tenualosa ilisha*

development is observed in *Liza parsia* (Fig. 1.1), *Mystus gulio* (Fig. 1.2), and *Tenualosa ilisha* (Fig. 1.3).

Oocyte Growth/Development

Major stages during egg development/growth involved the formation of primordial germ cells (PGCs), oogonia cells, primary oocytes, and secondary oocytes (Patiño and Sullivan 2002). During the early growth phase, oocytes are temporarily arrested for long in meiosis prophase I (diplotene) and uptake various substances (vitellogenin, Vtg) from the bloodstream by receptor-mediated endocytosis (Babin et al. 2007). At this stage, oocyte accumulates maternal RNA, and differentiation between cellular and non-cellular envelopes is completed. FSH regulates vitellogenic growth of follicles through E2, which are biosynthesize in ovarian follicles.

Table 1.1 Tabular representation of oocyte growth and maturation

Oocyte growth and maturation	
Egg development/growth	
Primordial germ cells	Present in the ovary
Oogonia	Produced after mitosis
Primary oocytes	• The first meiosis starts and gets arrested for **longer duration** at prophase I (diplotene) • At this stage, primary oocytes uptake various substances (esp. vitellogenin) from the bloodstream by receptor-mediated endocytosis
Oocyte maturation	
Secondary oocytes	• At the end of the first meiotic division, two cells (2n) of different sizes are produced. The first polar body is degenerated and the secondary oocyte is formed • Extrusion of the first polar body indicates the end of the first meiosis (Yoshikuni and Nagahama 1991) • Maturation processes characterized by slow or end of vitellogenin endocytosis, restart of meiosis, germinal vehicle breakdown (GVBD), appearance of cortical alveoli under the oolemma, yolk platelet dissolution. and hydration of pelagophilic oocytes • The second meiosis starts and proceeds to metaphase II and gets arrested at metaphase II
	• This arrest in meiosis is for a short time (at metaphase II) for *meiotic maturation* • The secondary oocytes (*metaphase II*, *haploid*) are ovulated out from the follicular cell and move to the ovarian lumen or abdominal cavity (depends on species)

Oocyte Maturation

Detail of oocyte growth and maturation is described in Table 1.1. Schematic representation of oocyte growth and maturation is given in Fig. 1.4. After

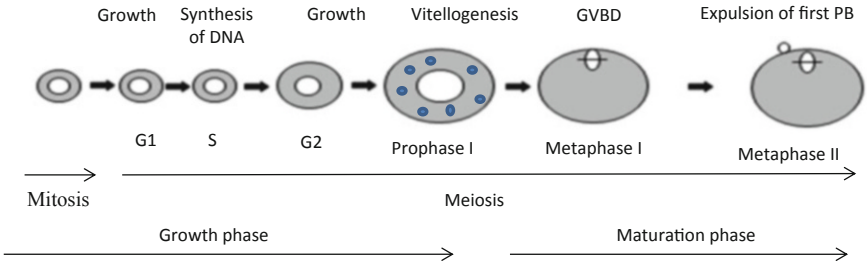

Fig. 1.4 Schematic representation of oocyte growth and maturation (Redrawn from source Lubzens et al. 2010)

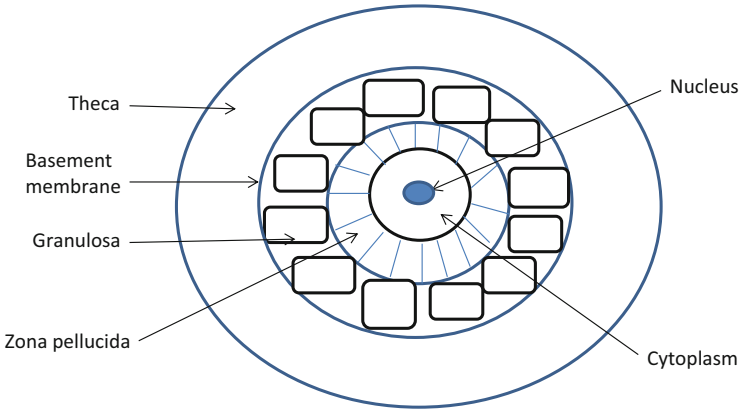

Fig. 1.5 Follicular envelop (theca, granulosa, and zona pellucida) of fish oocyte (Source: Redrawn from Hoar and Nagahama 1978)

vitellogenesis, meiosis is resumed and proceeds till metaphase II. This process is called meiotic maturation or oocyte maturation. The first polar body is extruded after the completion of the first meiosis. In other words, extrusion of the first polar body indicates the end of the first meiotic division (Yoshikuni and Nagahama 1991). LH regulate the final maturation of gametes, through the production of MIH or MIS (Nagahama et al. 1994). Through in vitro and in vivo systems, it is well established that oocyte maturation in fish is regulated by gonadotropin (GTH; LH), MIH, and maturation-promoting factor (MPF). A mature fish oocyte is enveloped with theca, granulosa, and zona pellucida cells (Fig. 1.5). Marine fish eggs are pelagic in seawater (pelagophils). The size of marine fish eggs increased due to water intake during maturation (Cerda et al. 2008).

Accumulation of free ions and free amino acids (result of hydrolysis of yolk protein) in oocyte leads to high osmotic pressure, which trigger the uptake of water through molecular water channels or aquaporins (Fabra et al. 2005; Finn and Kristoffersen 2007; Monsang et al. 2019). The higher the buoyancy, the higher the

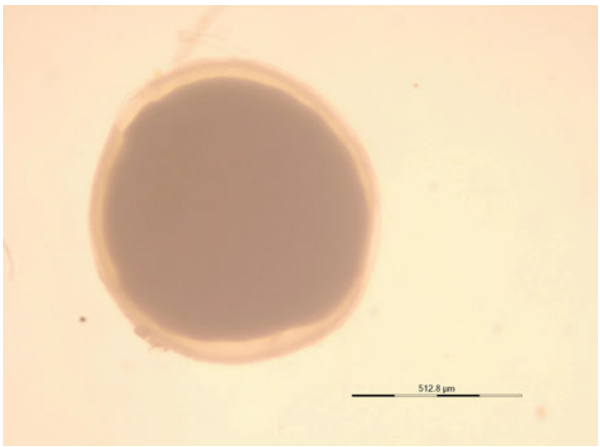

Fig. 1.6 *Mystus gulio* mature oocyte

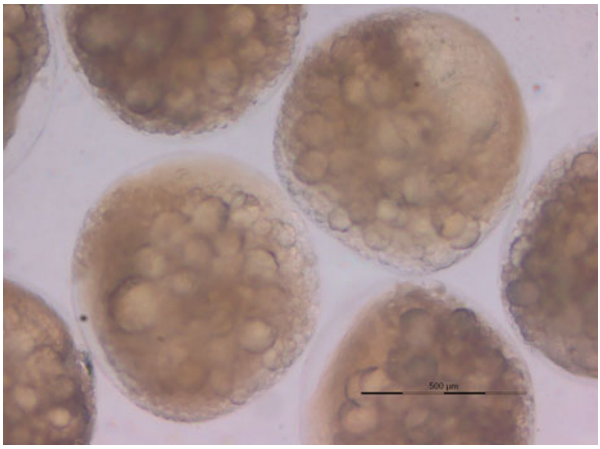

Fig. 1.7 *Tenualosa ilisha* mature oocyte

survival of the embryo; therefore, the egg buoyancy is used as a marker in marine fish embryo (Carnevali et al. 2001). Mature oocyte of *Mystus gulio*, *Tenualosa ilisha*, and *Liza parsia* is shown in Figs. 1.6, 1.7 and 1.8, respectively.

Testicular Development

Spermatogenesis and spermiation are regulated by FSH, LH, sex steroid hormones, and other growth factors. FSH triggers Sertoli cell proliferation and differentiation and the synthesis of insulin-like growth factor I (IGF-I) or activin B, which act as

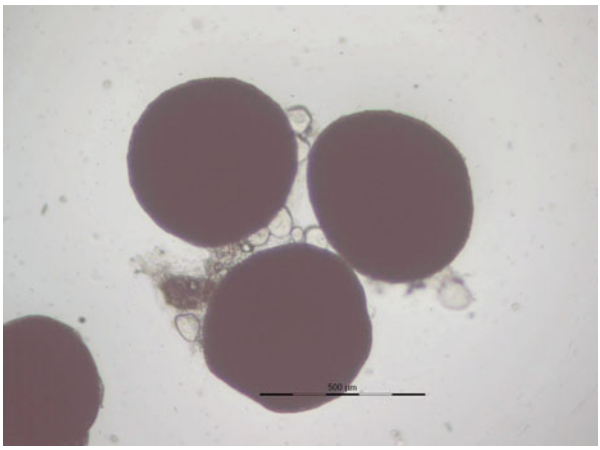

Fig. 1.8 *Liza parsia* mature oocyte

autocrine and paracrine factors (Schulz and Miura 2002). FHS and LH control the synthesis of 11-ketotestosterone (11-KT) and MIS from the Leydig cells of the testes, respectively. 11-KT regulates spermatogenesis, while MIS regulates sperm capacitation and spermiation (Miura and Miura 2003). Spermiation is the process of production of seminal fluid, which is required for the attainment of sperm motility and its movement through the sperm duct. Progestogen induces the sperm motility by increasing the pH of the sperm duct and cAMP levels in the sperm (Miura et al. 1992; Nagahama et al. 1994). Progestogen also induces hydration of milt (spermatozoa plus seminal plasma) (Vermeirssen et al. 2004). Fish (especially oviparous species) spermatozoa are immobile without aqueous environment (Billard et al. 1990). Aqueous medium ionic concentration, pH, and osmolality are vital for the attainment of sperm motility (Alavi and Cosson 2006). Spermatozoa intracellular parameters such as pH, ATP, cAMP, and Ca^{+} concentrations are also important for the mobility of fish spermatozoa (Coward et al. 2002; Alavi and Cosson 2006). Testes of *Mystus gulio*, *Tenualosa ilisha*, and *Liza parsia* are shown in Figs. 1.9, 1.10 and 1.11, respectively.

Brain-Pituitary-Gonad Axis (BPG Axis)

Similar to other vertebrates, pituitary gland activity is regulated by neurohormones (neuropeptides, neurotransmitters), which are synthesized in the hypothalamus of the brain. In all the vertebrates, the pituitary is attached to the hypothalamus by a short stalk, whereas in fish neurosecretory fibers connect the brain and pituitary. These neurosecretory fibers are axon from the neuron of the hypothalamus.

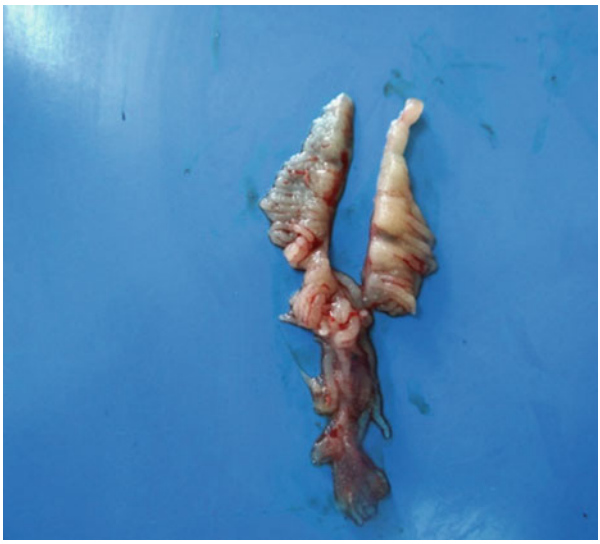

Fig. 1.9 *Mystus gulio* testis

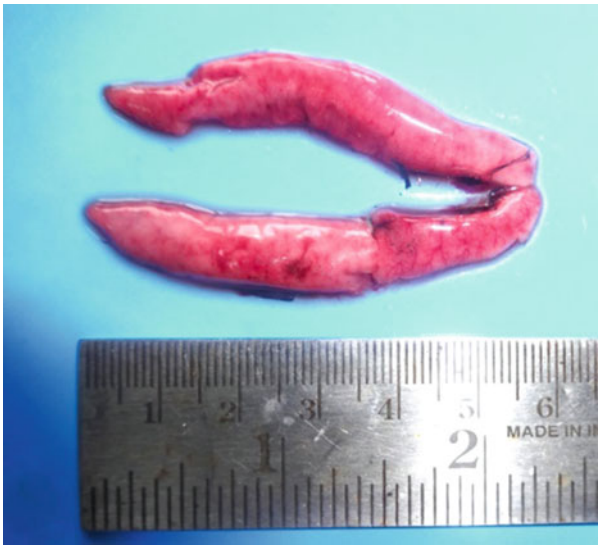

Fig. 1.10 *Tenualosa ilisha* testis

Pituitary Gland

The pituitary gland or hypophysis of teleost is located in the bony cavity, posterior to the optic chiasm (Frisen 1967). The pituitary gland, or hypophysis, comprises the adenohypophysis and the neurohypophysis. Glandular cells of the adenohypophysis (glandular part or non-neural part) secrete pituitary hormones. Neurosecretory fibers from different parts of the brain secrete different peptides in the neurohypophysis of the pituitary gland. In fish, the adenohypophysis has been divided into the pars

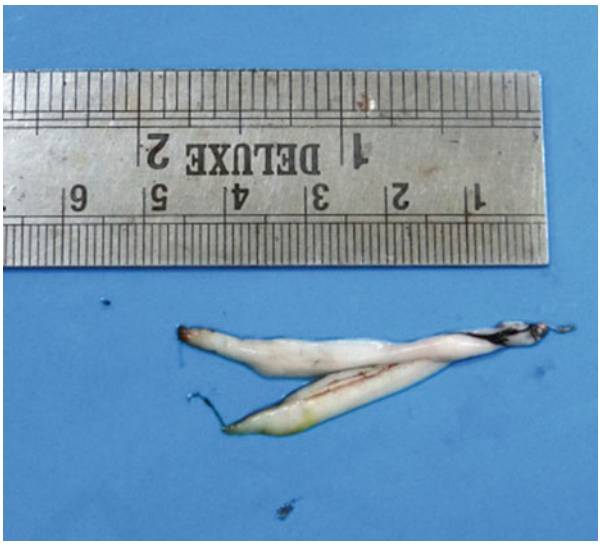

Fig. 1.11 *Liza parsia* testis

distalis (anterior lobe of terrestrial vertebrates) and pars intermedia (intermediate lobe of terrestrial vertebrates). The pars distalis is divided into the rostral and proximal pars distalis. In other words, the adenohypophysis is divided into three main parts: the rostral pars distalis (RPD), proximal pars distalis (PPD), and pars intermedia (PI). The corticotrophs (adenocorticotroph, ACTH), the mammotrophs (prolactin, PRL), and the thyrotrophs (TSH) are cells present in the RPD. The somatotrophs (growth hormone (GH) cells) and gonadotrophs (LH/FSH cells) are present in the PPD. In tetrapods, the hypothalamo-pituitary portal system is present whose primary plexus of capillaries is located in the median eminence. The median eminence is located in the floor of the hypothalamus. The neurohormones secreted from the hypophysiotropic neurons reached the target cells of the anterior lobe of the pituitary gland via blood. The posterior lobe of tetrapod pituitary is comprised of nerve fibers originating from the hypothalamus, which secrete oxytocin and vasopressin. This part of the pituitary is commonly called the neurointermediate lobe. Major pituitary hormones associated with reproduction (LH, FSH) directly regulate the gonadal development in vertebrates. Secondary hormones associated with reproduction (GH or TSH) directly regulate other physiological processes and indirectly influence reproduction.

The anterior lobe (pars distalis) of teleost pituitary differs from terrestrial vertebrates in two aspects: (1) Similar type of cells responsible for the production of a particular hormone remains together in one place of the pituitary gland. In mammals even similar types of cells do not form mass. (2) In fish, the hypothalamo-pituitary portal system is absent, and the pars distalis receives direct innervation from the hypothalamus.

Reproductive Hormones

Gonadotropin-Releasing Hormone

The team of Nobel laureates Guillemin and Schally in 1970 characterized hypothalamic hypophysiotropic deca-peptide and named it luteinizing hormone-releasing hormone (LHRH), which stimulates the secretion of LH from pituitary gonadotrophic cells. Later it is found that LHRH also regulates other gonadotropin, FSH. This changes the name of LHRH to GnRH. Relative observation in different vertebrates showed the existence of different molecular variants of GnRH deca-peptides, with similar function to stimulate the release of pituitary gonadotropin (Millar 2005; Kah et al. 2007). In the early 1970s, porcine and ovine hypothalamus LH-releasing factors are isolated by two research groups simultaneously from pig and sheep, respectively (Matsuo et al. 1971). The primary structure of this deca-peptide is pGlu-His-Trp-Ser-Tyr-Gly-Leu-Arg-Pro-Gly-NH2. It is found that in vertebrate there are two or three forms of GnRH (Kah et al. 2007; Morgan and Millar 2004). At present there are 24 variants of GnRH in vertebrates, protochordates, and invertebrates (Kah et al. 2007; Lethimonier et al. 2004; Morgan and Millar 2004; Okubo and Nagahama 2008). Among vertebrates, teleost has a maximum number of GnRH isoforms. Based on phylogenetic analysis, three forms of GnRH are found in vertebrates. The expression of GnRH-1 is seen in the hypothalamus of amphibians, mammals, and fishes. The expression of GnRH-2 is found in the synencephalon/mesencephalon of all vertebrates from fish to mammals. The expression of GnRH-3 is found mainly in the rostral forebrain of fish (salmon). Genes of GnRH have a common structure with four exons and three introns. The coding region of the GnRH genes is highly conserved, but the intron, upstream, and downstream regions are distinctively divergent (Chow et al. 1998).

Fish Gonadotropin (GtH-I/FSH and GtH-II/LH)

Gonadotropins are heterodimeric glycoproteins formed from two subunits of "a" and "b." Subunit "a" is common in both FSH and LH, which is non-covalently linked to b-subunit. The common a-subunit of both FSH and LH, b-sub unit of FSH, and b-subunit of LH are encoded by a distinct gene. The a-subunit is the most conserved among fish at the amino acid level (Li and Ford 1998). In fish, the a-subunit has two potential sites for N-glycosylation and ten conserved cysteines, which form five intra-molecular disulfide bridges that are similar to mammals. In tetrapods, both LH b-subunit and FSH b-subunit contain 12 conserved cysteines, which are linked by 6 disulfide bridges. This structure is not conserved in fish FSH b-subunit but conserved in LH b-subunit. During vitellogenesis, FSH induces steroidogenesis in two-cell model (outer theca and inner granulosa cells). During vitellogenesis, FSH induces steroidogenesis, the outer theca cell synthesizes T, which is transported to inner granulosa cells and converted to E2 with the aid of enzyme P-450 aromatase (Montserrat et al. 2004). During vitellogenesis, E2 regulate the synthesis of

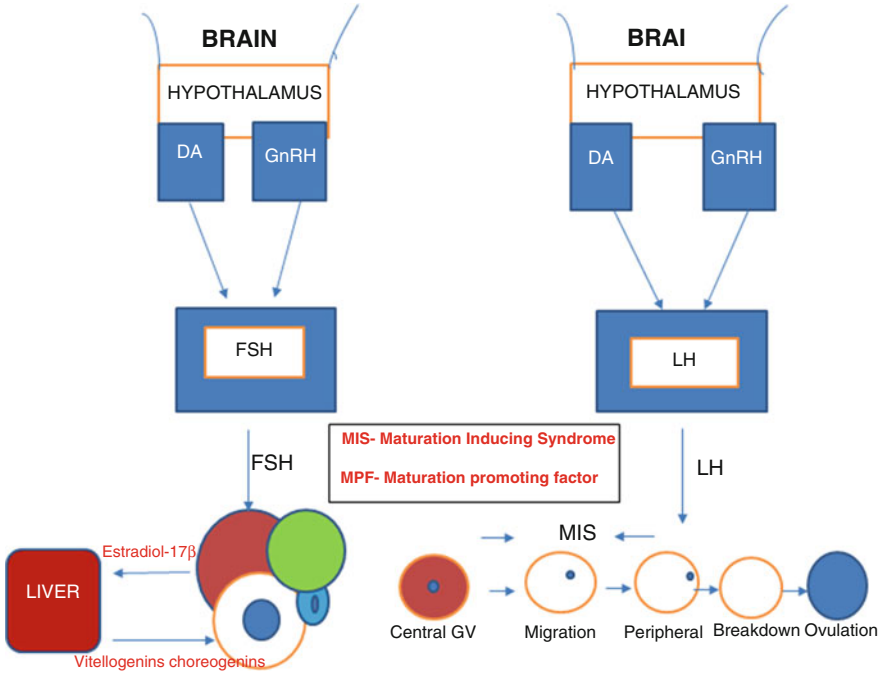

Fig. 1.12 Diagrammatic representation of hormonal control of oocyte development and growth

vitellogenin protein in the liver and oocyte development. The FSH stimulates the incorporation of Vtg into oocyte follicles (Jalabert 2005). At the end of vitellogenesis, LH acts on oocyte follicle and triggers the synthesis and secretion of MIH or MIS that regulate ovulation in female (Nagahama et al. 1994; Suwa and Yamashita 2007). Under the control of LH, the steroidogenic pathway shifts from the synthesis of E2 to dihydroxy-4-pregnen-3-one (DHP) or 20β-DHP in the ovarian follicles of fish (Nagahama and Yamashita 2008). FSH has a well-defined role in vitellogenesis in the synchronous ovarian development of fishes. Fishes with asynchronous ovarian development role of FSH in vitellogenesis are less clear, and the involvement of LH is also possible (Rosenfeld et al. 2007). The source and role of FHS and LH are shown in Fig. 1.12.

Hormonal Changes During the Reproductive Cycle

Gonadotropin is an important hormone, which regulates the gonad maturation; therefore, the estimation of this hormone in the pituitary and blood plasma is essential to investigate the reproductive physiology of fish. In fish, both FHS and LH are secreted differently during breeding/reproductive cycle.

FSH and LH in Female

FSH is released during the entire vitellogenesis, while LH remains low during vitellogenesis and attains peak before ovulation (Davis et al. 1995; Prat et al. 1996). Blood levels of FSH in immature and vitellogenic trout are higher than that of LH (Dickey and Swanson 1998). In trout, FSH increases only when ovulated eggs leave the body cavity (Breton et al. 1998). In female coho salmon, the level of FSH increases during vitellogenesis and declines during follicular maturation and spawning (Kawauchi 1989; Swanson et al. 1991). In the rainbow trout, FSH is seen during the entire vitellogenesis and attains peak during oocyte maturation, along with the rise of LH (Breton et al. 1998; Prat et al. 1996).

FSH and LH in Male

Both gonadotropins (FSH, LH) are equipotent to stimulate the synthesis of the androgens 11-KT and testosterone (T) in males (Planas and Swanson 1995). In males, levels of FSH are high during early spermatogenesis, attain maximum value during testicular growth phase, and decline after spawning. Alternatively, LH is low at early spermatogenesis, increases during spermiation, and attains peaks at the spawning season (Gomez et al. 1999; Mateos et al. 2003; Miwa et al. 1994; Mylonas et al. 1997). In male salmon, plasma FSH remains low in immature males and increases during spermatogenesis and just before spermiation, while LH is very low during early gametogenesis and rises significantly at spermiation. In males, the production of DHP reduces at later stages of spermatogenesis (Planas and Swanson 1995). Increase in LH just before spermiation defines the switch of steroidogenic and androgenic production to progestinic production, respectively, in the interstitial cells of the testis and the theca cells of the ovary (Simona and Ballestrazzi 2005).

Sex Steroids

Reproductive hormones play vital roles in many reproductive physiological processes of vertebrates. In teleost, the most common sex steroid hormones produced in gonadal tissue are E2, 11-KT, and 17α,20β-dihydroxy-4-pregnen-3-one (DHP). These hormones are produced under the control of pituitary gonadotropins, which are important for gametogenesis (Wallace and Browder 1985; Agahama and Yamashita 2008; Miura et al. 1991). Increase in plasma estrogen level during vitellogenesis is common in many teleosts. Steroidogenic pathway in female theca and granulosa cell is shown in Fig. 1.13.

Estradiol

The E2 level remains high during late-vitellogenic and vitellogenic stages and low during post-vitellogenic and hydrated stages in mullet (Kumar et al. 2015). A similar trend of E2 is noticed in other fish species (Matsuyama et al. 1988; Mylonas et al.

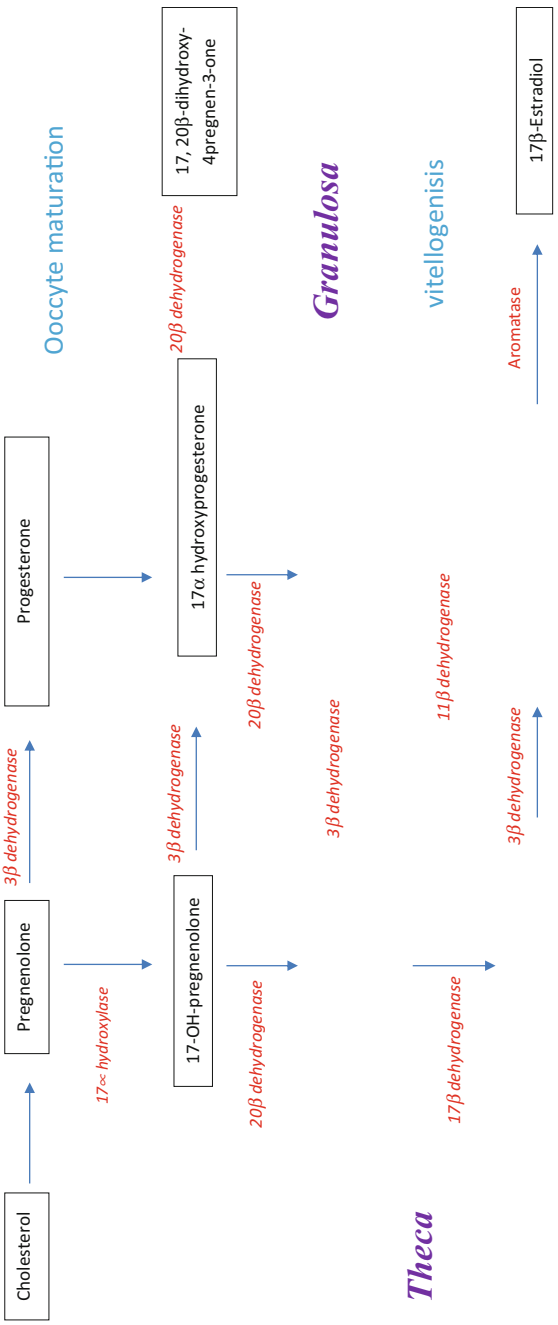

Fig. 1.13 Schematic representation of steroidogenesis in female fish

1998; Dahle and Swenson 2003). A sudden drop in plasma E2 level from vitellogenic to post-vitellogenic stage of maturation indicates the switching of aromatase (CYP19) activity (Das et al. 2014; Kumar et al. 2015).

Estradiols do not play any major role in spermatogenesis, but they stimulate the gonadotroph activity in immature male fish and inhibit the stimulatory effect of 11-KT spermatogenesis (Cavaco et al. 2001).

Testosterone

During annual reproductive cycle of female walleye, two distinct peaks of T are seen in the plasma (Malison et al. 1994). The first peak is noticed during vitellogenesis and at high E2 level. A similar trend of T as E2 is observed in *M. cephalus* (Das et al. 2014; Kumar et al. 2015); medaka, *Oryzias latipes* (Kobayashi et al. 1996); and Persian sturgeon, *Acipenser persicus* (Nazari 2010). The second peak of T is observed just before spawning (Malison et al. 1994). This type of bimodal pattern is observed in blue cod (*Parapercis colias*) (Pankhurst and Conroy 1987) and sea bass (Prat et al. 1990). In female of many teleosts, elevated T is observed during the pre-ovulatory period (Malison et al. 1994; Scott et al. 1980; Van Der Kraak et al. 1992; Fostier and Jalabert 1986; Pankhurst et al. 1986; Barry et al. 1992). This pre-ovulatory rise in T is to increase the pre-spawning GTH surge (Crim and Evans 1979; Kobayashi et al. 1989). A decreasing trend of T prior to spawning is probably to shift in the steroidogenic pathway from C19 to C21 steroid synthesis, which coincides with spawning (Barry et al. 1992).

In male fish, the annual reproductive cycle has two peaks of serum T. Pre-spawning peak of T is to stimulate secondary sexual behaviors, increase pituitary GTH levels, and serve as a precursor of 11-KT. This rise coincided with rapid increases in GSIs (Crim and Evans 1979; Kobayashi et al. 1989). This first rise is also to regulate male germ cell differentiation (Billard et al. 1978). The second rise of T is found just prior to the spawning season, which coincides with the increase in 11-KT level. Kumar et al. (2015) and Zaki et al. (1995) found that the serum T in male *M. cephalus* increased gradually with progress of maturity and reaches its peak during ripe stage. The higher T level during ripe stage is responsible for the release of mature spermatozoa from their cysts (Barry et al. 1990).

Ketotestosterone

In general it is assumed that 11-KT is a male-specific hormone; however, it is also found in female fishes, Pacific salmon (Schmidt and Idler 1962) and rainbow trout (Scott et al. 1980). The direct relation between 11-KT and plasma vitellogenin in Atlantic salmon (*Salmo salar*) indicates that 11-KT would have a role in oocyte development (Idler et al. 1981). The peak level of 11-KT is observed prior to spawning in male teleost, walleye (Malison et al. 1994), Pacific and Atlantic salmon (Schmidt and Idler 1962; Idler et al. 1971), brook trout (*Salvelinus fontinalis*)

(Sangalang and Freeman 1974), rainbow trout (Scott et al. 1980), and common carp (Barry et al. 1990). The level of 11-KT is at peak when the testis is full of spermatozoa. 11-KT also helps in maintaining viability of the spermatozoa. Both androgens T and 11-KT level decreased at the start of spawning in walleye (Malison et al. 1994), common carp (Barry et al. 1990), rainbow trout (Scott and Baynes 1982; Baynes and Scott 1985; Liley et al. 1986), and white sucker (*Catostomus commersoni*) (Scott et al. 1984). The drop in androgen level before spawning shifts the steroidogenic pathway from C19 androgen to C21 progestogen. This shift in steroidogenic pathway is to regulate spermiation (Ueda et al. 1985; Baynes and Scott 1985) and spawning behavior in male (Liley et al. 1986; Liley and Stacey 1983). During spawning of the carp, the shift from C19 to C21 inhibits the C-17,20-lyase activity by progestogens (17,20-P) (Barry et al. 1990).

Maturation-Inducing Steroid

Progesterone is the precursor of C21 steroid (Scott et al. 1983). This includes 17α,20β-dihydroxy-4-pregnen-3-one (17α,20β-DP), 17α,20β,21-trihydroxy-4-pregnen-3-one (20β-S), 20β-dihydroprogesterone, and 11-deoxycorticosterone (DOC). These are maturation-inducing steroid (MIS), which induces GVBD and FOM (Nagahama and Yamashita 2008). The level of progesterone is at peak during ripe stage of maturation in *M. cephalus* (Kumar et al. 2015) and *M. seheli* (EL-Gharabawy et al. 1994).

C21 steroids such as 20β-dihydroprogesterone (DP), 17α,20β-DP, 17α,20β,21-trihydroxy-4-pregnen-3-one (20β-S), and 11-deoxycorticosterone (DOC) are potent steroids inducing GVBD (Jalabert 1976; Sundararaj and Goswami 1977; Young et al. 1983; Goetz 1983; Nagahama et al. 1983). Among all, 17α,20β-DP is the most common steroid in the induction of GVBD in most teleosts (Nagahama et al. 1983). Testosterone and other C19 steroids induce GVBD only at high concentrations. E2 and other C18 steroids are generally not effective inducer of GVBD (oocyte maturation).

MIS of salmonids (*Oncorhynchus* and *Salmo* spp.) and a few freshwater and marine fishes is progestin 17α,20β-dihydroxy-4-pregnen-3-one (17α,20β-DP). The derivative of 17α,20β-DP, the 17α,20β,21-trihydroxy-4-pregnen-3-one (20β-S), is MIS in marine fishes (King et al. 1995; Schulz and Miura 2002; Thomas et al. 1991). In European sea bass (Asturiano 2000); striped bass, *Morone saxatilis* (Mylonas et al. 1997); and red seabream, *Pagrus major* (Suwa and Yamashita 2007), both 17α,20β-DP and 20β-S are MIH. The MIS bind to the receptor present on the plasma membrane of the oocyte and activate the maturation-promoting factor (MPF). This MPF helps in the resumption of meiosis and completion of oocyte maturation (Nagahama et al. 1994).

Reproductive Cycle in Multiple Spawners

In multiple spawner, the ovary develops asynchronously, and final oocyte maturation (FOM) and ovulation complete within 24 h. Therefore, steroid hormones in a multiple spawner will have a seasonal and diurnal rhythm, which is different from single spawner fish (Kashiwagi et al. 1984). In multiple spawner (blue tang, Japanese flounder, barfin flounder, red seabream, and turbot), T and E2 are low at low GSI (oocytes at chromatin and perinucleolar stage), increase with increase in GSI and oocyte diameter, and decline at late period of the spawning season or at regression phase (Sang et al. 2019). Two clear peaks of both gonadotropins (FSH and LH) are recorded in the plasma of female tilapia (multiple spawner) between two successive spawning. The first is evident 2–3 days after spawning, that is, the vitellogenic stage. The second peak of both LH and FSH is evident just before the next spawning. Parallel increase of FSH and LH level in tilapia suggests that apart from final oocyte maturation, LH may have a role in vitellogenesis and recruitment of a new generation of follicles for the next cycle (Aizen et al. 2007). Tacon et al. (2000) found that in tilapia, E2 levels peaked during vitellogenesis and DHP levels increased in parallel with LH levels, which suggests the role of DHP in the final maturation. In tilapia, there are two types of GnRH receptors (gnrhr): the first is gnrhr3, which is the reproductive type, and the other one is gnrh1, which is expressed in the somatotrophs (Chen and Fernald 2008). At the time of vitellogenesis, the female tilapia generally follows the following steps: (1) positive feedback of increased gnrhr3 and gnrhr1 mRNA levels on FSH release (Levavi-Sivan et al. 2004); (2) increased E2 concentration surge synthesis of dopamine receptor D2 (drd2), probably reflecting the negative feedback on LH release (Levavi-Sivan et al. 2003, 2005); and (3) at last inhibition of LH and FSH release due to decrease in drd2 mRNA levels concomitant with the increase in gnrhr3. This is for the recruitment of a new generation of oocytes for a new cycle (Aizen et al. 2007; Levavi-Sivan et al. 2006).

Conclusion and Future Direction

The knowledge on endocrine control of reproduction is required to understand the maturation pattern of captive reared stock. Further it helps in the hormonal intervention for induced maturation and spawning of fish. The hormone level in fish is used as a biomarker to understand the level of pollution in natural water bodies. Overall information on hormonal cycle will be helpful for the captive breeding of fishes.

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Vitellogenesis and Their Endocrine Control in Fishes

2

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Abstract

Due to the accumulation of yolk protein, the size of oocyte increased several times during oogenesis in teleosts; this process is known as vitellogenesis. Follicle undergoes final maturation by resumption of meiosis. Vitellogenin (Vtg) is a homodimeric phospholipoglycoprotein, the main precursor of yolk protein, which is synthesized in the liver under the stimulation of estrogen. Vtgs released into the blood and transported to the growing oocytes, and taken up by oocyte via receptor-mediated endocytosis. In their native state, purified Vtgs have a molecular weight of about 325 kilodaltons. Vtg splits into two major peptides having molecular weights of 190 and 160 kD on the treatment of sulfated sodium dodecyl. Vtgs include protein carbohydrates, lipids, minerals, vitamins, and other important materials, which are essential for embryogenesis. Yolk protein is made up of five different components such as heavy-chain lipovitellin, light-chain lipovitellin, phosvitin, β-component, and carboxy-terminal component. There are two forms of Vtgs, complete and incomplete. Incomplete Vtgs are further divided into those having phosvitin and phosvitinless (Pv-less) domain. Vtg C is another type of incomplete vitellogenin, without Pv and β-c and Ct domain but having lipovitellin (LvH and LvL). Lr8 is an important receptor having a molecular weight of 100–110 kDa. The Lr8 receptor has two variants Lr8+ and Lr8−, while Lr8− is dominant in the ovary. During the previtellogenic stage, the expression of Lr8− mRNA is highest, whereas its expression is lower or absent during the late- vitellogenic stage in ovulated eggs. Vtg in the blood of female fishes is an important biomarker of the onset of puberty and progression of gonad maturation. In some fishes, which don't display sexual dimorphism, the presence of Vtgs in their blood is an important indicator to identify the gender of fishes in aquaculture.

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Keywords

Vitellogenin · GnRH · Endocytosis · Yolk

Introduction

Among all the vertebrates, teleosts are the greatest diverse group. There are various reproductive strategies to overcome the adverse effect of their ecological niches. Oviparity is the most common reproductive method of fishes. In this method, the embryonic development does not take place inside the mother, and the yolk is the major source of nutrient to the developing embryo. In an oviparous animal, including fish, the development of embryos requires the synchronized transport of nutrients to growing oocytes. After spawning, the development of early life stages takes place within the eggs and depends on the nutrients stored in the eggs as yolk, till the beginning of exogenous feeding. In developing embryos and larvae, the egg yolk is the major source of nutrition (Arukwe and Goksoyr 2003). The process of accumulation of yolk in the oocytes takes place during the development phase of oocytes, termed as vitellogenesis. It is a seasonal or cyclic phenomenon and involves the sequestration and packaging of a hepatically derived plasma precursor, vitellogenin (Vtg), into yolk protein and deposited as yolk granules.

Uhlenhuth and Kodama (1914) first time reported a female-specific protein expressed in the blood of fishes during oocyte growth phase and termed as ovumin. Pan et al. (1969) coined the term vitellogenin (vitelline + genin, meaning source of egg yolk); they concluded that it is a female-specific protein in the hemolymph of the cecropia moth during oogenesis. Similarly, Hara and Hirai (1976) discovered an iron-binding female-specific serum protein (FSSP) in chum salmon (*Oncorhynchus keta*) and rainbow trout (*O. mykiss*); subsequently, in 1978, Hara and Hirai purified trout FSSP and identified it as Vtg for the first time in a teleost. Normally VTG is not produced in the male fish. However, on exogenous exposure of estrogens in male, it can be induced (De Vlaming et al. 1980). Hence, Vtg is a biomarker of the occurrence of estrogen-like chemicals in the aquatic environment (Sumpter and Jobling 1995).

Vitellogenesis is a sequential process incorporating the following events: (1) induction of vitellogenin synthesis and its release into circulation, (2) transport of vitellogenin in the bloodstream to the target tissue, (3) uptake of vitellogenin by the growing oocytes, and (4) the conversion of vitellogenin into storage forms (Ho 1987).

In general, Vtg proteins have the following features: they are (1) specific serum or plasma proteins of female, (2) precursors to yolk proteins, (3) induced by estradiol and exogenous estrogens, (4) phospholipoglycoproteins with molecular masses ranging from 300 kDa to 600 kDa, and (5) carrier proteins with both a lipid and ionic component (e.g., calcium, zinc, cadmium, iron, etc.). The growth of oocytes can be divided into two stages, previtellogenic (primary growth) and vitellogenic (secondary growth). During the vitellogenic stage, the major nutrients like protein,

lipid, vitamins, and minerals are stored within the oocytes, which are required for embryonic and larval growth.

The development of previtellogenic oocytes is induced by several environmental cues like changing day length, temperature, rainfall, and periodic surges of gonadotropins. In the ooplasm of oocytes, neutral lipids begin accumulating in the form of lipid droplet during previtellogenesis. During vitellogenic growth, phospholipid-rich yolk protein (YP) precursors termed as vitellogenins (Vtgs) start to accumulate in the oocytes. Presence of yolk vesicle in the cytoplasm of oocytes is the sign of the next stage. After the completion of vitellogenesis, yolk filled oocytes occupying the ovary consequently undergo maturation and ovulation.

Production of Estradiol-17

In all vertebrates, the fundamental physiological events of female reproductive cycles are controlled by estradiol, which is the key estrogen. Estradiol is a steroid hormone. Cholesterol is the precursor of steroid hormones; on the basis of their structure, steroid hormones are classified into five subgroups: estrogens, androgens, progestogens, glucocorticoids, and mineral corticoids (Guedes-Alonso et al. 2014). There is an important relationship between the plasma level of steroid hormones and gonadal development, and it has been proven that it is an important biomarker for understanding the endocrine control of reproduction in teleosts.

Gonadotropins and steroid hormones are secreted from pituitary and granulosa and theca cells of developing and mature oocytes, respectively (Taghizadeh et al. 2013). The synthesis of steroid in different cells of the ovary may be associated with different phases of oocyte progress. There is an increased estrogen level in plasma; principally, estradiol during vitellogenesis that is correlated with the growth of vitellogenic oocytes has been observed in many species. In the liver, estradiol regulates the synthesis of vitellogenins and choriogenins. In the postvitellogenic phase, plasma estradiol is low and undergoes a further decrease in numerous species including catfish, *Heteropneustes Fossilis* and *Clarias batrachus* (Joy et al. 1998) which indicates a shift in steroidogenesis in which the production of 17-alpha,20-beta-dihydroxy-4-pregnen-3-one (DHP) is enhanced (Nagahama 1994), leading to meiotic maturation (Senthilkumaran et al. 2004). The decline in other steroids and subsequently rise in DHP indicate the final maturation of oocytes (Kobayashi et al. 1987).

Synthesis of Vitellogenin

Depending on gonadotropin surge, vitellogenesis is a seasonal or cyclic process. Various factors, like water temperature, nutritional status, and photoperiod, induce the brain (hypothalamus) and discharge the gonadotropin-releasing hormone (GnRH), which further induce the pituitary gland and stimulate the secretion of gonadotropin hormones (Bhandari et al. 2003); subsequently, these gonadotropins

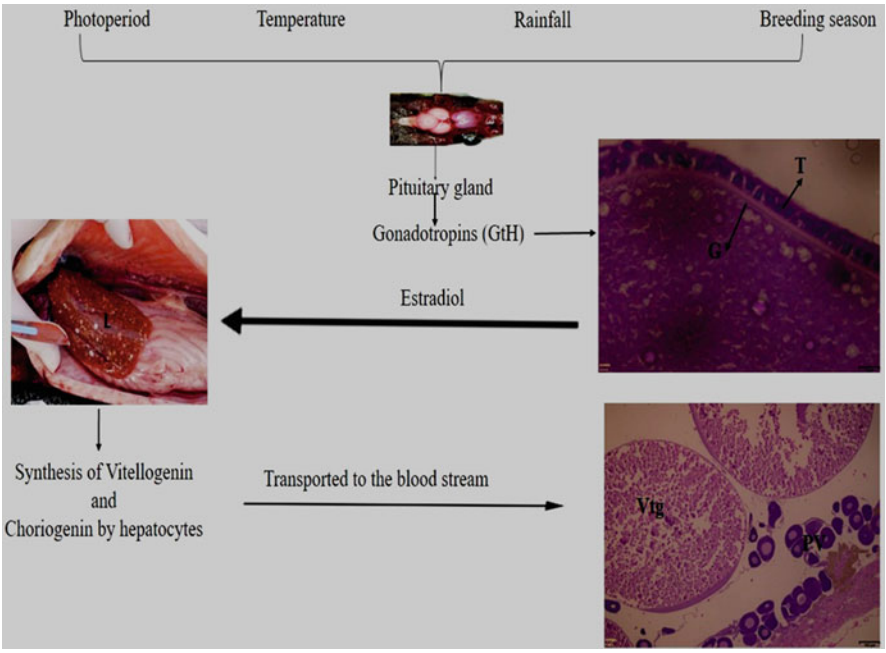

Fig. 2.1 Pathway of vitellogenin synthesis. Abbreviation: L liver, T theca layer, G granulosa layer, Vtg vitellogenic eggs, PV previtellogenic eggs

induce the ovarian follicle to secrete estradiol that binds to the hepatocyte cell of the liver by specific receptors. The binding of estradiol to Vtg receptor present on hepatocytes will lead to the transcription of Vtgs. Before the release of Vtg in the blood, Vtg is post-transnationally phosphorylated and glycosylated, and lipid groups are added. It is secreted in the blood in the form of homomeric complex and reached the growing oocytes, where it binds with a particular receptor in the plasma membrane and is incorporated in the oocytes by clathrin-mediated endocytosis (Anderson et al. 1996; Patiño and Sullivan 2002). Inside the oocytes, it is processed into their derivative YPs. In ovulated eggs of some species, the amount of Vtg-derived yolk is up to 80–90% of the total dry mass (Reading et al., 2017). Vtg synthesis is a gonadotropin-dependent phenomenon (Wallace 1985). The protein backbone of Vtg is synthesized first on membrane-bound ribosomes; afterward, some posttranslational modification like lipidation, glycosylation and phosphorylation (Arukwe and Goksoyr 2003). The pathway of vitellogenin synthesis is given in Fig. 2.1.

Tyler (1991) found that in partially denuded follicles of rainbow trout, GTH-I but not GTH-II induce the vitellogenin uptake (Tyler 1991). There is atresia of yolky oocytes after the removal of the pituitary gland; however, replacement therapy with gonadotropins of crude extract from fish pituitary stimulates vitellogenesis. Serum components like lipid, calcium, and phosphoproteins are elevated during

vitellogenesis in fish. There are two types of yolk, endogenous and exogenous. Endogenous yolk is synthesized in cytoplasm of oocytes by various organelles present in oocytes, while exogenous yolk, which is the main component of yolk synthesized by extra-ovarian tissues. Transported vitellogenins are cleaved into lipovitellin and phosvitin and deposited as yolk particles.

Structure of Vitellogenin

Vitellogenin is a female-specific large multidomain apolipoprotein but also exists in insignificant amount in males (Canapa et al. 2007). A complete Vtg consists of a signal polypeptide; a heavy-chain lipovitellin (LvH) having four subdomains termed as N sheet, α helix, C sheet, and A sheet; a phosvitin (Pv); a light-chain lipovitellin (LvL); and a von Willebrand factor type D domain (vWFD) including a β'component (β'-c) and a C-terminal coding region (Ct). The structure of *Catla catla* vitellogenin (VgB1) is given in Fig. 2.2.

Heavy-Chain Lipovitellin (LvH)

It is the largest YP that delivers amino acid and phospholipid to the growing offspring. The amino acid and phospholipid are involved in both anabolic and catabolic processes like substrate for energy and foundation of membrane and protein structure. LvH is having secondary and tertiary amphipathic structures, which form a pocket having hydrophobic residues which are suitable to enfold lipid molecules. This structure resembles apolipoprotein β of vertebrates. The *N* sheet, which is the subdomain of LvH having a receptor binding site, is responsible for the interaction with oocytes (Reading et al. 2017). The α helix subdomain is responsible for the binding of zinc. An alanine-rich sequence, which is present on the A sheet, is responsible for gluconeogenesis in the embryo (Mikawa et al. 2006). In most species, the average mass of LvH is about 114 Kd, as expected by amino acid sequencing and confirmed by acrylamide gel electrophoresis.

Phosvitin (Pv) Domain

It is an unusual, rich in serine, highly phosphorylated protein and capable for binding phosphates. The phosphate molecule has a negative charge, which is suitable for attracting multivalent cation like iron, calcium, magnesium, and zinc. Pv is rich in phosphorus while poor in lipid. In Pv phosphorus is present as esterified orthophosphate, and it accounts for about 10% of the phosphorus (Matsubara and Sawano 1995). It is important for freshwater fishes because freshwater is poor in these metal ions. Pv domain also contains glycosylation sites, which are suitable to bind carbohydrates with ions and increase the aqueous solubility of Vtgs.

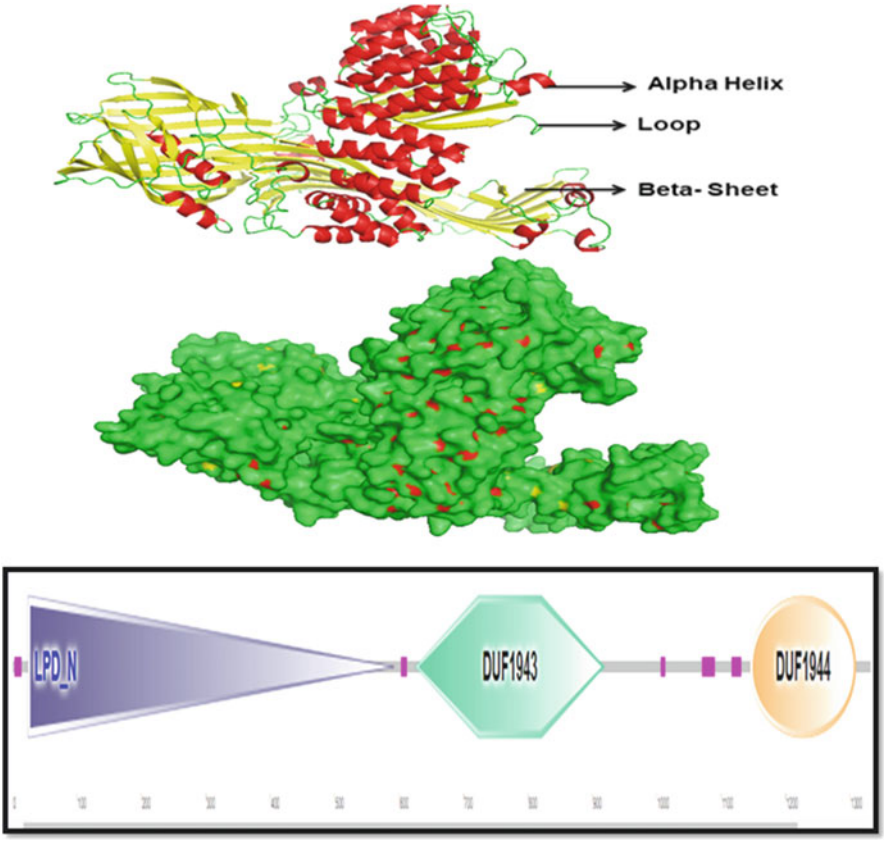

Fig. 2.2 Structure of *Catla catla* vitellogenin (VgB1). Lipoprotein N-terminal domain (LPD-N), domain of unknown function (DUF1943)

Light-Chain Lipovitellin (LvL) Domain

In native state, LvL is present as a dimer molecule, and it is a high molecular weight lipoprotein. It contains glycosylation sites and is also responsible for carrying lipid.

Von Willebrand Factor Type D Domain (vWFD)

Folding in Vtg is due to the presence of vWFD, which is involved in dimerization through disulfide linkage. It depends on extremely conserved cysteine residues (Finn 2007; Reading et al. 2009).

Vitellogenins are dimeric proteins and belong to a large lipid transfer protein superfamily, containing two identical subunits with protein, carbohydrate, lipid, and phosphate components. Vtg contains about 20% lipid by weight and has large lipoprotein molecules about 350–600 kDa. Phospholipid (specially phosphatidylcholines) is the major lipid of Vtgs; it accounts for around 80% of the total lipids. Vtg is the specialized carriers of vitamins like retinoids and carotenoids, minerals, and ions calcium, magnesium, iron, zinc, and copper inside the growing oocytes. Various hormones and regulatory compounds such as steroid and thyroid hormones are also delivered by Vtg inside oocytes. In marine fishes, which produce pelagic (floating) eggs having no or very minute oil droplets, phospholipids, triacylglycerides, and wax or steryl esters can account for 470, 8–12, and 4% of total egg lipids, respectively. However, some other teleosts that are producing pelagic egg contain large oil droplets, composed of neutral lipids (e.g., triacylglycerides and wax or steryl esters) that can occupy 50% of the ooplasm. In these species, Vtg is not a major source of natural lipid; it is transported to oocytes through a different mechanism. Natural lipid is delivered into the oocytes by VLDL as reported in cutthroat trout (*Oncorhynchus clarkii*), medaka (*Oryzias latipes*), and black skipjack tuna (*Euthynnus lineatus*). Natural lipid is transported in the form of free fatty acid, produced from triacylglyceride by a lipase-dependent, non-endocytotic pathway. Free fatty acid enters across the oolemma of oocytes and subsequently united into oil droplets inside the oocytes. The most abundant amino acid in Vtg is alanine, which accounts for 12% of the total residues. In embryonic gluconeogenesis, the alanine amino acid works as a substrate for the intermediary metabolism of carbohydrate.

Processing of Vitellogenin Inside the Oocyte

Vtg is present in the endosomes of ovarian follicle, and it is acidified with the help of proton pump. Subsequently, Vtgs are cleaved into lipovitellins, Pv, β'-c, and Ct by the action of cathepsin D (Finn and Kristoffersen 2007). In Vtgs, there are some specific cleavage positions that are present, which act as the construction of their constituents and show different levels of conservation. The cleavage site situated between LvH and Pv has the sequence KLKKIL, and the K(Y/F)LG consensus sequence is present between Pv and LvL in the majority of vertebrates (Finn 2007). There is a highly variable cleavage site, which is present between LvL and β'-c terminal. Apart from the major sites, additional cleavage positions are also existing in the Vtg peptides that are intricate in the progression of secondary dissociation by the action of various cathepsin enzymes (Reading et al. 2017).

Yolk Processing Enzyme

Processing of yolk takes place on various kinds of protease enzyme which is responsible for the early development and survival of fish embryos (Tingaud-Sequeira et al. 2011; Palomino et al. 2017). The major yolk processing enzymes are cathepsins and lipoprotein lipase. The major enzymes belong to the cathepsin family. On the basis of degradation of amino acid, cathepsin is divided into several subgroups such as cathepsins B and L, typical cysteine protease, and cathepsin D, an aspartate protease. Palomino and co-workers (2017) concluded that the egg quality of marine fish is determined by the yolk processing enzyme cathepsin. The survival of embryo and early development in yellowtail kingfish *Seriola lalandi* are associated with the enzyme activities and mRNA expressions of cathepsin B, cathepsin D, and cathepsin L (Palomino et al. 2017). Cathepsins B, D, and L are also having a key role during maturation, egg development, and embryogenesis in fish.

Gene Responsible for Vitellogenesis

During the vitellogenesis phase, there are 1046 differentially expressed transcripts that have been reported from the gene expression profiling of the liver of zebra fish, out of which more than 64% gene is controlled by estradiol. The key hormone of vitellogenin (Vtg) expression is the ovarian steroid hormone 17β-estradiol (E2), synthesized under the control of the hypothalamic-pituitary-gonadal axis (Polzonetti-Magni et al. 2004; Rather et al. 2016). The genomic action of estrogen mediated by through specific nuclear Estrogen receptors (ERs), which are present either in the cytosol or in the nucleus. Estrogens enter inside the target cells and bind with ERs. After the binding of estrogens and ERs, the formation of homo- or heterodimers of ERs takes place; subsequently, it binds with the promoter region of estrogen-responsive genes at specific palindromic estrogen response element (ERE) sequences (Gruber et al. 2004), which result in the recruitment of coactivators or corepressors to the promoter. Subsequently this leads to protein synthesis and physiological response by the up- or downregulation of mRNA levels (Klinge et al. 2004). There are two key ERs, i.e., ER alpha and ER beta, reported in fish, mammals, and birds. Estrogen receptor 1 (gene esr1), estrogen receptor 2 beta (esr2beta), and estrogen receptor 2 alpha (esr2alpha) are the three ER subtypes also reported in fish (Hawkins et al. 2000; Menuet et al. 2002). The non-genomic action of some estrogens is also reported, and the action is rapid and independent on RNA and protein synthesis. The non-genomic action is mediated by the activating protein kinase cascades through phosphorylation and activates the transcription factors (TFs) within the nucleus (Bjornstrom and Sjoberg 2005; Klinge 2001).

Pakdel et al. (1991) reported that the mRNA expression of Vtgs in the liver is closely related to the E2-dependent upregulation of esr1. Assessment of plasma Vtgs or Vtg gene expression is an important biomarker of female maturity status related to the gonadal steroid changes. Ziv et al. (2008) reported eight vtg genes from mature

ovarian follicle from zebra fish. There are three main classes of vtg protein vitellogenin 1 or VtgAo1 (with five corresponding genes, vtgs 1, 4, 5, 6, and 7), vitellogenin 2 or VtgAo2 (with two vtg2 genes), and vitellogenin 3 or Vtg C (encoded by vtg3). In zebra fish, 14 genes, which are responsible for vitellogenesis, are linked to chromosome 22, while phosvitinless gene (vtg3) is located at chromosome 11 (Finn and Kristoffersen 2007). Some important genes which are responsible for vitellogenesis in fish such as vtg; esr1; insulin-like growth factor 1 (igf1); zona pellucida glycoproteins (zps); choriogenin H; cytochrome p450, family 1, subfamily a (cyp1a; also known as cyp1a1); and peroxisome proliferator-activated receptors (ppars) (Arukwe and Goksoyr 2003; Menuet et al. 2004; Davis et al. 2008) are known to be regulated by estrogen. Estradiol also regulate apolipoproteins like apolipoprotein A-I (Apoa1), apolipoprotein A-II (Apoa2), and apolipoprotein E (Apoe), and it also controlled the modifications in the lipoprotein classes during vitellogenesis (Bon et al. 1997). There are eight important differentially expressed genes controlled by estradiol including vtg1 and vtg3, nots, syne1, fst1, nosip, grik1, and esr1. During vitellogenesis in female, the protein synthesis and secretion in the liver increase in the endoplasmic reticulum (Arukwe and Goksoyr 2003), and the cytoskeleton formation protein (syne1 and ank) showed higher upregulation (Olsson et al. 1989).

Conclusion and Future Direction

The final egg and seed quality depends on very specific regulatory mechanisms of vitellogenesis, choriogenesis. During the vitellogensis process, the size of oocytes increases several times by accumulating and synthesizing each and everything, which is required to support the embryogenesis. The status of circulating levels of estradiol and vitellogenin may be references of egg quality in the aquaculture sector. Estradiol and vitellogenin are the two important proteins during oogenesis in fish, and they are also the key biomarkers for assessing the effect of estrogen-like endocrine disrupting chemical in aquatic system.

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Estrogenic Regulation of Reproduction in Teleosts

3

Sherly Tomy

Abstract

Teleost exhibits extreme plasticity in its sexuality, retaining the conserved vertebrate neuroendocrine regulation of reproduction. The sex hormonal milieu is a major driver of sexual development in an organism. In teleosts, 17β-estradiol (E2) and 11-ketotestosterone (11-KT) act as natural inducers of ovarian and testicular differentiation, respectively, and alteration in the sex steroid milieu can reverse their sex. Estrogen also exerts pleiotropic effects on other physiological processes, in addition to its reproductive role. Different research strategies have indicated that estradiol, from either peripheral or central origin, exerts nucleus- and membrane-initiated signalling mechanisms which cooperate with other signalling pathways to modulate biologic responses of both reproductive and non-reproductive nature. Sex steroids also maintain a self-regulating feedback loop along the brain-pituitary-gonadotropic axis for a stage- and sex-specific steroidogenesis and reproductive behavior, though the underlying molecular mechanisms remain elusive. Understanding the estrogen signalling will enhance our understanding of fish reproduction and its regulation and will provide opportunities for the development of new strategies useful to aquaculture.

Keywords

Aromatase · Estrogen · Estrogen receptor · Sex differentiation · Sex change · Vitellogenesis

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Introduction

Teleosts exhibit a unique complex sexual plasticity with a labile genetic sex determination and differentiation process that can be altered by environmental factors, endocrine factors, or both (Devlin and Nagahama 2002; Liu et al. 2017). Coordinated interplay of a complex network of regulatory signals governs the differentiation of a bipotential gonadal primordium into an ovary or testis. Sex steroid hormones, which are small, hydrophobic, cholesterol-derived molecules synthesized in specialized steroid-producing cells in gonadal and non-gonadal tissues, are the main internal factors regulating gonadal differentiation and its functions (Nagahama and Yamashita 2008). In teleosts, 17β-estradiol (E2) and 11-ketotestosterone (11-KT) act as natural inducers of ovarian and testicular differentiation, respectively (Guiguen et al. 2010; Forsgren and Young 2012; Rather et al. 2017). Estrogen (predominantly 17β-estradiol) regulates female gonadal sex determination, ovarian development, maintenance of female characters, and hepatic synthesis of the egg yolk precursor, vitellogenin (Guiguen et al. 2010; Lubzens et al. 2010; Paul-Prasanth et al. 2013). On the other hand, they also stimulate spermatogonial proliferation (Schulz et al. 2010) and modulate sex change (Lee et al. 2002; Bhandari et al. 2005). They alter the organization of the brain during early development and coordinate various behaviors in a sex-dependent manner (Azcoitia et al. 2019). Additionally, they also exert their effects on the skeletal, cardiovascular, and nervous systems and stress and immune response (Manolagas and Kousteni 2001; Bouman et al. 2005). This review focuses on the biosynthesis of estrogen and their currently known receptor-mediated putative roles in teleost reproduction.

Estrogen Biosynthesis

The endocrine pathway regulating the synthesis and release of sex steroids into circulation from the gonads in teleosts, as in other vertebrates, is directly regulated by circulating gonadotropins (GtHs), namely, follicle-stimulating hormone (FSH) and luteinizing hormone (LH), from the pituitary. The secretion and activity of pituitary gonadotropins are, in turn, regulated by multiple neurohormonal factors, predominantly by gonadotropin-releasing hormone (GnRH) from the brain (Zohar et al. 2010). The gonadotropins, acting through their specific receptors, trigger the steroidogenic pathway of sex steroid biosynthesis where the precursor cholesterol is processed into pregnenolone and subsequently converted to progestins, androgens, and estrogens. Steroidogenic genes involved in the biosynthesis of sex steroids have been reported in several teleosts (Tomy et al. 2007; Rajakumar and Senthilkumaran 2020).

Estrogens are irreversibly biosynthesized from testosterone by the action of the rate-limiting enzyme, aromatase (encoded by *cyp19a1*). The expression and activity of aromatase determine the local balance between the two types of sex steroid, and thus it has been implicated in ovarian differentiation in fish (Wu et al. 2008; Guiguen et al. 2010). Gene editing (Lau et al. 2016; Yin et al. 2017; Zhang et al. 2017) and AI

treatment (Kwon et al. 2000; Komatsu et al. 2006) studies provide additional evidence supporting the role of E2 in ovarian differentiation, where the treated females developed as males. As a consequence of teleost-specific gene duplication, two isoforms of aromatase have been identified in teleosts, the brain (cyp19a1b) and the gonadal form (cyp19a1a) (Tomy et al. 2007; Rajakumar and Senthilkumaran 2020). Localization of aromatase-expressing cells (exclusively in proliferating radial glial cells, Takeuchi and Okubo 2013), together with studies on expression and activity of cyp19a1b, provides evidence that teleost brain, besides being a target site for E2 action, can also synthesize de novo an array of biologically active steroids including neuroestrogens (Tomy et al. 2007; Rajakumar and Senthilkumaran 2020). The activity of neural aromatase in teleosts is remarkably higher than any other vertebrates (100–1000-fold) and has been suggested to be a mechanism for continual neurogenesis and neuroplasticity in fish and also attributed to its neuroprotective role (Forlano et al. 2001; Tomy et al. 2007; Lin et al. 2016).

Estrogen Receptors

Nuclear Estrogen Receptors

Estrogens regulate several biological functions by interacting with nuclear receptors, ERα and ERβ (encoded by *esr1* and *esr2*, respectively), which act as ligand-dependent transcriptional factors directly regulating the downstream gene expression (Menuet et al. 2002; Heldring et al. 2007). They also mediate the autocrine action of estrogen on ovarian granulosa cells. Teleostean ERs, though sharing similarity to mammalian orthologs, differ in having a shorter ERα isoform with truncated N-terminal, in addition to the full-length ER, which is characterized by constitutive ligand-independent activity, probably relevant to hepatic vitellogenesis in oviparous species (Menuet et al. 2002). Furthermore, most teleosts have three estrogen receptors encoded by three separate genes, a single ERα gene (*esr1*) and two ERβ genes, ERβ1 (*esr2b*) and ERβ2 (*esr2a*), due to teleost-specific genome duplication (Filby and Tyler 2005; Chakraborty et al. 2011; Lafont et al. 2016; Hu et al. 2018). A second form of *esr1* (ERα2) is reported from some teleost species (Nagler et al. 2007; Nikoleris and Hansson 2015). Both ERα and ERβ are characterized by similar structure and functional domains but with distinct differential spatiotemporal expression patterns (Menuet et al. 2002; Chakraborty et al. 2011) and signalling properties depending on the estrogen response element (ERE) and binding affinities with various ligands (Sabo-Attwood et al. 2007). Significantly higher levels of Esr1 were observed in the liver during vitellogenesis, suggesting a regulatory role on the vitellogenin expression (Nelson and Habibi 2010; Chakraborty et al. 2011), while higher concentrations of Erβs, in particular Erβa, were reported in ovaries, supporting their probable role in early ovarian differentiation and development (Sabo-Attwood et al. 2007). The presence and distribution of ER in the brain have been reported in several teleosts augmenting their role in reproductive activities in teleosts (Menuet et al. 2002; Hawkins et al. 2005; Muriach

et al. 2008; Forlano et al. 2010). In medaka, pronounced sex-specific differences were reported in the distribution of ER (prominent expression in the forebrain of females and almost completely absent in male, Hiraki et al. 2012) and *cyp19a1b* (higher levels in the adult female brain, Okubo et al. 2011), suggesting sex-specific target sites for estrogen action in the brain.

Gene knockdown studies on ER have provided confirmatory evidence for their involvement in oocyte maturation. Knockout of *esr1* disrupted the hypothalamic/pituitary feedback regulation of LH secretion causing fertility defects in mice, while disturbance of *esr2* had no severe fertility defects. Similarly, premature ovarian failure (POF) syndrome in humans is also linked to a mutation in *esr1* (Yoon et al. 2010). On the other hand, double knockouts of ERs in mice resulted in viable, but infertile, females (Dupont et al. 2000). In contrast, evidence from teleost fish suggests *esr1* be dispensable for reproduction as *esr1* mutants displayed normal reproductive development and function compared to the homozygous *esr2a* and *esr2b* mutants with reproductive tract malformation and infertility. On the other hand, double and triple nER knockouts, especially *esr2a* and *esr2b*, resulted in infertility and/or female-to-male sex reversal (Kayo et al. 2019; Yan et al. 2019; Li and Ge 2020). These results indicate *esr2* to be more critical than *esr1* in reproduction in teleosts. Different endocrine hormones and paracrine factors modulate E2 activity during folliculogenesis, mainly through their regulatory actions on nERs (Liu et al. 2017).

Structure of Estrogen Receptors

The primary structural organization shared by the estrogen receptors mainly consists of six functionally distinct independent domains (A to F), with varying sequence homology, that is responsible for similar functional features (Fig. 3.1). The A/B domain at N-terminal is the most variable region in ER containing the activation function (AF-1) with a promoter- and cell-specific activity. The domain C or DNA-binding domain (DBD) is highly conserved with two zinc finger-like motifs which facilitate the receptor-specific, high-affinity binding to ERE in the promoter of the target gene. The hinge region or the D domain, located between the DBD and the LBD, is less conserved but essential for the maintenance of the receptor structure and modulates DNA-binding. The highly conserved ligand-binding domain (LBD, domain E) located toward the C-terminal region harbors a hormone-binding site, a dimerization interface (homo- and hetero-dimerization), and a ligand-dependent coregulator interaction function (activation function, AF-2) which are essential for specific ligand recognition and induction of conformational changes accompanying receptor dimer formation (Parker 1995; Ruff et al. 2000; Gruber et al. 2004; Thomas et al. 2007; Yaşar et al. 2017). A highly variable F domain, at the extreme carboxyl-terminus of the receptors, is suggested to have a role in transcription modulation and interactions with agonists/antagonists. Despite these similarities, both the estrogen receptors exert structural and functional distinctions in ligand recognition, receptor

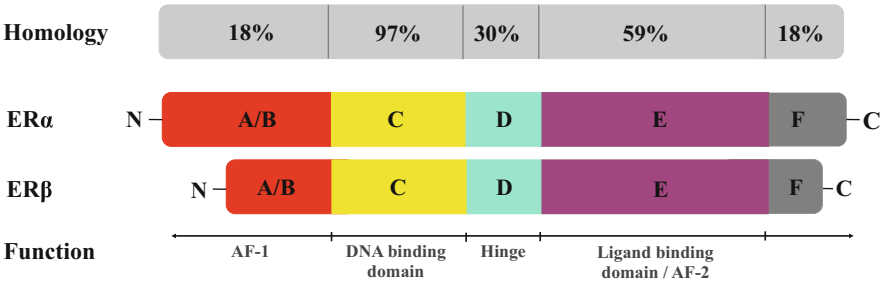

Fig. 3.1 Schematic diagram of human Estrogen receptors (ERs), ERα and ERβ. Both receptors consist of six functionally distinct domains, including the domains A/B at the N-terminal containing the activation function (AF-1) for protein-protein interactions and transcriptional activation of target gene expression. The conserved C domain, containing the DNA-binding domain (DBD) facilitates receptor specific binding to ERE in the promoter of the target gene. The domain D is essential for the nuclear localization signal, and the conserved domain E includes the ligand-binding domain (LBD) and the ligand-dependent activation function AF-2. The F domain, at C-terminus t suggested to have a role in transcription modulation. The ERs form dimers with or without the endogenous ligand, 17β-estradiol, the binding of which induces conformational changes in the receptors. The percentage homology between the two receptors is indicated

activation, and recruitment of coregulators and also in the target genes they regulate (Cui et al. 2013).

Membrane-Associated Estrogen Receptors

Evidence that estrogens could stimulate rapid cell responses via an extranuclear mechanism was first suggested by Pietras and Szego (1977). These membrane-initiated estrogen signalling, employing plasma membrane-specific receptors (GPER or ERα and ERβ themselves), rapidly alter cell signalling via modulation of intracellular downstream signalling cascades (Revankar 2005; Boonyaratanakornkit and Edwards 2007). Approximately 5–10% of nERs are trafficked to the cell membrane following palmitoylation (a post-translational modification resulting in the addition of a fatty acid chain to a cysteine residue which promotes the hydrophobicity of the protein and hence an increased association to the plasma membrane) by specific palmitoyl acyltransferases (Pedram et al. 2007, 2012). The inhibition of ERα palmitoylation constraints E2-induced extranuclear signalling and E2-dependent functions (Cipolletti et al. 2020). At the membrane, their association with other proteins (such as caveolins) helps in anchoring them into functional microdomains, facilitating their interaction with other receptors (growth factors, GPCR, etc.) for the rapid activation of many signalling pathways. These rapid and non-genomic membrane-initiated steroid signals (MISS) can affect post-translational modification of existing proteins and modify transcription of important genes by cooperating with nuclear steroid receptor pools, thereby influencing normal development and functions of multiple organs (Ascenzi et al. 2006; Levin 2018).

Homologs of GPER have been identified in several teleosts and are reported to be mainly involved with the maintenance of E2-induced meiotic arrest and maturation of oocytes (Thomas and Pang 2010; Thomas 2017), brain development (Shi et al. 2013), embryonic heart rate (Romano et al. 2017), and immunoregulation (Cabas et al. 2018). Two GPER genes, *gpera* and *gperb*, with differential tissue distribution, were reported in *Anguilla* species (Lafont et al. 2016; Morini et al. 2017) and European sea bass, *Dicentrarchus labrax* (Pinto et al. 2018). Evidence from in vitro and RNAi approaches demonstrated a GPER-mediated regulation of estrogen on ovarian follicle development and oocyte maturation in cultured teleost oocytes (Pang and Thomas 2010; Thomas and Pang 2010), contradictory to the observations reported from in vivo studies advocating a non-essential role for GPER in sex determination, ovary development, or fertility in zebrafish (Crowder et al. 2018; Li and Ge 2020).

Estrogen Signalling Pathways

Upon delivery to the target tissue, estrogens exert their biological functions by regulating the transcriptional processes through their specific cognate receptors (ERs—ERα and ERβ) in a genomic or a non-genomic manner. The classical genomic action (ER-dependent, nuclear-initiated estrogen signalling pathway) involves the passage of steroids through the plasma membranes of target cells due to their hydrophobic nature and binding to specific intracellular nuclear receptors (nER) (Hall et al. 2001) which initiates conformational changes in ER resulting in the release of inhibitory proteins (e.g., members of the heat shock protein family) associated with them in its inactive state. The activated ligand-receptor complex is then translocated to the cell nucleus, is dimerized, and subsequently binds to respective estrogen response elements (ERE) on target genes and initiates transcription (Gruber et al. 2004; Cui et al. 2013; Fuxjager and Schuppe 2018). The activated ligand-receptor complex can also act as a scaffold for several cofactors/coactivators and RNA polymerase required for transactivation (Shang et al. 2002). Additionally, in the non-classical pathway, the nuclear receptors indirectly regulate DNA transcription by interacting with other transcription factors like stimulating protein-1 (SP-1), activator protein 1 (AP-1), nuclear factor κB (NF-κB), and c-jun (Gottsch et al. 2009). These processes are typically slow and occur over a relatively long period (Fig. 3.2).

In addition to the classical E2 signalling, rapid, ER-dependent membrane-initiated estrogen signalling also exists which starts at the plasma membrane or cytoplasm and functions mainly through protein-protein interactions with other DNA-binding transcription factors with the downstream effects being only partially dependent on the translation or transcription (Vasudevan and Pfaff 2008). These pathways are mediated by G protein-coupled estrogen receptor (GPER; formerly known as G protein-coupled receptor 30, GPR30) or by the interaction of membrane ERs (ERα and ERβ) with G protein-coupled receptors (GPCR) linked to activation (Gq) and inhibitory (Gi/o) G protein pathways, which get triggered on estrogen

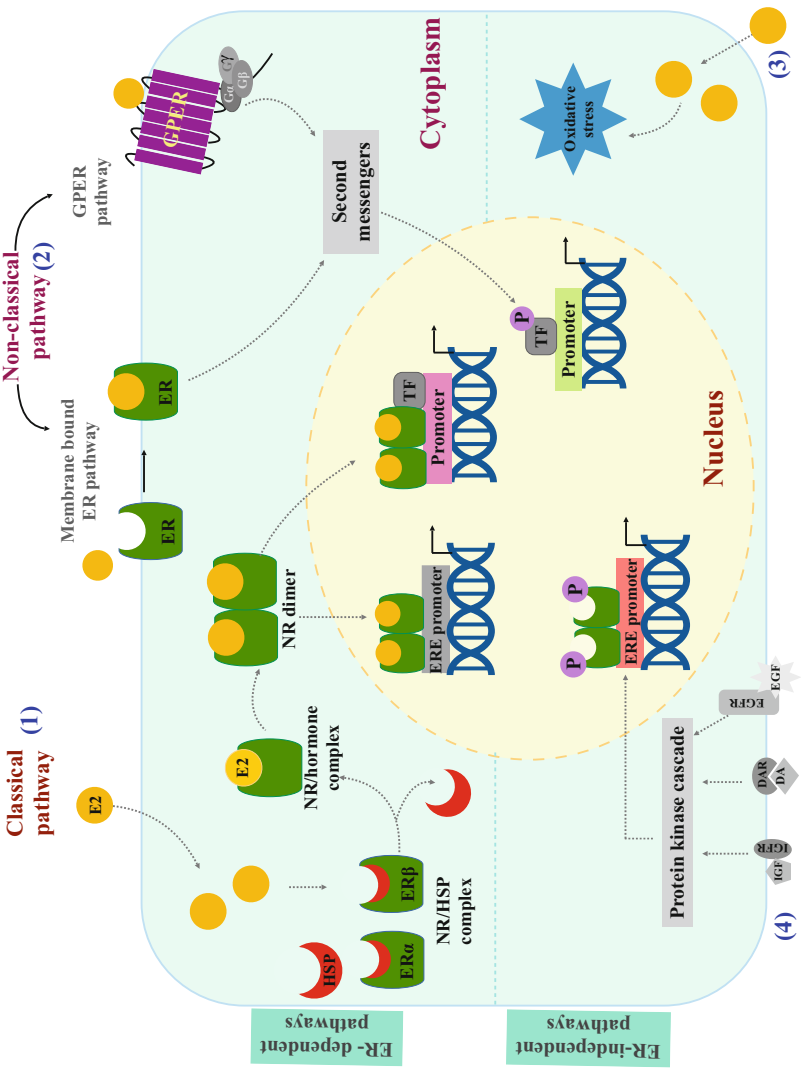

Fig. 3.2 Schematic representation of the signalling pathway mediated by E2 and ERs. Estrogens exert their biological functions through their specific receptors, ERα and Erβ, in a genomic or a nongenomic manner. The ER dependent nuclear-initiated genomic estrogen signalling leads to the transcriptional changes in

Fig. 3.2 (continued) estrogen—responsive genes with or without EREs (1). The ER activated, membrane-initiated estrogen signalling leads to diverse cytoplasmic effects, including regulation of second messenger systems leading to phosphorylation of transcription factors modulating gene expression (2). Estrogen can also exert antioxidant effects (3) or initiate genomic actions by interacting with other transcription factors (4) in an ER-independent manner. *E2* 17β-estradiol, *ER* estrogen receptor, *Erα* estrogen receptor alpha, *Erβ* estrogen receptor beta, *ERE* estrogen response element, *HSP* heat shock protein, *NR* nuclear receptor, *TF* transcription factor, *P* phosphorylation, *GPER* G-protein-coupled estrogen receptor, *Gα, Gβ and Gγ* G-alpha, beta and gamma subunits of G-protein, *DA* Dopamine, *DAR* DA-receptors, *EGF* epidermal growth factor, *EGFR* EGF-receptors, *IGF* Insulin-like growth factors, *IGFR* IGF-receptor

binding and rapidly activate the second messenger signal transduction cascades including the stimulation of adenylate cyclase activity, cAMP production and mobilization of intracellular calcium, and/or rapid activation of several signalling pathways involving MAPKs, ERK and PI3K (Filardo et al. 2002; Lösel and Wehling 2003; Schwartz et al. 2016; Thomas 2017), These ER-dependent membrane-initiated signalling pathways are characterized by shorter latency periods and transient nature (Fig. 3.2).

Estrogen exerts antioxidant properties and can suppress oxidative stress actions which are mediated through an ER-independent pathway (Haas et al. 2012). These actions are reportedly performed by the interaction of estrogen with other non-sex steroid receptors or by regulating the enzymatic activities to protect cell damage. Furthermore, a ligand-independent activation of ER is also reported where the signalling pathway is triggered by other factors including growth factors (epidermal growth factor, insulin-like growth factors) (Klotz et al. 2002), neurotransmitters (Power et al. 1991), or activators (kinases) of the intracellular pathways (Schreihofer et al. 2001) (Fig. 3.2).

Additional molecules, such as microRNAs (miRNAs), also participate in the transcriptional regulation exerted by estradiol (Azcoitia et al. 2019), which however needs further studies to gain a better understanding of their role in the process.

Significance of Estradiol During Sex Determination and Sex Differentiation

Endogenous sex hormones are regarded as natural sex inducers, and their levels during critical periods direct the undifferentiated gonad to develop into a testis or ovary (Devlin and Nagahama 2002; Blázquez et al. 2008; Kobayashi et al. 2013; Kitano 2018; Li et al. 2019). Thus, an abundance of 11-KT or 17β-estradiol induces masculine or feminine differentiation, respectively, in teleosts. The expression of estrogen receptors in the testis (Ito et al. 2007; Morini et al. 2017) and ovary (Chang et al. 1999; Piferrer 2001) suggests crucial roles for estrogen in the development and function of both ovarian and testicular tissues in teleosts. Lower expression levels of endogenous E2 were reported to be essential for male sexual differentiation and development in black porgy, *Acanthopagrus schlegeli* (Wu et al. 2008); Japanese eel, *Anguilla japonica* (Jeng et al. 2012); and Japanese huchen, *Hucho perryi* (Amer et al. 2001). Though the relevance of sex hormones on teleost reproduction is well studied, contradictory reports exist on the time of appearance of gonads and steroid-producing cells that secrete sex hormones. Some studies suggest that endogenous sex hormones are produced in the steroid-producing cells initially before or around the time of sex differentiation, which acts as a natural sex inducer (Nakamura and Nagahama 1993; Mosyagina and Zelennikov 2015). In contrast, observations supporting the appearance of steroidogenic cells after the completion of sex differentiation suggest sex hormones to not act as natural sex inducers (Kawahara and Yamashita 2000). Nevertheless, the importance of aromatase for both ovarian and

testicular differentiation has been confirmed in several gonochoristic and hermaphrodite fish species.

Unlike in mammals, teleosts retain the ability to change sex at different stages of development or even in adulthood through social manipulation, through inhibition of aromatase activity, or by steroid treatment (Liu et al. 2017; Imiuwa 2020). Research findings also corroborate that administration of exogenous androgen or aromatase inhibitor (AI) to undifferentiated fish induced maleness (Kwon et al. 2000; Bhandari et al. 2004, 2006; Navarro-Martín et al. 2009) while exogenous estrogen induced femaleness, in some fish, regardless of their genetic sex (Kobayashi and Iwamatsu 2005; Vizziano-Cantonnet et al. 2008; Wu et al. 2008). Contrarily, E2-induced feminization in seabass, *D. labrax* (Navarro-Martín et al. 2009), and the eel, *A. japonica* (Jeng et al. 2018), was attributed to the indirect association with *cyp19a1a*, due to the absence of ERE in the promoter region of cyp19a1a. Yamamoto (1969) suggested that the success of the treatment for complete and functional sex reversal depends on the time, adequate dose, and duration.

Role of Estrogens During Vitellogenesis and Gonad Maturation

The dynamic changes in E2 concentration play a pivotal role in regulating the female reproductive endocrine axis in teleosts. During vitellogenesis, ovarian follicles produce E2, primarily in response to FSH signalling, which exerts a receptor-mediated induction of hepatic vitellogenin synthesis and suppresses the pulsatile GnRH/LH release (Nagahama and Yamashita 2008; Lubzens et al. 2010; Kagawa 2013). As ovarian maturation progresses, the sustained elevations in estradiol during the late follicular phase of the cycle induce prolonged GnRH/LH surges, which, in turn, elicit a steroidogenic shift from the production of E2 to that of 17α,20-β-dihydroxy-4-pregnen-3-one (maturation-inducing steroids, MIS). The decline of E2 synthesis, together with the accompanying surge in maturational progestogens, results in an LH-dependent acquisition of oocyte maturational competence and subsequent ovulation (Nagahama and Yamashita 2008; Lubzens et al. 2010; Rajakumar and Senthilkumaran 2020). Estrogen receptors mediate the signalling pathways in oocyte maturation and E2-induced hepatic vitellogenin synthesis (Wintermantel et al. 2006; Chakraborty et al. 2011; Hu et al. 2018). Emerging evidences suggest that E2 exerts autoregulation on nuclear estrogen receptor expression, a mutual feedback between cyp19a1 and E2 (*cyp19a1b* transcripts were significantly upregulated by E2, while *cyp19a1a* was less responsive to E2 treatment) (Pal et al. 2018), and a feedback regulation along the brain-pituitary-gonadotropic axis providing homeostatic control of vitellogenesis and later maturational processes via autocrine, paracrine, and endocrine processes (Guiguen et al. 2010; Nelson and Habibi 2013; Rather et al. 2020).

A positive correlation between sex steroid hormone profile and gonad development has been documented in several teleosts, with the sex hormone levels increasing concomitantly during the pre-spawning period and reaching a peak at spawning (Manosroi et al. 2003; Li et al. 2007; Ismail et al. 2011; Xu et al. 2013). In most

species, testosterone and 17β-estradiol levels increased with the advancement of ovarian maturation and reached a peak in the mature stage, followed by a sudden decrease at post-vitellogenic stage. The decrease in E2 could be due to reduced aromatase (CYP19) activity as oocytes advance to final maturation (Lee and Yang 2002; Kumar et al. 2015; Mandal et al. 2020). However, in some teleosts, there was no decrease in E2 level during oocyte maturation (Prat et al. 1990). In males, E2 plays a role in spermatogonial stem cell renewal before the onset of spermatogenesis regulated by 11-KT and sperm capacitation and spermiation regulated by maturation-inducing steroid (MIS) (Miura and Miura 2003).

Role of Estrogens During Sex Change

Among vertebrates, teleosts have remarkable ability to change sex involving gonadal reorganization. In protogynous fishes, female-to-male sex reversal is coupled with a reduction in gonadal aromatase and E2 levels together with an increase in plasma 11-KT concentration as testicular tissue develops (Nakamura et al. 2003; Bhandari et al. 2006). The reciprocal pattern is observed in protandrous sex change (Piferrer 2001; Wu et al. 2010). High dose of E2 treatment induced female development, while low dose stimulated testis growth in black porgy, *A. schlegeli* (Wu et al. 2008). Treating females with an exogenous aromatase inhibitor (AI) induced sex change in protogynous fish (Higa et al. 2003) while inhibited sex change in protandrous fishes (Lee et al. 2002). Co-administration of E2 with AI or 11-KT suppressed sex change in protogynous fishes (Bhandari et al. 2006; Nozu et al. 2009). These results strongly suggest that drop in E2 levels below the threshold levels at the onset of sex change is a causative factor for a sex change (Bhandari et al. 2004; Göppert et al. 2016). These results also demonstrate that a change in the activities of steroidogenic enzymes together with the attainment of minimum threshold E2 levels can influence the direction and pace of gonadal transition in sex-changing fish (Devlin and Nagahama 2002; Liu et al. 2009; Godwin 2010). The predomination of E2 in teleost sexuality supports the concept of ovary being the ancestral gonadal state in hermaphroditic fishes (Shapiro 1992), and an inhibition in the mechanisms maintaining the female phase results in sex change. In protandrous fish, oocytes survive by creating a protecting microenvironment against the testicular environment until sex change (Wu and Chang 2013).

Conclusion and Future Direction

Sex steroids are key players regulating gonadal sex differentiation, maturation, and sex change in teleosts. The feedback regulation of E2 along the brain-pituitary-gonadotropic axis is complex and not well understood yet. The membrane and nuclear receptor-mediated multifunctional actions of E2 in genomic and non-genomic pathways are well recognized. Treatment strategies targeting endogenous estrogen pathways, mainly by exogenous aromatase inhibitors or antiestrogen

and knockout studies, have confirmed the significance of estrogen synthesis and signalling in both gonadal and extra-gonadal organs. Furthermore, the role of miRNA interaction and epigenetic regulation of essential genes related to steroidogenesis needs to be analyzed. Further studies to understand the potential crosstalk of E2 with other signalling pathways influencing the level of their expression and/or activity and confluence to modulate biologic response can contribute to the understanding of the complex molecular circuits in reproduction which can be beneficial in developing novel breeding protocols.

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Steroids and Its Receptors in Fish Reproduction

4

Partha Sarathi Tripathy, Janmejay Parhi, and Sagar Chandra Mandal

Abstract

Steroid hormones are associated with the regulation of various processes in fish, like embryonic development, sex differentiation, metabolism, immune responses, circadian rhythms, stress response, and reproduction. Steroids in fish are generally classified into C_{21}, $C_{19,}$ and C_{18} steroids based on their structure. These steroids like estrogens and androgens are used in fish farming to increase fish production based on sexual dimorphism. Progesterone (P4), 17,20β-dihydroxy-4-pregnen-3-one (17,20βP or MIH or DHP), 17,20β,21-trihydroxy-4-pregnen-3-one (20βS) and 11-deoxycortisol (S) are some of the major C21 steroids that are essential for gonadal maturation and production of other endogenous steroids. The C_{19} steroids, i.e. testosterone (T), 17α-Methyltestosterone (MT), and 11-Ketotestosterone (11-KT) classified as androgens help in fish spermatogenesis and C_{18} steroids, called as Estranes, are known as female hormones. Except for the role of steroids in fish reproduction they have a major role in immunity, puberty, and stress. Corticosteroids, a major C_{21} steroid, are associated with stress response in fish. Steroids like, 17β-estradiol (E2), 11KT, medroxyprogesterone, 17α,20β-dihydroxy-4-pregnen-3-one (DHP), are associated with fish adaptive and innate immunity response. Similarly, 11KT is a major steroid for fish puberty. At present, further insights are required in the field of synthetic steroids in fish and their impacts over various roles in fish physiology and future economic importance.

Keywords

Cortisol · Estranes · Spermiation · Puberty · Steroidogenesis · Testosterone

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Introduction

There are different reproductive strategies possessed by fishes with respect to their aquatic environment in which they adopt. But still some regulatory processes related to reproduction are conserved among fishes. In the early phase of females reproductive cycle, i.e. growth of oocytes is triggered by the estradiol hormone, whereas in the late phase, i.e. oocyte maturation prior to ovulation is influenced by the maturation inducing steroid (MIS) (Tokarz et al. 2015). In the case of males, 11 ketotestosterone (11-KT) induces spermatogenesis, whereas the maturation of spermatocytes by initiation meiotic cell divisions is done by MIS (Mananós et al. 2008). The pituitary hormones, i.e. follicle-stimulating hormone (FSH) and luteinizing hormone (LH) play an important role in reproduction by the downstream processes of gonadal steroid hormone synthesis. In the case of commercially important fish species, the ultimate goal for farmers is to produce year-round seed through induced breeding technique. So the development of induced breeding technique is always a challenge for fish breeders and researchers. Steroids like C_{21} steroids, C_{19} steroids, C_{18} steroids, and some endocrine disrupting chemicals (EDCs) are the key role players for gonadal steroidogenesis and ultimately ovulation/spermiation. Among these, some important EDCs are Kepone (Chlordecone), o,p-DDD, Diethylstilbestrol (DES), etc.

Synthesis of Steroid Hormones

Hypothalamus–pituitary–interrenal and hypothalamus-pituitary-gonadal axis regulate steroid biosynthesis in fishes (Fig. 4.1). The cholesterol acts as the precursor for the *de novo* synthesis of all classes of steroid hormones. The removal of the side chain of cholesterol by Cytochrome p450 enzyme (*Cyp11a1*) produces pregnenolone and this process is regulated by steroidogenic acute regulatory protein (StAR).

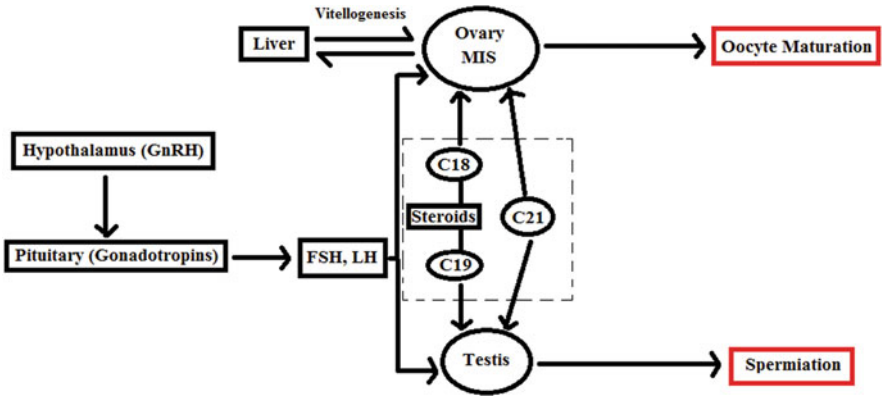

Fig. 4.1 Diagrammatic representation of the steroid effect in fish reproduction

The StAR also helps in transfer of cholesterol across the barrier of the outer and inner mitochondrial membrane. Several enzymes modify the steroid nucleus including side-chain cleavage, Δ5/Δ4-isomerization, hydrogenation, aromatization, hydroxylation, reduction, or oxidation, downstream of this synthesis pathway. The three important bottlenecks in the process of steroidogenesis are cytochrome p450 enzymes cholesterol side-chain cleavage (*cyp11a1*), 17α-hydroxylase/lyase (*cyp17a*), and aromatase (*cyp19a1*). The Cyp11a1 is the only enzyme that converts cholesterol to pregnenolone and that is why regarded as the entrance of the steroidogenesis pathway. Similarly, the Cyp17a is the only enzyme responsible for the conversion of C_{21} steroids to C_{19} steroids and that is why regarded as the next bottleneck in the pathway. This enzyme can use a variety of substrates, but the two most important products (17α-hydroxyprogesterone and androstenedione) cannot be synthesized by other enzymes. The Cyp19a1 i.e. responsible for the production of C18 steroids, is regarded as the most important enzyme for hormonal control of sexual development in teleost fishes (Tokarz et al. 2015).

17β-hydroxysteroid dehydrogenase type 3 (*hsd17b3*) is an essential enzyme for the synthesis of 11-ketotestosterone, the active androgen in fish, whereas type 1 (*hsd17b1*) converts inactive estrone (E1) to active receptor-binding estradiol (E2). 20β-hydroxysteroid dehydrogenase (Hsd20b) converts 17-α-hydroxyprogesterone and 11-deoxycortisol to maturation inducing steroids (MIS) 17α,20β-dihydroxy-4-pregnen-3-one (17,20β-P, or DHP) and 17α,20β,21-trihydroxy-4-pregnen-3-one (20β-S), respectively. The *hsd20b* has so far been the only gene identified in fishes among the genes responsible for MIS synthesis.

Steroid Hormone Receptors

Receptors for steroid hormones are either nuclear or membrane bound. These hormones may act in a genomic or a non-genomic manner depending on the receptor. In the case of nuclear receptors, steroid hormones bind to the respective cytosolic nuclear receptors and act in a genomic mode by binding to their respective response elements on the genomic DNA. The membrane-bound receptors on the cell surface control the non-genomic action of steroid hormones to initiate rapid intracellular responses. Some receptors with their ligand binding and mode of action have been given in Table 4.1.

C_{18} Steroids

C_{18} steroids are characterized by the presence of an aromatic ring in their structure. These are classified into the class of steroids called Estranes. Estradiol is the major C_{18} steroid used in the case of fish maturation studies in females (Fig. 4.1). During oocyte growth, follicular cells receive the pituitary hormone FSH by FSH receptor and secrete estradiol-17β into the circulation. The hepatic synthesis of yolk precursor, vitellogenin (Vg) is due to estradiol-17β. The estradiol-17β travels to the liver

Table 4.1 Important steroids with their ligand binding and mode of action

Receptor	Ligand binding	Mode of action	Reference
Nuclear progesterone receptor (PR or *pgr*)	17α,20β-Dihydroxy-4-pregnen-3-one, 17α,20β,21-trihydroxy-4-pregnen-3-one, progesterone, 17α-hydroxyprogesterone	genomic	Hammes and Levin (2007) and Tokarz et al. (2015)
Membrane-bound progestin receptor (mPR)	17α,20β-Dihydroxy-4-pregnen-3-one, 17α,20β,21-trihydroxy-4-pregnen-3-one, progesterone	non-genomic	
Androgen Receptor (AR)	Testosterone, 11-ketotestosterone, 5α-dihydrotestosterone, 11β-hydroxytestosterone, androstenedione	genomic	
Membrane-bound androgen receptor (mAR)	Testosterone, 11-ketotestosterone, 5α-dihydrotestosterone	non-genomic	
Estrogen receptor (ER)	Estradiol, estrone	genomic	
G-protein coupled estrogen receptor (GPER)	Estradiol	non-genomic	

via blood and helps in the production of Vg (Fig. 4.1). The Vg is then transported to the ovary via the bloodstream and is selectively taken up into the oocyte by specific cell surface receptors. The Vg helps in yolk globules increase and gonadotropin helps in the uptake of Vg by the oocytes. The oocytes acquire yolk protein after completion of the initial growth phase and become competent due to the action of gonadotropins, activins, and other factors (Busby et al. 2010). The estradiol levels are decreased or downregulated due to the downregulation of ovarian aromatase, when the process of oocyte growth and vitellogenesis completes (De Kime 1993).

In the case of males, estradiol-17β plays an important role in spermatogonial renewal like other vertebrates but has no role in proliferation and meiosis. Sex reversal or production of the mono-sex population has been achieved for commercial benefit using 17β-estradiol orally or through the water. The fish spawn is administered with 17β-estradiol to produce 100% female population. This is beneficial for Salmon and Carps, as female is more beneficial from the economic point of view in these groups. These steroids are also easy to administer through feed and gives 100% results. However, for inducing differentiated testis to the ovary, exogenous estrogen alone is not enough. So, the testicular trans-differentiation can be induced by the administration of E2 and simultaneous blockage of androgen synthesis. Recently, genome editing technologies have revealed the disruption of

Cyp19a1a in induced testicular development, which indicates this to be a key gene for estrogen synthesis and differentiation or maintenance of ovary.

C_{19} Steroids

The C_{19} steroids include androgens like testosterone (T) and 11-Ketotestosterone (11-KT). These are major male steroids and have been classified as Androstanes. These are especially male specific (Fig. 4.1). 11-KT was first identified as a major androgenic steroid in the male sockeye salmon (*Oncorhynchus nerka*). FSH stimulates the production of spermatogenesis-inducing steroid, 11-KT during the initiation of spermatogonial proliferation. FSH can induce the production of 11-KTin testis in vitro (Schulz et al. 2010). The 11-KT is involved in the initiation of spermatogonial proliferation towards meiosis. 11-KT initiates spermatogonial proliferation by two members of the TGFβ superfamily, anti-Müllerian hormone (AMH) (Miura et al. 2002) and activin B (Miura et al. 1995). AMH inhibits differentiation of spermatogonia and expression of AMH is suppressed by KT. Activin B induces the proliferation of spermatogonia. Synthesis of activin B is induced by 11-KT. The seasonal changes in the plasma level due to increased spawning maturation and peak levels at the onset of the spawning period is shown by 11 KT (Borg 1994).

The synthetic steroid 17α-methyltestosterone (MT) is commonly included in the fish feed to produce male populations. The administration of MT has stimulated spermatogenesis and steroidogenesis in a number of fish species. Moreover, sex reversal or production of the mono-sex population can be achieved for commercial benefit using MT orally or through the water. The fish spawn is administered with MT to produce the all-male population. This is beneficial for Tilapia and giant freshwater prawn economy.

C_{21} Steroids

Progesterone (P4), 17,20β-dihydroxy-4-pregnen-3-one (17,20βP or MIH or DHP), 17,20β,21-trihydroxy-4-pregnen-3-one (20βS), and 11-desoxycortisol (S) are major C_{21} steroids of this group which have been under research in fish reproduction. Progesterone is an endogenous steroid involved in oocyte maturation. It is also called Pregn-4-ene-3,20-dione and its chemical formula is $C_{21}H_{30}O_2$. It is an important metabolic intermediate in the production of other endogenous steroids and helps in brain function as a neurosteroid and is not a biologically active steroid in the case of fish. 17,20βP or MIH has its chemical formula as $C_{21}H_{32}O_3$ and is the most potent steroid for inducing final oocyte maturation in several species of fish. Similarly, 20βS and S have a chemical formula as $C_{25}H_{36}O_6$and $C_{21}H_{30}O_4$, respectively. S produces this 20βS with the help of 20β-hydroxysteroid dehydrogenase (20β-HSD) in ovarian follicular cells due to the action of pituitary gonadotropin

(Ogino et al. 2016) and stimulates the meiotic maturation of oocytes during GVBD. So C_{21} steroids are of importance in both male and female (Fig. 4.1).

Due to the downregulation of aromatase estradiol concentrations drops. LH stimulates the synthesis of *hsd20b* in the granulosa cells resulting in the synthesis of the MIS. MIS initiates the processes of oocyte maturation (Nagahama and Yamashita 2008). The substrate for Hsd20b, 17α-hydroxyprogesterone, is provided by the thecal cells. The orders of fish, i.e. Salmoniformes, Cypriniformes, Cyprinodontiformes, Siluriformes, Beloniformes, Esociformes, Osteoglossiformes, and Clupeiformes possess DHP, whereas in many Perciformes group fishes 20β-S acts as the MIS (Tokarz et al. 2015). The intracellular signaling is triggered and in turn stimulates all the processes of oocyte maturation when the MIS binds to mPRs. These processes include germinal vesicle breakdown with respect to the first meiotic cell division, spindle formation, chromosome condensation and formation of the first polar body. The MIS regulates ovulation by genomic mechanisms, thus utilizing a nuclear PR and this was earlier hypothesized by Nagahama and Yamashita (2008).

After gametogenesis, the 11-KT level decreases and this results in increased synthesis of the MIS under the action of the pituitary hormone LH. As seen in case of females, MIS stimulates the maturation of spermatocytes by initiating meiotic cell divisions. The production of seminal fluid by the efferent ducts along with the enhancement of sperm motility by alteration of pH and fluidity of the seminal fluid is done by MIS. The milt formation occurs when the matured spermatozoa are released into the seminal fluid and this is stored until the process of spawning (Mananós et al. 2008).

Steroids in Puberty

The transition or transformation from an immature juvenile to a mature adult in the reproductive cycle, i.e. when the fish becomes capable of reproducing is called puberty. The rapid proliferation of spermatogonia in males and the entry into the lipid droplet stage of oocyte development (Sundararaj et al. 1972) are the first stages requiring pituitary input in fish. While defining the endpoint of puberty process, it can be said that the first successful reproduction or the production of the first batch of fertile gametes, i.e. spermiation and sperm hydration in males; ovulation in females (Okuzawa 2002).

11-KT is a firm candidate for the regulation of the onset of puberty in teleosts (Cavaco et al. 1998). The stimulation of Sertoli cells to produce growth factors, thus promoting spermatogonial proliferation leading to meiosis and spermatogenesis is by this 11-KT. Whereas E2 helps in the first pituitary-dependent stage of ovarian development, the lipid droplet stage and increased cortical alveoli stage leading to puberty. The start of puberty is considered as the transition to the first wave of rapid spermatogonial proliferation or to the first batch of oocytes accumulating cortical alveoli, which is regulated by Fsh. The Fsh express the *fshr* gene and that is why can directly stimulate Leydig cells. The up-regulation of expression of pituitary *fshβ* and ovarian *fshr* gene starts prior to vitellogenesis followed by the accumulation of

cortical alveoli and continued through vitellogenesis (Guzmán et al. 2014). The Fsh signaling is important during the accumulation of cortical alveoli in oocytes in the early stages of puberty in fish (Taranger et al. 2010; Rather et al. 2016).

Steroids in Immunity

The immune system of teleost fish possesses both innate and adaptive immune responses. In the case of innate immunity, the epithelium and mucosal tissues act as the physical barriers for fish, while phagocytes, i.e. granulocytes and macrophages, non-specific cytotoxic cells, and eosinophilic cells, including mast cells, represent the cellular effectors. A variety of other molecules like the acute phase proteins, natural antibodies, cytokines, etc. act for the humoral immune response. The adaptive immune response comprises lymphocytes, i.e. B and T cells that acts as cellular components and immunoglobulins (Ig) acts as the humoral component.

Estrogens regulate the immune system of fish and several leukocyte functions. An increase in serum 17β-estradiol (E_2) levels promotes the movement of acidophilic granulocytes from the head kidney after an inflammation response (Chaves-Pozo et al. 2018). In the case of head kidney phagocytes, E_2 also enhances the inflammation by increasing the production of a pro-inflammatory cytokine and the interleukin-1β (IL1β). As compared to the effect of E_2, the androgens on the immune system of fish have been less studied. Moreover, testosterone causes a reduction in IgM secreting cells in peripheral blood, head kidney, spleen, and skin leukocytes. Similarly, 11-KT decreases the number and capability of IgM secreting cells of the spleen, head kidney, blood, and skin. Moreover, 11-KT alone, or in combination with testosterone, downregulates immunogenic expression in macrophages. Interestingly, the innate immune system of fish is stimulated by testosterone and upregulates expression of interleukin 1β, il1b, and some tlr genes.

The production of T-cell derived factors are inhibited by the action of progestins. The E_2 pro-inflammatory effects are seen in several tissues like injured vessels, endometrium, and cervix. The Progestin medroxyprogesterone (MPA) blocks this E_2 pro-inflammatory effect. The activity of both DHP and MPA inhibits the nitric oxide release by activated leukocytes and downregulates the transcription of immune related-factors of pro-inflammatory type I.

Another important aspect related to fish immune response and stress indicator for fish is the release of cortisol into the bloodstream. Cortisol regulates immunity as well as reproduction. The pathways, through which, steroids and cortisol work, are interconnected. The sensitivity of leukocytes to sex steroids is controlled by cortisol and stress conditions, by the regulation of transcription and production of nuclear estrogen receptors (ESRs), G protein-coupled estrogen receptor (GPER1), and local aromatase.

Steroids in Stress

Physiological responses of fish to stressors are of two types, i.e. primary responses and secondary responses. Primary responses include the initial neuroendocrine responses, i.e. release of catecholamines from chromaffin tissue and the stimulation of the hypothalamic-pituitary-interrenal (HPI) axis for the release of corticosteroid hormone. Secondary responses are related to the changes in plasma, tissue ion and metabolite levels, and heat-shock or stress proteins (HSPs), i.e. finally related to metabolism, respiration, immune function, cellular responses, etc. Primary responses are mainly related to steroids.

When fish are exposed to a stressor, the central nervous system (CNS) perceives the response. As a result of this the chromaffin cells, i.e. located in the anterior kidney, release catecholamines via cholinergic receptors. Later cortisol releases from the HPI axis with the help of corticotropin-releasing hormone (CRH) and adrenocorticotropin (ACTH). ACTH in turn stimulates the interrenal cells in the kidney to release corticosteroids. The cortisol and 1α-hydroxycorticosterone are the major corticosteroids in fish (Ruiz-Jarabo et al. 2019). Naturally occurring corticosteroids are cortisol ($C_{21}H_{30}O_5$), corticosterone ($C_{21}H_{30}O_4$), cortisone ($C_{21}H_{28}O_5$), and aldosterone ($C_{21}H_{28}O_5$). All the corticosteroids are C_{21} steroids. All these corticosteroids help in two ways, i.e. a glucocorticoid function for metabolism and growth, and a mineralocorticoid function for the transport of ions and water. But fish lacks aldosterone as a mineralocorticoid and instead 11-deoxycorticosterone is present. This 11-deoxycorticosteroid is an important part of ovarian or testicular steroidogenesis. The gonadal corticosteroidogenesis process is mediated by the presence of cortisol and 11-deoxycortisol in the ovary, sperm, and seminal fluid.

At present, pollutions from pharmaceutical companies are releasing synthetic corticosteroids to the aquatic environment which can have adverse effects on fish. Many synthetic corticosteroids, i.e. betamethasone, prednisone, prednisolone, triamcinolone, etc. need to be studied in fish for their impact on stress and reproduction.

Conclusion and Future Direction

The steroidogenesis process in case of fish and the steroids like C_{21} steroids, C_{19} steroids, C_{18} steroids, and some Endocrine Disrupting Chemicals (EDCs) are important for ovulation / spermiation. These steroid hormones are the key for induced breeding techniques in fishes. The C_{18} steroids in case of female, C_{19} steroids in case of male, and C_{21} steroids in the pathway of both oocyte maturation and spermiation are important. In the near future synthetic and eco-friendly use of steroids and the researches based on them are required in case of fish. Mono-sex culture of commercially important fish species and the successful application of synthetic steroids are of concern at present. Moreover, the residual effects from industrial synthetic steroid pollutants need to be stopped for conservation of riverine fish stocks by avoiding their sterility. Role of steroids in immunity, puberty, and stress needs to be studied further for new outcomes in the field of aquaculture.

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Hormonal Influence on Induced Maturation and Spawning in Striped Murrel, *Channa striata*

5

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Abstract

Striped murrel (snakehead) *Channa striata*, an air-breathing fish, is a delicacy among the consumers for its tenderness, less muscular spines and typical flavour. It is highly nutritive, recuperative and also known for its medicinal values. Unavailability of quality seed is a major constraint in murrel farming. The collection of murrel brooders for instant induced breeding trial from earthen pond is quite difficult and fishes incur considerable amount of stress which results in poor breeding response. Therefore, striped murrel brooders were raised in the concrete cistern with hormonal, habitat and feeding manipulations. Concrete cisterns were provided with soil base and floating aquatic macrophytes to simulate the natural environmental conditions. Fish are fed 2–3% of their biomass, 1% comprising of live insects/prawn/small fish and 1–2% of trash fish and rice bran (3:1). The hormone pellets of HCG were prepared and implanted intramuscular (500 IU/Kg body weight of fish) on dorsal side of fish for better maturation and synchronization of broodstock. Total replacement of water was done at weekly interval to maintain the water quality. It was observed that brooders attained full maturity in 3 weeks after implantation. Brooders in the weight range of 400–800 g were taken for breeding trials and fishes were injected intramuscularly with inducing agents (HCG, PGE and sGnRHa) for successful induced spawning. Then fishes were released into indoor circular breeding pools in 1:1 male to female ratio. They showed peculiar breeding behaviour before spawning. Spawning time was 16–18 h at 26–28 °C and eggs were free floating, spherical, non-adhesive and bright yellow in colour. The standardization of induced maturation and spawning of *C. striata* has paved the way for mass scale seed production of striped murrel.

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Keywords

Hormone · Maturation · *Channa striata* · Induced Breeding · Spawning · Striped Murrel

Introduction

Aquaculture has emerged as industry with huge potential for human consumption as well as for economy. The capture fishery production of world is declining day by day, the aquaculture production of world is climbing to new heights. According to FAO (2018) there is only 14% growth in capture fishery production, whereas 527% of growth has been recorded from aquaculture sector between the years 1990 and 2018. There are many factors which contributed to this trend, viz. implementation of new technologies, better biological information of species, feed supplementation, better water management practice, etc. But one factor that has contributed most effectively is the availability of seed of cultivable species in required quantity and quality. The availability of seed is made possible by induced breeding. Induced breeding is initiated with the help of synthetic hormones which are analogue of the natural hormones present in the fish body. Introduction of synthetic hormones for breeding fish opened a new horizon for farming of commercially important fishes.

The first attempt of using hormone was made in 1930 by Argentina. Brazil was the first country to achieve the successful induced breeding in 1934 and they used pituitary gland to carry out induced breeding. Russia successfully bred *Acipencer stellatus* (Sturgeon) in 1937. Khan in 1938 has tried to breed *Cirrhinus mrigala* by the mammalian pituitary gland extract administration, but it was Chaudhary H. who succeeded to breed *Esomus dandricus* with the pituitary gland extract of catla. Revolution in Indian fisheries started in 1957 when Hiralal Chaudhary and K. H. Alikunhi bred Indian major carps viz. *Labeo rohita, Cirrhinus mrigala, C. reba*, etc. with carp pituitary gland extract. Induced breeding has provided an option to produce the required number of seeds as and when required. From the first attempt of successful artificial breeding to the present day, the number of advances has been observed in this field. The numbers of synthetic hormones were successfully used for induced breeding of fishes. The advances in these hormones have brought more efficiency in the success rate, survival rate as well as total spawning.

The hormone manipulations play a vital role in commercial aquaculture, especially for fish that do not undergo final oocyte maturation and spermiation spontaneously. The hormonal manipulations for the induction of ovulation, spermiation and spawning will continue to play an important role in commercial broodstock management, even after various fish species become properly domesticated (Zohar and Mylonas 2001). The hormone supplementation has been the most effective and generally applicable means of induced maturation in aquaculture.

Hormonal Regulation of Reproduction

Environmental factors like rise in temperature and rhythmic light fluctuation during day and night impulse the central nervous system which regulates the secretion of gonadotropin releasing hormone (GnRH). This GnRH stimulates the production of gonadotropin from the gonadotrophs cells of pituitary gland. Gonadotropin is found in two forms: gonadotropin (GtH-I) and gonadotropin (GtH-II). Gonadotropin (GtH-I) initiates the secretion of estradiol from follicle cells, viz. theca and granulosa cells. Gonadotropin (GtH-I) is responsible for stimulating the theca cells to produce the testosterone from cholesterol precursor. Cholesterol molecules then go under side chain cleavage which results in pregnenolone which again turns into progesterone and followed by 17-alpha progesterone which is converted in androstenedione which finally becomes testosterone. Testosterone thus produced in theca cells enters in granulosa cell which also contains GtH-I receptor. Now in granulosa cells this GtH-I binds with the testosterone which initiates the process of conversion of testosterone to estradiol. Estradiol is catalysed by aromatase enzyme. Estradiol when reaches liver cell through blood circulation produces vitellogenin. When this vitellogenin enters into oocyte cytoplasm they convert into lipovitellin and phosvitin and get deposited as yolk granules or platelets.

The maturation of oocyte during post-vitellogenic phase is regulated by GtH-II, produced from gonadotrophs. The changes in oocyte are also brought about by various hormones like maturation inducing hormone (MIH) and maturation promoting factor (MPF). These hormones help oocyte to become mature and ready for fertilization.

Induced Breeding Through Hypophysation

Hypophysation is an induced breeding technique in which the pituitary gland extract is prepared and administered to the target mature fish (Fig. 5.1). These pituitary glands are collected from the fishes of same species or very close relative to the fish, i.e. same genus or same family. These glands are collected freshly or preserved ones can also be utilized for the purpose. Generally, two doses, i.e. primary and final doses, are required for the female brooders, whereas male brooders require only one during the final dose administration to the female fish.

The fishes like Indian major carps, common carp, tilapia, trout and the salmon are some major fish species who responded well to pituitary gland extract. Though the pituitary gland administration brought a revolution in fish seed production, the farmers faced late response and longer latency period with the pituitary gland extract administration. The collection of required number of pituitary glands at appropriate time of required species, lack of good quality of pituitary gland, adverse effects of pituitary hormones have been experienced by the farmers as well as researchers. Keeping this in mind, later the people moved and searched for some artificial or synthetic hormones which may act like the natural hormones.

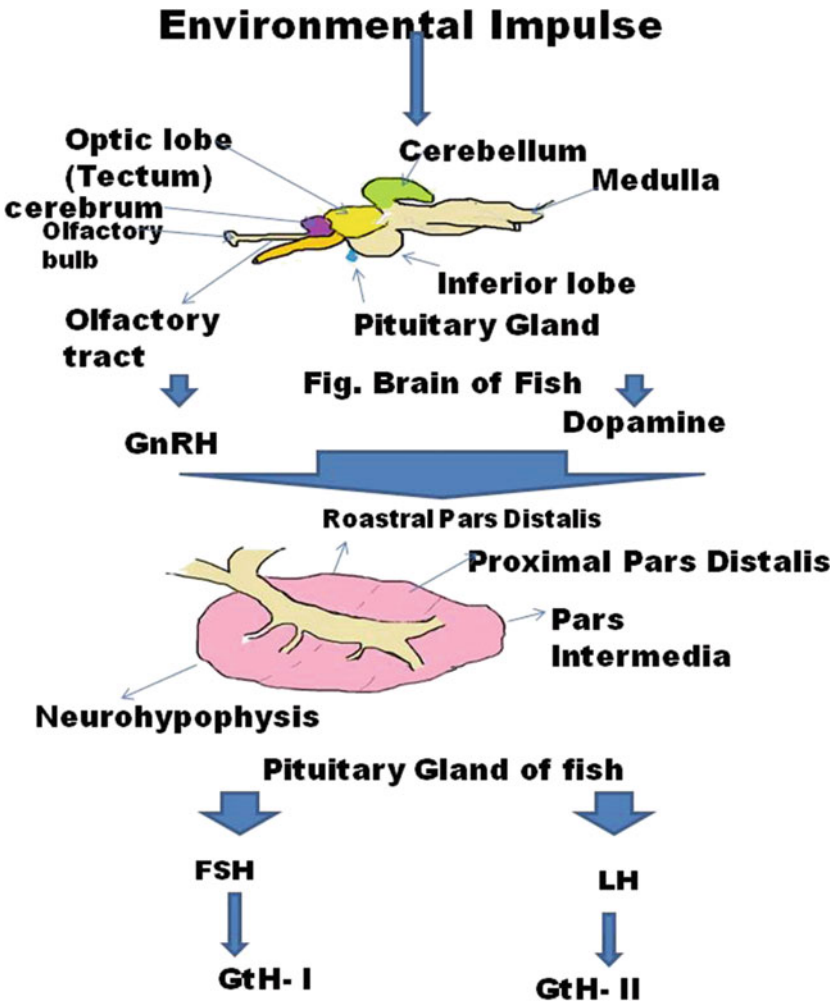

Fig. 5.1 Hypothalamic–pituitary–gonadal axis

Synthetic Hormones in Induced Breeding

Synthetic hormones or their analogues are the synthesized chemicals which has similar structure to the natural hormone. Sometimes there may be replacement of amino acids sequences to make it more receptor friendly and act faster. The various chemical had been used in inducing breeding in fish since its first sucessful breeding attempt and a number of advances evolved during this journey. The advances in these hormones have brought more efficacies in induced breeding (Thomas et al. 2003). They are categorized into four groups:

(a) Gonadotropins
(b) Gonadotropin releasing hormone analogue
(c) Steroids
(d) Prostaglandins

Gonadotropins

There are two types of gonadotropins reported in fishes: FSH and LH.

It has been observed, the plasma hormones were found in greater level which predominantly brought by the action of LH (Prasad et al. 2015; Schulz and Miura 2002). The main function of LH is to trigger the androgen production by Leydig cells through LH-receptor. FSH dosage systems with high specificity and sensitivity are available for only a few species. The function of FSH is involved in FSH-receptor binding mechanism associated with sertoli cells. FSH is associated with proliferation activity of sertoli cells. Another important thing about FSH is its association in modulation of growth factor. Reduced amount of milt is reported due to lower plasma levels of LH during spermiation period (Mananos et al. 2002; Mylonas and Zohar 2001). LH has exhibited its effect on PGF synthesis by the uterus and this is time bound response which appears in middle to late luteal phase in PGF synthesis (Shemesh et al. 2015). Gonadotropins used in fish induced breeding successfully are luteinizing hormone of mammals (LH), human chorionic gonadotropins (HCG), puberogen and pregnant mare serum gonadotropins.

Gonadotropin Releasing Hormone Analogue

To get more effective in achieving maximum fecundity in asynchronous species with long breeding season, GnRHa-delivery system is found to be more effective. GnRH analogues are being prepared by replacing the amino acids from 6, 7 and 10 position. GnRHa based hormones have been recorded as very successful for almost every species with very few exceptions. Various combinations of GnRH analogue and dopamine antagonist with various trade names are commercially available in the market. Among these combinations GnRHa and domperidone have been most successful. Ovaprim, Ovatide, Gonopro, Ovapel and Wova-FH are most commonly used synthetic hormones aquaculture.

Steroid Hormone

Steroids like, e.g. 11-deoxycorticosterone acetate (DOCA), antiestrogen tamoxifen (1-(p-Dimethylaminoethoxyphenyl)-1,2-diphenyl-1-butene), progestins such as 17a-hydroxy-20b-dihydro progesterone were successfully used for the induced breeding. Sunadarraj and Goswami have successfully bred the *Heteropneustes*

fossilis using DOCA. But its use is very limited as some of steroid need primer like pituitary extract for success and thus make the whole process again lengthy.

Prostaglandins

Prostaglandins, especially in goldfish and trout have shown positive results in effective induced breeding. Prostaglandins are found to be involved in follicle rapture during ovulation. Among these prostaglandins, F2 alpha has shown most prominent results.

Broodstock Management

General Management

In a simple term, the broodstock management is a combined effort majorly of environmental balance and feeding management. Environmental management involves majorly photoperiod, water temperature or spawning substrate. In favourable temperature range fishes respond positively, beyond the optimal ranges the reproduction process may behave abnormally. Collection of target brooders is also a huge task. Those fishes which might have already underwent one spawning may be considered as ideal brooders (Gupta et al. 1995). After collection from wild or culture ponds, they are conditioned in brooder ponds where they are kept in special care with special reference to feed and optimal water quality parameters. Mishandling of these fish may lead to stress which ultimately lead to breeding dysfunction. These fishes are fed with higher protein level with the ingredients of their feeding habit means if the fish is herbivorous the protein content must be of plant origin and if the fish is carnivore then the protein content must be added from animal origin. They are fed with usually @ 2–4% of their body weight. Some fish needs vegetation or hiding objects, accordingly they are provided with the necessary arrangements. The other factors which are point of consideration are age of fish, type of water body, availability of water in volume and depth, carrying capacity and stocking density, spawning behaviour of fish.

Induced Maturation

Hormonal treatments may be different in species with synchronous spawners (total spawners) and asynchronous ovarian development (multiple/batch/fractional spawner) (Tyler and Sumpter 1996) because of their different biology and management practice. A single or double GnRHa injection dose is found to be effective in synchronous fish (Mylonas et al. 1992), but for asynchronous species with a longer breeding season GnRHa-delivery systems may be more effective to obtain full fecundity (Barbaro et al. 1997; Larsson et al. 1997; Mugnier et al. 2000; Zohar

and Mylonas 2001). Hormone maturation practices in fish may involve priming or hormone pellet implantation. The successful induced maturation and breeding has been observed in European catfish, *Silurus glanis* (Leonardo et al. 2004); catfish "cachara", *Pseudoplatystoma fasciatum*; Japanese catfish, *Silurus asotus* (Kumakura et al. 2003); spotted sea bass, *Lateolabrax maculatus* (Lee and Yang 2002); Ocellated puffer, *Takifugu ocellatus* (Chen 2005); Pikeperch, *Sander lucioperca* (Zakes and Szczepkowski 2004); tench, *Tinca tinca* (Rodríguez et al. 2004) usually with single dose whereas two doses of GnRHa injections along with a DA antagonist have been used successfully in koi carp, *C. carpio* (Arabaci et al. 2004); lake mullet, *Chalcalburnus tarichi* (Arabaci and Sari 2004) and wild catfish, *Silurus asotus* (Wen and Lin 2004). These hormonal treatments may or may not require artificial stripping or artificial insemination. In asynchronous fish, GnRHa-delivery systems help in induction of multiple oocyte maturation and ovulation cycles in fishes like, seven ovulations in 10 days in the dusky grouper, *Epinephelus marginatus* (Marino et al. 2003), white bass, *M. chrysops* within 3 days two consecutive spawns (Mylonas et al. 1997) and greater amberjack, *Seriola dumerili* (Mylonas et al. 2004), five spawns in 7 days in the barramundi, *Lates calcarifer* (Almendras et al. 1988), in striped trumpeter, *Latris lineate* in 2 weeks five ovulations (Morehead et al. 1998), one to four ovulations within 7 days in the black sea bass, *Centropristis striata* (Watanabe et al. 2003). GnRHa-delivery systems have exhibited remarkable increases in fecundity, by increasing the number of brooders which is under oocyte maturation and the number of spawning in each breeding season (Barbaro et al. 2002; Larsson et al. 1997).

Importance of Murrel

Murrels are very important air-breathing indigenous freshwater fishes of India. They are commonly known as snakehead or serpent-headed fish due to the elongated and cylindrical body, flattened head and presence of eyes on the anterior part of head. The commercially important murrel species in India are *Channa striata*, *C. marulius* and *C. punctatus*; they fetch high price (Rs. 300–700/kg) in many states like Madhya Pradesh, Bihar, Uttar Pradesh, Haryana, Andhra Pradesh, Karnataka, Telangana, Tamil Nadu and all North-East States. They have high consumer preference because of nice flavour, meaty flesh with few intramuscular bones and medicinal value (Sahu et al. 2012a, b). These fishes are considered as high-value food fishes and marketed in live condition, as they can be kept alive for several hours outside water (Kumar et al. 2011; Kumari et al. 2018). The good growth rate, high consumer preferences, lucrative market value and their ability to withstand adverse water conditions make them suitable candidate species for freshwater aquaculture (Kumar et al. 2012).

Reproductive Biology of Murrel

Identification of Brooders

The males and females can be easily distinguished during the spawning season by their secondary sexual characters. Females exhibit slightly bulged abdomen, round and reddish vent, anal papilla is broad and blunt with reddish dots, whereas males exhibit pale vent, and the anal papilla is prominent with a pointed tip. *Channa striata* female weighing 300–600 g and male weighing 400–800 g gives better breeding performance under hatchery condition (Kumar and Mohanty 2018) (Figs. 5.2, 5.3, 5.4, 5.5, 5.6 and 5.7).

Broodstock Management of Murrel

Induced Maturation

Hormone supplementation has been the most effective and generally applicable means of induced maturation. Gonadal maturation has various stages and different sex steroids are important at each stage, but gonadotropins influence the production of all of them. Depending on the maturity of fish the gonad will be more or less responsive to gonadotropin. The mammalian gonadotropins have an edge over fish pituitary because of their easy availability, uniform potency, low cost and long shelf life. Among the mammalian gonadotropins, HCG has proven record for best results in fish breeding (Gupta and Gupta 2006).

Fig. 5.2 Male striped murrel

Fig. 5.3 Female striped murrel

Fig. 5.4 Hormone pellets

Pellets containing hormones are implanted in the fish for sustained-release of hormone to advance and synchronize maturation of gonads. Twenty parts of cholesterol and one part of binder (Gelatin:Gum Acacia 1:1) are mixed well with the help of mortar and pestle. The required quantity of HCG hormone is added and mixed thoroughly. Ethyl alcohol (50%) is added and continuously mixed to get a gel-like consistency. The HCG hormone pellets of 2 × 4 mm size (oval/rice-grain shaped)

Fig. 5.5 Hormone pellet implantation

Fig. 5.6 Striped murrel eggs

and weighing 15–20 mg are made by hand. The hormone concentration of the pellets is adjusted according to the requirements and it may range from 200 to 1000 IU of HCG. The GnRHa pellet is also made similarly except that the mixture is kept at 35 °C for drying to get a gel-like consistency. The pellet is implanted intramuscularly on the dorsal side of the female and male fish. Small incision is made on the

Fig. 5.7 Striped murrel spawn

skin and the sustained-release hormone pellet is pushed into the muscle manually. After proper implantation of pellet, antibiotic cream is applied on the incision. It has been observed that the wound gets healed completely in a week and only a tiny black scar could be seen after two weeks. The oval/ rice-grain shaped pellets are easy to implant and also it requires very small incision in the fish muscle. Therefore, fish are less stressed and the chance of rejection is also minimized. Brooder fish are implanted hormone pellet at the rate of 500–1000 IU HCG/kg body weight in the month of February. By the end of April gonads matured fully and remained mature till September. The gonad development was good and about 80–90% of brood fishes showed gonadal maturity. These brooders showed better spawning performance upon induced breeding under hatchery condition (Kumar and Mohanty 2018).

Feeding and Environmental Management

Fish are fed 2–3% of their biomass, 1% comprising of live insects/prawn/small fish and 2% of trash fish and rice bran (3:1). Recently, ICAR-CIFA, Bhubaneswar has formulated a sinking pellet feed for better maturation of striped murrel broodstock. The brood fishes fed with the broodstock diet showed better breeding performance. The concrete tank bottom is provided with 15 cm thick layer of soil. Floating aquatic macrophytes (water hyacinth/pistia) are added and maintained in about 20% water-spread area to simulate natural environment. Total replacement of water is done at weekly interval to maintain the water quality (Kumar and Mohanty 2018).

Induced Breeding

Striped murrel does not require water circulation in breeding pool and spawns successfully in stagnant freshwater. Breeding pool is filled with water (26–30 °C) and one-fifth of the water surface area is covered with floating aquatic macrophyte (water hyacinth). It is important to keep at least two feet of freeboard in breeding pool, which has a net covering, to avoid fish jumping out during spawning. The floating aquatic macrophyte is not essential but it probably helps in simulating a natural environment and this fish usually prefers to lay eggs in a nest made of aquatic vegetation. Any of the inducing agents can be injected intramuscularly to the female and male fish at the following doses: HCG @ 2000 IU/kg and 1500 IU/kg body weight; carp pituitary gland extract (PGE) @ 10–15 mg/kg body weight and 7.5–10 mg/kg body weight; Ovatide/Ovaprim @ 0.6 ml/kg body weight and 0.4 ml/kg body weight, respectively. Injection is given at the base of pectoral fins. Before spawning they move in pair and chase each other. Spawning occurs after 16–18 h. Eggs are spherical, non-adhesive, free floating and straw yellow in colour. Fertilized eggs are transparent and unfertilized eggs are opaque/white. The size of the fertilized eggs ranges between 1.1 and 1.4 mm. Eggs are collected with the help of plankton net and kept in FRP tanks for hatching. The average fecundity is in the range of 10,000–15,000 eggs/kg body weight. Incubation time is 16–18 h. The fertilization and hatching rate range between 75 and 98% and 70 and 95%, respectively; the success rate was better with carp pituitary gland extract. Size of the newly hatched larvae is 3.0–3.5 mm. Feeding starts 72 h after hatching. Microzooplankton, especially rotifer is the preferred food of hatchlings (Kumar and Mohanty 2018).

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Reproductive and Breeding Biology of Snowtrout *Schizothorax niger*

6

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Abstract

The information on the reproductive biology of *Schizothorax niger* (snowtrout) will help to know the inception and period of maturity stages, age at first maturity, sex ratio, spawning, and fecundity of this fish. These parameters are fundamental and will help in research, stock assessment, and management of this fish species. Size at first maturity of *Schizothorax niger* is 14.82 cm. The sex ratio of *Schizothorax niger* male:female is 1:2.44. During spawning season the gonadosomatic index (GSI) which is recorded is highest in the month of February for females (15.75) and particularly for males it is (12.5) signifying *Schizothorax niger* matures during the month of February and spawns during March–May and peak season in the month of March ensuring in an unexpected decline in GSI values of the fish. The above data claims that the fish is an annual breeder. Absolute fecundity is 11709 ± 8016 and at some time relative fecundity is 4645 ± 1804. The main plan of this chapter is to provide detailed information about the reproductive and breeding biology of snowtrout, which will be useful for devising the strategies for management of stock of this particular fish.

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Keywords

Reproductive biology · Snowtrout · Nigeen Lake · Kashmir

Introduction

Fish have been playing a pivotal role in the economy of the country as it helps as it helps in augmenting food supply, generating employment, raising nutritional level and earning foreign exchange by export. Fish is in particular vital in the rising world. The capture fisheries and aquaculture sector are of fundamental importance to the Asia-Pacific region in terms of food security, revenue generation and employment. The people from the countries like Bangladesh and Cambodia get 75% of the daily intake protein from fish (Mir et al. 2012). Fish is an important source of vital nutrients including quality animal proteins, u-3 polyunsaturated fatty acids (u-3-PUFAs) EPA and DHA (especially the marine fishes) and micronutrients (especially the small indigenous fishes), except carbohydrates. Since time immemorial, fish has been the staple food in South Asian countries including China, India, Bangladesh, Philippines, Korea and Japan. Fish is important source of food protein and single source of high quality protein with all essential aminoacids and essential fattyacids. Fish plays an imperative task in the fiscal development of countries by way of aquacultural practices and ornamental fisheries. Fisheries and aquaculture directly or indirectly play an indispensable role in the livelihood and food defense of millions of people approximately in the world. The capture fisheries and the aquaculture provided about 170.9 million tons of fish in 2016. The food fish supply increased from an average of 9.9 kg in 1960 and 18.5 kg in 2011 and a further increase in fish consumption to 20.3 kg according to world per capita. The world's freshwater catch during the last few decades from wild stocks has shown an incredible increase. The world's major fisheries continue to produce substantial yield, several of them have been severely overfished, and many more stocks emerge to be direction towards exhaustion. Appropriate fishery supervision is needed to have sustainable acquiesce from these water resources.

The aquatic resources of Jammu and Kashmir range from ponds, water flows, wetlands, springs, rivers to capacious lakes in the plains and the high altitudes. The waters of Jammu and Kashmir play an important role in the socio-economic status of the people. Kashmir is quite diverse from the rest of the country in terms of ichthyofauna and is mainly represented by the cold-water Schizothoracine group. From Kashmir division only forty species of fish have been reported, the majority of them belong to genus *Schizothorax*. Presently only 21 species of fish species are found water bodies of kashmir as resported by (Bhat et al. 2010). Jammu waters report about a hundred species of fish. Presently there is lack of data which need to clarify as what all the species are present in water bodies of Jammu and Kashmir. There should be some authority body who will look after what all the species to get extinct from water bodies of Jammu and Kashmir. The proper knowledge of extinct species will help to conserve fish biodiversity in the region (Balkhi 2007). Among

the inland fisheries in the country, the cold-water fisheries research is almost only three decades old. Jammu and Kashmir is blessed with innumerable cold-water resources and has tremendous potential for the development of fisheries. The state exhibits great diversity in its geo-climatic conditions. The Kashmir valley is situated in a midwestern Himalayas at an average altitude of 6000 ft. a. s. l. and exhibits temperate climate. The cold waters of Kashmir are generally located at 1500 m and above mean sea level. Fish fauna of the Kashmir valley is quite different from the rest of the country and is dominated by the Schizothoracine group. Fish fauna studies in Kashmir were previously made by Heckel (1838). During his study, he reported sixteen species of fish that were all new to science and published them in his book "*Fischeauscaschmir*".

The most important component of freshwater fisheries in India is the cold-water fisheries. The Kashmir valley is famous for its matchless picturesque beauty. It has been rightly called the paradise on the earth. It is spread over an area of about 39921.8 hectares (Bhat et al. 2010). It has been bestowed with enormous and cleanest resources of water in the shape of lakes, streams, rivers, etc. The peculiar natural conditions of the natural water bodies may become leading centers of cold-water fisheries which have a major contribution to make the blue revolution a success. These freshwater fishery resources are prime centers of cold-water fisheries.

The most beautiful natural resource of Kashmir is Dal Lake which forms the center of Kashmiri civilization. Nigeen Lake forms the western part of the famous Dal Lake (Qadri and Yousuf 2004) and is situated at a distance of about 9 km distance to the north of the city center, at an elevation of 1584 m (ASL). The area of Nigeen Lake is about 4.5 sq. km with a maximum water depth of 6 m (Siraj et al. 2004) and is connected with Dal Lake at Ashai Bagh, Saidakadal. The lake is known for aquatic sports and has been the focal point of tourist attraction. However, it is under great stress due to the influence of human settlements along the shore as well as within the basin itself. The freshwater area of the lake has got reduced as a result of the creation of floating gardens for vegetable cultivation. The unabated encroachment by way of floating gardens has not only squeezed most of the open water to narrow channels but has also led to the deterioration of the water quality, which has badly affected the fish population of Nigeen Lake. It has been reported that there was a mass fish kill during 2012 (FOFY, Report). The cause of the fish kill besides other physicochemical parameters is presumed to be due to an imbalance in fish stocks in the lake. So assessment of the fish stock population in Nigeen Lake is of immense importance.

Endemic fishes of Kashmir valley belong to genus *Schizothorax* which dwell in highland waters in all the three divisions of Jammu, Kashmir, and Ladakh. The *Schizothoracids* (Snowtrouts) are native fish species and are commercially important because of ample market claim (Yousuf et al. 2001; Bhat et al. 2010). *The Schizothorax niger* which is locally called as "Ale gad" is an esteemed home-grown freshwater teleost found in flat land lakes of Kashmir. This fish belongs to the family Cyprinidae and order Cypriniformes is the inexpensively most priceless and shows potential food fish of Kashmir. The food of *Schizothorax niger* is detritus, periphyton, and the associated invertebrate fauna (Das and Subla 1969).

Schizothorax species are leading in the torrential mountain streams of the Himalayas and Central Asia. They are restricted to cold regions and localities having snow-fed waters and are thus commonly called snowtrouts. They are supposed to have the Central Asiatic in origin. Such fishery resources are delimited by inner and southern slopes of Hindu Kush, Karakoram, and the inner ends of North-Western Himalayas and Suleiman range and ultimately enter the flat land lakes. The Kashmir valley possesses water bodies which are consecrated with Schizothorax species, viz. *S. niger, S. plagiostomus, S. esocinus, S. curvifrons, S. labiatus*. Among the Schizothoracids, *S. niger* is the most essential from the commercial point of view. While all other Schizothoracine species show breeding migrations, moving upstream to spawn, *Schizothorax niger* among them does not show any such migration and spawns in the shallow peripheral areas of the lakes. Schizothoracines play an imperative position in the capture fishery of flat land lakes of the valley, especially Dal Lake, Nigeen Lake, Manasbal Lake, and rivers Jhelum and Sindh (Bhat et al. 2010).

Schizothoracids are highly valued fish preferred to other fish species. Local people have developed a craze for *S. niger*. Most of the species of the fishes present are exploited in one way or the other and are facing immense pressure due to pollution and human interferences in their habitats at various levels. With the introduction of common carp which has invaded all the meandering rivers, floodplain lakes, and wetlands the Schizothoracids have been under tremendous pressure for survival. Furthermore, the fish population in the Kashmir lakes has shown a declining trend which is attributed to the encroachment of water bodies, urbanization, agricultural activities, eutrophication, and overfishing. The decline in the Schizothoracids population is also due to the destruction of breeding and feeding grounds especially shallow peripheral areas of the lake (Balkhi 2010).

The unrestricted fishery resource executive is based on complete knowledge of fishery biology and ecology (Greiner and Gregg 2010). A study on the reproductive perspective of fish is one of the most vital biological parameters for fish population estimation (Mekkawy and Hassan 2011). The breeding season, the age at first maturity, reproduction, and fecundity are the important parameters in reproductive biology (Jakobsen et al. 2009). Morgan recommended reproductive biology as a significant parameter in the determination of productivity. It displays the characteristic of the population to exploitation and also throws light on the turbulence caused by anthropogenic actions. Fecundity plays a solution function in the estimation of commercially important fish stock and provides a complete image of population dynamics. In order to evaluate the potential consequences of alternative harvest policies and its effects of fishing on fish populations the fishery stock assessment models are designed for the demographic analysis.

The biology of the fish is of prime importance in the field of fishery science. For understanding biology anatomy and morphology form its basis. The study of morphometric characters in fishes is very important from a taxonomic point of view (Balli et al. 2007). The study on length–weight relationship is of significant magnitude in fishery because it shows the significance of fish population dynamics and patterns of growth on fish stocks. The length-weight relationship of any fish is a requirement for the study of its population and has noteworthy importance in studying the growth, gonadal development, and general well-being of fish

population (Le-cren 1951). The life histories of fishes from different locations can be studied by comparing the L-W relationship of the fish species (Petrakis and Stergiou 1995).

For the nourishment and replacement of progeny in maintaining every living organism including fish is the reproduction. The fish stocks usually get exhausted in water bodies from unremitting cropping by the fishermen. Conversely, they get replenished through the process of reproduction (Bankole 1989). The size at first maturity and sequential variations in the gonadosomatic index and gonadal maturity are used to review the reproductive blueprint of fish species. The reproductive budding of a particular length/age group is very vital for its selection for breeding and seed production. The availability of feed is one of the factors which affects sexual maturation. The quantity of feed eaten largely determines the accessibility of the year classes, as well as the growth rate and sexual maturity. The main goal of this chapter is to provide comprehensive information about the reproductive and breeding biology parameters of snowtrout, which will be helpful for stock valuation and fisheries management of this fish.

Growth Curve

The increase in length and weight leads to physiological process called as growth. A growth curve is obtained, when the length or weight of a fish is plotted against a specified time known as vector diagram. This curve appears as a sigmoid one in different localities. The relationship between fish length and weight is considered to be of major importance. The length–weight relationship is often used to figure out the standing stock biomass (Smith 1996 and also to estimate fish weight by knowing its length. The growth rate is a serious component of the life history of all species (Musick 1999). For understanding population dynamics and maintaining sustainable yields in fisheries estimates of age and growth are the pre-requisites (Campana and Thorrold 2001). The growth parameters, viz. length infinity or asymptotic length (L_∞) and growth coefficient or curvature parameter (K) of the von Bertalanffy growth equation (VBGE) model are essential in fisheries science. The growth coefficient K parameter is closely related to the metabolic rate of fish. A high value of K indicates a high metabolic rate and such fishes mature at early age (T_{max}) or size at first maturity (L_m) which is large in relation to their asymptotic length (Qasim 1973). Determination of age is one of the most significant parameters for the explanation of population structure and its dynamic behavior (Mugiya 1968). Reliable estimation of growth parameters is a key issue in increasing sustainable fisheries stock assessment and management for a given fish species (Walters 1998).

For solving problems in fishery management studies on age and growth of fish are of prime importance (Polat et al. 2001). Awareness of age structure of fish populations allows estimation of growth, mortality, and recruitment and thus contributes to calculations of production rates of populations (Hilborn and Walters 1992). Depending on favorable or adverse ecological conditions the growth of fish is not consistent throughout the year in its lifetime and shows discontinuous fast and slow growth rate (Singh and Sharma 1995).

Annual Growth Rate of Fish

The longevity of the fish and its annual growth rate are two of the most important factors that determine the potential fish yield from a water body (Tandon and Johal 1996). Increase in length, or weight, or both with rising age is called as growth. Determination of growth at successive years of age is fundamental for assessing the rate of recuperation of a given stock under natural and exploited conditions (Bal and Rao 1984). Age and growth in fishes occupy a key position in fishery science. The good indicator of the health of individuals and the population as a whole is termed as growth and is intensively used to study aspects of fish biology. It varies with sex, age, and several other biotic and abiotic factors. Age gives an idea about sexual maturity, spawning time, catchable size, growth rate and longevity. Knowledge of all these parameters is essential in fisheries production. The estimation of the mortality rate helps to assess the sustainability of the fish stock (Bal and Rao 1990). Accurate and precise age are used to build reliable stock assessment.

Length at First Maturity

The minimum length at which particular fish attains maturity is termed as length at first maturity (L_m). The information of length at maturity (L_m) is vital in the supervision of fish stocks as berried females should not be caught during breeding season in order to allow them to spawn once in a year. The minimum size of maturity is essentially important in adjusting the mesh size of fishing gears to make certain that the smaller fish and berried females which have not spawned even once may have a chance to run away (Somnvanshi et al. 1998). The length at maturity (L_m) as described by Hodgkiss and Man (1978) is the minimum length at which at least 50% of the population originates to be mature. Maturation can be deliberate by the development of ova in unusual maturity stages which are notable by macroscopic and microscopic stages of the ovary. Throughout all the months fish are immature (I and II) except February and March, whereas fish mature (stages III, IV, and V) in rest of the months. Spent condition (VI and VII) is reported mainly during April of the study period. The *Schizothorax niger* spawns once in a year. The gonadosomatic index of S. niger is reported higher in month of March for females and for males in April and has been reported larger ova size of 2.5 mm in March is also in full conformity to the spawning season which was from March to mid-May. The fish attains maturity at 18.50 cm (Table 6.2). Sunder in 1984 reported length at first maturity in Schizothoracids to be 250 mm in *S. longipinnis*. Sunder observed that a small number of fish in ripe and spent conditions appeared in 210–220 mm size group, which may be roughly the minimum size at which the fish starts spawning. The common carp both the sexes are completely mature by age 2+ when they attain a weight range of 100–150 g was reported by Raina (1978). Matsui in 1957 reported that common carp in Japan matures in the third summer. The tropical fishes reach sexual maturity earlier than the temperate zones and observed length at first maturity in *Boops boops* as 130 mm by El-Agamy et al. (2004).

Age at first sexual maturity of *Schizothorax niger* is reached at size of 18.50 cm. The total length with corresponding age being 3 yrs (2+) of *Schizothorax niger* is characterized by comparatively late maturation compared to other cyprinid species like carps for whom size and age at first maturation are much smaller and younger (Rutaisire and Booth 2005). The reason is explained by the fiction that the temperature remains habitually low in water bodies of Kashmir. The rate of development and growth are very dependent on temperature as fishes are poikilotherms as explained by Pawson et al. (2000) and recommended that ecological conditions of water temperature and photoperiod significantly control the sexual maturity of fish and the same has been found proper for *Schizothorax niger*.

Sex Ratio

The parameter sex ratio of *Schizothorax niger* for males and females was calculated on monthly basis as shown in Table 6.1 and was obtained at 1:2.44. There was significant dominance by females in the months of March–November, and there was a preponderance of catch of males in the months of April which was done by chi-square values. The sex ratio was analyzed with respect to different months and was reported (male:female) as 1:2.44. There was significant dominance of females in the month of March indicated by chi-square (x^2) test. As per sunder et al. (1984) they found that proposition of more females than males in the same species of fish. There they found clear dominance of female species compared to male. The knowledge about the sex composition of catches helps in accepting whether any differential fishing exists and if so what probable bearing it has on the fishable stocks (Kesteven 1942). The proportion of male and female in natural environment should be in the ratio of 1:1. Segregation of fishes are based on certain factors viz., feeding habitat, breeding behavior and migration The variations may specify differences in the growth rate of the two sexes (Qasim 1966). As reported by Shafi and Yousuf

Table 6.1 The sex ratio of *S. niger*

Months	Males	% Males	Females	% Female	Total %
December-13	11	3.6	14	4.6	8.1
January-14	7	2.3	22	7.2	9.4
February-14	7	2.3	22	7.2	9.4
March-14	2	7	33	10.7	11.4
April-14	18	5.9	14	4.6	10.4
May-14	12	3.9	13	4.2	8.1
June-14	9	2.9	21	6.8	9.8
July-14	10	3.3	16	5.2	8.5
August-14	2	7	13	4.2	4.9
October-14	6	2	21	6.8	8.8
November-14	3	1	31	10.1	11.1
Total	87	28.3	220	71.7	100

(2012) sex ratio of *Carissus carissus* was found more female fish compared to male species and again observed that similar trend was also reported by Olurin and Aderihighe (2006) in case of African snakehead, *Parachanna obscura*, from River Oshun. Natural phenomenon is a powerful mechanism for population regulation. It regulates male and female ratio in equal propositions. Sex ratios can differ from an expected equal proportion of males and females, carrying substantial implications for our understanding of how mating systems evolve. Male:female sex ratio indicates the quantity of males and females in the population 261 and is expected to be 1:1 in nature. Any divergence in sex ratios across taxa carries substantial evolutionary relevance, influencing sexual selection and by playing a ubiquitous role in shaping population demographics and mating systems. Fishing, environmental factors and behavior of sexes etc. are the factors which will lead to sexual divergence (Bal and Rao 1984). Fagade et al. (1984) ascribed this natural phenomenon as a powerful mechanism for population regulation.

Similar reports from marine sector for sex ratio reported from Philippines may be 1:1 and also in the west coast of India (Muthiah 1985). It was greater than 1 in the west coast of Thailand (Yesaki 1994) and in Taiwan but was smaller than 1 in the South China Sea (Klinmuang 1978) and in Seychelles (Yesaki 1994). Chi-lu et al. (2005) reported a sex ratio of 0.559. Chen et al. reported that female and male ratio was estimated at 1:1. From Indian Ocean it was observed that male:female ratio was 1:1 but males predominate in the adult stage IOTC (2006).

Gonadosomatic Index

This is a periods which starts from an embryo and till period of senility, during which the organism slows down and eventually dies is the life cycle of a fish (Nikolsky 1963). During adult period, organism is able to reproduce to continue their life cycle. The link in the life cycle of a fish which, in connection with other links, ensures the continuation of the species is regarded as reproduction.

Moyle and Czech Jr. (2000) reported that success of any fish species is eventually determined by the ability of its members to reproduce fruitfully in a fluctuating environment. The four key components of reproduction are sexual maturity, reproductive period, gonadosomatic index, and fecundity which are vital characteristics essential for understanding life history of fish species (Cortes 2000). The mortality, the size, and age at first sexual maturity openly influence the reproductive potential of a species, partly determining the duration of the spawning period for each individual and also influencing the quantity of the spawning stock (Sinovcic and Zorica 2008).

The fish spawning in temperate and tropical region is an indicator of GSI. The gonadosomatic index (GSI) increases with the maturation of the fish and becomes maximum during maturity and decreases abruptly when the fish becomes spent. According to Biswas et al. (1984) maturity index is an indirect method for estimating spawning season of a species. The gonadosomatic index (GSI) of *Carassius carassius* was observed by Shafi and Yousuf (2012) and he reported higher values

Table 6.2 The gonadosomatic index of *S. niger* on monthly basis

Month	Male	Female
December-2013	9.2	9.45
January-2014	11.3	13.5
February-2014	12.5	15.75
March-2014	9.1	10.2
April-2014	5.51	5.34
May-2014	4.25	4.45
June-2014	4.1	3.3
July-2014	4.4	4.4
August-2014	6.65	4.76
October-2014	6.81	7.15
November-2014	9.25	9.32

in case of females than in males. The low values of GSI in case of males are due to low energy investment in gamete production than that used by females generally exhibited comparatively higher GSI than males (Khan 1945).

Table 6.2 indicates the month-wise GSI which is higher during March in the case of females and February in the case of males; conversely, it shows lower values during May in the case of males and in June in the case of females. In *Schizothorax niger* mean GSI values are reported higher in females (15.75) as compared to males (12.5). Thus these values indicate that the fish matures in the month of February and spawning starts in March when the GSI abruptly decreases to 3.3 in June for females and to 4.1 in the case of males.

It was reported from Garhwal Himalayan region hill-stream fishes spawn during summer and monsoon months as *Tor tor* and *Tor putitora* (April–July), *Labeo dyocheilus* and *L. dero* (March to June), *Barilius* spp. (April–June) (Badola and Singh 1984). The spawning season and periodicity exist because of varied ecological environments. *S. richardsonii* in Himachal Pradesh spawns from March to June, in Kumaon waters, it spawns from July to December Bisht (1974), Jhingran (1972), and in Garhwal Himalaya from July to September (Misra 1982). The Kashmir snowtrout, *S. niger* exhibits spawning from mid-April to May end (Malhotra 1965). The *S. plagiostomus* of Bhakra reservoir breeds twice in a year, i.e. from July to August and from December to January. Similarly, two breeding seasons (from September to October and February to March) in *S. plagiostomus* of Nepal waters have also been reported by Bhatnagar (1964). The breeding biology of *S. niger* studied from Dal Lake discovered that GSI recorded its highest value during February (14.35) which is the peak breeding season of the fish, then it decreased steadily during June attaining its lowest value in June (3.88), females exhibiting higher GSI value than males (Shafi et al. 2013). The maturation of the fish is maximum during the peak of maturity and decreases unexpectedly and sharply when the fish becomes spent and females generally exhibited comparatively higher GSI values than males. The mean GSI of females of *E. affinis* was observed low from September to February and in progress raised hastily from March before it reached a peak in July and declined in August and dropped to the lowest values thereafter

(Chiou et al. 2004). The values of GSI for males were also low from September to February and started to increase in March before it reached a peak in June. It declined in July and August and also dropped to the lowest values.

Spawning Season

The awareness of the spawning season is of paramount significance in the conservation and management of fishery resources. The spawning is defined as the emission of male and female gametes from the body of fish to the exterior, where fertilization occurs (Qasim 1973). The time of peak maturity and the period during which breeding occurs in a population signify the spawning season. It is based on the distribution of the different maturity stages. It was reported by Qasim (1973) along the east coast of India, spawning largely occurs during the pre-monsoon months, while on the west coast, fish largely spawn during the monsoon (June–September) and post-monsoon (October–January) months. The fish species reported from Indian marine waters are continuous breeders and protracted spawners.

Fecundity

Fish fecundity is defined as an adaptation that ensures the survival of the species under conditions in which it has originated and exists. The fish species have a higher fecundity or number of produced eggs compared to terrestrial vertebrates, the number of eggs spawned by various species of fish varies considerably. The fecundity, which is the number of eggs contained in an ovary, can be increased by fractional spawning, when groups of the eggs in an ovary are "ripe" at diverse times. Cambray (1992) described fecundity as number of eggs present in the ovary immediately before spawning. The fractional spawning which is the prolonged spawning periods is often feature of tropical and sub-tropical fish where no clear distinction between seasons is recorded. In fractional spawning, gonads are mature but the eggs are are within the ovaries are of the same size and mixture of large (fully developed) and smaller (undeveloped) oocytes, are often present at the same time. The fact that the "occurrence of small eggs together with large ones in the ovary is not always indicative of fractional spawning." The small eggs that stay in the ovary are reabsorbed. The diameter of ripe eggs can differ from a portion of a millimeter to as big as few millimeters (Helfman et al. 2000).

The biology and population dynamics of fish can be easily studied with the help of fish fecundity. When fecundity is pooled with other reproductive information such as age and size at maturity, spawning fraction, spawning season, fecundity is used to estimate spawning stock biomass and eventually recruitment and was reported by Murua et al. (2003). The fecundity is defined as the number of ripening oocytes and mature ova or eggs just before spawning (Sunder 1983). The data of fecundity data is used in estimation of survival rate, in order to determine the number of fishes required for broodstock and to help distinguish specific races, populations, or stocks

Table 6.3 Fecundity per gram body weight of *S. niger*

Total weight (g)	Weight of ovary (g)	Fecundity (Absolute)	Fecundity (Relative)
360	70.2	16243	4512
231	22.1	14213	6153
335	40.1	14531	4338
325	53.2	14207	4371
170	14.2	10897	6410
240	36.4	12654	5273
210	29.2	11543	5497
165	22.4	8630	5230
332	42.2	13759	4144
430	50.2	17645	4103
400	46.8	16539	4135
200	10.3	11174	5587
160	15.2	8431	5269
138	8.7	5841	4233
191	10.1	5691	2980
290	11.3	11438	3944
189	11.9	6481	3429
213	11.5	10984	5157
340	16.4	15418	4535
190	13.1	7451	3922
145	18.7	16415	4758
176	9.2	7418	4215

of fishes and for proper planning of hatchery and nursery populations (Bagenal 1978). The fecundity varies within species with latitude and location (Cushing 1968) and also with spawning time (Ware 1975). The different fish species reflect noticeable differences in their reproductive patterns and display dissimilar reproductive potentials in terms of fecundity. The information of fecundity is one of the most significant parts of reproductive biology (Nikolsky 1963; Naeem and Salam 2005; Jakobson et al. 2009). The fecundity is not a stable feature but it fluctuates with variations due to environmental conditions and specific factors (Khallaf and Authman 1991). The fecundity may vary annually even within a stock (Horwood et al. 1986).

The absolute fecundity of *S. niger* varied from 11709±8016 (Table 6.3). The relative fecundity per gram of body weight is 4645±1804. The average size of the ova (2.5 mm) is reported to be maximum during the month of March and minimum size during June (Table 6.4). The percentage frequency of the ova diameter is in the size of 0.2–0.6 mm (Table 6.5).

Das and koul (1965) reported that absolute fecundity of *S. niger* was found to be 11709 to 8016 whereas relative fecundity to be 1804 to 4645 eggs per gram of body weight. The fecundity of *S. longipinnis* in River Jhelum and fecundity per kg of body weight was estimated at 41,355 relative fecundity was found to vary from 25–71

Table 6.4 Ova diameter of *S. niger*

Month	Ova diameter
December-2013	1.4
January-2014	1.6
February-2014	1.9
March-2014	2.5
April-2014	2.3
May-2014	2.1
June-2014	0.2
July-2014	0.3
August-2014	0.5
September-2014	–
October-2014	0.7
November-2014	0.9

Table 6.5 Frequency of ova diameter of *S. niger*

Ova diameter	Frequency
0.2–0.6	3
0.7–1.1	1
1.2–1.6	1
1.7–2.2	2
2.2–2.7	2

Sundar and Subla (1985). Shafi et al (2013) reported absolute fecundity of *S. niger* from dal lake varied from 1550 to 3444 whereas relative fecundity ranged from 24 to 124 per gram body weight. The spawning biology of *S. richardsoni* (Gray) from River Gaula, Kumaon, Himalaya reported that the fecundity ranged from 2248 to 8726 in fishes of 160–245 mm TL and 40–110 g in weight (Mohan 2005).

From Indian Ocean the fecundity was reported as 0.21 million eggs of female weighing 1.4 kg female and 0.68 million eggs for 1.4 kg female per batch by Collette and Nauen in 1983. Farley and Davis (1998) reported that average spawning fecundity of 6.0 million oocytes per gram of body weight of *Euthynnus affinis*.

Conclusion and Future Direction

The review of the present status of the *S. niger* resource indicates that in Nigeen lake this fish species breeds once in a year but the breeding strength was not equally dispersed throughout the year. The management bodies of lakes and rivers should take proper strategies inorder to avoid catching fishes during breeding season.

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Reproductive and Breeding Biology of Tuna *Euthynnus affinis*

7

Kawkabul Saba, Faisal Rashid, Adnan Amin, and Tariq Hussain Bhat

Abstract

Reproduction process is important for fish population productivity and in its resilience to fishing and environmental changes. Reproductive potential is the ability to generate feasible amount of quality eggs in relation to the energy available and parental life expectancy. The important parameters for estimating the reproductive potential of the fish are maturity, fecundity, sex ratio, and fish condition which directly affect fish population productivity. *Euthynnus affinis* in the Veraval region of Gujarat is caught by using drift gillnets throughout the year with peak during December to March. The overall sex ratio is 0.82. The spawning occurs round the year with peak during December to March. The size at first maturity ranges from 55 to 60 cm, whereas absolute fecundity ranged from 171,550.4 to 827,734.35/kg body weight. The size of ova varied from 0.34 to 0.61 mm. Gonadosomatic index is highest during the month of December. Opportunistic predatory nature is in adults, and the most frequently encountered food was fishes, shrimps, *Decapterus*, squids, and digested fish.

Keywords

Reproductive biology · *Euthynnus affinis* · Veraval · India

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Introduction

India has a coastline of 7516 km, an exclusive economic zone (EEZ) of 2.02 million km^2, and more than 1 billion people; nearly 20% of the population live in the coastal areas. Fisheries and aquaculture play an important role in its economy and livelihood. Fisheries sector in India provides 13 million jobs with women representing 32% of the people employed in the sector (FAO 2019). In India, fish production reached an estimated level of 13.7 million tonnes (CMFRI 2018–2019). India's marine fish production in the year 2018 was estimated as 3.49 million metric tonnes registering a decline of about 3.47 lakh tonnes (9%) compared to 3.83 million tonnes in 2017 (CMFRI 2018–2019).

The total landing of tuna and tuna-like species (hereinafter referred to as tuna fishery) in India for 2018 was estimated at 208,928 tonnes, showing a marginal increase of 3.46 percent over the previous year (201,942 tonnes in 2017). Gillnets contributed 40.45 percent to the total landings of tuna fishery, followed by small purse/ring seines (12.42%) and trawls (10.01%). Pole and line fishing practiced exclusively in the waters of the Lakshadweep Group of Islands contributed 6.03 percent to the total tuna landings. Other gears like small longline and gillnet-cum-longline also contributed to tuna landings in small quantities during the year (IOTC 2019).

Considerable spatial variation has been observed in the tuna landings along the mainland coastline. The western coast of India contributed the major share to the landings (64%), while the east coast contributed mere 36 percent of the total landings. Tuna landings in 2018 were supported by seven species, four representing the neritic (27.76%) and three from the oceanic group (35.65%). Yellowfin tuna (*Thunnus albacares*) contributed the maximum (17.94%), followed by skipjack (*Katsuwonus pelamis*) (17.42%) and kawakawa (*Euthynnus affinis*) (15.89%) (IOTC 2019).

Tunas are marine water fishes belonging to the family Scombridae, among which majority are from the genus *Thunnus*. These are the largest and fastest swimming fishes in the marine water. Tuna keep on swimming and thus continuously generate heat within the body. Steady and efficient cruise swimming and capacity for powerful bursts are fundamental features for tuna biology (Block and Stevens 2001). Tuna species have been concisely described as energy speculators based on their high rates of energy return in a nutrient-poor pelagic environment (Korsemeyer et al. 1996). Tunas are predominantly dioecious, and sexual dimorphism is not seen in external morphological characters. Tunas are oviparous, having synchronous oocyte development, and are also as known as multiple or batch spawners (Block and Stevens 2001).

Coastal tunas are an important group of larger pelagic fish species along the Saurashtra coast, which are having huge domestic as well as export demand. *Euthynnus affinis* is one of the key coastal species among tunas. It represents a major group of food fish along the Saurashtra coast and constitutes an important component of exploited marine fishery resource.

Tuna species *Euthynnus affinis* is an epipelagic migratory fish which is mostly distributed in the tropical and subtropical waters along Indo-Pacific region and prefers water temperature ranging from 18 to 29 °C (IOTC 2006). *E. affinis* is widely rich in coastal areas over continental shelf. *Euthynnus affinis* is found on surface waters; however, it may range to the depth of over 400 m (Lee 1982).

In *Euthynnus affinis*, the anterior spines of the first dorsal fin are much higher than those in mid-way. *Euthynnus affinis* is mostly a cunning feeder which feeds on small fishes especially clupeids, squid, crustaceans, and zooplanktons. Size at first maturity is about 45–50 cm, and spawning occurs during summer (Collette and Nauen 1983). The fecundity of little tuna in India has been found approximately as 0.21 million eggs for female weighing 1.4 kg (48 cm fork length), whereas female weighing 4.6 kg (65 cm fork length) may spawn some 0.68 million eggs per batch (Collette and Nauen 1983).

Biology

Sex Ratio

The sex ratio of tuna in males and females is given in Fig. 7.1. In general, the sex ratio is 0.82. Chi square value points significant dominance by females in the months of January, February, and December, whereas preponderance is indicated for catch of males in the months of December-September and April (Fig. 7.1). The sex ratio of *E. affinis* stock differentiated mostly in various areas. In the Philippines, the sex ratio is 1 (Wade 1950), and it is similar in the west coast of India (Muthiah 1985). It is

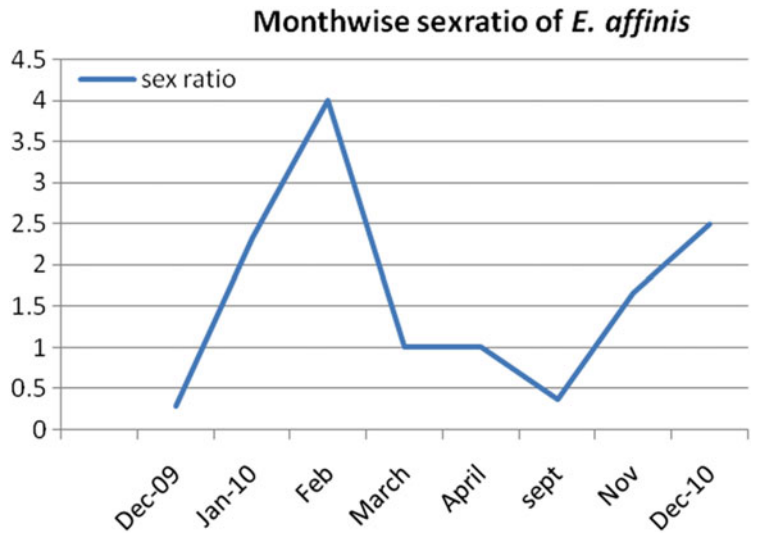

Fig. 7.1 Month-wise sex ratio of *E. affinis*

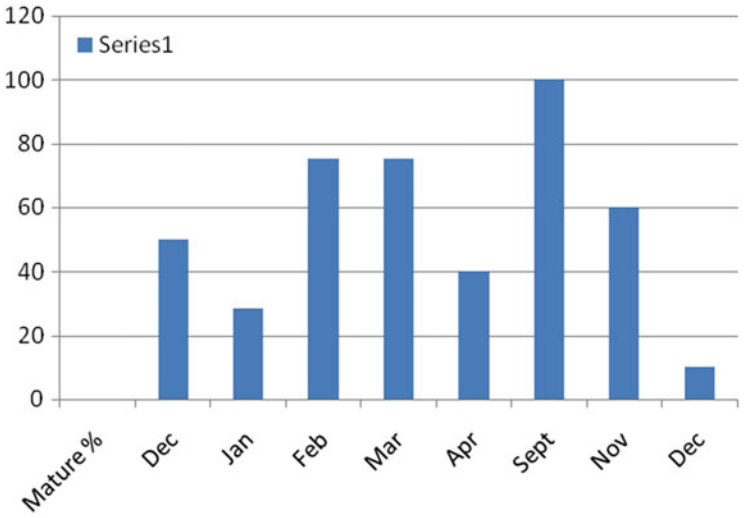

Fig. 7.2 Month-wise percentage of spawning females of *E. affinis*

greater than 1 in Thailand and in Taiwan (Yesaki 1994), but in the South China Sea, it is found to be smaller than 1 (Klinmuang 1978) and similar in the Seychelles (Yesaki 1994). Chi-lu et al. (2005) showed a sex ratio of 0.559. From the Indian Ocean, the male-to-female ratio is 1:1, but males predominate in the adult stage (IOTC 2006). It was found that the overall sex ratio is 0.82; however, females outnumbered males in most of the months (Fig. 7.2).

Maturity, Spawning, and Length at Maturity

The maturation can be observed by the development of ova into various maturity stages which are differentiated by macroscopic and microscopic stages of the ovary. Immature stages I and II are present in all the months except September and November, whereas mature stages (III, IV, and V) are in all the months (Fig. 7.3). Spent stages (VI and VII) are generally seen in the month of February. The fish spawned two times a year. Maximum spawning was attained in the month of September, in which 100% females were in mature state, and minimum spawning months were February–March, in which 75% of females were mature. In the month of November, the highest gonadosomatic index of 8.3 and the largest ova size of 0.61 mm were found which are also in full conformity to the spawning season. The 50% of the fish found mature at the size of 46.21 cm (Fig. 7.2).

Sivasubramanium (1970) revealed two peak seasons, i.e., from January to March and July to September, for troll line fishery of the southwest coast for *Euthynnus affinis*. It is contingent that there are seasonal spawning peaks for *E. affinis* differentiating according to the regions, i.e., from March to May in Philippine

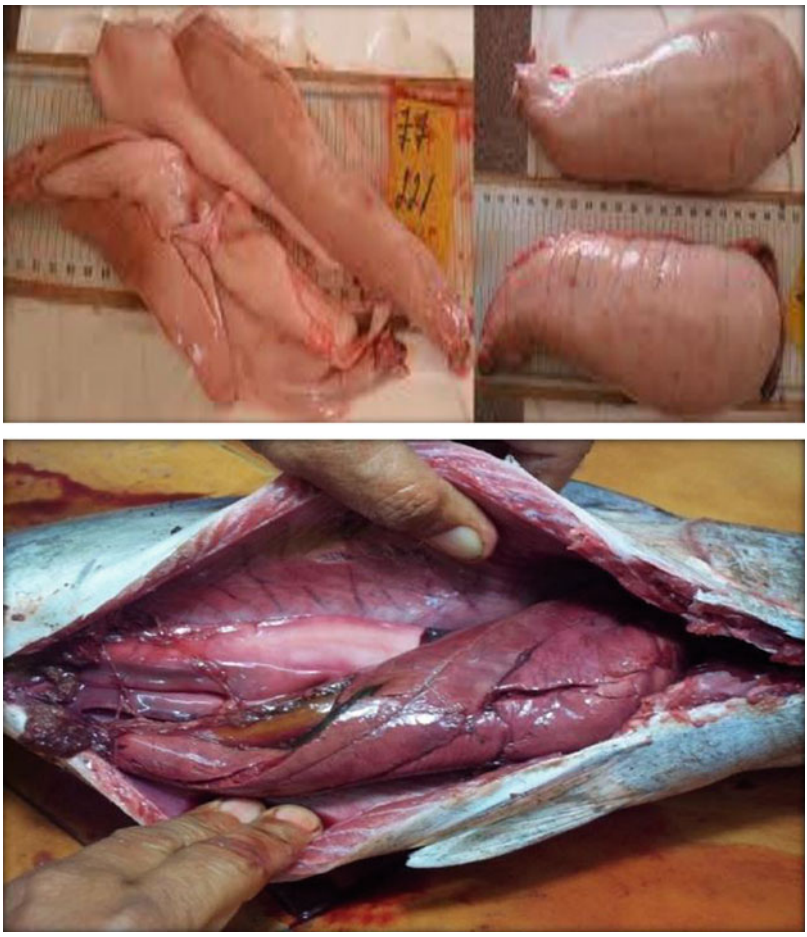

Fig. 7.3 V and VI maturity stages of *E. affinis*

waters, around October–November to April–May in the Seychelles, from January to July off East Africa, and probably from August to October off Indonesia (Collette and Nauen 1983). Joseph and Maldeniya (1985) revealed that the peak season for *E. affinis* is between May and July. Chiou et al. (2004) observed that based on the monthly change of GSI and development of oocyte and histological examination, the spawning season of *E. affinis* was reported from April to August with a peak in July. Darvishi and Behazadi reported peak season for *E. affinis* in May. According to IOTC (2006) on the Natal coast of South Africa, sexual maturity was attained at 45–50 cm for *E. affinis* and spawning occurred in summer. Chiou et al. (2009) reported that the size at first maturity for *E. affinis* was 48 cm FL for males; accordingly, for females, the size at first maturity (stage 4) was about 45 cm FL.

Fig. 7.4 Food items found in the gut of *E. affinis*

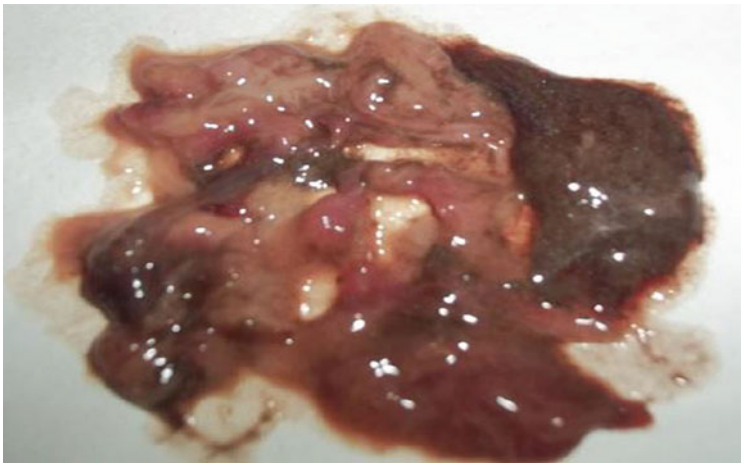

Fig. 7.5 Digested fish is a major food item in the gut of *E. affinis*

Chiou et al. (2004) observed the regularity of happening of food items of *E. affinis* and revealed that immature/smaller *E. affinis* feed on small-sized prey such as larvae, anchovy, and lantern fish. Mature and bigger fishes feed on larger-sized fish such as *Decapterus* spp. and *Scomber australasicus*. IOTC (2006) revealed that the *E. affinis* is a greatly cunning feeder and feeds on fishes, in particular on clupeoids, antherinids, squids, crustaceans, and zooplankton. Food items which were found in the gut of *E. affinis* mainly consisted of shrimps (mainly *Acetes*), squids, *Decapterus*, and digested fish, and it can be concluded that the diversity of food items found in the gut is in accordance to other researchers (Figs. 7.4 and 7.5).

Fecundity

Fecundity of fish *E. affinis* ranged from 171,550.4 during March to 827,734.35 in April 2010 (Fig. 7.6). In the month of December, higher fecundity per gram of body weight (292.56) was observed, and lower fecundity was found in the month of March (80.96). The average fecundity per gram body weight is 210.346. The average fecundity for a period of 8 months is 5,563,525.09. Fecundity ranged from 790,000 to 2,500,000 (Rao 1964) and from 202,000 to 1,570,000 (Muthiah 1985) in Vizhinjam, India, and from 585,000 to 2,593,000 with an average of 1,730,000 in the South China Sea. Farley and Davis (1998) indicated the average spawning batch fecundity of 6.0 million oocyte per gram of body weight. Fecundity of *E. affinis* ranged from 171,550.4 in March to 87,734.35 in April. The average fecundity for a period of 8 months was 5,563,525.09. In warmer less productive environments, smaller batch fecundity because of small size is partly compensated by longer spawning seasons (Fig. 7.7).

Ova Diameter

The size of the ova varied from 0.34 to 0.61 mm (Table 7.1). The average bigger size of ova is found in the month of November (0.61 mm) and small size during the month of March (0.37 mm). The percentage frequency of the ova diameter was found in the size of 0.39 mm (Table 7.1). Chiou et al. (2004) revealed the size of ova in *E. affinis* of various stages of maturity and observed that egg sizes measured bigger than 0.6 mm with a mean (+SD) of 487,205 + 161,191. The size of the ova in *Euthynnus affinis* at Veraval varied from 0.34 to 0.61 mm. The average size of the

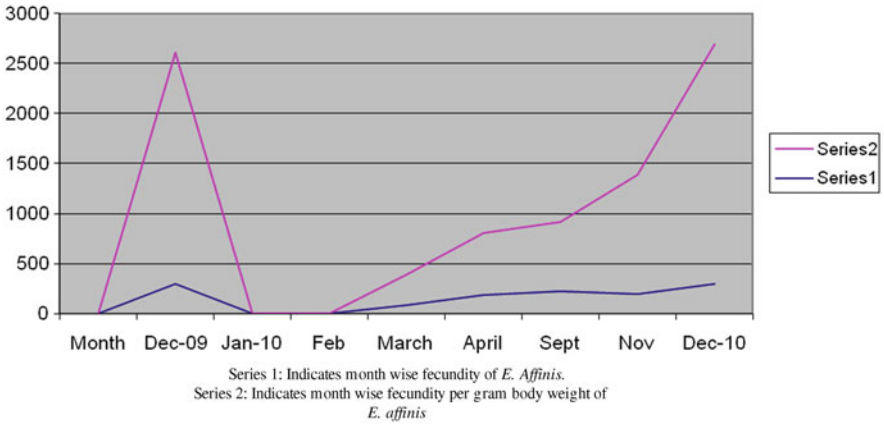

Fig. 7.6 Month-wise fecundity per gram body weight of *E. affinis*. Series 1: Indicates month-wise fecundity of *E. affinis*. Series 2: Indicates month-wise fecundity per gram body weight of *E. affinis*

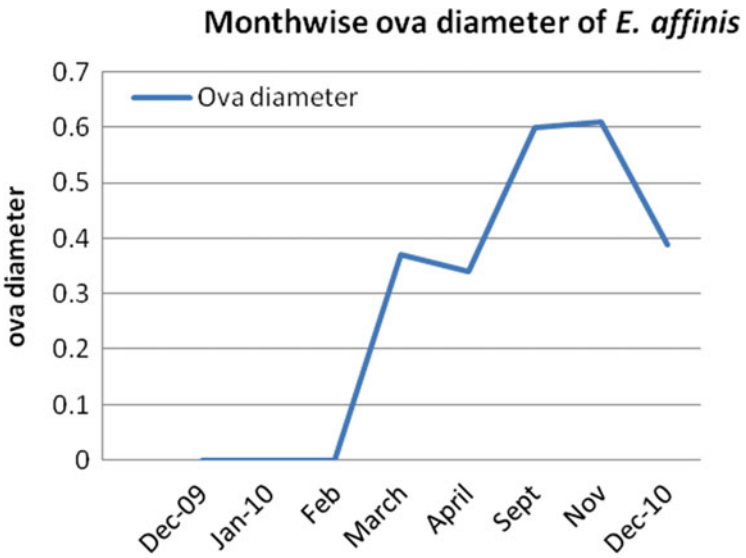

Fig. 7.7 Month-wise ova diameter of *E. affinis*

Table 7.1 Frequency of ova diameter of *E. affinis*

Ova diameter	Frequency
0.2–0.55	2
0.18–0.5	2
0.2–1.00	3
0.23–1.00	5
0.18–0.6	1

ova is maximum in December (0.61 mm), and the minimum size was in March (0.37 mm).

Gonadosomatic Index (GSI)

Month-wise GSI is given in Fig. 7.8, and it is higher during the months of December (8.3) and January (6.3), whereas it has lower values during September (3.1) and April (3.4). Chiou et al. (2004) showed that the mean GSI of females of *E. affinis* were minimum during the months of September and February, whereas it started to increase rapidly from March before reaching its peak in July. GSI started declining in August and dropped to lowest values thereafter. The GSI values for the males were found minimum in the months of September and February, whereas it started to increase in March before reaching its peak in June. GSI gradually decreased in July and August and also declined to the lowest values thereafter. GSI of females in all the months were >3. The highest GSI of 8.3 was found in December.

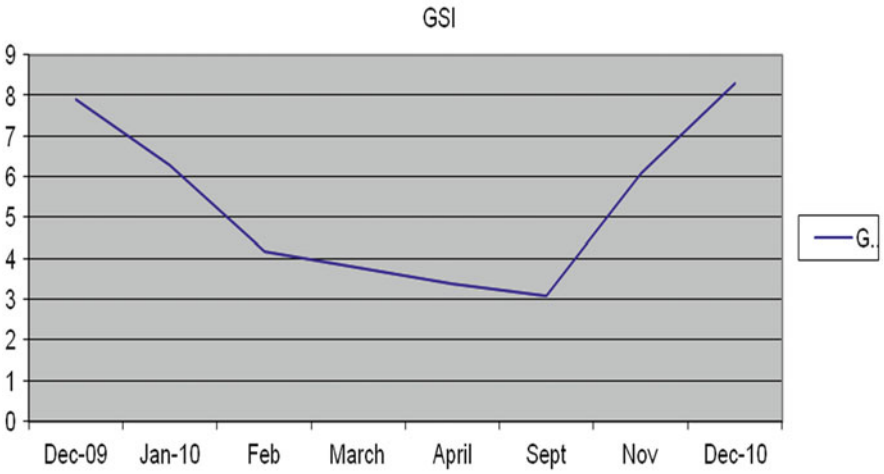

Fig. 7.8 Month-wise gonadosomatic index of *E. affinis*

Table 7.2 Length-wise feeding intensity percentage of *E. affinis*

Class interval	Empty	Trace	One fourth	Half	Three fourth	Full
410–470	21.73	0	16.66	0	0	0
470–520	18.8	45.5	16.66	0	28.57	100
520–590	31.88	55.5	33.32	60	0	0
590–670	13	0	33.32	40	71.42	0

Table 7.3 Month-wise feeding intensity percentage of *E. affinis*

Month	Empty	Trace	One fourth	One half	Three fourth	Full
December 2009	89.89	11.11	0	0	0	0
January 2010	0	0	0	0	0	0
February	70	10	10	10	0	0
March	75	0	12.5	12.5	0	0
April	20	0	30	0	50	0
September	54.5	0	9	18.1	9	9
November	50	0	25	25	0	0
December	78.57	0	7.1	14.25	0	7.1

Feeding Intensity

The length of *E. affinis* caught in the Veraval waters during 8 months ranged from 410 to 673 cm total length (TL). The length-wise and month-wise feeding intensity is given in Tables 7.2 and 7.3. Most of the specimens were found with empty stomachs. The stomach volume consisted of empty (68.5%), trace (12.35%), one quarter (6.74%), one half (5.62%), three quarter (7.86%), and full stomach (1.12%) (Figs. 7.4 and 7.5). Narasimham (1976) observed the food and feeding habits, size

at first maturity, spawning season, and fecundity of the *Eupleurogrammus muticus* from Kakinada during 1966–1971 and reported that its diet components included bony fishes and crustaceans. The size at first maturity was 51 cm, the spawning season extended from February to November, and the fecundity varied from 19.3 to 35.5 ova per gram weight of fish.

Conclusion and Future Direction

The huge demand for tuna in both local and export markets along with multiday fishing operations has also enhanced the capability to tuna catches. *E. affinis* breeds twice a year with peak in September and minimum in February. The species composition of tuna in recent years revealed a change in fishing areas to more offshore areas because of continuous exploitation. The need of the hour is to formulate policies and place suitable measures to contain overexploitation of marine fishery resources at Veraval. The fishermen should be educated to implement and practice the policies and measures at his fishing.

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Zebrafish (*Danio rerio*): A Versatile Model for Reproductive Biology

8

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Abstract

Animals are used as model organisms to understand various important biological processes as well as to acquire information which can give an idea how other organisms work. Different organisms are used including rat, guinea pig, rabbit, goat, squirrel, hamster, and fish (*Catla catla*, *Labeo rohita*, *Channa punctatus*, and Danio rerio) as well to study various biological aspects. For the validation of drug and chemical, it is important to study their toxicity tests at biochemical, serological, histological, and molecular. Generally rat or mice are used to study these toxic natures of any drug or chemical, but these mammalian models have long gestational period, are expensive, and also need special conditions for rearing. Development of embryo occurs outside the mother womb and is optically clear, and the duration of blastula stage ranges up to 3 h; however, gastrulation ranges up to 5 h, and when the embryo becomes 18 h old, the ears, eyes, segmented muscles, and brain are found well developed and are transparent. Due to these specific features, zebrafish is widely used to study the reproductive toxicity of various chemicals, drugs, and endocrine-disrupting chemicals (EDCs), to unravel their effect on body physiology, histoarchitecture, as well as molecular mechanism. Moreover, the use of zebrafish as a model is increasing day by day to different genetic aspects in aquaculture species and in toxicogenomics and to establish a zebrafish disease model applicable in human biomedicines. Therefore, the present review summarizes the silent features of zebrafish to predict its use as a model organism to study various reproductive processes.

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Keywords

Zebrafish · Model organism · Reproductive toxicity · EDCs

Introduction

Animals are used as model organisms to understand various important biological processes as well as to acquire information which can give an idea how other organisms work. Different organisms are used including rat, guinea pig, rabbit, goat, squirrel, hamster, and fish (*Catla catla*, *Labeo rohita*, *Channa punctatus*, and Danio rerio) as well to study various biological aspects. Zebrafish is one such emerging model used in developmental biology, cancer, toxicology, drug discovery, reproductive biology, and molecular genetics. Moreover, the use of zebrafish as a model is increasing day by day to different genetic aspects in aquaculture species and in toxicogenomics and to establish a zebrafish disease model applicable in human biomedicines. In many research fields, this tiny adaptable fish is used as a model organism because of its easy maintenance and breeding and because during early development its body looks transparent.

In 1970, George Streisinger (University of Oregon) first used zebrafish as a biological model organism, due to its simple genomic organization in comparison to mouse and because it was found easy to manipulate genetically. During the 1990s, the zebrafish has been widely used to establish a genetically mutant model, one by Nobel Prize winner Christiane Nusslein-Volhard in Tubingen, Germany, and second by Wolfgang Driever and Mark Fishman in Boston, USA. The mutant identification is one of the big strategy to study different areas of biology. In addition to this, zebrafish possesses similarities in physiology as well as in genetics with human beings, such as the brain, digestive tract, musculature, and innate immune system (Gore et al. 2012; Kanungo et al. 2014; Kalueff et al. 2014; Guyon et al. 2007; Weinstein 2002; Lieschke et al. 2001; Zhao et al. 2015). It has been reported that 70% human disease genes are functionally similar with those of zebrafish (Santoriello and Zon 2012).

Salient Features of Zebrafish as a Model Organism

Scientists recommended zebrafish as a model organism due to its different characteristics that make its use as a model organism. Development of embryo occurs outside the mother womb and is optically clear, and the duration of blastula stage ranges up to 3 h; however, gastrulation ranges up to 5 h, and when the embryo becomes 18 h old, the ears, eyes, segmented muscles, and brain are found well developed and are transparent. The segmentation gets completed in 18 h, and important primary organ systems are formed. The embryo hatches out from the

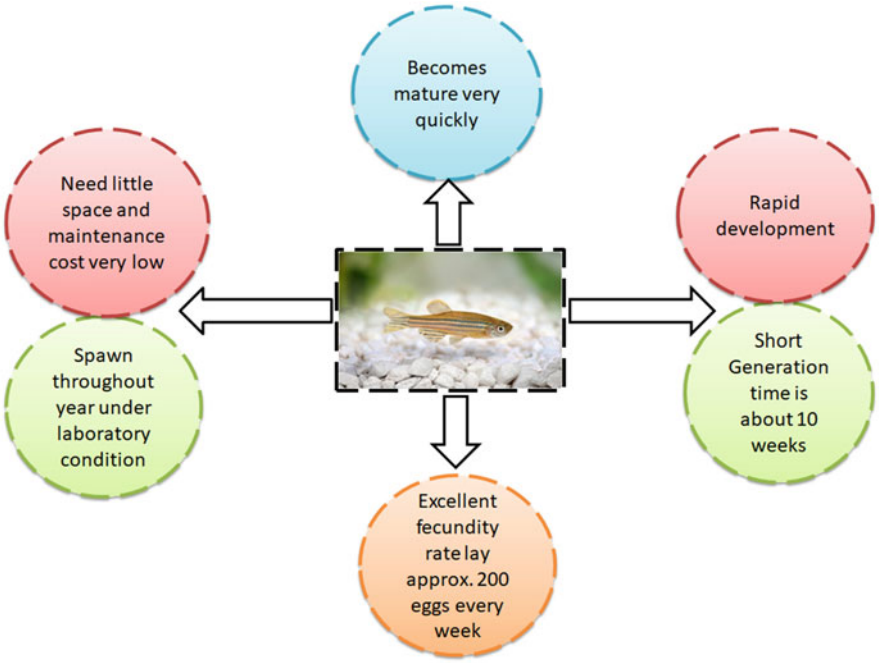

Fig. 8.1 Representative diagram showing features of zebrafish

eggshell by 72 h, and after 2 days, it starts hunting food. The conversion of embryo into adult occurs within 4 days. Due to this rapid development, it is justified to study genetic processes. The adult zebrafish becomes mature very quickly, with generation time 10 weeks approximately and having excellent fecundity rate. Zebrafish can lay approximately 200 eggs every week under optimum conditions (Brand et al. 2002; Carpio and Estrada 2006). Zebrafish has the ability to spawn throughout the year under laboratory conditions that evidences the invariable supply of offsprings from a particular pair that makes this tiny transparent fish an important choice for the study of genetic aspects at large scale, to recognize novel genes, as well as to discover their particular purposes in vertebrates (Pelegri 2002). Further, it needs little space, and its maintenance costs are very low. Considering these features into mind makes the zebrafish as an eye-catching model organism for developmental, toxicological, reproductive, and transgenic studies (Lele and Krone 1996). This chapter summarizes the use of zebrafish in the research of reproductive biology and can provide a flavor on recent developments in the reproductive biology (Fig. 8.1).

The chorion and embryo of zebrafish look transparent that makes clear visualization of the internal processes occurring during various early larval stages, which include the formation and functioning of internal organs within the living organism. The transparent body also allows to track the expression of fluorescently tagged transgenes as well as to monitor the activity of reporter genes (e.g., GFP and its

derivatives, luciferase) and laser manipulation (e.g., cell ablation, uncaging experiments) (Gilmour 2002).

Zebrafish (*Danio rerio*) is a tropical freshwater fish, inhabitant of rivers (Ganges mainly) of Himalayan region of South Asia especially India, Nepal, Bhutan, Pakistan, Bangladesh, and Myanmar. It is a bony fish (teleost) that belongs to the family Cyprinidae under the class Actinopterygii (ray-finned fishes). Zebrafish is a well-established model organism due to the following features: the relative ease of rearing and breeding in captivity, high fecundity, rapid development, short generation time, and availability of genomic resources, including the complete zebrafish genome sequence (Gore et al. 2012; Kanungo et al. 2014; Kalueff et al. 2014; Guyon et al. 2007; Weinstein 2002; Lieschke et al. 2001; Zhao et al. 2015; Santoriello and Zon 2012). The familiarity of this model is due to its ability to reproduce under wild as well as laboratory conditions demanding its importance for husbandry. In the present era, use of zebrafish as a model organism to understand various aspects of reproductive biology as well as for behavioral studies has increased day by day. Development has been made in recent years to unravel the gonadal development and social interactions during reproduction such as mate choice and courtship (Gerlach 2006; Gerlach and Lysiak 2006; Skinner and Watt 2007; Spence and Smith 2005; Spence and Smith 2006).

Zebrafish as a Genetic Tool to Study the Function of Different Genes

Since the last 25 years, zebrafish was projected to be a potential model organism to study embryonic development (Streisinger et al. 1981); the tools and techniques required to study zebrafish are increasing continuously. The first protocol developed was to study the process of mutagenesis, by which different mutations were identified and characterized. Various other genetic techniques which include reverse genetic techniques (RNA interference, morpholino knockdown, and tilling) were established in zebrafish to target known genes. Various other mutagenesis protocols include employing N-ethyl-N-nitrosourea, ENU, as a mutagen; radiation, mainly gamma rays; and insertional mutagenesis using retroviruses or transposable elements (Dahm and Geisler 2006), whereas ENU mutations have been studied in zebrafish (Driever et al. 1996; Haffter et al. 1996; Frohnhoefer 2002). In addition to mutational studies, zebrafish is used to study in vitro fertilization (Pelegri 2002).

Reproductive Gender and Biology of Zebrafish

Mammals are sexually distinguished (dimorphic) having dimorphic sex chromosomes, in which female is having XX (homogametic) and male is having XY (heterogametic). SRY (sex-determining region of Y chromosome) gene is having great effect in determining the sex of an individual by acting like a genetic switch that starts male development pathways in bipotential gonads (Kashimada and

Koopman 2010; Piprek 2010). However, zebrafish lacks sex determination pathways. Complex pattern of sex determination is due to the genetic as well as environmental factors including temperature of surroundings (Uchida et al. 2004), exposure to sex hormones (e.g., estrogen and androgen), and availability of oxygen (Shang et al. 2006) which have been showed by steady works in gonad ontogenetic differentiation of zebrafish. From the genetic point of view, current research reports showed that chromosome number 4 is the potential sex-determining chromosome in zebrafish under natural conditions; however, this sex-determining mechanism was found very weak under domesticated strain of zebrafish (Anderson et al. 2012; Wilson et al. 2014). However, a similar condition is observed in humans, in which various autosomal genes have proved considerable roles in the development and differentiation of gonads and reproductive cells. Anti-Müllerian hormone (AMH) is one of the principal hormone imparting its role in sex differentiation during fetal development. During the transcriptional regulation through SOX9, steroidogenic factor 1 (SF-1), Wilms' tumor suppressor gene 1 (wt1), and GATA4, AMH are discharged from the Sertoli cells in fetal testis (De Santa Barbara et al. 1998; Lourenço et al. 2011; Barrionuevo and Scherer 2010). In addition to degenerated Müllerian ducts, a pair of ducts further develops into fallopian tubes and uterus, because AMH suppresses the expression of a P450 aromatase enzyme (CYP19a1); this enzyme converts the androgens into estrogens (Rodríguez-Marí et al. 2005). In zebrafish, similar mechanistic processes occur during the development of gonads by having the expression of AMH in the gonads in addition to the identification of gene binding sites for the identical transcriptional factors in the promoter region of AMH gene sequence (Wang and Orban 2007). Zebrafish has a short generation time due to the development of precursor organs after 24 hours of fertilization and also attains reproductive maturity within 3–6 months after fertilization along with maturity period which is proportional to its body length of about 23 mm (Spence et al. 2008). Zebrafish has an identical anatomy of gonads with human beings (Van den Hurk and Resink 1992); male zebrafish possesses a pair of testis having tubular organization; walls of each tubule are lined with Sertoli cells functioning as nutritional cells to promote the morphogenesis as well as process of spermatogenesis; however, Leydig cells which are confined within the interstitial spaces produces testosterone (Van den Hurk and Resink 1992; Siegfried and Nüsslein-Volhard 2008). One distinguished feature in the spermatogenesis process is the presence of spermatogenic cyst which has been observed in zebrafish; these spermatogenic cysts comprise a group of sertoli cells enclosing the germ cells that develop synchronously; however, in higher vertebrates, there are only few germ cells under various developmental stages in sertoli cells (Schulz et al. 2015). Male zebrafish shows the presence of accessory sperm duct gland; it secretes mucosubstances and also produces tail of sperm (Kemadjou Njiwa et al. 2004). Female zebrafish possesses various key similarities in the structure reproductive system and function of ovaries. Zebrafish encompasses paired bilateral ovaries, situated between the swim bladder and abdominal wall (Gupta and Mullins 2010). Wall of ovaries are lined by a thin layer of epithelial cells having a number of oogonia and oocyte follicles surrounded by interstitial tissues and somatic cells observed. Lobulated structure having

interlobular spaces and joining with oviduct are observed under histological section (Menke et al. 2011). In vertebrates, female gametes are produced and developed within the ovaries (Gerlach 2006; DeFalco and Capel 2009). In zebrafish, the development of ovaries takes place in four stages, viz., primary oocyte stage having comparatively small spherical cells, cortical-alveolar stage with big oocytes filled with cortical alveoli, vitellogenic stage which is specified by the presence of egg yolk in oocytes, and the final stage which is the maturation of oocytes characterized by asymmetrical layer (Çakıcı and Üçüncü 2007). Similar to the teleost fish and humans, the zebrafish oocyte is surrounded by the zona radiata along with follicular layer having inner granulosa cell and outer thecal cell layer (Clelland and Peng 2009). During the ovulation process, male gonadal pheromones induce the rupturing of layers (Spence et al. 2008; Gerlach 2006). There are also some striking homologies in the reproductive system of zebrafish and humans at anatomical, physiological, as well as genetic levels.

Reproductive Behavior and Performance of Zebrafish

Zebrafish is an early morning breeders and group spawners (Spence et al. 2008; Hutter et al. 2010). Females are potentially capable of spawning repeatedly but in an irregular manner with various hundreds of eggs in a spawning session (Nasiadka and Clark 2012). The frequency of interspawning is about 1 to 6 days (Uusi-Heikkilä et al. 2012). Eggs spawned by zebrafish are optically translucent and are normally large in comparison to other fishes, having about 0.7 mm in diameter (Spence et al. 2008). In addition to having healthy sexual organs as well as morphological sexual features, developmental and regular steroidogenesis (Liu et al. 2013; Deng et al. 2010), normal courtship behavior is one of the important condition for a successful reproduction in zebrafish (Spence et al. 2008). Male and female members of zebrafish show different mating behaviors; male shows five special mating behaviors such as chasing/following the females (chase), contact with female through its nose or tail (tail-nose), moving around the female in a circular manner (encircle), circling around the female in the form of "figure eight" pattern (zigzag), and quick movement of tails against the body of females (quiver) (Darrow and Harris 2004; Spence et al. 2008; Larsen et al. 2008). The sexual behavior in female start with moving towards the male by swimming immediately towards the males (approach), swimming alongside males or residing still when being chased (escort), swimming alongside males or residing still when being chased (escorts), swimming around the males and being fluent in front of males (present), swimming in a particular location within its habitat (lead), and oviposition (egg-lay) (Darrow and Harris 2004; Spence et al. 2008).

During courtship, chase, tail-nose as well as carries three initiatory mating activities showed by male and female zebrafish followed by presenting and then escorts from female as receptive behavior (Darrow and Harris 2004; Spence et al.

2008). Nonetheless, sometimes female move away from male aggressively when male partner shows unfavorable behavior (Darrow and Harris 2004; Spence et al. 2008); after showing the receptive behavior, female zebrafish begins to swim toward a particular direction for three times (Hutter et al. 2010). Finally, male zebrafish swims and extends caudal as well as dorsal fins around the females in order to align their genital pores; quick oscillation of tail has been observed, which encourages the spawning (Darrow and Harris 2004; Hutter et al. 2010; Larsen et al. 2008). The simultaneous discharge of sperms and eggs has been studied, or sperms are discharged prior to the discharge of eggs (Kemadjou Njiwa et al. 2004). Generally the peak time for the males to discharge sperms is approximately 30 minutes; however, it may be continued for 1 hour (Darrow and Harris 2004).

Different environmental factors such as photoperiod (Blanco-Vives and Sanchez-Vazquez 2009; Lawrence 2007), tank volume (Goolish et al. 1998), temperature, pH (Lawrence 2007), topography, fish densities, and presence of natural habitat items including aquatic plants and substrates affect the reproductive performance of zebrafish (Sessa et al. 2008). In zebrafish, rhythm generated inside the body (endogenous), is affected by photoperiod, and depends on the light-dark cycle (10-hour light and 14-hour dark) (Blanco-Vives and Sanchez-Vazquez 2009; Lawrence 2007). In wild as well as laboratory conditions, zebrafish normally spawn in the first few hours of daylight (Darrow and Harris 2004; Spence et al. 2008).

Zebrafish: An Efficient Model to Study Reproductive Disorders

Infertility can be defined as the inability of an organism to achieve pregnancy despite having regular sexual intercourse for 1 year or more. Epidemiological studies reported that approximately 20% of couples in the whole are suffering from infertility (Turchi 2015). The primary reasons for infertility in males are poor quality and quantity of sperms, while in females it includes irregular ovulation and tubal pathology (Parikh et al. 1997; Laven et al. 2002). Hormonal imbalance is considered as the main cause of infertility because of unhealthy and stressful lifestyles (Chidrawar et al. 2011; Lynch et al. 2014), and long-term exposure to harmful chemicals and adverse environmental conditions (Snijder et al. 2012; Zafar et al. 2015) are evidenced to be responsible for the pathogenic cause of infertility. Therefore, zebrafish has been found to be a suitable model organism to study the impact of various factors on reproductive potential.

Stress-Induced Infertility

Exposure of stress given to the zebrafish showed that nucleus preopticus (NPO) (regions homologous to the paraventricular nucleus (PVN) in the hypothalamus of mammals) begins to secrete CRH, corticotrophs in APG starts releasing ACTH, helps in the stimulation of cortisol biosynthesis in the interregnal gland (Alsop et al. 2009. The effect of HPI on the reproductive axis in zebrafish is similar to that in

mammals; the secretion of hormones including CRP, ACTH, and cortisol under stress usually causes impairment in the reproductive system by inhibiting the release of hormones important for reproduction as well as for gametogenesis (Alsop et al. 2009). The adverse effects of ACTH and cortisol on gametogenesis and successful fertilization in zebrafish have been revealed by the identification of damage in oocyte as well as decrease in nucleic acid through disruption of protein synthesis (Sousa et al. 2015). In addition to this, ACTH induce higher vacuolization in ooplasm in zebrafish; a similar situation has been observed in adrenal glands of mammals when they were exposed to ACTH (Sousa et al. 2015; Volkova et al. 2015). It has been reported that ACTH represses the gonadotropin-stimulated estradiol discharge from follicles of the ovary (Alsop et al. 2009). Inhibition of steroidogenesis through exposure to stress might be associated with binding of ACTH to melanocortin 2 receptor (MC2R), which is a specific receptor identified in the ovary of zebrafish in addition to the presence of inhibitory G protein in MC2R signaling (Alsop et al. 2009). Hoo et al. (2016) reported that the effect of ACTH has not been observed on the reproductive system of male zebrafish; however, MC2R receptors have been identified in the gonads of male zebrafish; therefore, it might be predicted that ACTH is associated with the modulation of steroids in the gonads of male zebrafish.

Chemical-Induced Infertility

In the 1750s, the development of industrialization era has been started, and the production of chemicals and their accumulation increased in the environment (Han et al. 2002). Exposure to chemicals also poses harmful health threats in all organisms, which elevates the demand for robust as well as cost-efficient methods to estimate their effects on human health as well as in other living beings, especially on growth as well as development along with effects on gonads (Wuttke et al. 2010; Fucic et al. 2012). Toxicity assessment of chemicals and their effect on the reproductive system or any other system, generally mammalian models such as rat or mice are used. However, these mammalian models and the assays to carry out the examination of reproductive toxicity are time-consuming, costly, complex and also require higher-scale experimental investigation (Arora et al. 2011). In addition to this, higher doses are required, which leads to various toxic manifestations (Myers et al. 2009). In comparison to this, use of zebrafish to examine the exposure of these chemicals might be helpful to decrease the toxic effects. Various aspects for the study of different reproductive functions in zebrafish are given in Fig. 8.2.

Environment-Induced Infertility

From the since past decades, the effect of various environmental factors including availability of oxygen and external exposure of heat on gonads and their functions has become a focal area of research for the scientific community, because of the diversity in living places and their jobs, for example, some people live and work at

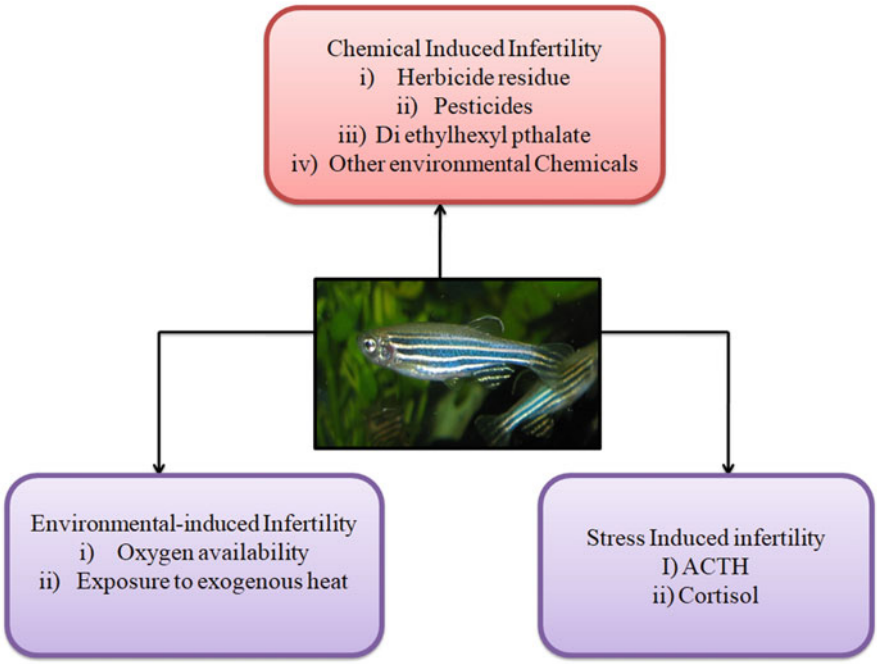

Fig. 8.2 Representative diagram showing various aspects for the study of different reproductive functions

high altitudes, while some work in areas of higher temperature (Figa-Talamanca et al. 1992; Mur et al. 1998; Vargas et al. 2011). The effect of low oxygen availability in aquatic ecosystem influences the reproductive function of the dwelling living creatures, due to the increase in the occurrence of eutrophication as well as organic pollution; all these adverse changes have been deeply studied in different fishes and in zebrafish as well (Shang et al. 2006; Wu et al. 2003; Landry et al. 2007; Thomas et al. 2007; Yu et al. 2012). The hypoxic condition does not affect the reproductive system/functions directly, but is having an indirect effect by altering the sex steroid level in circulatory plasma, especially testosterone and estradiol along with the molecular as well as genetic mechanism involving the genes associated with HPG axis (Martinovic et al. 2009); such factors include hypoxia-inducible factor 1 (HIF-1) (Yu et al. 2012; Martinovic et al. 2009), cellular lipids and steroid hormones (Thomas et al. 2007; Martinovic et al. 2009), and leptin (Yu et al. 2012). Hormonal imbalance is one of the major factor which lags the growth of gonads, masculinization of the ovary, and sex ratio alteration in favor of males, and gametogenesis becomes arrested (Shang et al. 2006; Yu et al. 2012). Under hypoxic state, the process of ovulation in female gets predominantly decreased which can be correlated with alteration in steroid and contractile gene expression. The hypoxic condition mainly affects the migration of primordial germ cells (PGC) that might be one of the cause of infertility (Lo et al. 2011). Thus, it can be summarized that

hypoxic condition causes impairments such as abnormal development of gonads, decreased quantity as well as quality of germ cells and qualities, fertilization and hatching success, and larval and juvenile viability has been successfully evaluated by using zebrafish as the animal model. In addition to this, temperature of testicles is also another vital factor which reveals the quality and quantity of sperm in humans and in other mammals as well (Bujan et al. 2000; Lue et al. 2000). Temperature in testis is about 2 to 40 °C lower than the normal temperature of the body that is required for the normal functioning of testis for successful spermatogenesis; fundamentally in the testis, temperature is regulated through two mechanisms, viz., first the dissipation of heat through the surface of the scrotum and second the loss of heat from the blood coming through the arteries into the testis to the outgoing venous blood (Sheiner et al. 2003). In zebrafish, when the temperature of water increases, chromosomal anomalies in sperms increases (Poss et al. 2004). The aneuploidy condition in germ cells occurs because of the mutation in monopolar spindle 1 (Mps1), which is a vital mitotic checkpoint kinase factor (Poss et al. 2004).

Reproductive Toxicity Assessment in the Zebrafish

Generally, for the assessment of reproductive toxicity of drugs and chemicals, mammalian models such as rat or mice are used. These chemicals generally affect the histoarchitecture of tissues such as the testis or ovary, sperm quality, and developmental stages of offsprings during their early embryonic life (Kuriyama et al. 2005; Lilienthal et al. 2006; Stoker et al. 2005; Tseng et al. 2006). However, these mammalian models are very complex, costly, and time-consuming having small possibility for high great output or analysis at large scale. Moreover, higher doses are required for the testing of these chemicals through oral route in mammalian models, which makes them unsuitable for analyzing their reproductive toxicity aspects, because these chemicals might come from environmental pollutants via water-soluble pollutants dissolved in the aquatic system. Zebrafish has been found to be a suitable model organism to study the toxicity of various chemicals coming from environment through water, oral exposure, or any other medium. Zebrafish being simple has been widely used for in vitro fertilization and embryogenesis and also for examining reproductive toxicity as well as teratogenicity (Deng et al. 2010; Du et al. 2009; He et al. 2011; Heiden et al. 2005; Van den Belt et al. 2001). Use of zebrafish will minimize the time duration, decrease cost, and increase the output. Zebrafish is used to study the toxicity of various toxicants such as metals, organochlorines, pesticides, halogenated aromatic hydrocarbons, substituted anilines, synthetic as well as natural estrogens, and also industrial chemicals. Zebrafish has been widely used to study the toxic effect of endocrine-disrupting chemicals (Spitsbergen and Kent 2003). The toxicity index formulated by OECD 229 for the assessment of reproductive toxicity using zebrafish focuses on the following points: (1) weight index of gonads (gonad weight, GSI) (Brion et al. 2004; Deng et al. 2010; He et al. 2011); (2) reproductive capability: the egg-laying amount (Brion et al. 2004; Deng et al. 2010) and quality of sperms (He et al. 2011; Jing et al., 2009; Wang et al.

2010); (3) histoarchitecture of the gonad; (4) the vitellogenin (Vtg) expression assay (Brion et al. 2002; Fenske et al. 2005); and (5) the hypothalamus-pituitary-gonadal (HPG) axis gene expression observation. Some studies reported that EDCs (endocrine-disrupting chemicals) alter the expression of HPG axis genes (Liu et al. 2009, 2013; Wang et al. 2011). These alterations in gene expressions are very important to study and to unravel their toxic manifestation (Deng et al. 2010; Shi et al. 2009). The assessment of HPG axis expression of genes is one of the key methods to find out the endocrine functions and how these EDCs disrupt the endocrine system.

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Sex Determination in Teleost Fish

9

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Abstract

Sex determination (SD) is the fundamental developmental process crucial for the survival of biological species. Fishes are the only class of vertebrates which show a larger plasticity in gonadal development and are represented by both gonochoristic (one sex at a time) and hermaphrodite (more than one sex) species. In teleosts, SD is either regulated by the genetic mode (GSD), where male and female have different sets of alleles that specify their reproductive fate and morphology, or determined by environmental variables (ESD) such as temperature, pH, salinity, or social conditions. Male-restricted master regulators like *Dmy*, *Gsdf*, *Amhy*, *SdY*, *Sox3*, and *Dmrt1* or female-specific $Foxl_2$ and $Foxl_3$ have been well documented in different teleost species till date. However, the critical balance between the turnover rates of testosterone (T) to either estrogen (E_2) or 11-ketotestosterone (11-KT) regulated by either aromatase enzyme (coded by *Cyp19a1a*) or 11β-hydroxylase enzyme (coded by *Cyp11b*) and 11-β-hydroxysteroid dehydrogenase enzyme (coded by *Hsd11b2*), respectively, finally determines the sexual development and gonadal output. This chapter precisely discusses various SD mechanisms like the environmental conditions including social cue, endocrine factors, and genetic regulatory network(s) that collectively determine the gonadal fate and function in teleosts.

Keywords

Sex determination · Sexual development · Gonadal development · Teleosts

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Introduction

Biological species which reproduce sexually require an assembly of two types of haploid gametes, i.e., eggs and sperm. These sex cells have specialized functions and are produced in the female and male gonads, respectively (Capel 2017). Sex determination (SD) is the fundamental developmental process which leads to a binary gonadal fate decision (either ovary or testis) with specific sexual characteristics and therefore becomes critical for the survival of biological species (DeFalco and Capel 2009). Interestingly, fishes are the only class of vertebrates which show a larger plasticity in gonadal development and are represented by both gonochorism (one sex at a time) and hermaphroditism (more than one sex) species (Devlin and Nagahama 2002). The only known exception of unisexual species is *Poecilia formosa* (Devlin and Nagahama 2002). Gonochoristic teleosts develop as either males or females only and retain such uniform sexual identity throughout their life span. On the other hand, in hermaphrodite fishes (protogynous or protandrous), the developmental pathways leading to the formation of either testis or ovary are plastic and susceptible to the sex reversal signals significantly beyond embryogenesis and early larval stages. In gonochoristic species, such developmental decision toward sex is irreversibly taken long before adulthood is reached (DeFalco and Capel 2009; Devlin and Nagahama 2002). Teleosts exhibit natural hermaphroditism where an individual changes from one sex to the other during adulthood. Some teleosts have gonads containing both mature ovaries and testes (synchronous hermaphroditism). In sequential hermaphroditism, some fishes change sex from male to female (protandrous sex change, e.g., black porgy *Acanthopagrus schlegeli*), others change from female to male (protogynous sex change, e.g., bluehead wrasse *Thalassoma bifasciatum*), and a few change sex in both directions for multiple times (bidirectional sex change, e.g., monogamous coral-dwelling gobies *Gobiodon* and *Paragobiodon*) (DeFalco and Capel 2009; Devlin and Nagahama 2002). In many cases of sequential hermaphroditism, sex change is controlled by social cues such as the disappearance of the dominant male or female from a group (Capel 2017).

In endothermic vertebrates like birds and mammals, the trigger for the sex-determining pathway is exclusively genetic (known as genetic sex determination GSD), whereas in poikilotherms, in addition to such GSD, an environmental cue, mostly temperature, also acts as the trigger, which is called environmental-/temperature-determined sex (ESD/TSD) (Capel 2017; DeFalco and Capel 2009).

Teleosts are classically categorized as ray-finned bony fishes with homocercal tails which represent almost half of all living vertebrate species (Devlin and Nagahama 2002) and show a wide variety of sex determination (both GSD and ESD mode) mechanisms (Capel 2017; DeFalco and Capel 2009; Devlin and Nagahama 2002). In many fishes, sex determination is genetic (GSD), i.e., males and females have different alleles or even different sets of genes that specify their reproductive fate and morphology (Devlin and Nagahama 2002). Additionally, sex may also be determined by environmental (ESD) variables such as temperature, pH, salinity, and social conditions (Devlin and Nagahama 2002; Baroiller et al. 2009).

Thus, fishes are a very interesting group among the vertebrates for the study of sex determination and differentiation.

The mechanisms of sex determination (SD) and differentiation in fishes are highly diverse and plastic which have been extensively reviewed from time to time (DeFalco and Capel 2009). This chapter aims to briefly discuss the environmental, endocrine, and genetic aspects of sex determination (SD) in teleost fishes.

Environmental Mode of Sex Determination (ESD)

ESD system is mediated by temperature, pH, population density, and visual-endocrine cues all together (Capel 2017; DeFalco and Capel 2009; Baroiller et al. 2009; Editorial 2009).

Temperature

Temperature-induced SD or TSD is the major environmental factor in teleosts (Baroiller et al. 2009; Editorial 2009). It was first demonstrated in Atlantic silverside *Menidia menidia* (Baroiller et al. 2009). However, in most species, both GSD and ESD coexist, and either of the two or both mechanisms may be cooperative to drive SD. Despite having a strong GSD, temperature-induced sex reversal has been reported in the Nile tilapia *Oreochromis niloticus* and pejerrey *Odontesthes bonariensis* where high temperature prefers the male progenies (Baroiller et al. 2009). Other reported species include Japanese flounder *Paralichthys olivaceus* and medaka *Oryzias latipes* having male heterogametic GSD system or blue tilapia *Oreochromis aureus*, turbot *Scophthalmus maximus*, and half-smooth tongue sole *Cynoglossus semilaevis* having female heterogametic GSD system and finally seabass *Dicentrarchus labrax* or domestic strains of the zebrafish *Danio rerio* having a polygenic GSD system (Baroiller et al. 2009; Editorial 2009).

Visual-Endocrine Cue

In bluehead wrasse *Thalassoma bifasciatum* which shows a protogynous sex change, the behavioral, ecological, and neuroendocrine bases have been well studied (Capel 2017; Baroiller et al. 2009; Todd 2016). Female bluehead wrasses show rapid aggression during courtship leading to the behavioral sex change independent of their gonads. Such male-typical behavior is associated with the neuroendocrine axis which includes the expression of arginine vasotocin, whereas estrogen (E_2) implants block such behavioral sex change (Todd 2016). Social interactions and environmental stimuli operate together through both hypothalamus-pituitary-gonadal (HPG) and hypothalamus-pituitary-interrenal (HPI) axes via neuroendocrine factors including kisspeptin (KP), dopamine (DA), gonadotropin-releasing hormone (GnRH), and arginine vasotocin (AVT) and steroids like 17β-estradiol (E_2) and testosterone (T).

Both follicle-stimulating hormone (FSH) and luteinizing hormone (LH) stimulate the survival, proliferation, or maturation of the germ cells (Capel 2017; Baroiller and D'Cotta 2006; Todd 2016). Corticosteroids produced from adrenal glands act on the gonads to block the aromatase enzyme (encoded by *Cyp19a1a*) that produces E_2 from T in the females. However, cortisol activates the enzymes encoded by *Cyp11c1* and *Hsd11b2*, which convert T to its bio-active form 11-ketotestosterone (11-KT) in the males. Therefore, cortisol seems to be the major player in fishes for sex reversal/determination by regulating the balance between the function of *Cyp19a1a* and *Cyp11c1* or *Hsd11b2*, thereby fixing the E_2:11-KT ratio (Capel 2017; Todd 2016).

Population Density or Size

Sex change in gobiid fish is determined by either behavior or size. For example, in a fixed population of Okinawa rubble *Trimma okinawae*, larger male fishes remain as male, while the smaller males become females. Intriguingly in the absence of males, the largest female can even change its sex to male (Capel 2017; Todd 2016).

Endocrine Factors

The key regulation of the production of two gonadal steroids, i.e., estrogen (E_2) and androgen (T or 11-KT), determines the sexual fate in fishes (Capel 2017; Baroiller and D'Cotta 2006; Todd 2016). In teleosts, E_2 and 11-KT are the major steroids that promote ovarian or testicular differentiation and function, respectively. The production of either E_2 or 11-KT depends on the bioconversion T via the aromatase enzyme coded by *Cyp19a1a* gene or the 11β-hydroxylase enzyme coded by *Cyp11b* gene and 11β-hydroxysteroid dehydrogenase (11β-HSD) enzyme coded by *Hsd11b2* gene, respectively (Capel 2017; Todd 2016).

Administrations of non-aromatizable androgens disrupt the female developmental pathway, and supplementations of aromatase inhibitors further suppress ovarian E_2 production in female fishes. Interestingly, it is essential to note here that sex reversal is not fully sustained following the withdrawal of hormonal stimuli suggesting that, although sex steroids potentially induce the gonadal transdifferentiation, a robust regulatory genetic network is required further for the switch to shift the sex-specific hormonal production and maintain the gonadal fate (Capel 2017; Baroiller and D'Cotta 2006; Todd 2016).

During gonadal transdifferentiation in sex-changing fishes, a dramatic shift in plasma sex steroids has been reported (Todd 2016). For example, in protogynous sex change, a severe decline in plasma E_2 leads to ovarian degeneration followed by a gradual rise in 11-KT production with spermatogenic onset. Alternatively, in protandrous sex change, plasma E_2 rises with decline of 11-KT level (Todd 2016). However, in bidirectional sex change, only plasma E_2 (but not 11-KT) level follows such sexual directional pattern. Poor circulatory 11-KT in gobiid fishes results into lack of secondary male characteristics in these species and facilitates the rapid

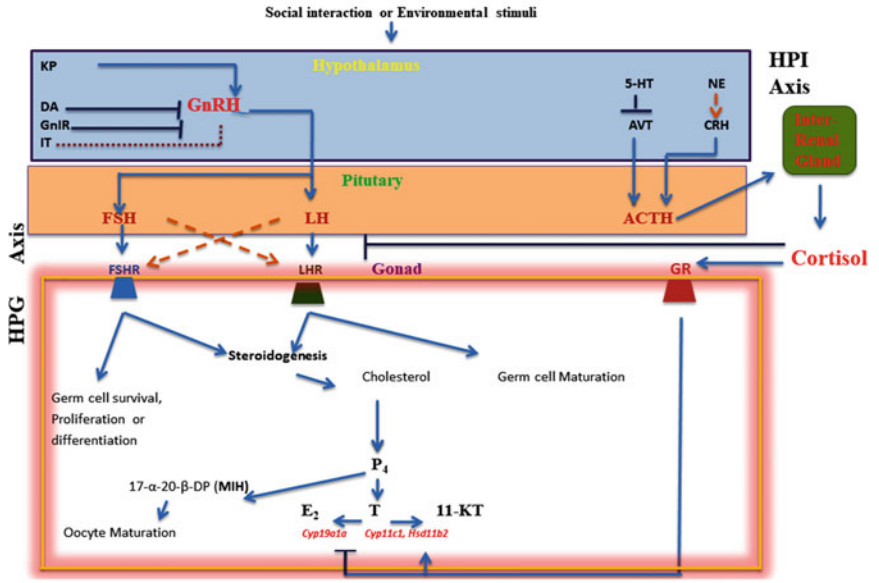
switching between sexual phenotypes (Todd 2016). Exogenous stimulation of sex steroids are reported to induce either masculinization or feminization in many teleosts such as Nile tilapia, rainbow trout, and Japanese flounder (Baroiller et al. 2009; Todd 2016).

The critical roles of kisspeptin (KP) and isotocin (IT) have also been well documented in sex-changing fishes (Todd 2016). KP stimulates GnRH release, whereas IT, the teleost ortholog of mammalian oxytocin, is associated with social and sex-specific reproductive behaviors. Alterations in the expression of either KP (coded by *Kiss2*) or its receptor (coded by *Kiss1r*) are reported in orange-spotted grouper *Epinephelus coioides* during sex reversal (Todd 2016). In the forebrain of bluehead wrasse, increased expression of IT has been found during sex change (Todd 2016). However, in bluebanded goby, poor IT activity has been observed in the pre-optic area (POA) of males as compared to that of the females (Todd 2016).

The stress response is regulated through the hypothalamic-pituitary-interrenal (HPI) axis and thereby modulates the transitional changes in behavior, metabolism, and growth during sex reversal. The corticotropin-releasing hormone (CRH) and glucocorticoid steroids (GCs) like cortisol respond to the environmental stress and potentially determine the gonadal fate (Capel 2017; Baroiller et al. 2009; Editorial 2009; Todd 2016). Elevated temperature induces gonadal masculinization via increased cortisol levels leading to downregulation of aromatase enzyme (Todd 2016). Furthermore, cortisol also stimulates *Hsd11b2* expression, which catalyzes the production of both 11-KT and cortisone, the deactivated metabolite of cortisol (Baroiller and D'Cotta 2006; Todd 2016). A transient surge in serum cortisol has been recorded during both protandrous (cinnamon clownfish) and protogynous (bluebanded goby) sex change. However, long-term cortisol supplementation has shown to promote protogynous sex change in three-spot wrasse (Todd 2016). A schematic illustration of the neuroendocrine crosstalk between the HPG and HPI axes regulating steroidogenesis and sexual behavior/fate in teleosts has been described in Fig. 9.1.

Genetic Mode of Sex Determination (GSD)

The GSD mode of sex determination is best understood in mammals and our knowledge has largely emerged from the mouse. After the discovery of the *Sry* gene in the 1990s, the molecular basis of mammalian sex determination (SD) and differentiation has been investigated in greater details (Capel 2017; DeFalco and Capel 2009; DeFalco 2014). In mice, during embryonic (E) age of 11.5, the SD results from the initial switch of either the *Sry*-dependent testis differentiation (in XY gonad) or *Sry*-independent ovary differentiation (in XX gonad). However, recent advancement in this field has indicated that *Dmrt1* and $Foxl_2$ are the two key transcription factors that, respectively, maintain the masculinity and femininity intact in adult mammals (DeFalco 2014). For example, the loss of $Foxl_2$ in adult female mice ovaries results in reprogramming of the granulosa and theca cell lineages into Sertoli-like and Leydig-like cell lineages (Matson et al. 2011).



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ACTH = Adrenocorticotropic hormone;
AVT = arginine vasotocin;
CRH = corticotropin-releasing hormone;
DA = dopamine;
E_2 = 17β-estradiol;
GnIH = gonadotropininhibitory hormone;
GnRH = gonadotropin-releasing hormone;
HPI= hypothalamic-pituitary-interrenal Axis;
HPG= hypothalamic-pituitary-gonadal Axis;
FSH = follicle-stimulating hormone;
FSHR = FSH receptor;
IT= Isotocin;
LH = luteinising hormone;
LHR = LH receptor;
MIH = maturation-inducing hormone;
NE = norepinephrine;
T = Testosterone;
5-HT = serotonin;
11-KT = 11-keto-testosterone.

Fig. 9.1 A schematic illustration of the neuroendocrine crosstalk between the HPG and HPI axes regulating steroidogenesis and sexual behavior/fate in teleosts

Similarly, the conditional ablation of *Dmrt1* results into a female reprogramming in adult testes (Matson et al. 2011). Therefore, the evolutionary significance of these two genes is of great interest and now being studied even in non-mammalian vertebrates like fowl, frog, and fishes (Capel 2017; Herpin and Schartl 2015; Bertho 2016).

In fishes, GSD could be monofactorial having a single master SD gene such as *Dmy* in medaka *Oryzias latipes* and/or could be polyfactorial involving several genes on multiple chromosomes such as in zebrafish *Danio rerio* (Capel 2017; Herpin and Schartl 2015). Other than *Dmy*, *Amhy* in the Patagonian pejerrey *Odontesthes hatcheri*, *Gsdf* in *Oryzias luzonensis*, *Amhr2* in fugu *Takifugu rubripes*, *SdY* in rainbow trout *Oncorhynchus mykiss*, and *Sox3* in *Oryzias dancena* have been identified as candidates for GSD (Herpin and Schartl 2015). Here we discuss some of the key molecules identified till date that regulate GSD in teleosts.

$Foxl_2$/$Foxl_3$

$Foxl_2$ is a member of the large family of Forkhead Box (Fox) domain transcription factors (Bertho 2016). Since their discovery, some Fox genes like *$Foxc_1$*, *$Foxl_2$*, *$Foxl_3$*, and various *FoxO*s have been reported to regulate the ovarian function and *$Foxj_2$*, *$Foxp_3$*, or *$Foxo_1$* for spermatogenesis, respectively (Bertho 2016). The *$Foxl_2$* gene is a highly conserved transcriptional factor expressed in the somatic cells of ovary and is essential for ovarian development and maintenance in mammals and fishes (Uhlenhaut et al. 2009; Bertho 2016; Nishimura and Tanaka 2016; Bhat et al. 2016a, b). In female gonads, *$Foxl_2$* is considered to suppress *Dmrt1* or its orthologs and upregulates the expression of female programming genes including *Cyp19a1a*, *Rspo1*, and *Wnt4/β-catenin*, thereby ensuring the production of E_2 (Bertho 2016; Nishimura and Tanaka 2016). In teleosts, two paralogs of *$Foxl_2$*, *$Foxl_2a$*, and *$Foxl_2b$* are present originated from teleost-specific genome duplication (Bertho 2016). However, recent phylogenetic analyses revealed that *$Foxl_2b$* is found in tetrapods, including reptiles, birds, and marsupials (Bertho 2016; Nishimura and Tanaka 2016). Therefore, *$Foxl_2a$* and *$Foxl_2b$* are currently renamed as *$Foxl_2$* and *$Foxl_3$*, respectively (Nishimura and Tanaka 2016). In salmonids and seabass, *$Foxl_2$* displays a clear sexually dimorphic expression pattern in the differentiating and adult gonads with elevated expression in ovaries as compared to testes (Nishimura and Tanaka 2016). During adulthood, *$Foxl_2$* mainly present in follicular ovarian cells, i.e., granulosa cells and theca cells, surrounding the oocytes (Bertho 2016).

In medaka *Oryzias latipes*, *$Foxl_2$* is also restricted to ovarian tissues only (Bertho 2016; Nishimura and Tanaka 2016). Throughout the transition of germline stem cells to oocytes, the FOXL2 protein is first present in the germline stem cells and thereafter maintained during meiosis until oogenesis is completed (Bertho 2016). During oocyte formation, no FOXL2 protein is detected in the ovarian cord/follicular cells. However, FOXL2 protein is localized in the surrounding cells progressively with maturation of the oocyte (Bertho 2016). Unlike mammals, FOXL2 protein is

localized in the nuclei of all granulosa cells only not in theca cells in medaka (Bertho 2016).

On the other hand, *Foxl*$_3$ is expressed in germ cells and acts as a major determinant of sexual fate decision in medaka *Oryzias latipes* (Nishimura et al. 2015). In the undifferentiated gonads, the expansion of germ cells is reported by stem cell-like self-renewal proliferation denoted as type I division. By the hatching stage of the female embryos, a subset of germ cells undergoing type I division initiates a type II cystic division leading to the meiotic onset. *Foxl*$_3$ transcript and $FOXL_3$ protein are initially detected in type I germ cells of both male and female embryos. Thereafter, $FOXL_3$ is only detected in a subset of mitotically active type I germ cells in female embryos, but not in mitotically quiescent germ cells in males (Nishimura et al. 2015). Both *Foxl*$_3$/$FOXL_3$ are expressed in type II germ cells but gradually start disappearing with meiotic progression and are completely lost in oocytes (Nishimura and Tanaka 2016). However, by 10 days post-hatching, such expression pattern of *Foxl*$_3$/$FOXL_3$ becomes undetectable in male fishes (Nishimura and Tanaka 2016).

Dmrt1

Doublesex- and mab-3-related transcription factor 1 (*Dmrt1*) is a critical inducer of testicular morphogenesis mostly conserved in metazoan organisms (Herpin and Schartl 2011 Zafer et al. 2019). It suppresses the transcription of female programming genes like *Cyp19a1a*, *Rspo1*, *Figla*, *Gdf9*, and *Wnt4/β-catenin* and further promotes the expression of male-specific genes like *Gsdf*, *Cyp11c1*, *Sox9/3*, *Amh*, etc. in the testes (Herpin and Schartl 2011; Dar et al. 2020). Male-restricted expression pattern of *Dmrt1* has been reported in African catfish *Clarias gariepinus*, rare minnow *Gobiocypris rarus*, Nile tilapia *Oreochromis niloticus*, medaka *Oryzias latipes*, olive flounder *Paralichthys olivaceus*, lake sturgeon *Acipenser fulvescens*, zebrafish *Danio rerio*, Atlantic cod *Gadus morhua*, pejerrey *Odontesthes bonariensis*, rainbow trout *Oncorhynchus mykiss*, shovelnose sturgeon *Scaphirhynchus platorynchus*, rohu *Labeo rohita*, and southern catfish *Silurus meridionalis* (Herpin and Schartl 2011; Sahoo et al. 2019). In gonochoristic annual breeders like *Clarias gariepinus*, *Oncorhynchus mykiss*, and *Silurus meridionalis*, *Dmrt1* has the key role during testicular recrudescence and spermatogenic onset (Herpin and Schartl 2011). Furthermore, in hermaphrodite teleosts like protogynous; black porgy *Acanthopagrus schlegeli*, gilthead seabream *Sparus aurata*, protandrous; grouper *Epinephelus coioides*, wrasse *Halichoeres tenuispinis*, rice field eel *Monopterus albus* the expression dynamics of *Dmrt1*s are shown to be consistent with the testicular development (Herpin and Schartl 2011). Additionally, in pejerrey *Odontesthes bonariensis*, which shows a strong TSD system, the developmental expression pattern of *Dmrt1* coincides perfectly with the rearing temperature (up at male-determining temperatures and down at female-determining temperatures) (Herpin and Schartl 2011). However, in *Gadus morhua* and *Danio rerio*, *Dmrt1* expressions are detected in the ovarian germ cells too (Herpin and Schartl 2011). Fish *Dmrt*s (*Dmrt*2, *Dmrt*3, *Dmrt*4, *Dmrt*5) show conserved

expression during embryonic development in undifferentiated gonads (Herpin and Schartl 2011). Both male and female gonadal expressions have been reported for *Dmrt2* in medaka and *Dmrt3* or *Dmrt*5 in zebrafish (Herpin and Schartl 2011). Male-specific gonadal expression has been observed for *Dmrt3/4* in medaka (Herpin and Schartl 2011) and *Dmrt4* in olive flounder (Herpin and Schartl 2011). On the contrary, *Dmrt*4 expression is restricted to ovary in Nile tilapia (Herpin and Schartl 2011).

Dmy

Medaka *Oryzias latipes* employs an XX/XY SD system in which the Y chromosome bears the master SD gene, the duplicated copy of *Dmrt1a* on the Y chromosome *Dmy/Dmrt1bY* (DM domain gene on the Y chromosome/doublesex- and mab-3-related transcription factor 1b on the Y chromosome) (Nanda et al. 2002; Matsuda et al. 2002). It is the only functional gene in the whole Y-specific region of the medaka sex chromosome (Kikuchi and Hamaguchi 2013). Mutations affecting this gene result in male-to-female sex reversal (Kikuchi and Hamaguchi 2013). Furthermore, *Dmy* transgene-induced testis development in genetic females (XX) specifically indicates that *Dmy* is not only necessary but also sufficient for triggering male developmental programming (Herpin and Schartl 2011). In XY medaka males, *Dmy*-driven primordial germ cell proliferation and the determination of pre-Sertoli cells are the primary events together leading to the male-specific primordial germ cell mitotic arrest (Herpin and Schartl 2011). *Dmy* is also reported to downregulate the hedgehog pathway in differentiating testes by suppressing its receptor *Pitch-2* and upregulating its antagonist *Hhip* in medaka (Herpin and Schartl 2011). *Dmy* is a transcription factor, and its transcription is suppressed by P element like DNA transposon named *Izanagi* within its own promoter (Herpin and Schartl 2011). During testicular differentiation in XY gonads, an 11-nucleotide protein-binding motif located in the 3″-UTR of *Dmy* mediates unique gonad-specific mRNA stability (Herpin and Schartl 2011). Interestingly, such motif is conserved in the 3-UTRs of a wide range of *Dmrt1* orthologous genes from *Drosophila* to mice, suggesting that different taxa may employ an evolutionary conserved RNA regulatory mechanism for this gene (Herpin and Schartl 2015; Kikuchi and Hamaguchi 2013; Herpin and Schartl 2011). In medaka *Oryzias latipes*, the insertion of transposable element *Rex1* into the promoter of *Dmy* gene results in the binding of transcription factor *Sox5*, which downregulates *Dmy* expression. XX mutants for *Sox5* thereby show a complete female-to-male sex reversal (Schartl et al. 2018).

Gsdf

Like *Oryzias latipes*, *Oryzias curvinotus* too retains the *Dmy*, as its SD gene (Herpin and Schartl 2015). However, in *Oryzias luzonensis*, although *Dmy* is lost, the SD mode follows a simple Mendelian trait (Kikuchi and Hamaguchi 2013). In 2012, an

advanced genetic mapping has identified *Gsdf* (gonadal soma-derived growth factor) as a strong candidate for the master SD gene in this species (Myosho et al. 2012). *Gsdf* encodes a secretory protein belonging to the transforming growth factor-β (TGF-β) superfamily. *Gsdf* is present on both chromosomes (X and Y) in *Oryzias luzonensis* (Myosho et al. 2012). Transgenic experiments have demonstrated that the allele of this gene residing on the Y chromosome (*GsdfY*) was sufficient to induce female-to-male sex reversal in XX *Oryzias luzonensis* (Myosho et al. 2012). Interspecific transgenic experiment between *Oryzias latipes* and *Oryzias luzonensis* indicated that *GsdfY* of *Oryzias luzonensis* is capable to produce a male phenotype in XX *Oryzias latipes* in the absence of *Dmy* (Schartl et al. 2018). The spatial and temporal expression pattern of *Gsdf* is closely correlated to that of *Dmy* in *Oryzias latipes* (Kikuchi and Hamaguchi 2013), and the expression patterns of the further downstream genes in gonadal differentiation, such as *Sox9a*$_2$, *Dmrt1*, and *Foxl*$_2$, are comparable between *Oryzias latipes* and *Oryzias luzonensis* (Kikuchi and Hamaguchi 2013). In *Oryzias latipes*, *Gsdf* is the target of *Dmy* and both of them get co-localized in pre-Sertoli cells. Knocking down of *Dmy* resulted in male-to-female sex reversal induced by suppressing *Gsdf* or *Sox9a* and upregulating *R-spondin1* or *Rspo1* (Chakraborty et al. 2016). Complete female-to-male sex reversal is observed in *Oryzias sakaizumii* but not in *Oryzias latipes* by *Gsdf* supplementation (Kikuchi and Hamaguchi 2013).

Gsdf is also found to be the SD gene in the sablefish *Anoplopoma fimbria* (Kikuchi and Hamaguchi 2013). GSDF protein has been found as a somatic factor controlling the proliferation of primordial germ cells and spermatogonia in rainbow trout (Kikuchi and Hamaguchi 2013), and its expression in gonads is also observed in medaka *Oryzias latipes* and zebrafish *Danio rerio* (Kikuchi and Hamaguchi 2013).

In XY tilapia *Oreochromis niloticus*, *Gsdf* action is reported to suppress the E_2 production probably via the inhibition of ovarian-differentiating genes (Jiang et al. 2016). The upregulation of *Gsdf* mRNA was found to be comparable with *Dmrt1* in the somatic cells surrounding germ cells (Jiang et al. 2016). In vitro co-transfection of Dmrt1 and *Sf1* also activates *Gsdf* in a dose-dependent manner in this fish (Jiang et al. 2016).

Sox9

The Sry-related HMG box (*Sox*) gene(s) encode a variety of transcription factors and have been implicated in male SD across the vertebrates (Capel 2017). In mammals, *Sox9* is the only known direct target of the SRY transcription factor and in the testis is expressed exclusively in Sertoli cells (Capel 2017, DeFalco and Capel 2009; Bhat et al. 2016a, b). In Nile tilapia *Oreochromis niloticus*, by 25 days of post-hatching, *Sox9* mRNA level is shown to be higher in XY gonads, whereas such expression becomes undetectable in XX gonads (Siegfried 2010). Two paralogous forms of *Sox9*, namely, *Sox9a* and *Sox9b*, are reported in medaka *Oryzias latipes* and zebrafish *Danio reri*o. However, in their developing gonads, no such sexual

dimorphic expression pattern is observed (Herpin and Schartl 2015; Kikuchi and Hamaguchi 2013).

Sox3

In Indian ricefish *Oryzias dancena*, *Sox3* gene is present in the XY sex chromosome (Takehana et al. 2014). The male-specific region of the Y chromosome bears a cis-regulatory DNA segment that induces the expression of the Y-chromosomal *Sox3*. Targeted deletion of the Y-chromosomal *Sox3* results in developing females, whereas XX fish transgenic for that regulatory segment matures as males confirming the role of *Sox3* as a master SD gene in *Oryzias dancena*. *Sox3* has been shown further to initiate the testicular differentiation by upregulating expression of *Gsdf* (Takehana et al. 2014). However, the back clone of *Sox3* from *Oryzias dancena* fails to induce testicular development in *Oryzias latipes* (Herpin and Schartl 2015).

Amhy/Amha

In the Patagonian pejerrey *Odontesthes hatcheri*, SD is directed by a Y copy of the anti-Müllerian hormone (*Amhy*) gene that codes for TGF-β superfamily protein (Hattori et al. 2012). Despite having a strong TSD, another pejerrey *Odontesthes bonariensis* also expressed *Amhy* at early stages of male-promoting temperatures followed by the expression of the autosomal *Amha*, showing the coexistence of both GSD and TSD mode in this species (Yamamoto et al. 2014). In the Nile tilapia *Oreochromis niloticus*, two tandem copies of *anti-Müllerian hormone* (*Amh*) gene have been identified *Amhy* and *AmhΔY* (a truncated *Amh* gene lacking the TGF-β domain due to 233-bp deleted region in exon VII and a 5-bp insertion in exon VI). However, knocking down of only Amhy (but not *AmhΔY*) demonstrated a male-to-female sex reversal, whereas the overexpression of only *Amhy* in XX fish resulted in female-to-male sex reversal (Li et al. 2015). Very recently, a male-specific duplicate copy of *AmhbY* (Y chromosome-specific anti-Müllerian hormone paralog b) showing differential expression pattern from its autosomal paralog *Amha* has been reported as a master regulator SD gene in northern pike *Esox lucius* (Pan et al. 2019).

Amhr2

The tiger pufferfish *Takifugu rubripes* employs a XX/XY SD system. A missense single-nucleotide polymorphism (SNP) in the kinase domain of the anti-Müllerian hormone receptor type II (*Amhr2*) has been found to be associated with the sexual phenotype in this fish (Kamiya et al. 2012). Two *Amhr2* subtypes are present that differ by one amino acid (H384D) in the kinase domain. The *384His* mutation causes lower activity to the receptor and is encoded on the X chromosome. Females are homozygous for the mutant, whereas the males keep one allele of the wild-type

receptor on their Y chromosome. Therefore, a quantitative difference in *Amh* signaling becomes critical for the male development. Similarly *hotei* mutation observed in *Amhr2* of medaka *Oryzias latipes* with a compromised *Amh* signaling shows a male-to-female sex reversal (Capel 2017, Kikuchi and Hamaguchi 2013). In Nile tilapia *Oreochromis niloticus*, knockout of *Amhr2* results in complete (100%) male-to-female sex reversal, but in contrast, with *Amhy*, only 60% of such effect has been observed (Li et al. 2015). Knockdown of both *Amhy* and *Amhr2* in XY tilapia shows upregulation of the aromatase *Cyp19a1a* gene as well as higher E_2 levels; however, no such rise in *Cyp19a1a* expression has been observed in *Amhy*/*Amhr2* overexpressed XX fish (Li et al. 2015).

SdY

Rainbow trout *Oncorhynchus mykiss* belongs to salmonid family and native to tributaries of the Pacific Ocean in Asia and North America. SD in this fish is strictly genetic, with an XX/XY system controlled by a single SD locus. In 2012, Yano and colleagues have identified a male-specific gene, *SdY*, expressed in the somatic cells surrounding germ cells (Yano et al. 2012). This gene encodes a novel protein that displays sequence homology with the carboxy-terminal domain of interferon regulatory factor 9 (*Irf9*). IRF9 is a transcription regulatory factor that mediates signaling by type I interferon in mammals. Microinjection of the *SdY* into eggs resulted in female-to-male sex reversal in XX fish, whereas the targeted inactivation of *SdY* induced ovarian differentiation in F_1 XY fishes (Yano et al. 2012).

Table 9.1 describes the major master regulator gene(s) of SD in teleosts.

Germ Cell Number

Beyond the several environmental cues and genes, the germ cell numbers seem to be a key determinant of sex in fishes. In fact, fishes are the only class of vertebrates where germ cell number plays a key role in SD (Capel 2017; DeFalco and Capel 2009; Baroiller and D'Cotta 2006; Todd 2016). In teleosts, the germline stem cells express *Nanos2* and support gametogenesis in both the sexes. Therefore, unlike mammals, germ cells in fishes show high sexual plasticity even in the matured testes and ovaries (Nishimura and Tanaka 2016). In zebrafish, goldfish, medaka, and rainbow trout, germ cells obtained from mature testes and/or ovaries can colonize in larval gonads and can differentiate into either sperm or eggs according to the sex of gonadal somatic cells (Nishimura and Tanaka 2016). In medaka *Oryzias latipes*, transplantation of genetically male (XY) somatic cells is sufficient to induce spermatogenesis in female (XX) fishes (Nishimura and Tanaka 2016). Emerging evidences from zebrafish *Danio rerio* (Uchida et al. 2004; Slanchev et al. 2005 Siegfried and Nüsslein-Volhard 2008) and medaka *Oryzias latipes* (Kurokawa et al. 2007) suggest that germ cell number may drive SD in these species. In zebrafish, if germ cells are depleted during the first day of development, all fishes develop as

Table 9.1 Master regulator genes of SD in teleosts

S. No.	Master SD gene	Organism(s) studied	SD system	SD ancestor gene	SD gene generated from ancestor gene	Ancestor gene function(s)	Key references(s)
1	*Foxl$_2$*	Many teleosts	XY/ WZ/ others	*Foxl$_2$*	Gene duplication/ allelic diversification	Transcription factor inducing female developmental programming and ovarian function	Bertho (2016) and Nishimura and Tanaka (2016)
2	*Foxl$_3$*	Many teleosts including medaka *Oryzias latipes*	XY/ WZ/ others	*Foxl$_2$*	Gene duplication/ allelic diversification	Transcription factor regulating female germ cell specification for meiotic entry	Nishimura and Tanaka (2016) and Nishimura et al. (2015)
3	*Dmrt1/ 2/4/4/5*	*Danio rerio*, *Oryzias latipes*, *Paralichthys olivaceus*, *Cynoglossus semilaevis*	XY/ WZ/ others	*Dmrt1*	Allelic diversification	Transcription factor inducing male developmental programming and testicular differentiation	Herpin and Schartl (2011)
4	*Dmy*	Medaka (*Oryzias latipes*, *Oryzias curvinotus*)	XY	*Dmrt1*	Gene duplication	Transcription factor inducing male developmental programming and testicular differentiation	Nanda et al. (2002) and Matsuda et al. (2002)
5	*GsdfY*	Luzon ricefish *Oryzias luzonensis* Sablefish *Anoplopoma fimbria*	XY	*Gsdf*	Allelic diversification	TGF-β factor, important role in fish gonad development	Myosho et al. (2012), Chakraborty et al. (2016), and Jiang et al. (2016)
6	*AmhY/ AmhbY*	Pejerrey *Odontesthes hatcheri* *Odontesthes bonariensis* Nile tilapia *Oreochromis niloticus* Northern pike *Esox lucius*	XY	*Amh*	Gene duplication	Anti-Müllerian hormone (AMH), growth factor	Hattori et al. (2012), Li et al. (2015), and Pan et al. (2019)

(continued)

Table 9.1 (continued)

S. No.	Master SD gene	Organism(s) studied	SD system	SD ancestor gene	SD gene generated from ancestor gene	Ancestor gene function(s)	Key references(s)
7	*Amhr2Y*	Fugu *Takifugu rubripes*	XY	*Amh receptor 2*	Allelic diversification	Type II receptor for AMH ligand, important function in gonad development	Kamiya et al. (2012)
8	*SdY*	Rainbow trout *Oncorhynchus mykiss*	XY	*Irf9*	Gene duplication	Interferon response factor with unknown gonadal function	Yano et al. (2012)
9	*Sox9a/ Sox9b*	*Oreochromis niloticus*, *Danio rerio*, *Oryzias latipes*	XY/ others	*Sox9*	Allelic diversification	Transcription factor inducing male developmental programming and testicular differentiation	Siegfried (2010) and Kikuchi and Hamaguchi (2013)
10	*Sox3Y*	Indian ricefish *Oryzias dancena*	XY	*Sox3*	Allelic diversification	Transcription factor inducing male developmental programming and testicular differentiation	Takehana et al. (2014)
11	*Sox5Y*	*Oryzias latipes*	XY	*Sox5*	Allelic diversification	Transcription factor binds to *Rex1 cis*-regulatory sequence in the promoter of *Dmy* gene and suppresses its expression leading to major regulator of germ cell number	Schartl et al. (2018)
12	*Gsf6Y*	Turquoise killifish *Nothobranchius furzeri*	XY	*Gsf6*	Allelic diversification	Growth differentiation factor 6, a member of the TGF-β family	Reichwald et al. (2015)
13	*Runx1*	Rainbow trout *Oncorhynchus mykiss*	XY	*Runt*	Allelic diversification	Transcription factor inducing female developmental programming and pre-granulosa cell specification/differentiation	Nicol et al. (2019)

sterile males (Capel 2017). However, if germ cells are depleted in later stage, as occurs in *Fanconi anemia* mutation (*Fancl*), adult females undergo sex reversal to phenotypic males and can further become fertile if some of the germline stem cells persist and populate the testis (Rodriguez-Mari et al. 2010). On the other hand, in medaka, germ cell-depleted fishes develop with male phenotype; by contrast, when the number of germ cells is amplified (e.g., in the *hotei* mutant, the Amhr2 gene results into a compromised *Amh* signaling on the mitotic self-renewing germ cells leading to massive germ cell proliferation), fishes show a male-to-female sex reversal (Nakamura et al. 2012). Similarly, loss of germ cells induced by high temperatures in fugu *Takifugu rubripes* induced sex reversal of females into males (Baroiller and D'Cotta 2006). However, the depletion of germ cells fails to affect the sexual fate of gonadal somatic cells in goldfish *Carassius auratus* (Goto et al. 2012) or loach *Misgurnus anguillicaudatus* (Fujimoto et al. 2010).

Conclusion

Various laboratories across the globe for the past five decades have substantially contributed toward our present knowledge regarding the environmental, endocrine, and genetic control on SD mechanisms in fishes. Briefly at the molecular level, in females, the transcription factors like *Foxl*$_2$ and *Sf1* (controlled by gonadotropins like FSH via cAMP pathway) upregulate the expression of *Cyp19a1a* to produce the aromatase enzyme for the bioconversion of E_2 from T. This estrogenic environment further maintains an auto-regulatory feed-forward loop for supporting the ovarian function via promoting female-specific gene expression while suppressing male programming genes. In males, *Dmrt1* directly inhibits *Cyp19a1a* promoter activity, while *Dax1* negatively modulates the expression of *Cyp19a1a* through its suppression of *Sf1* and *Foxl*$_2$. Therefore, such inhibition of aromatase action promotes the male-specific genes for testicular differentiation and function. Furthermore, the environmental stress induces cortisol production, which regulates the balance between the turnover rate of T to either E_2 or 11-KT (Capel 2017; Baroiller and D'Cotta 2006; Todd 2016). In summary, a complex antagonistic crosstalk between neuroendocrine signaling and genetic regulatory network promotes either an estrogenic or an androgenic milieu to determine the sexual fate in teleosts. Figure 9.2 schematically represents such antagonistic regulation toward gonadal development and function in fishes.

Future Direction

Emerging data in the last decade further indicate that unlike mammals, SD in fishes is not brought by a simple linear cascade, but a complex network of multiple regulatory pathways is involved with apparently diversified upstream inducers and relatively conserved downstream effectors (Herpin and Schartl 2015; Kikuchi and Hamaguchi 2013; Herpin et al. 2013). However, in-depth comparative analyses in

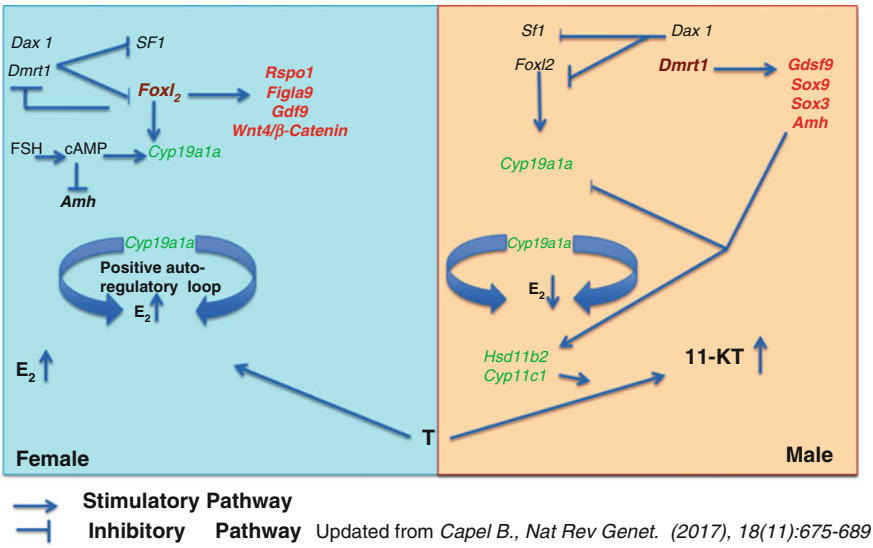

Fig. 9.2 The antagonistic regulatory pathways controlling the differential steroidogenesis and genetic networks determining the sexual fate in teleost fishes

diverse non-model species are necessary for the better understanding of such regulatory networks. The advancement in next-generation high-throughput sequencing technologies and *omics* approach may potentially reveal the gonadal transcriptome, miRNome, or methylome for future discovery/identification of other novel putative factor(s) and establish their interaction(s) with known sex-determining loci (Qian et al. 2014; Pan et al. 2016).

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The Involvement of Gonadotropin-Inhibitory Hormone (GnIH) in Fish Reproduction

10

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Abstract

Gonadotropin-inhibitory hormone (GnIH) is a hypothalamic neuropeptide that was discovered from the brain of Japanese quail. GnIH belongs to the RFamide peptide family having LPXRFamide (X = L or Q) sequence at C-terminal. GnIH has an inhibitory effect on the reproductive axis in birds and most of the mammals, but in fishes, it showed both inhibitory and stimulatory effects depending on species. Even in single fish species, it can have different impacts during different breeding seasons. GnIH also showed involvement in other functions in fishes affecting growth, stress, and behavior. All these functions are mediated via G protein-coupled GnIH receptors, GPR147. Fishes even with more than half of the known vertebrate species, the study about GnIH, its physiological effect are very limited, and even results obtained in some studies are conflicting. So in this chapter, we summarize the available information about the GnIH and its distribution, evolutionary origin, ontogeny, interaction with other molecules of the HPG axis, and physiological effects in fishes. With GnIH, as a fascinating molecule of the HPG axis having different types of impacts, further study is required in different groups of fishes.

Keywords

GnIH · HPG axis · GPR147 · Fish reproduction · Neuropeptide

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Introduction

Hypothalamus-pituitary-gonadal (HPG) axis plays a significant role in vertebrate's reproduction. Gonadotropin-releasing hormone (GnRH) stimulates the synthesis and release of luteinizing hormone (LH) and follicle-stimulating hormone (FSH) from the pituitary gland (Rather et al. 2017, 2020). Before the discovery of gonadotropin-inhibitory hormone (GnIH), it was believed that GnRH is the only neuropeptide that regulates the HPG axis in vertebrates. GnIH was reported in the year 2000 from the brain of Japanese quail as a novel hypothalamic peptide that acts as the negative regulator of the HPG axis by inhibiting the effect of gonadotropins (Tsutsui et al. 2000). It is an RFamide neuropeptide, containing arginine-phenylalanine-NH2 motif at C-terminal. GnIH also regulates the GnRH and kisspeptin expression by its receptor, GPR147, present in the GnRH and kisspeptin neurons (Ubuka et al. 2013; Tsutsui 2009, 2016). GnIH is influenced by environmental factors like photoperiod, social interactions, and stress as well as by internal factors like melatonin hormone (Tsutsui et al. 2015). GnIH and its orthologs have been identified in many species of avians, fishes, amphibians, reptiles, and mammals. GnIH is named because of its negative impact on reproduction-related genes in birds and mammals; however, in other vertebrates, this function is not yet clearly established (Di Yorio et al. 2019a, b). In birds, it has an inhibitory effect on GnRH and gonadotropin release and synthesis. In the mammalian brain, GnIH acts on the pituitary and GnRH neurons to inhibit the reproductive functions by decreasing gonadotropin release and synthesis (Ogawa and Parhar 2014), while in amphibians like frog, GnIH homologs stimulated the release of growth hormone (GH) and prolactin (PRL) but failed to have any effect on the gonadotropins, so it is called a "frog growth hormone-releasing peptide" (fGRP) (Koda et al. 2002).

In fishes, GnIH has been reported to exhibit both inhibitory and stimulatory effects on GnRH neurons and gonadotropin release and synthesis and is called GnIH or LPXRF. In fishes, LPXRF was named because of the presence of a common LPXRFamide (X=L or Q) motif at the C-terminus (Tsutsui et al. 2012; Moussavi et al. 2012, 2013). GnIH is translated into a long polypeptide (prepropeptide), which generates different numbers of mature peptides in different species. In birds three while in mammals, only two peptides were produced. In fish, GnIH polypeptide encodes three GnIH peptides (GnIH/LPXRF-1, 2, 3). GnIH receptors (GnIHRs) are the G protein-coupled receptors with seven transmembrane domains, designated as GPR147 (Ubuka et al. 2013). Birds and mammals have only one type of GnIH receptor (Dardente et al. 2008; Ubuka et al. 2008), while some fishes have up to three receptors (Qi et al. 2013a; Peng et al. 2016). GnIH receptors were detected from the hypothalamus to gonads on the whole HPG axis, which indicates the regulation of GnIH on all levels of the HPG axis (Bentley et al. 2008). Even though the GnIH has been characterized and studied in many fish species, its role is still ambiguous, so more studies need to be conducted to confirm the reproductive stage-specific role of this hormone.

Discovery of GnIH/GnIHRs

GnIH is a member of the RFamide peptide family, which contains the Arg-Phe-NH2 motif at their C-terminal. The first RFamide peptide (FMRFamide) was isolated from the ganglia of Venus clam, *Macrocallista nimbosa*, as a cardioexcitatory peptide back in the 1970s (Price and Greenberg 1977). Since then, many RFamide peptides had been identified in various invertebrates that act as neurotransmitters, neuromodulators, and neuroendocrine hormones (Ubuka et al. 2013). In the vertebrates, the immunohistochemical studies by different researchers suggested the presence of an unknown hypothalamic RFamide peptide that may regulate the secretion of anterior pituitary hormones (Raffa 1988; Rastogi et al. 2001). Tsutsui and his colleagues searched for the novel RFamide peptide in the brain of the Japanese quail, *Coturnix japonica*, and got the first breakthrough in 2000 when they isolated a novel RFamide peptide (SIKPSAYLPLRFamide) possessing Arg-Phe-NH_2 sequence at C-terminal that actively inhibited gonadotropin release in the pituitary of quail. Later, a cDNA encoding the precursor polypeptide for GnIH was identified in the brain of quail (Satake et al. 2001). Following this, GnIH gene was reported from other avian species (Bentley et al. 2003; Ciccone et al. 2004; Ubuka et al. 2008; Tobari et al. 2010) and mammals (Fukusumi et al. 2001; Ukena et al. 2002; Yoshida et al. 2003; Ubuka et al. 2009a, b). In fishes, Sawada et al. (2002) first amplified GnIH peptide precursor cDNA from the brain of goldfish by a combination of 3' and 5' rapid amplification of cDNA ends and also purified the endogenously occurring GnIH peptide by immunoaffinity purification combined with mass spectrometry. Later, the GnIH was identified in several fish species from freshwater to marine water zebrafish, common carp, tilapia, *Catla catla* orange-spotted grouper, grass pufferfish, European sea bass, sole fish, and lampreys (Zhang et al. 2010; Shahjahan et al. 2011; Osugi et al. 2012; Biran et al. 2014; Wang et al. 2015; Paullada-Salmerón et al. 2016b; Peng et al. 2016; Aliaga-Guerrero et al. 2018; Kumar et al. 2019).

GnIH receptors (GnIHRs) have been identified in different species from fish to humans (Ubuka et al. 2006; Tsutsui et al. 2010; Biran et al. 2014; Ogawa and Parhar 2014). GnIH receptors belong to a subfamily of G protein-coupled receptors (GPCRs), called RFamide receptors, that contains neuropeptide FF1 (NPFF1R/GPR147), neuropeptide FF2 (NPFF2R/GPR74), prolactin-releasing peptide (PrRP/GPR10), kisspeptin (GPR54), and QRFP (GPR103) receptors (Quillet et al. 2016). All the receptors bind with their endogenous neuropeptides that have a conserved Arg-Phe-NH_2 (RFamide) motif at their carboxyl-terminal end. This motif was mandatory for the affinity and the activity of these peptides toward their receptors. GnIH/RFRP has the highest binding relationship with NPFF1R/GPR147 receptor, while NPFF and neuropeptide AF (NPAF) have the high binding affinity for NPFF2R/GPR74 receptor, which suggested that GPR147 is the candidate receptor for GnIH (Ayachi and Simonin 2014; Elhabazi et al. 2017). GPR147 receptor has been identified in the zebrafish (Zhang et al. 2010), grass pufferfish (Shahjahan et al. 2011), goldfish (Moussavi et al. 2013; Qi et al. 2013a), tilapia (Biran et al. 2014), orange-spotted grouper (Wang et al. 2015), and common carp (Peng et al. 2016). In

most teleosts, only one GnIH receptor had been identified, while in the zebrafish, three different GnIH receptors (GnIHR1, GnIHR2, and GnIHR3) were detected (Zhang et al. 2010). The binding affinities of teleost GPR147 GnIH peptides had not been much studied, but Biran et al. (2014) showed that tilapia GPR147 (tiLPXRFa-R) had a strong affinity to tilapia LPXRFa-2 peptides through both cAMP/PKA and CaC2/PKC pathways. By in situ hybridization, Qi et al. (2013a) characterized three GnIHRs from the goldfish brain and reported their localization in the hypothalamus and pituitary. In the hypothalamus, GnIHRs were found in the NPP, NPO, and NLT, while in the pituitary, only two GnIHRs were localized in the pars intermedia. Further, it was demonstrated that goldfish GnIHRs are localized in the oocytes before the cortical alveolus stage and in the interstitial tissue of the testis apart from the brain.

Evolutionary Origin of GnIH

GnIH and neuropeptide FF (NPFF), also known as PQRFamide peptide, are pain-modulatory neuropeptides and paralog genes. Both genes may have diverged from a common ancestral gene through the whole genome duplication during vertebrate evolution (Osugi et al. 2014a, b) and were identified in the most basal vertebrates, agnathans (Osugi et al. 2006, 2011, 2012). To clarify the evolutionary origin of GnIH and NPFF gene, Osugi et al. (2014b) conducted a study in the protochordate amphioxus *Branchiostoma floridae* and found three RFa peptides (PQRFa peptides) that have the C-terminal PQRFa motif identical to that of vertebrate peptides of the GnIH and NPFF group. Further, phylogenetic analysis of PQRFa peptides in amphioxus showed that PQRFa peptide precursor was present before the splitting of the GnIH and NPFF group, suggesting PQRFa peptide as a common ancestor of GnIH and NPFF precursors. Besides, the GnIH gene is located near the HOXA clusters, while the NPFF gene has been present near the HOXC clusters on the chromosomes. It is believed that the HOX clusters have been duplicated from a common ancestral gene during whole genome duplication through vertebrate evolution (IKEMOTO and PARK 2005; Osugi et al. 2012, 2014a). Yin et al. (2005) observed high sequence similarity between the receptors for GnIH (GPR147) and NPFF (GPR74). All these pieces of information led to the strong hypothesis that GnIH and NPFF genes have diverged from a common ancestral gene through chromosome duplication (IKEMOTO and PARK 2005; Osugi et al. 2012, 2014b).

Distributions of GnIH/GnIHRs

Brain and Pituitary

GnIH neurons have been studied and reported in different species. In birds, GnIH neurons were observed in the paraventricular nucleus (PVN) and axonal terminals in the median eminence (ME) (Tsutsui et al. 2000; Bentley et al. 2003; Ukena et al.

2003; Osugi et al. 2004) which also influences GnRH neurons. In European starlings, GnIH neurons project to GnRH-I and GnRH-II neurons and inhibit the action of these two types of GnRH via the GnIHR GPR147 (Ubuka et al. 2008).

In mammals, GnIH neurons are widespread in the brain, where they are present in the dorsomedial nucleus (DMH), PVN, and the mediobasal hypothalamus (Kriegsfeld et al. 2006; Clarke et al. 2008). Soga et al. (2014) detected the GnIH expression in the ventromedial hypothalamus (VMH) in transgenic rats. In humans, GnIH-immunoreactive neuronal cell bodies were identified in the dorsomedial region (DME) of the hypothalamus with axonal projections to GnRH neurons in the preoptic area (POA) as well as to the median eminence (ME) by immunocytochemistry (Ubuka et al. 2009a). In the brain of European green frog, RFa-containing neurons are localized in the anterior preoptic area (POA), the suprachiasmatic nucleus (SCN), and the dorsal and ventral hypothalamic nuclei of the hypothalamus (Chartrel et al. 2002). In bullfrog, fGRP neurons are located in the telencephalon and the diencephalon, including the medial septum, nucleus of the diagonal band of Broca, anterior POA, and the SCN (Koda et al. 2002; Chowdhury et al. 2007). In the brain of newt, nLPXRFa mRNA and the peptide are expressed only in the SCN in the hypothalamus, whereas immunoreactive fibers were detected in the mesencephalic and rhombencephalic regions and terminated in the ME (Chowdhury et al. 2011).

The study related to the distribution of GnIH neurons in fishes is scanty due to limited GnIH gene sequences and a lack of specific antibodies to non-mammalian GnIH orthologous peptides. In the sea lamprey, the LPXRFa-immunoreactive cells have been detected in the bed nucleus of the tract of the postoptic commissure (nTPOC) in the hypothalamus, and some fibers are also seen in the neurohypophysis of the pituitary. Some immunoreactive fibers in lamprey were detected close to GnRH-III neurons (Osugi et al. 2012; Ogawa and Parhar 2014). By using antibodies to avian GnIH, the distribution of GnIH orthologs has been examined in the brain of several teleosts including goldfish, sockeye salmon, and rohu (Sawada et al. 2002; Amano et al. 2006; Biswas et al. 2015). In goldfish, GnIH mRNA was detected in the hypothalamus of the brain with the intense expression in nucleus posterioris periventricularis (NPPv) (Sawada et al. 2002), while in grouper, a strong expression of GnIH mRNA was detected in the nucleus posterioris periventricularis (NPPv) of the hypothalamus (Wang et al. 2015). In tilapia, lpxrf mRNA expression was identified in the proximal pars distalis of the pituitary; especially LH-positive cells expressed tilapia lpxrf mRNA, whereas there was no localization of lpxrf in GH- or FSH-positive cells (Biran et al. 2014). Biswas et al. (2015) showed GnIH immunoreactivity in the CNS and pituitary during the development of *Labeo rohita* and compared it with the localization of GnRH. The localization of GnIH and GnRH in the pituitary suggested the involvement of these neuropeptides in the regulation of pituitary hormones. In zebrafish, Lpxrfa-ir fibers were localized in the medial region of the neurohypophysis in the pituitary, while in the brain, it was found in the forebrain, midbrain, and hindbrain (Spicer et al. 2017). In sole fish, GnIH-ir fibers were reported from almost all forebrain areas, like olfactory bulbs, telencephalon,

diencephalon, mesencephalic tectum and tegmentum, medulla oblongata, and spinal cord (Aliaga-Guerrero et al. 2018).

Gonads

The gonadal GnIH system was discovered in European starlings and Japanese quail (Bentley et al. 2008). In quail testes, GnIH immunoreactivity was observed primarily in Leydig cells and germ cells (spermatocytes and spermatids). In addition to that, the intense immunoreactivity was also detected in the pseudostratified columnar epithelial cells of the epididymis (Bentley et al. 2008). In female starling, GnIH-ir was observed in the thecal and granulosa layers of the ovary (Bentley et al. 2008). In situ hybridization and immunohistochemical analysis confirmed the presence of GnIH peptides in the thecal and germ cells of the ovary and testis along with the pseudostratified columnar epithelial cells of the epididymis (Bentley et al. 2008; McGuire and Bentley 2010). In European starlings, melatonin increases the expression of GnIH in the gonads, and treatment of both GnIH and melatonin significantly decreased the testosterone secretion from testes (McGuire et al. 2011). In the mammalian species, the expression of GnIH and its role in gonadal maturation have been well demonstrated (Bentley et al. 2010; Ogawa and Parhar 2014; Ubuka et al. 2014). In the Syrian hamster, GnIH was detected in spermatocytes and spermatids but not in the Leydig cells of the testis (Zhao et al. 2010), while in rhesus macaque, it was spotted in the Leydig cells (McGuire and Bentley 2010). Researchers confirmed the expression of GnIH in both testes and ovary of different mammals (Singh et al. 2011; Li et al. 2012; Oishi et al. 2012; Anjum et al. 2014), but in amphibians, the expression of GnIH in gonads was not observed (Ogawa and Parhar 2014).

The expression of GnIH neurons was detected from the testes and ovary of different fish species (Zhang et al. 2010; Osugi et al. 2012; Qi et al. 2013b; Biran et al. 2014). In sea lamprey, zebrafish, and tilapia, LPXRFa mRNAs were expressed in the testis and ovary (Zhang et al. 2010; Osugi et al. 2012; Biran et al. 2014), which indicated that GnIH is not limited to the brain alone. In goldfish, the in vitro treatment of GnIH peptides with ovarian cell culture did not have any effect on the mRNA expression of genes involved in steroidogenesis, while in testicular cell culture, it significantly increased the expression of genes involved in testosterone biosynthesis (Qi et al. 2013b). However, in the grass puffer, the expression of LPXRFa mRNA was not detected in the gonads (Shahjahan et al. 2011).

GnIH Sequences Identified in Fishes

GnIH orthologs were discovered in all vertebrates from fish to mammals, including humans (Tsutsui et al. 2012; Ubuka et al. 2012). The name of GnIH orthologs varies in different groups like GnIH in birds, RFRP in mammals, LPXRFa/GnIH in fish, and fGRH in amphibians depending upon the role in that species. In fishes, GnIH

precursor polypeptide cleaved into two or three mature peptides, GnIH/LPXRF-1, GnIH/LPXRF-2, and GnIH/LPXRF-3, summarized in Table 10.1.

Ontogeny and Tissue Distribution of Fish GnIH System

In fishes, very few reports are available about the function of GnIH during development. Studies had shown some impressive results indicating that many of these peptides have different roles in the early stages and adults. To date, only five reports have examined GnIH expression pattern during fish development, showing that this peptide is detected from early developmental stages (Zhang et al. 2010; Biswas et al. 2015; Paullada-Salmerón et al. 2017; Di Yorio et al. 2018: Wang et al. 2019). In zebrafish, the GnIH transcript was detected from the prime-5 stage, while all three GnIHRs could be distinguished from all the embryonic and early larval developmental stages examined (Zhang et al. 2010). In sea bass, GnIH and GnIHR transcripts were detected from 5 dpf, and although the authors did not quantify the expression in stages before hatching, two temporal increases in the GnIH messengers were observed: one from 5 days post-hatching (dph) to 25 dph, when the larva starts exogenous feeding and the gonad is still undifferentiated, and the other by 150 dph during the onset of gonadal differentiation (Paullada-Salmerón et al. 2017). In *Cichlasoma dimerus*, GnIH mRNA was first detected at 1 dph, with its expression increasing from 12 dph and reaching a peak at 20 dph (Di Yorio et al. 2018). In tongue sole, both LPXRF and LPXRF-R mRNAs were detected in unfertilized eggs and during embryogenesis stage, but with different expression profiles. However, the expression of LPXRF mRNAs during larval development did not vary significantly, while LPXRF-R mRNAs showed a differential expression pattern (Wang et al. 2019). The GnIH neurons or GnIH-immunoreactive (GnIH-ir) fibers were observed in different species with one or more clusters. The spatial-temporal expression pattern of these nuclei could suggest different origins or functions during development (Di Yorio et al. 2019a, b). Biswas et al. (2015) observed the GnIH and GnRH immunoreactivities from the olfactory system to the spinal cord in *Labeo rohita.* In the brain, both neuropeptides were localized in the telencephalon; diencephalon, including the preoptic area; and rhombencephalon. The localization of GnIH and GnRH in the pituitary suggests that these neuropeptides are involved in the regulation of pituitary hormones by an autocrine manner during development, while in *C. dimerus*, GnIH neurons in the NOR were detected by 3 dph, while NPPv cells by 14 dph (Di Yorio et al. 2018). The cells in the NOR increase in number from 5 dph, coinciding with the time when larvae start to feed exogenously and continue to increase in number during the development and differentiation of gonadal primordia.

Table 10.1 Amino acid sequences of mature GnIH and its homologous peptides (LPXRFamide peptides) in fishes

S. No	Species name	Name of peptides	Sequences	Distribution	References
1.	Protochordate amphioxus (*Branchiostoma japonicum*)	PQRFa-1 PQRFa-2 PQRFa-3	WDEAWR**PQRFa** GDHTKDGWR**PQRFa** GRDQGWR**PQRFa**	–	Osugi et al. (2014a, b)
2.	Sea lampreys (*Petromyzon marinus*)	PQRFa PQRFa-RP-1 PQRFa-RP-1	SWGAPAEKFWMRAM**PQRFa** AFMHF**PQRFa** AGPSSLFQ**PQRFa**	B	Osugi et al. (2006)
		LPXRFa-1a LPXRFa-1b LPXRFa-2	SGVGQGRSSKTLF**QPQRFa** AALRSGVGQGRSSKTLF**QPQRFa** SEPFWHRT**RPQRFa**	B, H, T, O	Osugi et al. (2012)
3.	Brown hagfish (*Paramyxine atami*)	PQRFa-RP-1 PQRFa-RP-2 PQRFa	AFSNT**PQRFa** ADTSHFFQ**PQRFa** NSQETVPAYVWMRAF**PQRFa**	–	Osugi et al. (2011)
		PQRFa-RP-1 PQRFa-RP-2 LPQRFa	AFSNT**PQRFa** ADTSHFFQ**PQRFa** AL**PQRFa**	–	
4.	Goldfish (*Carassius auratus*)	gfLPXRFa-1 gfLPXRFa-2 gfLPXRFa-3	PTHLHAN**LPLRFa** AKSNIN**LPQRFa** SGTGLSAT**LPQRFa**	B	Sawada et al. (2002)

5.	Zebrafish (*Danio rerio*)	zfLPXRFa-1 zfLPXRFa-2 zfLPXRFa-3	PAHLHAN**LPLRFa** APKSTIN**LPQRFa** SGTGPSAT**LPQRFa**	B, E, T, O, M, K, SP, G	Zhang et al. (2010)
6.	*Takifugu niphobles*	LPXRFa-1 LPXRFa-2	SLDMERINIQVSPTSGKVSLPTIVRLYPPTLQPHHQHVN**MPMRFa** DGVQGGDHVPNLNPN**MPQRFa**	B, P, E, K, SP	Shahjahan et al. (2011)
7.	Nile tilapia (*Oreochromis niloticus*)	LPXRFa-1 LPXRFa-2 LPXRFa-3	TLLSSNDGTYSVRKQPHQETKNEIHRSLDL ESFNIRVAPTTSKFSLPTIIRFYPPTVKPLHLHAN**MPLRFa** QSDERTPNSSPN**LPQRFa** APNQL**LSQRFa**	B, P, T, O	Biran et al. (2014)
8.	Orange-spotted grouper (*Epinephelus coioides*)	gGnIH-III gGnIH-III gGnIH-III	LFPPTAKPFQLHAN**MPMRFa** ESVPGDDSAPNSTPN**MPQRFa** EAQNPI**LPQRLa**	Hyp	Wang et al. (2015)
9.	Common carp (*Cyprinus carpio*)	GnIH-III	SGTGLSAT**LPQRFa**	Hyp, T, O	Peng et al. (2016)
10.	Flatfish (*Solea senegalensis*)	ssGnih-1 ssGnih-2 ssGnih-3	PHRHAN**MPMRFa** SPNSPN**MPQRFa** VCAECDEELNPA**LPQRFa**	P, R, Tel, Die, Mes	Aliaga-Guerrero et al. (2018)
11.	European sea bass (*Dicentrarchus labrax*)	sbGnIH-1 sbGnIH-2	PLHLHAN**MPMRFa** SPNSTPN**MPQRFa**	T, R, Tel, Die, OT, Cer	Paullada-Salmerón et al. (2016a, b, c)
12.	Half-smooth tongue sole (*Cynoglossus semilaevis*)	tsLPXRFa-1 tsLPXRFa-2	SLDLERLNMRVTPTASKSSLPTIIKLYPPTVNPHIHAN**MPMRFa** EVEPEDDQSHNTPN**MPQRFa**	B, O, H, S, K, M	Wang et al. (2018)

(continued)

Table 10.1 (continued)

S. No	Species name	Name of peptides	Sequences	Distribution	References
13.	*Cichlasoma dimerus*	cd-MPLRF cd-LPQRFa-1 cd-LPQRFa-2	SLDLESFNVHVAPTTSKFSHPTIIRFYPPTVKPLHLHAN**MPLRFa** TPNSSPN**LPQRFa** APNQV**LPQRFa**	NPPv, NOR, NLT	Di Yorio et al. (2016)
14.	Catla (*Catla catla*)	GnIH-I GnIH-II GnIH-III	PNVSVATNPLLLKAH**LPIRFa** ERASKSNIN**LPQRFa** SVNGPSAT**LPQRFa**	B, G, I, H, L, Gi, S	Kumar et al. (2019)

B brain, Hyp hypothalamus, P pituitary, O ovary, T testis, H heart, S stomach, K kidney, G gonads, I intestine, L liver, Gi gill, R retina, E eye, Sp spleen, M muscle, Tel telencephalon, Die diencephalon, Mes mesencephalon, OT optimum tectum, Cer cerebellum, NPPv nucleus posterioris periventricularis, NOR nucleus olfacto-retinalis, NLT nucleus lateralis tuberis

Regulatory Mechanism of GnIH in Fishes

GnIH is regulated by photoperiod, temperature, stress, etc. in different species of photoperiodic mammals, birds, and amphibians (Ubuka et al. 2005, 2012; Chowdhury et al. 2011; Tsutsui et al. 2013; Kriegsfeld et al. 2015), but in fishes, the regulatory mechanism of GnIH is very less studied. In general, photoperiodic regulation of reproduction in fish is mediated by plasma melatonin release from the pineal gland and retina (Muñoz-Cueto et al. 2017). Melatonin levels increase at night and decrease during the day. Melatonin receptors (MTs) mediate the actions of melatonin, which in some species decreases the sexual maturation by inhibiting FSH and LH release (McGuire et al. 2011; Sébert et al. 2008). Fishes have three subtypes of MTs, MT1, MT2, and MT3, and MT3 is the primary receptor through which melatonin affects the expression of GnIH (Shin et al. 2011). The expression of MT3 was detected in the GnIH neurons of some fishes, which support that at least some part of melatonin is acting on reproduction through the GnIH system (Choi et al. 2016). In addition, both GnIH and MT receptor proteins were co-expressed in the hypothalamus of cinnamon clownfish. In sea bass, GnIH neurons were detected in the brain regions known to exhibit melatonin-binding sites (Herrera-Pérez et al. 2010; Paullada-Salmerón et al. 2016b). Further, in grass puffer, the expression of GnIH and its receptor showed diurnal and circadian rhythmicity at the spawning stage, in association with melatonin receptor expression, suggesting that the action of GnIH is cyclic possibly due to regulation by melatonin (Muñoz-Cueto et al. 2017). In sea bass, pinealectomy (Px) reduced night-time plasma melatonin levels and GnIH gene expression in the mid-hindbrain, a region with mutual connections to the pineal organ, which showed that the GnIH expression is regulated by the pineal gland (Cowan et al. 2017). All these studies gave concrete evidence that melatonin plays a vital role in the regulation of the GnIH system in fish. Paullada-Salmerón et al. (2017) first checked the effects of rearing temperature, low temperature (LT, 15 °C) and high temperature (HT, 21 °C), in sea bass during the thermosensitive period (5–60 days post-fertilization, dpf) on the expression of the GnIH and its receptor (GnIHR). Results showed significant effects of temperature on GnIH and GnIHR expression during the thermosensitive period, with higher transcript levels under LT condition. There are several findings in birds and mammals which suggest that apart from photoperiod and temperature, stress may also act through GnIH neurons to inhibit reproductive function (Tsutsui et al. 2015; Ubuka et al. 2016). In the rat, both acute and chronic stress upregulate hypothalamic GnIH expression, and this stress-induced increase of GnIH is blocked by adrenalectomy (Kirby et al. 2009). But in fishes, there is no report indicating the involvement of GnIH in stress response.

Interactions of GnIH with GnRH and Kisspeptin

In the vertebrates, three GnRH variants are expressed by different neurons: GnRH1, GnRH2, and GnRH3. GnRH1 is the main hypophysiotropic variant in most fishes. In zebrafish, Lpxrfa neurons were localized to the ventral hypothalamus, with fibers extending throughout the brain and to the pituitary and in the preoptic area. And these fibers interact with gonadotropin-releasing hormone 3 (Gnrh3) neurons. Also, Lpxrf-immunoreactive fibers were observed, interacting with kisspeptin receptor-1a-expressing neurons in the preoptic area (Spicer et al. 2017). In sea bass, GnIH terminals contacted GnRH1 cells in the preoptic area (Paullada-Salmerón et al. 2019). In *C. dimerus*, double-labeling immunofluorescence analysis did not show axo-somatic or fiber-fiber contacts in GnIH and GnRH1. However, the fiber-fiber connection between GnIH and GnRH2 was observed in the nucleus lateralis tuberis (NLT) and midbrain tegmentum (MBT) (Di Yorio et al. 2018). On the other hand, in tilapia, the study showed that both LPXRFa-immunoreactive fibers and LPXRFa-R are not co-expressed with GnRH1, GnRH3, or kisspeptin (Kiss2) neurons. Further, in the pituitary, LPXRFa fibers are closely associated with gonadotrope-expressing cells, which suggest that LPXRFa and LPXRFa-R signaling acts directly on the pituitary cells independent from GnRH or kisspeptin in this species (Ogawa et al. 2016).

The Physiological Effect of GnIH

In Fish Reproduction

Brain

In fish, administration of GnIH exerts both inhibitory and stimulatory effects on synthesis and release of GnRH as well as GTH. In some fish, GnIH negatively regulates the HPG axis by decreasing the GnRH or GTH mRNA expression, serum level, or sometimes both (Zhang et al. 2010; Umatani et al. 2013; Qi et al. 2013a; Wang et al. 2015, 2017a; Choi et al. 2016; Paullada-Salmerón et al. 2016a; Peng et al. 2016; Spicer et al. 2017; Aliaga-Guerrero et al. 2018). On the contrary, in some fish species, it positively regulates the expression and secretion of FSH and LH (Amano et al. 2006; Shahjahan et al. 2011; Osugi et al. 2012; Biran et al. 2014), while in goldfish it shows both stimulatory and inhibitory actions on LH and FSH mRNA and serum level (Moussavi et al. 2012, 2013).

The administration of the zebrafish LPXRFa peptide-3 in mature goldfish could significantly decrease the serum LH levels at a dose of 1.0 μg/g BW (Zhang et al. 2010). In goldfish, intraperitoneal (ip) injection of GnIH-2 and GnIH-3 peptide significantly decreased the GnRH, FSH-β, and LH-β mRNA levels (Qi et al. 2013a). Umatani et al. (2013) found out that RFRP-2 in dwarf gourami decreased the firing frequency of terminal nerve gonadotropin-releasing hormone (TN-GnRH) neurons, which indicates its adverse effect on reproduction. In orange-spotted grouper, all three GnIH peptides significantly decreased the expression of GnRH-1

in the brain, while GnIH-II peptide significantly decreased LH-β mRNA levels in the pituitary (Wang et al. 2015). In clownfish, ip injection of goldfish GnIH-3 peptide significantly reduced the expression of GTH and GnRH mRNAs, while the expression levels of GnIH, GnIHR, and melatonin receptor were increased in all the experimental groups (immature, male, and female) (Choi et al. 2016). In common carp, GnIH-III peptide at a dose of 10 μg/kg and 100 μg/kg body weight of fish significantly decreased both FSH-β and LH-β mRNA levels in vivo (Peng et al. 2016). Further, in cultured pituitary cells, GnIH peptide with a high dose of 100 μM significantly decreased the GTH mRNA level. In sea bass, ICV injection of GnIH-2 significantly decreased the GnRH-2 mRNA in brain and FSH-β and LH-β mRNAs in the pituitary, whereas GnIH-1 only downregulated the brain GnRH-1 mRNA level. However, both GnIH-1 and GnIH-2 significantly decreased the LH plasma levels (Paullada-Salmerón et al. 2016a). Zebrafish Lpxrfa-3 peptide downregulated the LH expression in the pituitary explants and GnRH-3 expression in brain slices (Spicer et al. 2017). Administration of GnIH-3 (1.0 μg/g BW) significantly reduced the expression of LH-β and GnRH-3 transcripts, while the injection of GnIH-2 did not have any effect on the above genes in sole fish (Aliaga-Guerrero et al. 2018). In sockeye salmon cultured pituitary cells, all three goldfish LPXRFamide peptides stimulated the release of FSH, LH, and GH (Amano et al. 2006). In primary pituitary cultures of grass pufferfish, the treatment of goldfish LPXRFa-1 significantly increased the expression of FSH-β and LH-β mRNAs (Shahjahan et al. 2011). Female lampreys treated with LPXRFa-2 at 100 μg/kg BW showed a significant increase in the expression of GnRH-I, GnRH-III, and gonadotropin β-subunit (Osugi et al. 2012). Tilapia LPXRFa-2 peptide positively regulates the LH and FSH release both in vivo and in vitro (Biran et al. 2014).

Gonads

The gonads are also the source of GnIH production apart from the hypothalamus but in lower quantity. Different studies like in tilapia, sea bass, and zebrafish showed the basal expression of GnIH in the ovary and testis (Zhang et al. 2010; Biran et al. 2014; Paullada-Salmerón et al. 2016b; Corchuelo et al. 2017). In zebrafish, GnIH transcripts were detected in the cortical vesicles of previtellogenic oocytes as well as in the follicular cells and the zona radiata of the vitellogenic oocytes (Corchuelo et al. 2017). Although the expression of GnIH has been reported in gonads of some fish species (Zhang et al. 2010; Qi et al. 2013b; Biran et al. 2014; Paullada-Salmerón et al. 2016b, c; Corchuelo et al. 2017), even from that the effect of GnIH on gonads has been studied in very few species (Qi et al. 2013b; Paullada-Salmerón et al. 2016c; Wang et al. 2017b). In goldfish, implanted GnIH peptides significantly increased the serum testosterone levels in males, but do not have any effect on estradiol level in females. Further, ip injection of GnIH peptides increased the expression of StAR and 3bHSD mRNA and decreased the expression of CYP19 mRNA significantly in the testis. In vitro study in goldfish testicular cells showed the higher expression of StAR, 3bHSD, FSHR, and LHR after the administration of goldfish GnIH peptide, but failed to have any effect in ovarian cells (Qi et al. 2013b). In contrast, in female grouper ovarian cells, GnIH-II peptide increased the LHR

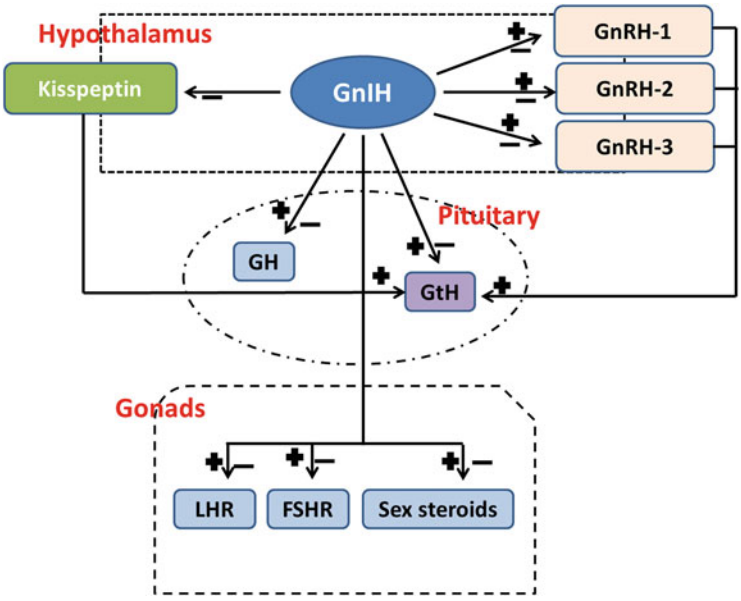

Fig. 10.1 Schematic diagram of GnIH action on the fish reproductive axis

mRNA level and GnIH-I peptide significantly increased the mRNA levels of StAR and 3βHSD1 (Wang et al. 2017b). Further, in male sea bass, the peripheral implantation of GnIH-I and GnIH-II decreased testosterone (T) and 11-ketotestosterone (11-KT) plasma levels during the non-breeding season and increased the number of spermatogonia in testicles and enhance the spermatogenesis process during the breeding season. In addition, both GnIH peptides decreased the gene expression and plasma level of LH, while plasma FSH level was only reduced by GnIH-I (Paullada-Salmerón et al. 2016c). All these findings suggest the regulation of the reproductive axis by GnIH not only one at the brain and pituitary levels but also on the gonadal level in fishes (Fig. 10.1).

Other Roles of GnIH

Most of the studies till now analyzed the effect of GnIH peptide on the reproductive axis, with very few reports on the possible role of this peptide in the regulation of other functions. Different neuroanatomical localization studies showed that GnIH fibers are distributed not only in the preoptic hypothalamic area, which controls the reproductive axis, but also in the retina-optic tract and midbrain, suggesting a potential role of GnIH as a neuromodulator or neurotransmitter. In sea bass, GnIH-implanted animals exhibited a significant increase in diurnal activity from late spermatogenic to early spermatogenic stages (Paullada-Salmerón et al. 2016c). There is some evidence about the role of GnIH on growth hormone (GH) synthesis

and release. In cultured pituitary cells of sockeye salmon, the goldfish GnIH stimulated GH release (Amano et al. 2006). In *C. dimerus*, GnIH administration increased the GH level in culture medium (Di Yorio et al. 2016), while in grass pufferfish, GnIH increased the GH and PRL expression in the primary pituitary cultures (Shahjahan et al. 2016). However, its administration of GnIH decreased GH in sea bass (Paullada-Salmerón et al. 2016a), whereas intraperitoneal administration of GnIH did not affect GH release in tilapia (Biran et al. 2014). In goldfish, gGnIH is not merely a stimulatory or inhibitory peptide for GH release and GH mRNA expression, but the effect is dependent on the mode of application, and duration of exposure, as well as reproductive season/gonadal recrudescence status (Moussavi et al. 2014). In conclusion, these results indicate that GnIH exerts complex effects on basal and GnRH stimulated GH in a seasonal-reproductive manner, and thus, this peptide could be involved in the regulation of somatic growth and/or in the interaction between growth and reproduction (Di Yorio et al. 2019b).

Conclusion and Future Direction

Since the discovery of GnIH, a hypothalamic RFamide neuropeptide, it has been characterized in different fish species. Studies showed that the number of mature GnIH peptides varies from two to three in fish species, which may be due to the whole genome duplication in fishes. The GnIH and GnIH receptor fibers are widely distributed in various areas of the brain and pituitary to regulate gonadotropin release and synthesis. In addition, the fibers are also present in cells of testis and ovary in birds and mammals, but due to lack of studies, the information is not available in fishes. However, the tissue distribution study showed the presence of GnIH in gonads of fishes. Further in the brain, the GnIH fibers are also seen in close association with other neurons expressing neuropeptides such as GnRH and kisspeptin, confirming the regulating effects on other genes of the reproductive axis. The role of GnIH has been observed not only in reproduction but also in non-reproductive functions like the stress response, socio-sexual behavior, body growth, etc. At the reproductive axis, it showed the effect on gonadotropin release and synthesis as both a negative and a positive regulator in fishes. The impact of GnIH in other species of birds and mammals is only inhibitory, so in fishes, further studies are much necessary to conclude its function and distribution in different body parts apart from the brain and pituitary.

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Gene Regulation on Spermiation of Catfish

11

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Abstract

Catfishes are commercially important for fisheries and aquaculture industry due to several attributes such as good taste and nutritional and medicinal value. The present catfish production in India is 279,684 tons. The production data of the country is not yet published for the year 2017–2018. Catfishes, being air-breathing in nature, can survive in water conditions that are unsuitable for carps. Synthetic hormones are key to induced breeding and are widely used for seed production in carps. However, catfish like the *Clarias magur* male does not release milt even when the hormone is administered and has to be sacrificed for milt collection. Thus, spermiation is the major problem in several catfish. Identification and expression analysis of different genes that are directly or indirectly related to spermiation will help in understanding the molecular mechanisms underlying maturation and spawning. Therefore, essential genes can be identified and plentiful researches can be performed so that the problems in the spermiation process can be easily solved.

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Keywords

Catfish · Spermiation · Ectoplasmic specialization · Tubulobulbar complexes · Focal adhesion

Introduction

Spermiation is a complex process that involves many alterations in both the spermatid and the Sertoli cell that are ready for the release of spermatids from the supporting Sertoli cell at the end of spermatogenesis (Fig. 11.1). The pivotal events are the renovation of the spermatid nucleus and cytoplasm to secrete the streamlined spermatozoan, withdrawal of Sertoli cell "ectoplasmic specialization" (ES) junctions and cytoplasm, and elongation of the spermatid into the lumen. Spermiation ends with the release of the spermatid (spermatozoan) from the lumen and the phagocytosis of the remaining residuals by the Sertoli cell.

An extensive cytoplasm is shown by the spermatid around the flagellum during the stage VII of spermiation and is enfolded by tube-like projections of the cytoplasm of Sertoli cell's apical (apical process). In the ventral curvature of the spermatid head, in areas deficient in ES, tubulobulbar complexes (TBCs) are formed.

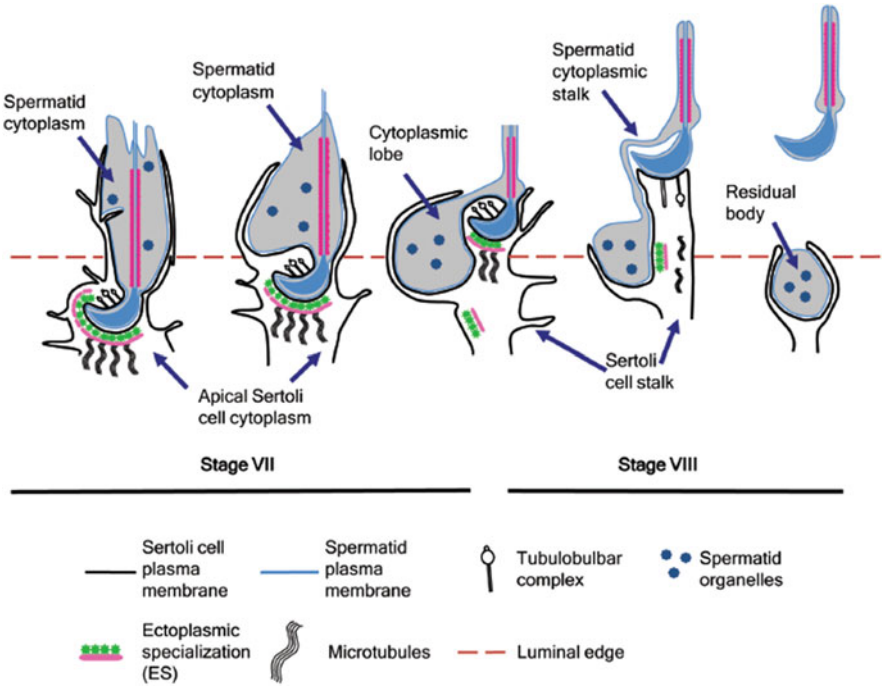

Fig. 11.1 Spermiation process of fish

As spermiation progresses, the Sertoli cell cytoplasm moderately de-escalates until it contacts only the dorsal surface in stage VIII. The head and flagellum of the spermatid are extended into the lumen of the tubule through the lengthening of the Sertoli cell stalk. As the head of the spermatid extended, the cytoplasm remains stationary within the epithelium and condenses in volume. Ultimately the cytoplasm starts flowing downward below the level of the head (spermatid cytoplasmic lobe). Here, organelles of the spermatid become concentrated and finally after insularism form the residual body. The ES structure disappears from the Sertoli cell plasma membrane opposite the spermatid head, and tracts of ES can be observed adjacent to the Sertoli cell plasma membrane within the Sertoli cell stalk during the progression from stages VII to VIII (Source: O'Donnell et al. 2011).

Ectoplasmic Specialization

Prior to spermiation, the Sertoli cell interacts with elongated spermatids through a complex structure ectoplasmic specialization. As step VIII begins, the ES is first seen in the Sertoli cell cytoplasm opposite round spermatids when the spermatid nucleus polarizes to one side of the cell. Association of ES with elongated spermatids persists till the beginning of spermiation. At the time of spermiogenesis, the ES interacts with the motor proteins and microtubules to transfer spermatids over the epithelium. During the elongation, the downward movement of spermatids occurs in deep "crypts" within Sertoli cells. As the spermiation begins, the spermatids are quickly transferred from crypts to the luminal edge through the microtubule-dependent mechanism. A significant aim of spermiation is to dismantle the apical ES for the preparation of the release of spermatids over the lumen.

Figure 11.2(A) shows the late spermatid in rats at stage VII during spermiation. The cytoplasm of spermatid begun to condense; the arrow indicates the predicted route through which the cytoplasmic contents may secrete into the region of perinuclear and, possibly, into TBCs. TBCs are formed in the area deficient in ES probably the ventral of the spermatid head. TBC formation occurs with a tiny pit coated with clathrin and finally becomes elongated as a tubular structure, followed by the development of the dilated bulbous region. Vesicles are visible close to TBCs' ends.

Figure 11.2(B) shows a mature TBC morphology which contains firmly opposed Sertoli cell plasma membranes and spermatid. Dendritic actin surrounds the long proximal tube. However, the actin is not visible near the dilated bulbous region which is enclosed by the endoplasmic reticulum. A small distal tube ends in a clathrin-coated pit. A double-membrane vesicle is formed near the TBCs through the budding of the bulbous portion. Figure 11.2(C) is an electron micrograph of a rat's late-stage VII and depicts the origin of TBC from a spermatid head. A focal area of ES is also visible (Source: O'Donnell et al. 2011).

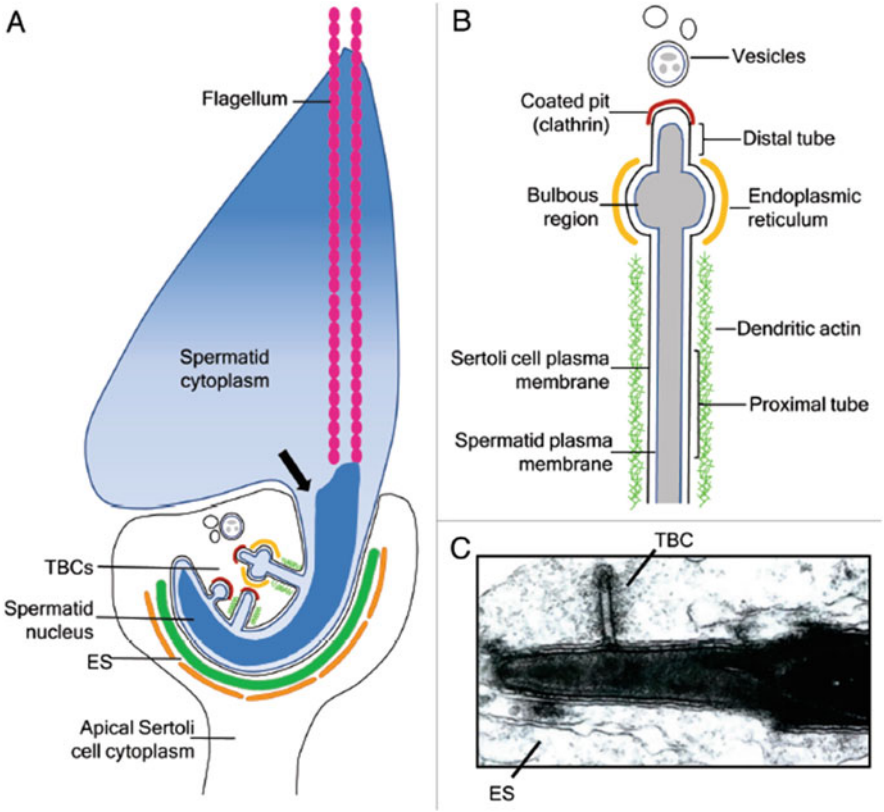

Fig. 11.2 Tubulobulbar complexes formation and morphology during spermiation

Regulation of Spermiation

Failure of spermiation might be an intimation of incorrect happening in the testis. At several levels, the spermiation is controlled and regulated through a variety of signal transduction pathways.

Endocrine and Paracrine Regulation

It is well known that changes in testicular hormones alternate the spermiation process. The primary endocrine regulators of spermiation are FSH and androgen, acting on their receptors in Sertoli cells. Spermiation failure during androgen and FSH suppression indicates the occurrence of ES and spermatid cytoplasm removal during the early phase of spermiation. Still, at the end of spermiation, spermatids fail to disassociate from the Sertoli cells. Spermatids remain associated with

phosphorylated FAK (FAKTyr397) and α6β1 integrin at the late-stage/early-stage VIII (Siu et al. 2003), suggesting that this spermatid dissociation failure may happen due to the inability of an integrin-based focal adhesion (FA) to "let go" of the spermatid. These results suggest that within the Sertoli cells, androgens and FSH act on signaling pathways to modify the function of the FA at the spermiation process.

There is evidence to suggest that an integrin-based junction achieves adhesion during TBC formation, ES structure withdrawal, and spermatid remodeling with correspondence to a focal adhesion. Focal adhesions are colossal, macromolecular complexes that mediate adhesion between cells and the extracellular matrix (ECM). They are capable of prompt adhesion formation and disassembly (such as observed in migrating cells) and also of tight, stable adhesion. In between Sertoli cells and elongating spermatids, the ES adhesion domain is present throughout spermiogenesis that appears to comprise some different adhesion junction components, including adherens junction proteins (Cheng and Mruk 2002) and FA components (Beardsley et al. 2006). As the ES structure forms opposite step VIII round spermatids, α6β1 integrins (Mulholland et al. 2001), integrin-linked kinase (ILK) (Mulholland et al. 2001), and phosphorylated FAK can be seen in the vicinity of the developing ES, and α6β1 integrins are present opposite elongating spermatids throughout spermiogenesis.

Sertoli cells express α6β1 integrin, and the likely ligand on extending and elongated spermatids is laminin 333 (Yan and Cheng 2006). Thus, the ES adhesion domain includes junctions with properties of FAs throughout spermiogenesis. This FA-type junction is also expected to be an integral constituent of the spermiation machinery while the ES structure is removed. At the beginning of spermiation when spermatids are translocated to the luminal edge, α6β1 integrin becomes concentrated on the outer dorsal curvature of the spermatid head, along with phosphorylated FAK and laminin subunits as well as other FA-related proteins. Integrins can be visualized in TBCs, connoting that they are internalized during ES removal (Young et al. 2009). However, after ES removal, α6β1 integrin immunostaining also persists and remains at the dorsal surface of the spermatid head until insularism.

By binding to the nuclear retinoid receptors (RARα, β, and γ) and retinoid X receptors (RXRα, β, and γ), retinoic acid (RA) which is metabolized from retinol (vitamin A) exerts its effects. To control the expression of RA-responsive genes, these receptors heterodimerize (Chambon 2005). An elegant series of studies on some transgenic mouse models have unveiled that RA acting on a RARα/RXRβ heterodimer expressed in Sertoli cells is imperative for spermiation (Ghyselinck et al. 2006; Vernet et al. 2006). RA activates rarα, but ligand-dependent activation of RXRβ is not statutory for spermiation. Spermiation failure is an acute feature of and is highly susceptible to a lack of RA signaling and is corroborated by retained spermatids in stages IX–X and a curtailed epididymal sperm count. Some spermatids fail to actuate spermiation in phase VII, whereas others may fail to disengage. RARα/RXRβ in Sertoli cells may cooperate to some degree with AR signaling and regulate the expression of adhesion junction components (Vernet et al. 2008).

Initiation Spermiation

The spermiation commencement failure is commonly observed at the initiation of stage VII. Translocation of spermatids to the luminal edge is the beginning of spermiation responsive to microtubule dynamics in the Sertoli cells. Translocation of spermatids to the luminal edge is prevented by some agents such as colchicine and taxol which impair microtubule dynamics. Spermatid translocation is supported by microtubules and some associated motor proteins, through their interaction with the ES cytoplasmic face within the Sertoli cell. Adenoviral-mediated overexpression of γ-tubulin in Sertoli cells facilitates an increase in tubulin protein around the heads of rat's spermatids in step 19 was disclosed with a failure to initiate spermiation in stage VII (Fleming et al. 2003). Furthermore, exogenous estradiol administration caused a crash to start spermiation and coincided with disturbances in microtubule localization in phase VII (D'souza et al. 2008). Hence, pathways within Sertoli cells that regulate microtubule dynamics, polarity, and association with proteins and molecular motors are critical for the successful initiation of spermiation. Ehd1-deficient mice are infertile and exhibit spermatid retention and incomplete removal of ES and spermatid cytoplasm (Rainey et al. 2010). It is possible that Ehd1 is a new component of TBCs and that this will be a useful model for investigating the regulation of TBC formation and adhesion junction dynamics during spermiation.

Spermatid Cytoplasm Elimination

A complex series of events, including endocytic pathways, regulation of actin dynamics, signal transduction events, and regulation of adhesion structures, are involved in the dissolution of the ES and TBC formation in the initial phases of spermiation. By the Sertoli cell, excess cytoplasm is removed principally during spermiation, insinuating that spermatids may influence cytoplasm removal by producing proteins imperative for dynamic changes in their cytoskeleton. For example, during spermiation, a missense mutation in the Capza3 gene in mice results in infertility due to abnormal sperm morphology and a failure to shed excess cytoplasm (Geyer et al. 2009). Capza3 is localized in extended spermatid cytoplasm and is an actin-capping protein involved in the regulation of F-actin dynamics.

In mice, SPEM1 is localized in the cytoplasm of late spermatids. However, its function is unascertained (Zheng et al. 2007). Due to abnormal sperm morphology arising during the final steps of maturation, likely during spermiation, the ablation of this gene results in infertility. Sperm in the epididymis evince gross cytoplasmic abnormalities, with the cytoplasm remaining attached to and connecting the head and the middle piece of the tail, so that sperm heads are bent back onto the flagella (Zheng et al. 2007). In a subset of mice deficient in a variety of genes important in late spermiogenesis including Tarbp2, this phenotype of sperm abnormality is also espied (Zhong et al. 1999), raising the intriguing possibility that various defects during spermiogenesis may contribute to the failure of cytoplasmic removal during spermiation (Zheng et al. 2007). It is thus possible that abnormalities in the spermatid physically impede the maneuver of the cytoplasm and hinder the Sertoli cell's ability to "strip off" the cytoplasmic lobe (Table 11.1).

Table 11.1 List of genes can be targeted for future researches

Gene	Function	Cell	Model	Spermiation the phenotype
γ-Tubulin	Microtubule nucleation	All	Adenoviral vector-mediated overexpression in SC	• Stages VII–VIII showed the retained spermatids indicative of spermiation failure. Increased numbers of retained spermatids and residual bodies • In stages IX–XIV, defective dissociation and residual body processing are indicated by an increased number of spermatids and residual bodies
Spem1	Unknown	elST	Null	• Abnormal removal of spermatid cytoplasm leads to infertility in late spermatids
Capza3	Regulates actin dynamics	elST	Missense mutation (Repro23)	• Delayed spermiation • Abnormal removal of cytoplasm during spermiation • Other defects in sperm morphology • Infertility
RARα (retinoic acid receptor α)	Receptor for retinoic acid	SC	Null and SC-specific ablation	• Failure of spermatids to "line up" at the luminal edge • In stages VIII and IX, the retention of spermatids indicates the failure of spermiation initiation by at least some sperm • Epididymis shows reduced sperm content • Other defects in spermiogenesis • Infertility
RXRβ (retinoid X receptor β)	Cellular response to retinoic acid heterodimerizes with other nuclear receptors	SC	Null (the whole body and SC-specific ablation)	• Some of the spermatids retained in stages VII–IX, responsive to initiate spermiation failure • Some defects in sperm morphology • Infertility

(continued)

Table 11.1 (continued)

Gene	Function	Cell	Model	Spermiation the phenotype
Rbp4; retinol binding protein	Bioavailability of retinol to tissues	NA	Retinol deficiency when fed vitamin A-deficient diet	• Some of the spermatids retained in stages IX–X indicate partial spermiation failure • Reduced sperm content in the epididymis • Other spermiogenic defects
Tarbp2	Encodes Prbp protein; in spermatids this controls translational activation of protamines	rST	Null	• Some of the spermatids retained in stages VII–XI and few mature sperm in the epididymis • Other dysfunctions in spermiogenesis including defective shaping of the head and loss of immature spermatids • Infertility

Conclusion and Future Direction

Many catfish are there such as *C. magur*, which do not ooze out sperm while stripping the male. This is because Sertoli cells are in abutting contact with the germ cells, especially in the testis compartment. Many future aspects may ease out the spermiation process in male catfish such as removing the tightness between the Sertoli cells and germ cells junction through some chemicals like oxytocin, ovatocin, etc. which will ease out the spermiation and the male fish doesn't need to be sacrificed. Since other catfish show some positive results, there is a probability of getting better results in *C. magur* using a somewhat similar chemical like oxytocin or it can be a novel one. As it is not incumbent that the same drug will work similarly in different catfish, another approach can be the application of an antagonist which will block the receptor responsible for the establishment of the tight junction between the Sertoli cells and germ cells. Through some genetic engineering tools the receptor can be internalized below the cell membrane so that it will not be available to interact with the protein/signal responsible for spermiation failure.

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Ghrelin and Its Role in Reproductive Physiology of Fish

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Abstract

The endocrine regulation of vertebrate reproduction is achieved by the synchronized actions of a large number of endocrine elements, largely produced from the brain, pituitary, and gonads. The hypothalamic region of the brain plays a crucial role in the regulation of neuroendocrine and reproductive functions. GRL (ghrelin) is a gut-brain hormone that plays a vital role in the regulation of mammalian reproduction. Ghrelin is a peptide hormone with species-specific effects and was first identified in fish 20 years ago as a 28 amino acid hunger-stimulating and GH-releasing peptide hormone released from the stomach and intestine. Ghrelin hormone receptor called as growth hormone secretagogue receptor (GHSR) is an endogenous ligand first discovered in rats. Recent studies revealed that ghrelin and ghrelin receptor mRNAs are expressed in the ovary and testis of mammals, including fishes suggesting a direct effect of ghrelin in the control of reproduction. Ghrelin has shown a stimulatory effect on the pituitary of fish, as reported so far, while giving in vitro treatment with ghrelin stimulates LH release in common carp and goldfish. Synthetic ghrelin stimulates LH release by directly acting on dispersed pituitary cells of goldfish. Ghrelin controls fish reproduction by regulating hormone secretion indirectly from the brain and pituitary and by acting directly on the gonads to cause reproductive tissue

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development and steroid hormone release. The aim of this review is to do an outlook analysis of the relative aspects of role of ghrelin in fish reproduction.

Keywords

Ghrelin · Reproduction · Growth hormone secretagogue receptor · Fish

Introduction

GRL, a gut-brain peptide hormone, was originally discovered in the stomach of rat as an endogenous ligand for the (GHS-R1a) (Kojima et al. 1999) and has opened new horizons to understand the regulatory mechanisms of neuroendocrine systems, including growth and energy homeostasis (Kojima et al. 2001; Kojima and Kangawa 2005; Dar et al. 2018b). GRL undergoes a posttranslational acyl modification; a medium-chain fatty acid is attached to the peptide at the third serine residue of the N-terminal region. This modification is catalyzed by GOAT (ghrelin O-acyltransferase) (Gutierrez et al. 2008; Yang et al. 2008) and is vital for receptor binding. In vertebrates, the first seven amino acids on N-terminus are highly conserved and are designated as "active core" (Unniappan and Peter 2005; Kaiya et al. 2008), and fish GRL possesses an amide modification on the C-terminus (Kaiya et al. 2008). The key site for the synthesis of ghrelin in all the vertebrates is the stomach or its equivalent (Dar et al. 2018a), although gene expression of ghrelin by PCR shows an extensive tissue distribution, with low levels of expression in peripheral tissues and brain in both mammals (Gnanapavan et al. 2002) and fish (Unniappan et al. 2002; Kaiya et al. 2003; Feng et al. 2013).

GHS-R1a, a seven-transmembrane protein GPCR (G protein-coupled receptor) is responsive to GRL in regulating metabolic, neuroendocrine, and non-endocrine actions. In contrast, GHS-R1b is a five-transmembrane nonfunctional receptor. GHS-R1a heterodimer facilitates translocation of GHS-R1a to the nucleus declining the constitutive signaling of GHS-R1a, resulting in inhibition of ghrelin's actions, thus acting as a dominant-negative mutant of the active receptor (Leung et al. 2007). So far, multiple isoforms of GRL have been identified 4 in rainbow trout, 11 in goldfish, and 2 in tilapia. Among various isoforms, ghrelin-C8 is prevalent in goldfish and eel, while ghrelin-C10 is predominant in tilapia.

Role of GRL in Maintaining Energy Balance

Availability of energy is a critical factor to determine the reproductive capacity; GRL as an orexigenic substance plays a vital role in energy acquirement (Unniappan 2010). Interestingly, the role of GRL varies in different species of fishes. It acts as orexigenic in goldfish, sea bass, and zebrafish (Unniappan et al. 2002; Matsuda et al. 2006; Terova et al. 2008; Amole and Unniappan 2009; Miura et al. 2009); on the other hand, it acts as anorexigenic in turbot and rainbow trout (Nieminen et al. 2003;

Jönsson et al. 2007, 2010).GRL circulates in the blood and serves as a stimulatory signal on CNS for feed intake. In contrast, in goldfish, the afferent vagus nerve predominantly mediates the action of GRL rather than the bloodstream (Matsuda et al. 2011). In the brain, the primary site for ghrelin's appetite-stimulating activity is the "hypothalamic arcuate nucleus." Central administration of ghrelin stimulates food intake in goldfish (Unniappan et al. 2002; Unniappan and Peter 2004).

Central and peripheral injections of des-acyl GRL (lacking acyl modification) are ineffective in regulating the food intake of goldfish (Matsuda et al. 2006). Moreover, GRL levels (mRNA and protein levels) are altered in feeding conditions (Kaiya et al. 2008). Preproghrelin mRNA levels increase at 3, 5, and 7 days post-feeding (dpf) and decrease during refeeding, suggesting a role in feed intake and energy balance (Amole and Unniappan 2009). Once feed intake is resumed, the GRL levels remain elevated in some species, including sea bass, until body energy stores are restocked, by stimulating appetite and adipogenesis (Terova et al. 2008). In goldfish, 3 dpf, serum GRL levels increase significantly achieving a peak at 5 dpf which further decreases at 7 dpf (Unniappan and Peter 2004). Instead, in the case of Nile tilapia, the GRL mRNA levels remain unchanged after 7 dpf (Parhar et al. 2003); that may be because of the compensatory effect of one or more different sets of appetite regulatory peptides (Kaiya et al. 2008). Ghrelin is partially involved in carbohydrate-glycogen metabolism in zebrafish (Cruz et al. 2010).

Role of GRL on Different Reproductive Hormones

In fishes, the endocrine control of spermatogenesis is mostly regulated by the pituitary, which secretes hormones that regulate gonadal steroid hormones (Hatef and Unniappan 2019). On one hand, early testicular development (spermatogenesis) is regulated by FSH, while on the other hand, testicular maturation (spermiation) is controlled by LH (Nagahama 1994). Leydig cells are stimulated by FSH to secrete 1-ketotestosterone which further stimulates Sertoli cells to produce activin B, which in turn acts on spermatogonia to induce mitosis leading to the development of spermatocytes and successively spermatogenesis (Miura and Miura 2003). At spawning, LH induces the production of MIS (maturation inducing steroid), which eventually activates specific enzymes to increase seminal plasma pH, and cAMP in sperm. Eventually, spermatozoa capacitation and spermiation are induced (Nagahama et al. 1994; Cavaco et al. 2001; Zohar et al. 2010). GRL and GOAT mRNAs have been detected in the ovaries and testes of several fishes. Besides, GRL has a direct effect on the HPG axis to modulate reproduction. Interestingly, GRL has a stimulatory effect on the pituitary of fish, as reported so far, e.g., in vitro treatment with GRL stimulates LH release in common carp and goldfish. Synthetic ghrelin stimulates the release of LH in goldfish by acting directly on dispersed pituitary cells (Grey et al. 2010). Furthermore, both intraperitoneal and intracerebroventricular injections of GRL in goldfish increase the plasma level of LH. The effect of GRL on the release of LH from the pituitary is mediated by Ca- and PKC-mediated mechanisms. GLR not only induces LH from pituitary but also potentiates

sGnRH-A (salmon gonadotropin-releasing hormone)-induced LH (Shepperd et al. 2012).

On culturing testicular fragments of eel for 30 days with various concentrations of rGH (recombinant growth hormone), induced type A spermatogonia proliferation, suggesting a role of GH in early spermatogenesis (Miura et al. 2011). The stimulation of pituitary by ghrelin to release GH has been well characterized in mammals, amphibians, birds, and teleost fish such as rainbow trout (Shepherd et al. 2007), sea bream (Chan and Cheng 2004), tilapia (Riley et al. 2002; Kaiya et al. 2003; Fox et al. 2006), hybrid striped bass (Picha et al. 2008), channel fish (Kaiya et al. 2005), and goldfish (Unniappan et al. 2004). Further, GRL has potent stimulatory actions on GH release (Unniappan and Peter 2004). In Nile tilapia, GRL stimulates the release of GH from the pituitary via hypothalamic growth hormone-releasing hormone neurons (Ogawa et al. 2018). Kojima et al. (1999) reported that by intravenous injection of ghrelin in mammalian and nonmammalian vertebrates, potent GH release is induced. In addition, the treatment of cultured pituitary cells with GRL leads to the release of GH, suggesting direct stimulation of pituitary somatotrophs. GH stimulates sex steroid production in the testes and ovaries of fish (Van Der Kraak et al. 1990; Schulz et al. 2010). Moreover, in catfish, GH treatment promotes spermatogonia proliferation (Gopal et al. 2014). At the spermatogonia stage of the testes, a 19 kDa band of GH protein can be noticed by western blotting but not at later stages of spermatogenesis (Miura et al. 2011). However, GH did not induce 11-KT, E2, and dihydro-progesterone (DHP) production in testicular fragments of eel, suggesting that the GH-induced proliferation of spermatogonia is independent of steroid hormones, in vitro. Furthermore, steroid hormone inhibitors do not affect GH-mediated spermatogonia proliferation. Overall, these studies specify that GH in Sertoli cells of testes has a paracrine role in the renewal of type A spermatogonia via their communication with GHR (growth hormone receptor) in germ cells. On the other hand, GH transgenic fish are fast-growing organisms for potential human consumption, but, unfortunately, they show reproductive impairment. The reproductive problems include late sexual maturation and decreased gonadal size in goldfish (Cao et al. 2014), reduced ovarian size and sperm quantity in Nile tilapia (Rahman et al. 2001), reduced courtship and spawning in salmon (Bessey et al. 2004; Fitzpatrick et al. 2011), reduced nest fidelity, quivering frequency, and spawning participation (Moreau et al. 2011).

GRL, whose receptors are also found in granulosa and theca cells surrounding follicles, can act directly on ovaries of zebrafish and goldfish (Shepperd et al. 2012). During the first three stages of follicular development, both ghrelin and ghrelin receptor mRNA expression are highest and significantly decrease during the last two stages in zebrafish. In addition, GRL inhibits both basal and MIH-induced stage IV GVBD (germinal vesicle breakdown) of oocyte maturation, in vitro (Shepperd et al. 2012). Furthermore, the addition of GRL receptor antagonist, D-lys3-GHRP-6 abolishes the inhibitory effect of GRL, suggesting that GRL might be acting directly on the follicular receptors. Since GRL shows a stimulatory effect on LH but an inhibitory effect on stage IV GVBD. Ghrelin may have potential stage-specific effects on follicle development which needs further investigation. Furthermore,

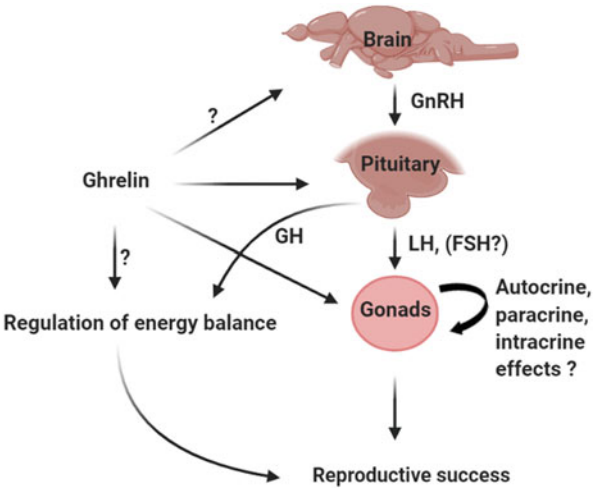

Fig. 12.1 General role of ghrelin in reproductive functions. Where GH stands for growth hormone, GnRH means gonadotropin-releasing hormone, and LH and FSH stand for luteinizing and follicle-stimulating hormone, respectively

hGRL (human ghrelin) increases the number of mature ovarian follicles and reduces the average oocyte diameter in *Barbus sharpeyi* (Mabudi et al. 2011). The representational model showing the effect on GRL on reproduction is depicted in Fig. 12.1.

Conclusion and Future Direction

The availability of energy is a critical factor that determines reproductive capacity of fishes. Ghrelin, primarily as an appetite regulatory peptide benefits to maintain a stable energy balance. Hormonal regulation of reproduction is multifactorial, and reproductive success is dependent on energy availability. Ghrelin as an orexigenic plays a vital role in energy acquirement. In addition, ghrelin regulates luteinizing hormone release from the pituitary in vitro and in vivo and can also directly act on gonads. In addition to this, numerous recent studies indicate that ghrelin has direct effects on the HPG axis to modulate reproduction. Upcoming studies should have intention to brighten the tissue-specific mechanisms of actions of ghrelin and the exact role of ghrelin in regulating reproduction under numerous metabolic states of nonmammalian vertebrates.

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Melatonin and Seasonal Reproduction in Teleosts

13

Mehak Hafeez and Irfan Ahmad

Abstract

N-acetyl-5-methoxytryptamine or melatonin is prepared and discharge by the pineal gland andretina of fish. This tiny but versatile tryptophan-derived hormone serves as an internal biological clock by conveying the information on the time of day and the time of year to the fish via the rhythmicity in its daily and seasonal levels in serum. Apart from that, melatonin is also involved in the regulation of diverse physiological functions in teleosts including their seasonal breeding. Numerous studies to demonstrate the mechanism of how exactly plasma melatonin rhythmic levels control the yearly act of spawning in various fish species have been done, but the clear-cut picture is still not there. This chapter aims to convey the existing understanding on the regulatory action of melatonin on the fish reproduction chiefly focussing on the hypotheses explaining the possible interplay between this hormone and hypothalamic-pituitary-gonadal (HPG) axis or gonadal development of teleosts.

Keywords

Melatonin · Hypothalamic-pituitary-gonadal · Biological clock · Teleosts

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Introduction

An indoleamine compound, melatonin (*N*-acetyl-5-methoxytryptamine), was first isolated by Lerner et al. in 1958 from the bovine pineal gland extract. Since then numerous studies have revealed the presence of melatonin in non-pineal organs/tissues like the retina (Iuvone and Besharse 1983) and gut (Bubenik 1980) in most of the vertebrates and the Harderian gland (Menendez-Pelaez et al. 1987), gonads (Itoh et al. 1999) and placenta (Iwasaki et al. 2005) in mammals, in addition to a wide variety of organisms ranging from invertebrates to different plants and even in some microbes (Chowdhury and Maitra 2012). However, it still remains cynical whether melatonin found in these tissues [except the retina in fish species, which is capable of both synthesising as well as secreting the hormone (Falcón et al. 2010)] is produced within the tissue itself or somewhere else and then incorporated from the blood circulation.

Melatonin is a small lipophilic molecule formed from the essential amino acid L-tryptophan via a biosynthetic pathway involving five enzymatic steps (Klein 2007) (Fig. 13.1). The precursor L-tryptophan, through diet, is released into the blood circulation from where it is taken up by the pineal gland cells and is converted to 5-hydroxy tryptophan (5-HTP) in the mitochondria by tryptophan-5-mono-oxygenase/hydroxylase which afterwards undergoes decarboxylation to form

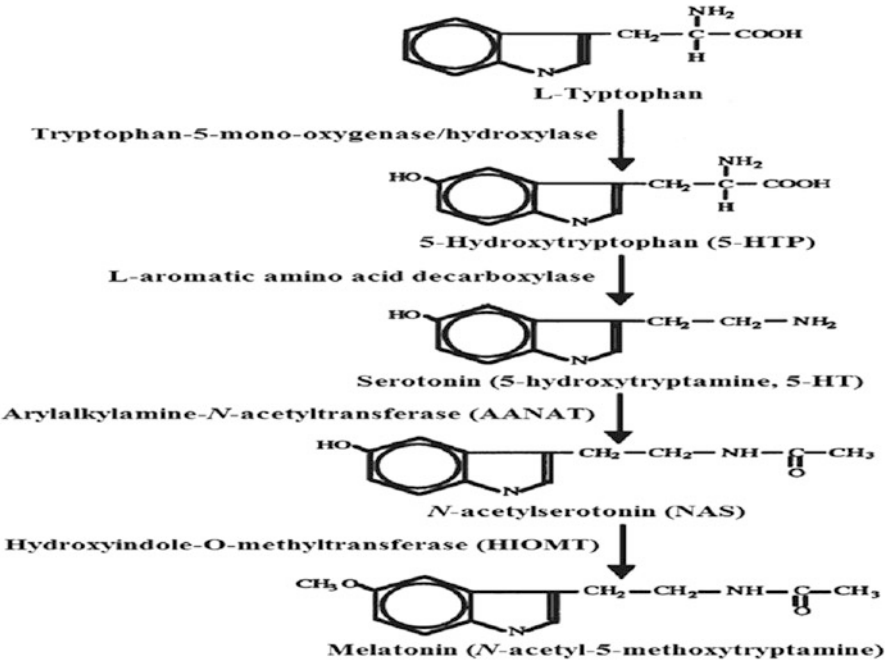

Fig. 13.1 Biosynthetic pathway of melatonin

serotonin (5-hydroxytryptamine, 5-HT) by L-aromatic amino acid decarboxylase in the cytosol. Serotonin is then acetylated (*N*-acetylation) by arylalkylamine-*N*-acetyltransferase (AANAT) into *N*-acetylserotonin (NAS) which is finally O-methylated by hydroxyindole-O-methyltransferase (HIOMT) to form melatonin (*N*-acetyl-5-methoxytryptamine) (Axelrod and Weissbach 1960). The penultimate enzyme, AANAT is the rate-limiting enzyme which regulates the biosynthesis of melatonin (Klein 2007).

The synthesis and liberation of melatonin into the blood circulation in a rhythmic manner in all vertebrates including fish are done by the pineal gland which is the main photoneuroendocrine organ of the central nervous system. The mechanism is controlled by the daily light-dark cycle with peak levels of plasma melatonin detected in absolute darkness (nighttime) under normal environmental conditions (Collin et al. 1989). The diversity in the nomenclature of melatonin is largely attributed to its conservative trait among living organisms, and various names like *zeitgeber* "signal of darkness" or "time-keeping hormone" have been coined (Arendt and Deacon 1997; Hardeland 2008). Three variants of nocturnal melatonin profiles, viz., type A, B and C, have been recognised in different vertebrates, including fish (Falcón et al. 2010; Migaud et al. 2010). A-type profile shows a discrete peak of plasma melatonin titre in late dark phase, e.g. Atlantic cod *Gadus morhua* (Porter et al. 2000), haddock *Melanogrammus aeglefinus* (Davie et al. 2007), whereas B-type profile shows the same in the mid dark phase, e.g. Nile tilapia *Oreochromis niloticus* (Martinez-Chavez et al. 2008). In C-type profile, a rapid rise in melatonin levels is observed immediately after the onset of the darkness, e.g. Atlantic salmon *Salmo salar*, rainbow trout *Oncorhynchus mykiss*, Atlantic halibut *Hippoglossus hippoglossus* and majority of the teleosts (Falcón et al. 2010; Migaud et al. 2010). The reason behind these different melatonin profiles is not yet clearly understood and might be possibly correlated to the differential ability of diverse fish fauna to respond to the photic signals via the circadian clocks (Martinez-Chavez et al. 2008). A couple of studies made on Indian major carp, *Catla catla*, have, however, shown that there exist both profiles of nocturnal melatonin peaks, with A-type during the preparatory phase and B-type during the remaining part of the reproductive phase (Maitra et al. 2005; Chattoraj et al. 2009a).

Role of Melatonin in the Regulation of Fish Reproduction

Majority of the fish species are irregular or seasonal breeders, i.e. showing a peak of reproductive activity or produce for a diminutive time phase which is then pursued through a protracted preparatory phase in an annual cycle. The periodic/rhythmic reproductive events are often synchronous with seasonal changes in one or more environmental parameters (photoperiod, temperature, rainfall/water current, nutrients, pH, turbidity, salinity, dissolved oxygen, total alkalinity and lunar periodicity) so as to ensure that spawning occurs at the most favourable time (in terms of food availability) of the year (Maitra 2011). Thus, during evolution these organisms must have developed certain mechanisms to respond and adapt physiologically to

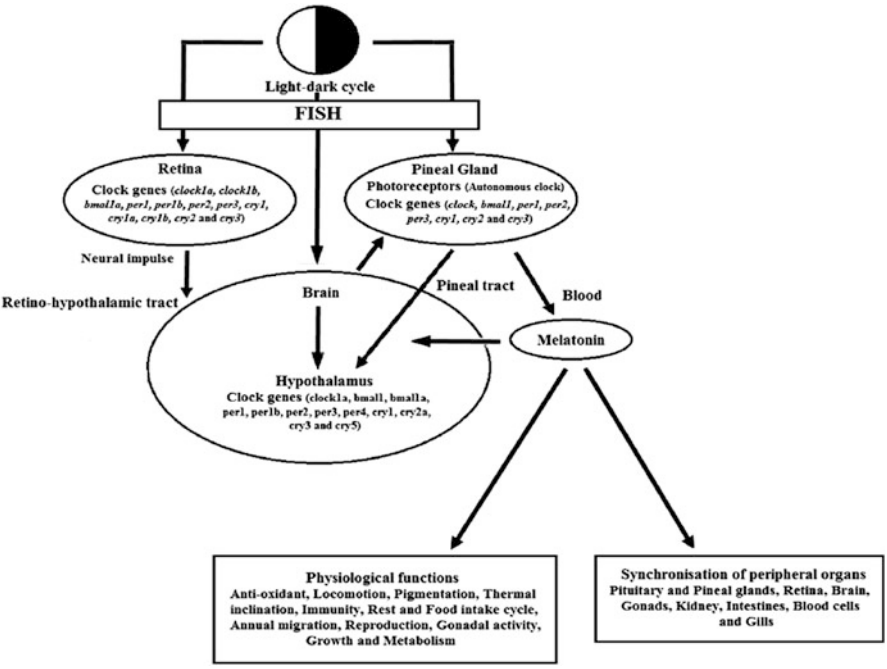

Fig. 13.2 Schematic representation of melatonin regulation of various functions in fish

different environmental and seasonal variations and consequently calculate the episode seeking the help of circadian clock/natural rhythms. Over the past few decades, numerous studies in fish under natural and varied experimental conditions have emphasised that melatonin, from pineal gland and eye retina, plays a potent role in the regulation of diverse periodic/rhythmic body functions by measuring and predicting the daily and the seasonal time in their annual cycle (Falcón et al. 2010; Maitra et al. 2013).These functions include locomotion, thermal inclination, rest, food intake, vertical migration and shoaling behaviour, skin pigmentation, osmoregulation and metabolism, annual processes like smoltification in salmonids, growth and especially the seasonal reproduction in fish (Fig. 13.2). Apart from several temperate fish and air-breathing fish species (Maitra et al. 2013), most studies have been conducted on non-air-breathing carp, *Catla catla* in order to understand the photoperiodic control mechanism of melatonin on fish reproduction (Maitra 2011).

The first evidence that melatonin has indeed an effect on fish reproduction came from an in vitro study on the Atlantic croaker (*Micropogonias undulatus*) (Khan and Thomas 1996). The mechanism by which melatonin regulates reproduction in fish species is not yet clearly understood since the data available is insufficient. Possibly, melatonin acts directly on the hypothalamic-pituitary-gonadal axis and ovaries of the fish (Carnevali et al. 2011; Lombardo et al. 2012; Lombardo et al. 2014).

Effect of Melatonin on the HPG Axis in Fish

This theory came out as a result of various studies and in broad sense states that the regulatory action of melatonin on the fish reproductive system occurs due to its interaction with the HPG axis. This phenomenon becomes more important in seasonal breeders like fish where melatonin provides photoperiodic and seasonal information to the organism (Falcón et al. 2007). The mechanism by which melatonin regulates reproduction via the HPG axis-mediated pathway may vary from organism to organism depending upon their neuroendocrine system. In the case of fish, the nervous signals from the retina and the pineal gland are received by the preoptic area (POA) of hypothalamus to assimilate photoperiodic data from diverse resource; however, the pineal melatonin secretion plays a significant function in the photoneuroendocrine control of their reproduction (Falcón et al. 2010). POA neurons convey monoaminergic (i.e. dopamine, 5-hydroxytryptamine) or peptidergic indication to the pituitary gland, analogous to peptides (isotocin and arginine vasotocin) of the neurohypophysis or releasing/inhibiting hormones via varied adenohypophyseal cells in order to control production and discharge of diverse hormones allied with seasonal growth of gonads in the annual cycle of fish. Furthermore, the identification of melatonin receptors (both MT1 and MT2 subtypes) and demonstration of 125IMel-membrane fastening in fish might control neuroendocrine functions by affecting the pituitary gland itself (Gaildrat and Falcón 2000). The varied effects of melatonin in different phases of the fish reproductive cycle depend on the type of melatonin receptors expressed in the pituitary glands (Falcón et al. 2007). Melatonin by itself is neither antigonadotrophic nor progonadotrophic, but the varying daily and seasonal melatonin levels in blood (with nocturnal peak) might operate as a submissive signal by which the hypothalamic-pituitary-gonadal axis predicts the episode of year (Hazlerigg 2001; Reiter et al. 2009). As its consequence, HPG axis in fish adjusts the timing of reproduction to ensure maximum survivability of the spawn. It is a well-established fact that the hypothalamus gland regulates most of the pituitary gland functions; therefore quite a lot of studies have laid emphasis on the effects of melatonin on this gland.

GnRH and GnIH in Melatonin-HPG Axis Interaction: Possibilities of Role Played by Duo

The neurosecretory cells of the hypothalamus gland synthesise and secrete gonadotropin-releasing hormone (GnRH), which plays a vital task in regulating reproduction in vertebrates. The binding and activation of the GnRH receptor on the gonadotrope cells of the pituitary gland occur with the help of this decapeptide which then stimulates the synthesis and secretion of gonadotropins (GtHs). Thirty structurally different forms of GnRH have been identified so far in the living organisms of which 18 are found in vertebrates (Roch et al. 2014). In fish species, there are several evidences of the presence of only three variants, namely, GnRH I,

GnRH II and GnRH III, with GnRH III, a conserved trait present only in teleosts (Kochman 2012). The GnRH receptor belongs to the rhodopsin-like G protein-coupled receptor (GPCR) superfamily having a typical seven-transmembrane (TM) domain structure (Cui et al. 2000). A study on European sea bass (*Dicentrarchus labrax*) revealed the inhibitory effects of the diurnal variation in melatonin levels on the expression of GnRH receptors in the brain (Servili et al. 2013). Therefore, interactions between melatonin and GnRH receptors could likely embody the photoperiodic effects on the physiological events including reproduction, where the melatonin hormone is directly involved in regulating seasonal reproduction of fish.

The novel finding of GnIH from hypothalamus gland of various vertebrates, including fish, has updated the present insight of hypothalamic control of reproduction (Tsutsui 2009, 2013, 2015). GnIH acts negatively in the avian and mammalian reproductive axis by hampering the release of luteinising hormone (LH) after fastening to GnIH receptor (GnIHR). A few studies on the GnIH/GnIHR system in different fish species have led to the discovery of the orthologous *gnih* genes in stickleback, medaka and *Takifugu* and three orthologous *gnih* genes (*gnihr1*, *gnihr2* and *gnihr3*) in zebrafish (Zhang et al. 2010). A prior study has shown that the goldfish gnih peptide could actually stimulate LH and follicle-stimulating hormone (FSH) release from cultured salmon pituitary cells (Amano et al. 2006). In contrast to it, additional studies on mature female goldfish have revealed the inhibitory effect of GnIH on gonadotropin release (Zhang et al. 2010; Moussavi et al. 2012), hence demonstrating the power of GnIH and its orthologs over the HPG axis in fish, though it still needs further study. Melatonin seems to stimulate hypothalamic GnIH neurons to synthesise and release GnIH in photoperiodic birds and mammals (Ubuka et al. 2005; Chowdhury et al. 2010; Tsutsui et al. 2013, 2015). Similar is the case for teleosts where it has been experimentally demonstrated that melatonin shows stimulatory effect on GnIH mRNA expression within brain cells of goldfish (*Carassius auratus*) (Choi et al. 2015). However, it still remains anonymous how melatonin acts on GnIH neurons of the hypothalamus gland to control seasonal reproduction in teleosts.

Possible Role of Kisspeptin in Melatonin-HPG Axis Interaction

The kisspeptins are a family of neuropeptides which stimulate GnRH neurons to release GnRH and control the HPG axis in most vertebrates including fish (Pinilla et al. 2012; Rather et al. 2017; Rather et al. 2020). They form an important part of the environmental and seasonal regulatory mechanism of reproduction. In addition to this, kisspeptins transduce the feedback mechanism of gonadal steroids and have an independent or nonsteroid-dependent circannual rhythm (Kanda et al. 2008; Kitahashi et al. 2009; Akazome et al. 2010; Elakkanai et al. 2015). Most non-mammalian vertebrates are known to possess up to three kisspeptin genes (Mechaly et al. 2013). In zebrafish (*D. rerio*), medaka (*O. latipes*) and goldfish (*C. auratus*), two kisspeptin genes (*kiss1* and *kiss2*) have been identified (Lee et al.

2009), whereas in green-spotted puffer (*Tetraodon nigroviridis*), torafugu (*Takifugu rubripes*) and three-spined stickleback (*G. aculeatus*), only *kiss2* gene has been found (Akazome et al. 2010). Kisspeptin-10 (Kp-10) encoded by both *kiss1* and *kiss2* genes is able to make activation of the HPG axis in zebrafish and sea bass, though the kiss2 decapeptide appears to be more effective (Kitahashi et al. 2009). More studies in goldfish also point out that the *kiss1*-encoded decapeptide is more potent in secreting gonadotropin (Li et al. 2009). The literature available strongly supports the view that kiss proteins play a dominant role in controlling the HPG axis in fish via melatonin signalling both by regulating *kiss1* expression and changing sensitivity of *kiss1* to sex steroid feedback mechanism (Grieves et al. 2008; Akazome et al. 2010). A study on Nile tilapia (*Oreochromis niloticus*) for the first time provided an indication of a direct link between kisspeptin and GnRH by the expression of *gpr54* gene which plays a vital role in the normal onset of puberty in the species (Martinez-Chavez et al. 2008; Elizur 2009). A similar study on zebrafish demonstrated that the receptor-mediated action of melatonin via the kisspeptin/GPR54 system in hypothalamic GnRH neurons stimulates GnRH secretion (Carnevali et al. 2011). This finding was supported by the transcriptional activity examination of GnRH, luteinising hormone receptor (lhr) and melatonin receptor (mtnr) in the brain of female killifish (Lombardo et al. 2014). In spite of reproductive stage-dependent regulation of the kisspeptin genes by photoperiodic signals, no expression of melatonin receptor by kisspeptin cells leads to the unidentification of seasonal changes in the kisspeptin gene activity (Pinilla et al. 2012). Conversely, a study on goldfish suggested that the photoperiod-mediated regulatory action of melatonin on the sexual maturity in fish might occur as a result of interactivity among melatonin, GnIH and kisspeptins (Choi et al. 2015). These functional interactions among GnRH, GnIH, kisspeptins and melatonin in the hypothalamus gland in the regulation of seasonal breeding in fish could become an important area of research in coming years.

Effect of Melatonin on the Gonadal Development of Fish

The first evidence on the presence of melatonin receptors on teleost gonads was provided in Western blot analysis of the ovarian homogenate of carp by using anti-MT1 goat polyclonal antibody in which a band of 37 kDa equivalent to Mel1a melatonin receptor (Mel1aR) proteins was detected (Chattoraj et al. 2009b). Insight on the presence of Mel1aR proteins in unlike cellular portions of the ovary and their day-night profiles in different phases of an annual reproductive cycle is provided by this analysis (Maitra et al. 2013; Chattoraj et al. 2009b). In ovaries of carp homogenates, presence of Mel1aR proteins in membrane and cytosolic portions has been revealed, relative band intensity (a ratio of the band intensity of Mel1aR to β-actin) of ovarian Mel1aR being greater in the membrane portion than in its cytosolic equivalent. The membrane-attached Mel1aR plays a functional role to mediate the intracellular effects of melatonin (Gaildrat et al. 2002; Dubocovich et al. 2010), while the function of cytosolic Mel1aR remains unknown. Carp ovarian

Mel1aR protein exhibits maximum activity at midnight and least at midday in a circadian cycle. This rhythm in ovarian Mel1aR does not change with the reproductive standing of the carp or photothermal environmental settings, but its nocturnal expression changes according to the reproductive phases in circannual cycle, with peak levels observed throughout the generation phase. The serum and ovarian contours of melatonin and Mel1aR in carp, respectively, show the same outline of diurnal disparities in the reproductive phase as against preparatory phase which remains different, when diurnal peaks of serum melatonin (in late dark phase) and of ovarian Mel1aR (at midnight) are completely independent (Moniruzzaman and Maitra 2012). Furthermore, findings like a daily injection of melatonin at 25 μg/100 g body wt. for 30 days in male (Bhattacharya et al. 2007) and female (Maitra et al. 2005) carp show progonadal reaction throughout the preparatory phase, but antigonadal reaction in the rest of the reproductive cycle could indicate an unexplained effect of melatonin on carp gonads. A possible hypothesis which still remains to be proven experimentally states that the reproductive phase-reliant consequences of exogenous melatonin on carp gonads might be as a result of the relevant season-related diurnal variations of endogenous melatonin level and/or its interface with other hormones, instead of nocturnal outline of ovarian Mel1aR (Moniruzzaman and Maitra 2012).The location and nocturnal outline of melatonin receptors on the carp oocytes proposes new insights in the control mechanisms of its rhythms and reaction to photoperiods in order to recognise the functioning of melatonin vis-à-vis seasonal reproduction in fish.

Conclusion and Future Direction

The circadian and circannual system in teleosts via the expression of melatonin is far from being fully understood. This time-keeping hormone of fish is known to directly or indirectly affect the production of various hormones and thus impacts time-regulated functions such as growth, feeding, immunity and reproduction. Past few decades have witnessed several studies in order to understand the complete mechanism of controlling of melatonin on seasonal reproduction in fish. The investigations elucidating the relationship between the melatonin and the neuroendocrine system in fish are just at their beginnings, with a few studies showing the action of melatonin on HPG axis and gonads. This hormone plays a decisive role in the control of HPG axis beginning from the kisspeptins which stimulate hypothalamic GnRH neurons to generate GnRH and/or GnIH to control gonadotropic functioning of adenohypophysis. While as the hormone acts directly on gonads and may control transcription of genes and their expression products involved in the production of gonadal steroids for the control of oocyte maturation, the melatonin regulation on fish reproduction is so far limited mostly to *Catla catla*; therefore more elaborate studies need to be conducted as the picture probably differs among the 25,000+ fish species. It is imperative that in future studies on diversified taxa of fish species would probably supplement our present knowledge of this hormone in regulating the conserved seasonal breeding in teleosts.

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14

Emerging Role of Small Non-coding (MicroRNAs) During Regulation of Endocrine Function in Fishes

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Abstract

The genome complexity and its duplication is a serious concern in fishes while understanding the fish physiology and other biological activity, etc. However, non-coding RNAs such as microRNAs are playing a vital role in regulating genes at a post-transcriptional level associated with developmental biology, metabolism, physiology, and reproduction, etc. Earlier studies depicted that microRNAs are highly conserved and regulates multiple genes by inducing mRNA degradation or translational repression via targeting at complementary sites of mRNA. Advancement in the next-generation sequencing technology and bioinformatics field have been helpful in identifying or solve biological questions in fishes using genome and/or transcriptome sequencing. In this paper, we have deliberated the role of microRNAs in fish nutritional physiology and reproduction. We have given a detailed account of advances in the investigation of miRNAs and their crucial role in the metabolic activity as well as gonadal development in fish species. The identified miRNAs can be utilized as biomarkers for elucidating the complex network of metabolism and reproductive physiology in fishes. This can be a further important source of miRNAs database for the development of antagomir (miRNA inhibitors) for functional level studies during nutritional programming or modulating the reproductive biology of fishes.

Keywords

MicroRNA · Teleost · Metabolism · Reproduction · Next generation sequencing

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Introduction

The aquaculture is one of the fastest-growing sectors and provides a sustainable source of protein diet to humans. However, due to recent development in the technologies and their intensification, aquaculture facing a serious concern and/or challenges such as increased input cost (Feed, fertilizers, and chemicals, etc.), lack of inadequate quality seeds, the emergence of diseases, etc. The recent development in sequencing technologies has been utilized in aquaculture species for understanding their biological complexity through genome/transcriptome sequencing. Till now, more than a dozen aquaculture species draft genome has been sequenced and published for tackling several key issues of fishes. Although selective breeding techniques have been utilized to upscale quality seed production in aquaculture, the demand gap exists regarding the availability of fish seeds, lack of species-specific feeds, and proper remedies for disease problems. The increase in the human population and aquatic pollution, aquaculture sector facing serious economic and ecological problems. The aquatic pollution hampered the reproductive efficiency of candidate aquaculture species. Thus, in order to ensure sustainable aquaculture production, new and novel technologies need to utilize for undertaking those challenges.

Now a day, sequencing technologies have been applied in the aquaculture fish species for deciphering their genome and/or transcriptome sequence and published in several public databases. Those valuable databases could be utilized by any researchers in the globe for understanding or solving biological questions in aquaculture species. These may be utilized to identify molecular markers (SSR/SNPs) associated with commercial important traits for production enhancement or functional level studies in fish species. Thus, the field of fish biology is now experiencing a transformative phase with the evolution of advent in the genomics as well as transcriptomics. The transcriptome sequencing has been used to detect differentially expressed transcripts/genes associated with various physiological events such as stress, metabolism, and reproduction, etc., as evidenced by several research groups (Biju Sam Kamalam and Panserat 2017). In addition to this, multiple gene regulation controlled by small non-coding RNAs such as microRNAs has been revealed in several fish species. The miRNAs are small non-coding RNAs (19-25nt), mainly found in the 3' UTR region of the gene. The multiple genes were regulated by those miRNAs at the post-transcriptional level via targeting or binding mRNAs based on complementary sequence sites (Kaeuferle et al. 2014). Several researchers depicted the biogenesis of miRNA and their role in fish physiology (Rasal et al. 2016; Rasal et al. 2019a). The role of miRNAs has been discovered in several organisms, such as animals, including fishes (Herkenhoff et al. 2018). The studies depicted that miRNAs are highly conserved across species and within miRNA families, which is an important criterion used for its discovery. One single miRNAs can target several genes for regulation of its expression. Altogether, evidence suggested that microRNAs play a vital role in developmental biology, such as cell progression and differentiation, apoptosis, signaling, organogenesis, etc. (Lau et al. 2014).

In the aquaculture sector, feed and seed are the major constraints which ensure the sustainability of fish production. The increase in feed cost has become a major hurdle to enhance aquaculture production. Fish feed mostly comprises 30–35% protein, which in turn results in the inflation of feed cost and ammonification of water. The aquaculture sector dominated by omnivorous carps, which supposed to be utilized more carbohydrate in their regime. Thus, attempts were made to understand fish physiology toward metabolism via the inclusion of a higher level of carbohydrates as evidence by several groups (Biju Sam Kamalam and Panserat 2017). We have reported details of these studies, where advanced technology has been used to decipher modulation in gene expression activities associated with metabolism in aquaculture fish species. In addition to this, we have also deliberated that miRNAs are involved in germ cell development and proliferation in male and female gonads of fishes and which affects the regulatory network of key genes associated with reproductive function. The differential expression of miRNAs in the gonadal tissues (germ and somatic cells) depicted their role and adequacy in spermatogenesis or oogenesis. We have discussed advances technologies used in the identification of key miRNAs and their targets associated with the development and function of male / female gonads. This could be an important resource material for understanding key issues in fish reproduction and reference for other species to solve reproductive disorders.

MicroRNAs Modulates Metabolic Activity in Fishes

As the feed cost is the major concern in the aquaculture sector, which accounts for more than 50% of the input cost, which in turn influences economic returns. Several research groups attempted to investigate the nutritional requirement of cultured species (NRC 2011). However, the protein component is the major contributor to increasing feed cost. The utilization of proteins in fishes as the primary energy source for the synthesis of glucose impairs protein retention and increases nitrogen release into the aquatic environment. Researchers attempted to decipher alternate ways for reducing feed cost by using locally available cheap feed ingredients, thereby to enhance economic gain/return. The use of carbohydrates in the feed will increase the protein-sparing effect/efficiency as well as reduce the feed cost. The nutritionally balanced regime is a pre-requisite for sustainable aquaculture production. Biotechnological tools used to understand protein, lipid, and carbohydrate metabolism at the molecular level and reveal its regulatory processes in fishes during the inclusion of feed components in their regime. Although the nutritional requirement of fishes has been reported by several groups, the physiological and molecular basis of this apparent glucose intolerance in fish is not fully understood. To date, the number of studies on metabolism in fish related to nutritional status is scarce. However, many studies have focused on the comprehension of the metabolic phenomena toward the improvement of nutrient utilization and fish performance in aquaculture systems.

In order to understand glucose homeostasis, Zhang and their colleagues compared all possible genes associated with glucose metabolic pathways in five species and

identified 66% conservation of these genes (Zhang et al. 2018). It has been revealed that miRNAs play as a key regulator in the metabolism (Bizuayehu and Babiak 2014). In rainbow trout, liver-specific miRNAs such as miR-33 and miR-122 were discovered, which linked with lipid as well as glucose metabolism (Mennigen et al. 2014a; Mennigen et al. 2014b; Miao et al. 2017). Using NGS technology, a total of 124 miRNAs in the liver linked with glucose metabolism in Blunt Snout Bream (*M. amblycephala*) were investigated and detected regulatory miRNAs of genes such as miR-128, miR-192, miR-205-5p, and miR-100-5p, etc. Mennigen and Zhang (2016) reported the MicroTrout, a comprehensive database, which was developed to implement an algorithm to predict relationships among miRNA-mRNA targets (Mennigen and Zhang 2016). It has been reported that eight miRNAs such as miR-1a, miR-181a, miR-133a, miR-214, miR-133b, miR-206, miR-146, and miR-26a are involved in myogenesis process in grass carp based on response to refeeding and fasting (Zhu et al. 2014). MiR-122b found to be link with the lipid biogenesis mechanism (Mennigen et al. 2012). In rainbow trout fingerlings, modulated expression of miRNA-33 and miRNA-122a/b isomiRs were reported using qPCR upon switching from endogenous to exogenous feeding (Mennigen et al. 2013). The microRNAs, such as MiR-33 and miR-122b, have been reported to be involved in lipogenesis (Fernandez-Hernando et al. 2011). The key microRNAs such as miR-103, miR-107, and miR-143 involved in post-prandial regulation of lipid as well as glucose metabolism, as reported in several fishes (Deiuliis 2016). Other microRNAs such as miR-29 and miR-103/107 are being reported to be involved in the regulation of insulin signaling (Vienberg et al. 2017). In Atlantic halibut, expression of miR103/107 shown their association with metabolism and miR-122, which is highly expressed in the liver of fish species tambaqui (Gomes et al. 2017). It has been depicted that miR-122 is highly conserved among vertebrate species linked with lipid/glucose metabolism (Mennigen et al. 2012, 2013; Bizuayehu et al. 2012). In rohu, *Labeo rohita,* a total of 138 known and 161 novel miRNAs were identified using deep sequencing upon fed with a high carbohydrate diet, and those studies indicated that miR-22, miR-122, miR-365, miR-200, and miR-146 involved in carbohydrate metabolism (Rasal et al. 2019b).

In rainbow trout, *Oncorhynchus mykiss* modulated the expression of miR-122 reported to be involved in lipid/glucose metabolism using LNA-122 inhibitor (Mennigen et al. 2014a). In this study, the altered expression profile of metabolic genes such as *glycolytic (GK), srebp1c, acc* was observed upon injection of antagomir. During feeding and refeeding, modulation in expression of key miRNAs such as miR-1, miR-206, miR-199, and miR-23a were investigated in trout, consequently negative level of correlated expression of associated genes IGF-1 for miR-1, miR-206, and miR-199; mTOR for miR-199; and MFbx and PGC1a for miR-23a were detected (Paula et al. 2017). Miao and group have reported that key miRNAs such as miR-34a have been involved in glucose metabolism using antagomir (Miao et al. 2018) and observed a suppressed expression profile of *SIRT1 (Sirtuin 1)* and *p53 (Tumor protein p53)* and other key genes associated with metabolic pathways.

Reproduction Controlled by Small Non-coding RNAs

In aquaculture, breeding of candidate species in a sustainable manner is a pre-requisite to provide seeds to the farming community. Recently, diversification of species has been given priority to enhance aquaculture production. Thus, to understand complex and diverse sexual behaviour of fish species, identification of breeding pairs, spawning habits, and developments in the gametes is essential to fish breeders undertake the breeding program for fish. However, certain challenges in the breeding industry have occurred, such as climate change, human embankment, pollution, alteration in sexual behavior, reduction in reproductive fitness and availability of quality broodstock, this has put forward to sustain reproductive efficiency of fish.

Several researchers are involved in understanding the complex behaviors of brood fishes using genetic and molecular tools at the population level. The NGS approaches using transcriptome sequencing have been utilized to investigate biological issues in several aquaculture species (Qian et al. 2014). It has also been reported that microRNAs are playing a vital role in sexual differentiation and maturation in fish species (Jing et al. 2014; Wang et al. 2017a). In Nile tilapia, *Oreochromis niloticus* miR-456 and miR-138 have shown altered expression profiles by targeting AMH gene, thereby inhibited the testis differentiation (Eshel et al. 2014; Wang et al. 2016). Those studies reported that miRNA (miR-4585) could be acts as a biomarker for sex determination and understating sex reversal in tilapia, which leads to producing monosex populations. The novel and known miRNAs were observed in the brain, pituitary, and gonadal tissues of the reproductive axis of several fishes such as Nile tilapia Atlantic halibut and yellow catfish, etc. Interestingly, those studies reflected miRNAs act as a key regulator in reproduction, which controls the expression of several key genes associated with spawning and maturation. The NGS technology, i.e., deep sequencing has been used in some of the fish species for identifying miRNAs and their regulatory network during gonad development (ovary and embryo stages) in Yellow River carp. Those studies suggested an important role in driving sexual development and differentiation in various fishes. The differentially expressed miRNAs such as MiR-200b and miR-726 were detected in the ovary of flounder *Paralichthys olivaceus* (Gu et al. 2014). miR-2184 observed in the testis of medaka and involved in the maturation process compared to other tissues. In yellow catfish, several unique tissue-specific (ovary, testis) miRNAs were identified (Xiao et al. 2014). miR-375 and miR-499 reported a key role in testicular differentiation in *Xenopus* and tilapia, respectively. In addition to this, the expression profile of miR-9, miR-192 miR-27d, miR-29b, miR-92b, miR-144-5p, and miR-455 reported to be involved in testes development, suggested their critical role during sex differentiation in tilapia. The miR-141 and miR-429 shown a crucial role during spermatogenesis and testis development in yellow catfish. Similarly, miR-143, miR-145, let-7a, and miR-202 identified as modulators for the induction of testicular differentiation in Atlantic halibut (Bizuayehu et al. 2012).

In common carp *Cyprinus carpio*, a total of 8765 miRNAs comprising 2155 known and 6505 novel miRNAs linked with ovarian development stages were

detected using deep sequencing (Wang et al. 2017b) and 150, 628, and 431 differentially expressed miRNAs were detected in different stages of ovary such as primordial gonad, ovary (juvenile), and ovary (adult), respectively. The ovarian stage-specific miRNAs were also detected based on their expression profile and targeted genes (*dmrt1*, *atm*, *gsdf*, and *sox9*) such as miR-6758, miR-2985, miR-3050 in gonad, miR-3544, miR-6877, and miR-9086 in Juvenile ovary and miR-154, miR-3958 and miR-5307 in adult ovary, etc. The earlier studies revealed that miR-430 family involved in embryonic development, as reported in Yellow River carp and zebrafish, shown to target chemokine signaling pathways associated with germ cell development. In this study, regulatory microRNAs, such as miR-21, MiR-143, and Let-7 depicted their involvement in ovarian development, gonadal differentiation, and endocrine regulation. In Yellow River carp, miRNAs such as miR-430, miR-21, let-7, miR-181a, and miR-143 represented as a vital role in ovary differentiation and development (Wang et al. 2019). In yellow catfish, a total of miRNAs 384 conserved and 113 novel putative were detected using high-throughput sequencing, among which miRNAs of total 23 in XX ovary, 30 in XY testis and 14 YY testis were identified, respectively (Xu et al. 2013). The let-7 and miR-21 involved in egg development in rainbow trout. In zebrafish, modulatory level of expression of the miRNA in follicular cells at different stages (IIIa and IIIb) were studied using next-generation sequencing and 44 novel and 31 conserved miRNAs in zebrafish vitellogenic ovarian follicular cells (Zayed et al. 2019). The differentially expressed miRNAs (DFM) such as miR-16a, miR-22a, miR-29a, and miR-181a were investigated to be associated with ovarian follicular development.

Concluding Remarks

The advancement in the sequencing technology has resulted in huge data with regard to genome and transcriptome, including small RNAs of fish species. Till now, a total of 3687 microRNAs (known and novel) are being characterized from 16 fish species (teloest) and available in the miRBase database (miRBase 22.1, http://www.mirbase.org/). Those data will help to analyze miRNAs structure and abundance in other fish species, assist in their expression profiling linked with physiological events. However, studies of miRNAs functions in fish physiology, and growth are not adequate yet. The environmental adaptation and teleost plasticity are yet too understand and their possible mechanism to combat biotic and abiotic stress. In aquaculture, deep sequencing technology has been immensely used to decipher the role of miRNAs in fish linked with metabolism, reproduction as discussed above. Those reports will be helpful to undertake studies in other species and analyzing expression patterns of miRNAs associated with different stages of gonadal development. Earlier studies clearly indicated that DFE miRNAs involved in testis and ovary development (oogenesis and spermatogenesis). Thus miRNAs could act as a biomarker for identification of developmental stages of gonads and could be model for other species. The ovarian development and testicular regulation can be studied using identified miRNAs retrieved from the database. Due to the vital property of

multigenic regulation by miRNAs, single key miRNAs can be used to control specific biological activity, i.e., alter the expression of several target genes. However, this is challenging to obtain the desired phenotype using manipulated miRNAs or its antagomir, and those studies required deep screening of modulation in gene expression patterns in different tissues. The detection of off-target effects of manipulated miRNAs could be problematic to decipher in new species, whose well-characterized genome is not available. Here, cutting-edge genome editing technology such as CRISPR-Cas9 can be used to perform gene knock-in or knockout for understanding gene/miRNAs function specifically. The SNPs linked with miRNAs need to study, as their interaction could have an impact on the expression of targeted genes. The miRNA-seq and Degradome sequencing technology need to apply together to assess the impact of SNPs and miRNAs in fish species for revealing biological implications. As the miRNAs are highly conserved among vertebrates, those generated data facilitate to study metabolic activities and reproduction in the teleosts. The miRNAs have a potential to be used as a molecular marker in breeding programs of fish species that can enhance global aquaculture production.

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Conflict of Interest The authors declare that they have no conflict of interest.

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Roles of Bioinformatics in Reproductive Science

15

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Abstract

The area of bioinformatics emerged as a method to promote biological research more than two decades ago. Bioinformatics is a multidisciplinary field of study composed of biology, mathematics, and computer science. It has emerged as a smoothing biological science instrument and saves findings as far as possible. Every day, enormous biological data are accessible to the science community by

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producing high throughput sequencing (HTS) technologies. Bioinformatics and algorithm approaches are now being used to evaluate such significant results. More scientifically, bioinformatics can be used to retrieve, mine, interpret and sort information with cross-linking references, which are accessible to help grasp biological complexities in this comprehensive natural archive and offer new insight.

Bioinformatics tools will provide scientifically valid information about the structural biology of different genes and proteins which are related to the reproductive traits of animals. Bioinformatics is integrated with reproductive science to provide key influence for the production of wide-ranging species like livestock productivities, agriculture production, fisheries production, wildlife conservation and development, and special environmental management. This chapter will provide comprehensive information about bioinformatics tools and their applications in reproductive science.

Keywords

Bioinformatics · Reproduction · Big data · Tools · Fish

Introduction

Bioinformatics is an interdisciplinary scientific field of life science and involves the amalgamation of computers, statistics software tools, and database science in order to address biological questions (Fulekar 2009). Bioinformatics has been incorporated with numerous diverse disciplines like drug development, molecular medicine, personalized medicine, preventative medicine, gene therapy, microbial genome applications, antibiotic resistance, evolutionary studies, waste cleanup, biotechnology, forensic analysis, climate change studies, crop improvement, development of drought resistant varieties, bio-weapon creation, insect resistance, improve nutritional quality, alternative energy sources, veterinary sciences, and reproductive science. In 1956 first protein sequencing was done which was insulin peptide. More technically, in this field large biological databases are involved for retrieving, mining, analyzing, and sorting data using recently developed computational tools and software like NCBI, Ensemble, SMART, STRING databases, which are accessible for better understanding of biological complexity with advances to thrift place space, time, cost, and wet lab practice issues. With the immense development in information technology, currently bioinformatics has broader scope for assessment of valuable data to encompass modeling and image analysis via involving classical methods for comparative study of three-dimensional protein structures and linear sequences (Gu and Bourne 2009) mentioned in Fig. 15.1.

Human Genome Project was started in 1988 and it was a big challenge for the scientific community on how to analyze such a huge data. After the continuous progress in the field of computational biology, the analysis of such data has become easy. This type of problem can only be possibly handled by using bioinformatics

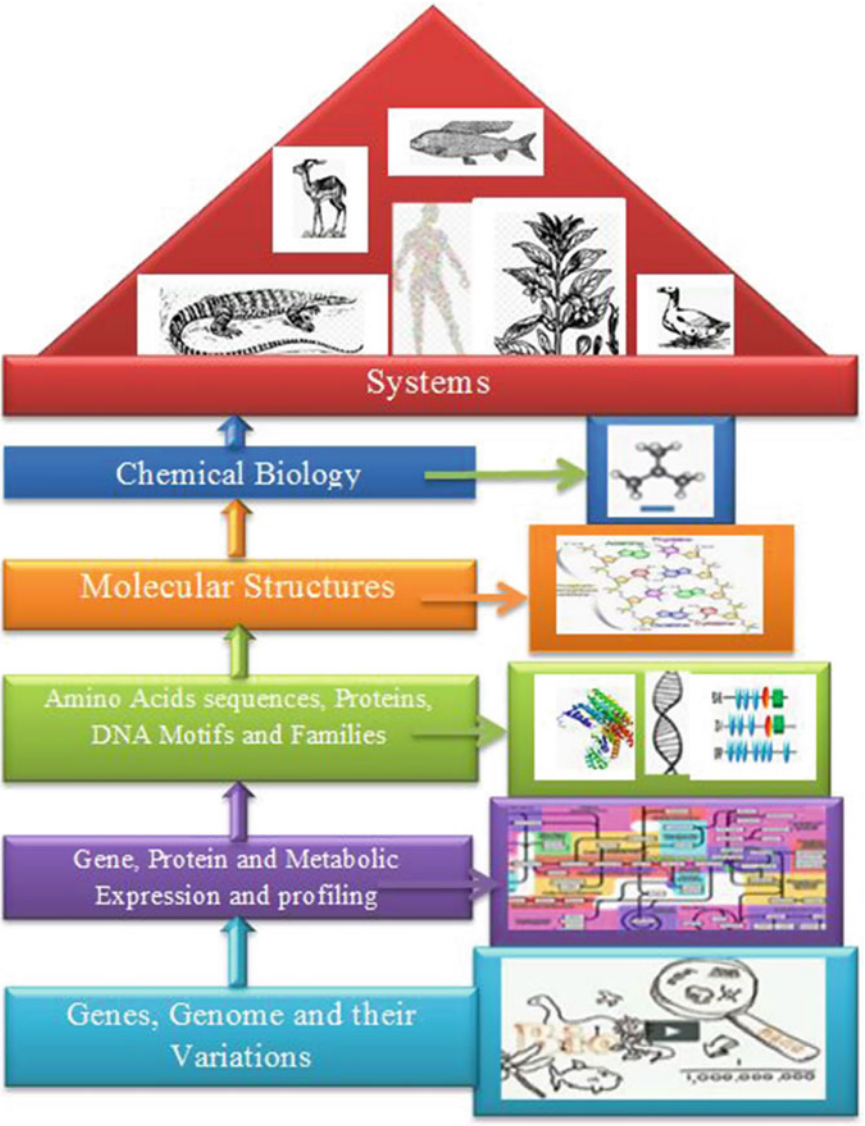

Fig. 15.1 A general overview for scope of bioinformatics and broad types of data fall under other integrated sciences for sorting, retrieving, updating, analyzing, and managing data. According to current demand of bioinformatics now it is used in very broad ranging fields including system biology, antibiotic confrontation, evolutionary studies, waste cleanup, biotechnology, forensic analysis, climate change studies, crop improvement, structural and computational biology, bio-weapon design and formulation, insect resistance, increase nutritional value, development of substitute energy sources, veterinary sciences, molecular medicine, personalized medicine, preventative medicine, gene therapy, microbial genome applications, gene therapy, development of drought resistant varieties and reproductive science, etc

(Jhala et al. 2011). A progress was made in the development of computer algorithm and database management system paralleled with this type of growth and providing a motivation for the field of bioinformatics for further working and understanding.

Bioinformatics is growing rapidly in the field of research and development to become a principal technology in all life disciplines or it provides an easy insight for the future among worldwide researchers to communicate with primary data and let information be oriented models to address the complex problems. Worldwide multidimensional collaboration is more extended among researchers to develop genome sequencing projects of human, rice, yeast, zebrafish, corn, etc. for all bioinformatics provide multiple insights to analyze data via informatics tools and technologies to determine broad range concepts through including silico approaches about structure prediction, homology, molecular docking, and dynamic simulation and molecular modeling of all biological systems which are involved in high throughput technology (Kaufman 2004). The terms of bioinformatics, data mining and knowledge discovery in databases have attracted a significant amount of attention from the scientific and research community for the last two decades (Piatetsky-Shapiro 1996). For example, data manipulation comprises web-based online molecular tools (Han et al. 2011) and large databases associated with physical servers for exchanging information. The individual researcher can perform a broad range of experiments daily, which are freely available for public domains like NCBI, DDB, EMBL (Chen et al. 1996).

Fields of Bioinformatics

Genomics

The study of gene on expression level is called genomics. This field provides an immense quality of information from genome via gene sequencing, their correlation and functions. To handle this huge data, bioinformatics performs significant roles to handle the whole genome sequence on gene level for increasing the number of organisms; bioinformatics is also involved to ensure both theoretical and realistic techniques in the field of structural genomics, functional genomics, and nutritional genomics for identifying systematic functions and behaviors of different organisms on complex level of interactions (Bradbury & Pongor 2012; Kanehisa and Bork 2003).

Proteomics

Proteomics is a study of protein structure, functions, properties, and their interconnected relations at particular stage of the cell or tissues from individual or group of organisms. Proteomics is linked with multiple field of sciences like molecular biology, genetics, and biochemistry. With the aid of bioinformatics many tools have been developed to deal with this wide range of datasets, for

example, algorithms for image analysis of electrophorese gel, peptide fragmentation, and peptide mass fingerprinting (Tyers and Mann 2003).

Transcriptomics

Transcriptomics is a branch of science in which a set of mRNA molecules are studied at cellular level. It is also known as expression profiling technique where DNA microarray is selected to find the expression degree of mRNA in a provided cell inhabitant. The microarray techniques and transcriptome analysis with the aid of bioinformatics produce a large amount of datasets or analyze them with multiple algorithm-based packages. RNA sequencing and determination of quantity of RNA in sample are also considered as a part of transcriptomic and carry out using next-generation sequencing techniques (Aplin and Singh 2008).

Cheminformatics

Cheminformatics also known as chemoinformatics is the usage of informational and computational techniques to understand problems and challenges of chemistry. With the aid of bioinformatics all applications of cheminformatics become easy to resolve the issues about the analysis of therapeutic effects, properties, searching resemblance, virtual screening, clustering, and QSAR modeling (Fourches et al. 2010).

Drug Discovery

Bioinformatics is a computational field of drug designing including all aspects of assessment and development at clinical levels (Katara 2013). Different approaches of drug designing face many issues like time, cost, and complexity to find new promising drugs, so the interest of bioinformatics is more remarkable such as computer-aided drug design. Bioinformatics also help to provide wide ranging tools, software, and databases for improving the processes of drug developments (Wishart 2005).

Evolutionary Studies or Phylogenetics

Evolutionary biology is the subfield of biology among the various species for monitoring the evolution mechanism (natural selection, common descent, and speciation) and phylogenetic relationships, using bioinformatics methodologies (Allaby and Woodwark 2004).

Crop Improvement

Crop improvement is a crucial field of OMICS research in the agriculture sector, and in this field bioinformatics provides novel insight on urgent issues in response to climate change. Bioinformatics also provides online public access to large biological databases based on newly developed algorithmic techniques for experimenting with increasing plant productions (Mochida and Shinozaki 2010).

Biotechnology

Biotechnology is a branch of biology and deals with applications for mankind via involving a broad range of terms like disease characterization, pharmaceutical discovery, molecular modeling and designing, forensics investigations, agriculture functionality and improvements, clinical healthcare, and other natural sciences including different worldwide social issues. In some context bioinformatics involved in every step of biotechnology to increase the productivity based on time, space, and complexity advantages like gene documentations for novel identifications of genes, genes proteins, whole genome sequencing based on automated systems, extrapolation of gene and protein function and issues, phylogeny linage and analysis, drug designing and improvement, sympathy of organisms via developing model organism, vaccine designing and development, informative visualization and better involvement about gene and genome complexity, informative visualization about protein structure, functions, and folding. Conversely, bioinformatics has a great importance among all natural sciences like environmental biotechnology, genomics, proteomics, transcriptomics, and other fields to improve or innovate in mankind applications (Rao et al. 2008).

Forensic Science

Forensic science is an explanatory field of science integrated with bioinformatics to investigate the information about individual identification and relationship based on DNA profiles for future evidence via computer developed techniques and statistical methods. Bioinformatics also help to store, retrieve, investigate, and analyze molecular data in different databases (Bianchi and Liò 2007).

Veterinary Science

Veterinary science is the study of livestock for improving the quality and quantity of demanded products for human needs in the way of food production, competent animal production, and reproduction system in animals for selective breeding. In the field of bioinformatics tools and techniques are involved in veterinary science to

improve breeds of livestock via involving with large biological datasets to understand the systems genetics of complex traits (Kadarmideen 2014).

Biodefense

Biodefense is a way of employing medical procedures to protect bio-lives from various interventions, including bioterrorism and bio-war attacks. In this way, bioinformatics has improved organisms' biosafety, such as forensic science and intelligence operations based on evolved algorithms. Bioinformatics in biodefence contexts guides the operation of numerous biodefense countermeasures like interoperability among the genome sequence and the similarity between different entities to examine forensic behavior. However, existing algorithms have not excellently interpreted available pathogen genomic data into standardized diagnostics, rational vaccine development, or broad-spectrum therapeutics (valdivia-Granda, 2010).

Waste Cleanup

Clean up the world is an international concern, how to deal with environmental and other pollutions which are generated from different sources and are dangerous to human health. The natural biochemical cycles introduced biodegradation techniques, which are more important to deal with this type of issues. With the aid of improved technologies, bioinformatics tools and techniques deal with organisms on genomic and proteomic levels to enhance the microbial potentiality and provide a better bio-degradative pathway (Sadraeian and Molaee 2009).

Climate Change Studies

Climate Change Research is a global concern to continue tracking climate change updates, such as heatwave processing, sea ice depletion, and sea-wave acceleration. The simplest way to address this type of issue and problems by sequencing the genome of numerous species using high through put technologies that effectively eliminate carbon dioxide and other greenhouse gases to control global climate change (Sinha 2015).

Bioenergy/Biofuels

Bioenergy is a field of science in which biofuels are studied for promising global demands of energy via providing different sources of renewable energy. Recent advancement in algal genomics, in combining with other "omic" methods, has accelerated the ability to identify metabolic pathways and genes that are possible targets in the progress of genetically engineered microalgal strains with optimum lipid content (Mitra et al. 2013).

Personalized Medicine

Precision medicine also commonly referred to personalized medicine is a new model for healthcare merging genomics, big data analytics, and population health. The method depends on scientific breakthroughs in the understanding of how a person's unique molecular and genetic profile makes them vulnerable to certain diseases (Fernald et al. 2011).

Bio-Weapon Creation

Biological weapons commonly "bio-weapons," "biological threat agents," or "bio-agents" are microorganisms like bacteria, virus, fungi, or other toxins that are created and released intentionally to cause disease and death in humans, animals, or plants. Biological weapons are a subsection of a larger class of weapons including chemical, nuclear, and radiological weapons which will be a major threat for living organisms including human beings in the coming decades (Selvi 2012).

Molecular Medicine

Molecular medicine is a field of medicine where many fields are involved to understand the response of genomic, proteomic, and transcriptomic relations among organisms at molecular structures. In increased clinical adoption, bioinformatics has been involved with molecular medicine to provide the opportunity of next-generation sequencing (NGS) for identification of genetic errors with developed *molecular* interventions to cure the diseases (Altman 1998).

Gene Therapy

Gene therapy is the study of treating genetic diseases via introducing RNA molecules into a host cell to alter the genes within the body. In current situation bioinformatics provides a new insight about exploration of genes via speeding the processes for future incredible role in medicines (Giordano et al. 2007).

Antibiotic Resistance

Antibiotic resistance is a field of medicine where professionals only focus on antibiotics to treat infectious diseases without a clear division among the therapeutic and toxic doses. Nowadays infectious diseases are rapidly growing with genomic alterations of microbes and antibiotics are major challenge to develop within time. Bioinformatics provides numerous applications for solving previous issue based on molecular and structural understanding of microbes systems via in silico or in vivo

methods for drug discovery to develop potent antibiotic medicines such as quinolones, cephalosporin's, penicillin, macrolides, tetracycline, sulphonamides, aminoglycosides, and glyco-peptides, to treat severe bacterial infections (Ndagi et al. 2020).

Improve Nutritional Quality

Like other biological sciences, nutrition science can benefit immensely from the techniques of computational biology. With the help of bioinformatics or computational biology, nutritional value data of each plant, fruit, or vegetable can be easily available (Edwards et al. 2009).

Reproductive Science

In reproductive science; bioinformatics emerges with genetics and molecular biology to create a better understanding of life including their suggested solutions at the initial stages of infertility and it gives a vision about the status of pathological gametogenesis or gamete functions. Bioinformatics provides a quantum leap for database projects and online analysis tools to manage the flow of high throughput data from genomics, transcriptomics, and proteomics to deal with reproductive biology (Primig 2012). It will also deal with structural biology and computational analysis of proteins which are related to reproductive traits of animals.

Role of Bioinformatics in Animal's Reproduction and Breeding

Bioinformatics is the field of science in which interpretation and analysis of biological information of various disciplines including biotechnology, statistics, computer sciences, molecular biology, mathematics, and information engineering are done (Wong 2004). It is actually the application of computational approaches to handle large amounts of information concerning molecular biology, biotechnology, genetics, and various other biological sciences. This technique was firstly utilized in 1979 by Paulien Hogeweg and Ben Hesper (Lesk 2013).

In animal breeding and reproduction, bioinformatics covers various aspects of computational technology including the development of algorithms, software packages to align and compare DNA and protein sequences and visualizes the creation and prediction of three-dimensional structure of protein Sjakste & Grislis 2006. The human genome project, the prediction of gene expression, and determination of protein-to-protein interaction were also the major achievements of bioinformatics. In the field of genomics, bioinformatics plays a vital role in the identification of single nucleotide polymorphism and novel genes in order to analyze the genetic variations in different animal species (Londin et al. 2013).

Different bioinformatics approaches and databases on proteins and nucleotides are available in order to store, compare, analyze, and retrieve the biological information related to breeding and reproduction of various animal species. Mixing of these predicted techniques with the experimental research leads to the discovery of modern livestock genetics and animal breeding (Jonas and Koning 2015). It also helps to detect various sequence variants such as single nucleotide polymorphisms, copy number variations, insertions, and deletions in order to discover functionally related traits which could lead to genetic diversity (Daetwyler et al. 2013).

Reproductive research is also one of the well-recognized roles of bioinformatics which helps to understand the diseases associated with reproductive system of particular species as the reproductive diseases are most common these days due to a number of reasons, and it is very important to understand the cellular mechanism involution in regulating the mother to fetus interaction and to study the factors associated with infertility in male and females (Cheong et al. 2002). These defects are mainly associated with the continual practice of chemical modulators and increased contact of certain toxic chemicals, i.e. mercury, cadmium responsible for certain mutation at DNA level. Various techniques such as microarray technique and cell signaling pathway analysis approach help to detect these defects of premature pregnancy loss and inappropriate uterine reception (Vannuccini et al. 2016).

Bioinformatics has also played an essential role in human reproduction studies. This includes the use of various software programs and algorithms in order to improve the diagnosis of genetic disorders, to figure out the proper molecular therapy, and also is helpful to check the appropriate response that could be generated. It also helps researchers to analyze the tremendous amount of biological data in order to solve various biological problems and molecular based prediction and diagnosis in the field of animal reproduction and breeding (Anagnostopoulos et al. 2011). Various proteins such as MARK4, TSSK, BRCP, and FAK are involved in regulation of steriodogenesis and spermatogenesis in male. These proteins are potential candidates for certain environmental toxins, drugs, and contraceptives. The protein such as P-glycoprotein is the key target for environmental toxins such as bisphenol A and cadmium and for the formation of contraceptive (Nya-Ngatchou and Amory 2013). The chemical modulators are involved in morphological variations in sperms, affecting the blood–testis barrier, disturbing the motility of sperm which ultimately results in premature sperm release (Mathur and D'cruz 2011). Detailed knowledge of underlying mechanism of action is required to determine the relation between target and chemical modulators. These interactions could be better understood by the use of various bioinformatics techniques such as molecular simulation, docking, and molecular modeling technique. In this way bioinformatics plays a very important role in the reproductive studies research by understanding the interactions of protein responsible for reproduction between chemical modulators and drug compounds (D'Cruz et al. 2012a, b).

The contraceptives based on steroids in female have been extensively used to target various hormonal receptors including progesterone, androgen, and estrogen receptors. In silico interaction analysis and molecular mapping help to recognize the interactions of drugs targeting these hormones and for the identification of specific

functional groups responsible for their conformational changes. The pharmacophore maps serve as library for all available natural and synthetic source that could be a possible candidate to design drugs showing relevant pharmacophore properties (Jenardhanan et al. 2014). The information obtained from these screening libraries did not only save our time and cost but also contributed in drug designing and discovery which could be further studied for their effectiveness in the target receptors. The information of these maps have been studied and verified against various kinases such as FYN, FAK, and MARK to treat certain type of cancers of reproductive system (Xiao et al. 2014).

Reproduction-related biomarkers identification is also one of the major achievements of bioinformatics. One such proteomic database is Swiss-Prot which provides high throughput identification and analysis of protein markers which subsequently help in understanding and diagnosis of reproductive problems including infertility, fetal aneuploidies, premature pregnancies, and preterm labor. Proteomics databases hence serve as essential tools to study and evaluate reproduction-related biomarkers and understanding the molecular pathways involved in reproductive diseases (Kolialexi et al. 2010).

Bioinformatics tools and databases such as whole genome regression predictive software statistics help in better understanding of the complicated phenotypic and genotypic traits where small-n and large-p values are applied to regress the phenotype with various markers at a time. This as whole genome regression predictive software had crucial role in animal breeding and reproduction studies. Various statistical programs such as BigPSD, GEMMA, Synbreed, BGLR, SIGENAE, and BrrBLUP are available for further prediction analysis. SIGENAE is an animal genome analysis database commonly used to analyze animal genomics and breeding studies in trout, pig, sheep, rabbit, and chicken (De los Campos et al. 2013). Bioinformatics have huge potentials to study the inherited diseases and disorders which affect animal health and decrease productivity. Thus, animal breeders rely on the use of various databases such as genetic control databases (GDC) for early identification of possible carriers of inherited diseases and for investigating and distinguishing the genotypes into affected, carriers, and normal which would prove to be beneficial for their experimental studies (Jovanović et al. 2009).

Bioinformatics have also a crucial role in fish management and reproduction. Fish reproductive studies have a fundamental role in the management of fishery where it plays a vital role in determining the inception of breeding season, total interval of spawning season, and managing the size at the start of maturation (Dias et al. 2010). But in the emerging countries due to limited amount of available data about fisheries management and because of misuse of available stockfish management faced severe downfall (Alves and Minte-Vera 2012). Moreover subsidization facilities provided by the government could limit further exploitation to some sustainable level. It is important to track the reproductive level and breeding scale since some fishes have a year or two breeding time (Cushing 1990). However, proper handling includes the connection between breeding and the required climate to ensure larval growth

(Lowerre-Barbieri et al. 2011a, b). The availability of valid fishing data is therefore essential for the provision of adequate fisheries.

The huge repository of databases does not only provide information about all possible genes and genetic material of particular species but also serves as a reliable source to study specific tissues and even specific organs including male and female reproductive organs (Zhang et al. 2011). These databases provide authenticated knowledge about gene expression level of the organ under studies or biological system under investigation. The Ovarian Kaleidoscope database covered about 3400 human genes concerned with female reproductive organ serves as a quick annotation database providing high-throughput results (Hsueh and Rauch 2012).

Bioinformatics also plays an important role in identification of certain environmental toxins and chemical compounds that are carcinogenic and responsible for various defects in reproductive organs and certain infertility in these organs; therefore, the structural analysis helps in the recognition of genes in reproductive organs, designing anticancer drugs and possible solution to inhibit binding of these environmental toxins with these proteins (Jenardhanan et al. 2014). In the field of animal breeding various bioinformatics techniques such as transcriptome analysis, genome annotation, microarray data analysis, genome-wide association studies, protein structure prediction, phylogenetic analysis, and genetic diversity analysis help breeders to increase livestock production by selecting the animals with improved and desirable traits and to develop disease resistance traits in animals. It also has an appreciable role in the preservation of rare and extinct animal species (Singh et al. 2018). The field of bioinformatics has a great opportunity for animal breeders to develop new and improved breeds with high quality reproductive traits and stress tolerant properties. This may also help them to formulate proper management schemes that would be helpful in marking future prediction. Bioinformatics helps them to process, store, and retrieve large amount of sequence information, assemble the predictive molecular maps and markers, and access different databases in order to take fruitful decision to increase cattle yield quality (Hu et al. 2013). Different databases on animal genetics are interlinked covering all the quality assurance programs, animal disease control program, and implementation of novel breeding approaches. These databases also organize all types of data regarding animal breeding including breeding zones, breeding information about livestock and farm animals, and information about the reproductive traits of the particular animal selected for breed (Wickham 2013).

Bioinformatics Tools for Reproductive Biology

Bioinformatics is integrated with reproductive science to provide a major contribution for production of broad categorized species like livestock social standing productivities, human health, macro and microclimate changes, fisheries production, pest animal control, wildlife development, and other environmental managements. For future prospective of rational improvement of policies on reproductive topics reproductive scientists are known by the community and collaborate with other

sciences to provide scientifically valid information for capturing fertility of welfare of animals, humans, and other potential lives. Bioinformatics is an interdisciplinary science that merges with other disciplines for unraveling or adding meaning to different biological problems. Based on computational tools bioinformatics is being continuously retrieved, analyzed, and annotated the large datasets related with simulation of living cells to verify problems and their solutions among interaction of biomolecules (Mount 2004). Its applications are very broad for underrating the functionality of gene characterizations and their potential physiochemical properties of proteins and phylogenetic investigation among different species. Bioinformatics provides in silico facility to perform experiments via decreasing the efforts; cost and time of scientists for drug discovery process based on screening large datasets stored in chemical databases and also help for annotation of structural and functional properties of proteins, identification of cellular functions among cellular partners and many other key roles in reproduction as functional modulators.

In earlier reports approximately 50 fish species whole genome sequences were reported and submitted in different genome databases for public access (Huang et al. 2016). In research, different numbers of reviews have been written on many specialized aspects of bioinformatics (Ouzounis 2012) in the field of computational study for researcher who does not have knowledge about computational biology how integrated with reproductive sciences. Here we take the opportunity to introduce various computational tools for reproductive areas of science in a single platform for non-specialist reader to help for extracting useful information regarding their task. The domains of particular areas included whole genome sequence databases, tools for prediction of protein 3D structures, dynamic simulation and modeling, analysis of amino acids and nucleotide sequences, phylogenic relation and computational studies, molecular pathways, interactions and drug designing, etc. We organized tabulated information mentioned in Tables 15.1, 15.2, 15.3, 15.4, 15.5, 15.6, 15.7, 15.8, and 15.9 for simplistic overview of bioinformatics tools followed from review and literature as a key report for particular task of reproductive biology.

Conclusion and Future Direction

In the field of life science, bioinformatics is an interesting scientific field and has used computerized methods to manipulate biological data in a shorter time. It offers the opportunity to examine large-scale scientific data from data integrated into different science disciplines to solve complex problems by designing new algorithms, software, tools, especially pilot data for genome sequencing and analysis on expression level. Further theoretically, in bioinformatics, the large biological databases are interconnected to retrieve, extract, analyze, and classify data with cross-references such as the National Center for Biotechnological Information (NCBI), Ensemble, Simple Modular Architecture Retrieval Tool (SMART), STRING database, Therapeutic Target Database (TTD), and Protein Data Bank (PDB). Data is available for a better understanding of biological complexity and to

Table 15.1 Gene prediction, annotation, and sequence analyses

Name	Description	References
GenScan	Input as genomic sequence of DNA and identify the gene structure, intron–exon regions from different organisms	Aggarwal and Ramaswamy (2002)
GeneMark	GeneMark is a package of gene predictions program including DNA genomic sequence analysis of prokaryotic (small genome), eukaryotic organisms and also provides the facility of EST and cDNA analysis	Besemer and Borodovsky (2005)
Gene Finder	Find the protein coding exon, splice sites, recognize promoter, build protein model, and detect poly-A region	http://rulai.cshl.org/tools/genefinder/
GENECODIS	Find the gene annotation with statistical significance	Carmona-Saez et al. (2007)
Easy Gene	Utilize DNA sequence of prokaryotic organisms to identify the list of predicted genes	Schou Larsen and Krogh (2003)
NetGene 2	Predict splice sites based on neural networks techniques	Hebsgaard et al. (1996)
Gene Publisher	Received Affymetrix CEL files as input or gene table for forming statistical and numeral analysis	Knudsen et al. (2003)
GLIMMER	Used Markov models to identify the coding regions from microbial DNA and separate the genes from noncoding regions	Aggarwal and Ramaswamy (2002)
BLAST	Computational tool used to search similarity among different sequences of DNA and proteins	Madden (2013)
Softberry Tools	Package of several tools for annotation, structure, and function prediction of RNAs and proteins among diverse ranging cell types	http://www.softberry.com/
Clustal Omega	Used for MSA and phylogenetic analysis	Sievers and Higgins (2014)
AUGUSTUS	Used to predict the gene of eukaryotic organisms	Keller et al. (2011)
HMMER	Used HMM for local and global searching	Durbin et al. (1998)
novoSNP	Used to find the SNP variations in DNA sequences from different organisms	Weckx et al. (2005)
WebGeSTer	During transcriptional process, predict the termination sites of genes	Mitra et al. (2013)
LAMBDA	Faster with the support of SAM/BAM formats and performed local alignment among large species	Byma et al. (2017)
Sequerome	Simple tool for sequence profile	112
Geneid	Used input as a DNA sequence to predict genes, exons, splice sites, and other signals	Alioto et al. (2018)
mGene.ngs	Used to find genes based on SVM formats	Gan et al. (2011)

provide new insights into reproductive science with advances in the management of space, time, cost, and wet laboratory issues. There are many bioinformatics tools, application, and approaches available with in silico or in vivo facilities including broad sense of object modeling, image analysis, 3D protein structure prediction and analysis, dynamic simulation and modeling, sequence and alignment analysis, and

Table 15.2 Structure–function analyses of proteins

Name	Description	References
RaptorX	Provide the facility of protein prediction based on single or multi-template threading	Källberg et al. (2012)
CATH	Used to categorize the protein association based on manual or auto methodology	Sillitoe et al. (2012)
ROSETTA	Used to predict the 3D structure of proteins	Rohl et al. (2004)
SWISS-MODEL	Automatic server for homology modeling	Schwede et al. (2003)
3D-JIGSAW	Automatic program for enhancing protein modeling	Bates et al. (2001)
Expasy	Contain a set of tools to predict secondary and tertiary structure of proteins	https://www.expasy.org/proteomics/protein_structure
MODELLER	Utilized comparative modeling techniques to predict protein 3D structure	Eswar et al. (2006)
Phyre and Phyre2	Both are automatic web-based protein structure prediction servers	Kelley et al. (2015)
JPRED	Method to predict protein secondary structure	Cuff et al. (1998)
HMMSTR	Used the HMM for finding protein correlations based on sequence structure	Bystroff et al. (2000)

Table 15.3 Phylogenetic analysis tools and software

Name	Description	References
TreeAlign	TreeAlign uses distance matrix and approximate parsimony which are efficient hybrid methods to predict tree	
MEGA	Desktop based software used to build phylogenetic tree with multi-parametric details for evolutionary studies	119
Dendroscope	It is used for graphical representation of tree with several optimistic options like re-rooted tree and formatting of tree branches, etc.	Huson and Scornavacca (2012)
Treefinder	Treefinder is very fast based on scripting language extensions to deal with tree visualization from DNA and proteins via using maximum likelihood, distances, and other hybrid methods	Jakobsen (2007)
TREE-PUZZLE	TREE-PUZZLE is a program package for statistical analysis of tree based on maximum-likelihood methods	Schmidt et al. (2002)
iTOL	iTOL is online tool for professional researcher to give many opportunities or options to do tree visualization	Letunic and Bork (2019)
JStree	Tools for attractive representation of phylogenetic tree with advanced setting for viewing and editing	Boc et al. (2012)
TreeView	Developed for viewing the phylogenetic tree with facilities of attractive changing	Page (1996)
MOLPHY	Tool for molecular phylogenetic tree based on maximum-likelihood method	Adachi and Hasegawa (1992)
Jalview	Online editor used to align the multiple sequences with refined methodology	Waterhouse et al. (2009)
PAML	Online web-based tool used for phylogenic tree prediction using maximum likelihood methodology	Yang (1997)
PHYLIP	Web-based package for molecular phylogenetic studies	Felsenstein (1993)

Table 15.4 Genome and nucleotide databases

Name	Description	References
Ensembl	Online database for many vertebrates and other model organisms like zebrafish, human, mouse, etc.	Flicek et al. (2012)
GenBank	Online public domain database freely available for nucleotide collections	Bilofsky et al. (1986)
NCBI	Mash linked online database for standard genome resources and provide online access for retrieval of protein, genes sequences	Pruitt et al. (2005)
EMBL	EMBL online database offers many fields of public study with the scope of molecular biology sported by intergovernmental organizations	Kanz et al. (2005)
UCSC Genome Browser	Web-based database, provide up to date large collection of genome sequences with annotated features	Karolchik et al. (2003)
DDBJ	Contains multiple online nucleotide sequences for public domains	Tateno et al. (2002)
OMIM	Online Mendelian inheritance in man provides inherited diseases information for all public domains	Hamosh et al. (2000)
1000 Genomes	1000 Genomes project publicly available and consists of individual 1000 genomes	
Zebrafish Information Network	Provide complete information of model organism like zebrafish and linked with other mesh related database	Sprague et al. (2006)
KEGG	Kyoto encyclopedia of genes and genomes	Kanehisa (2002)
HapMap	Online project database free available for public domains and provide information about genetic variations and other same linked factors	Thorisson et al. (2005)

Table 15.5 Protein sequence databases

Name	Description	References
SWISS-PROT	SWISS-PROT is a wide library includes manually annotated protein sequences	Boeckmann et al. (2003)
Uniprot	Public access online protein sequence database	Consortium (2015)
InterPro	InterPro used to predict the presence of domains and sites and also provide information on protein families based on classification	Hunter et al. (2009)
Pfam	Pfam is online protein families database	Bateman et al. (2004)
PROSITE	Protein families and domains database for public accesses	Hulo et al. (2006)
NCBI	Provide large domain protein sequence and knowledgebase information in open accesses	Jenuth (2000)
PDB	PDB is an online database for three-dimensional structural data of large biological molecules and provides the navel insight to researcher based on link with many other databases	Berman et al. (2000)
SCOP	Online database for structural classification of proteins	Lo Conte et al. (2000)
SUPERFAMILY	Database of superfamily annotations for all completely sequenced organisms	Wilson et al. (2007)

Table 15.6 Molecular interactions

Name	Description	References
SMART	SMART is an online tool used to explain the multiple interactions of protein based on given query from user	Letunic et al. (2012)
MOE	An integrated package used for drug discovery. It combines multi-protocols for visualization, modeling, and drug discovery on single platform	Maginn et al. (2013)
Graemlin	Used functional evolution model for generalization of existing alignment scores to introduce metabolic pathways based on conserved network topologies	Micale et al. (2012)
CFinder	Used to find the clustering groups of genetic networks and microarray based on quantitative descriptions for evolution	Adamcsek et al. (2006)
AutoDock	AutoDock is efficient tool for prediction of protein–ligand interactions	Cosconati et al. (2010)
MIMO	MIMO used to create biological networks with efficient way of dynamic graph	Di Lena et al. (2013)
IntAct	It is an open source database system and provides analysis tools for molecular interaction data. All interactions are derived from literature, books, or direct user submissions and are freely available for public domains	Kerrien et al. (2012)
STRING	STRING is an online database system for prediction of functional partner of proteins with their exclusive networks among different organisms supported by other databases	Szklarczyk et al. (2017)
HADDOCK	HADDOCK is an online system for modeling and biomolecule interactions	De Vries et al. (2010)
BIND	BIND is a biological database for molecular interaction and bio-complexes freely available for public domains	Bader et al. (2003)

Table 15.7 Signaling and metabolic pathway databases

Name	Description	References
MetaCyc	MetaCyc is a comprehensive reference database of metabolic pathways and enzymes from all domains of life	Caspi et al. (2018)
Reactome	Reactome is an open source database for public to perform analysis supported by pathways knowledge of system biology, genome and proteomic analysis, education and modeling	Fabregat et al. (2018)
PID	PID provides information about human molecular signaling and regulatory processes at cellular level For including cellular pathways and cancer research	Schaefer et al. (2009)
KEGG	KEGG is an open source embedded suit linked with multiple software for understanding the genomic information at functional behavior of cell	Wrzodek et al. (2011)
CMAP	CMAP used to discover or explore complement networks and their new connections for research community	Yang et al. (2013)
HMDB	HMDB contains a large collection of human metabolism in entire world. It also contains a large number of metabolites information coming from different books, research articles, journal and electronic databases in the field of natural science	Wishart et al. (2018)
PathBank	Pathways of model organisms	Wishart et al. (2020)
SGMP	SGMP is an online database containing signaling pathway to provide highly structured data on signal transductions of proteins	Dinasarapu et al. (2011)

Table 15.8 Drug designing and targeting databases

Name	Description	References
ChEMBL	Database consists of collection with many bioactive drug like molecules with many computational functions	Gaulton et al. (2012)
Therapeutic Target Database (TTD)	Consists of known therapeutic molecules and link with other targeted databases	Chen et al. (2002)
TDR Target Database	Used to identify the gene of interest via ignoring disease targeted sites	Magariños et al. (2012)
DrugBank	This database is contained with many drug entries and protein sequences associated with these entries	Law et al. (2014)
TB Drug Target Database	Special database for targeted protein and drugs for treatment of tuberculosis	Sandgren et al. (2009)
DrugPort	This database is linked with PDB to provide structural information of drugs including drug Bank database	Sharvit et al. (2012)
MATADOR	Provide details of protein chemical interactions, list of binding sites via linking with OMIM and PubMed	Günther et al. (2007)
Potential Drug Target Database (PDTD)	Large database and widely assessable for drug targeting consisting of more than 1250 entries	Gao et al. (2008)

Table 15.9 Molecular dynamics simulation tools

Name	Description	References
Ascalaph	Molecular modeling tool to accomplish quantum mechanics calculations. It has a built-in ability to intermingle with external molecular modeling packages	https://omictools.com/ascalaph-tool
AutoDock3.0	Used to predict the small molecules, substrate, or other receptors of known 3D structures	https://www.scripps.edu/pub/olson-web/dock/autodock
Discovery Studio	Used for modeling, simulation to optimize the drug discovery process	Studio (2008)
Amber	Amber is the collection of programs that facilitate users to perform molecular dynamics simulations with an emphasis on biomolecules	Case et al. (2006)
GROMOS	General tools for studying biomolecular systems based on computer molecular dynamics and simulations	https://www.igc.ethz.ch/GROMOS/index
Abalone	Modeling program for simulations and also ability to interact with external quantum programs	http://www.biomolecula-modeling.com/Abalone/
FoldX	Provide molecular interaction based on quantitative approximation for stability of different proteins	http://foldxsuite.crg.eu/

phylogenetic relationships. In Reproductive biology, bioinformatics engages in a computer-based analysis that provides scientific ways of comprehending, coupling, reflecting, and creating pharmaceuticals used to speed up studies in reproductive medicine using traditional approaches. Furthermore, bio-industries are incorporated into reproductive science to affect processing techniques significantly. They include various methods for wildlife control, including agricultural productivity, anthropology, macro-and micro-climate changes, fish production, pest management, and wildlife development. In the logical advances of reproductive politics, scientists are known to the world, collaborating alongside other sciences such as bioinformatics, biotechnology, genomics, proteomics, and transcriptomics to provide relevant scientific evidence on the fertility of vast databases of organisms, humans, and other future life on earth.

Bioinformatics is an interdisciplinary field of life science and has huge potential to study the inherited diseases and disorders which affect animal health and decreased productivity. Thus, animal breeders rely on the use of various databases such as genetic control databases (GDC) for early identification of possible carriers of inherited diseases and to investigate and distinguish the genotypes into affected, carriers, and normal which would prove to be beneficial for their experimental studies. So for further understanding of big data scientist should focus on high-throughput methodology to develop tools, applications, and rational databases for OMICS analysis in the field of reproductive science. Exploration of genomics, metagenomics, proteomics, and transcriptomics is very important with knowledge of cross-linking fields for a better understanding of how researchers can achieve data retrieval using sophisticated operating systems, software, algorithms, and database, how it is done first with minimal time, space, and complexity to manage, analyze, and interpret complex biological data.

In current situation of reproductive science bioinformatics is globally separated for community to manage, analyze, and interpret complex genetic data. Therefore, bioinformatics training techniques, learning, workshops, conferences, educational material, and high-performance computing systems should be available for developing countries to accomplishing this goal. Ideas for whole genome sequencing based on third generation technologies are very important for reproductive science to understand the basic to complex functions of genomic data. There is a demand to make sequenced genome data more functional and integrated by designing more organized, user-friendly, cell-width biological networks, and metabolic pathways with improved imagining properties, graphical outputs, and knowledge base assemblies.

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16 Modulation of Hypothalamic-Pituitary-Gonadal (HPG) Axis by Phytotherapy Using Different Delivery Approaches

Ishfaq Nazir Mir and Irfan Ahmad Bhat

Abstract

Reproduction is arguably the most vital process in animals including fish that is being controlled by the brain through hypothalamic-pituitary-gonadal (HPG) axis. Several bioactive compounds from plants were recognized to be having a great role in promoting a variety of biological activities such as growth, immunomodulation, feed consumption and antioxidant activity or can act as antiviral agents. Moreover, phytochemicals have been used for several hundred years as additions to energize, vitalize and eventually curb the male and female reproductive functions. There can be different approaches to administer the plant-based bioactive compounds such as oral, immersion or injection methods. However, the nanodelivery approach forms an effective way to achieve the delivery to target tissues with less or no impediment. The nanodelivery approach improves the efficiency of targeted delivery of plant-based bioactive compounds. The phytochemicals can also be involved in phenotypic sex manipulations that sometimes become necessary in fisheries and aquaculture. The aim of this chapter is to provide an overview of available studies on the reproductive control via HPG axis along with different phytochemicals and their delivery approaches for reproductive modulation.

Keywords

HPG axis · Phytochemical · Nanodelivery · Reproduction

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Introduction

A fishery in India is a very important economic activity and a flourishing sector with varied resources and potentials. Fisheries contribute significantly towards the Indian agricultural economy, with a share of about 5.3% in agricultural GDP and about 1.1% in the overall GDP. Human population is increasing continuously and is about to reach 10 billion by 2050, which led to an increase in demand for the food. Fish is an important source of quality and beneficial nutrients. Fish consumption has undergone major changes in the past four decades. It has been projected that the country's fish requirement would be high in the upcoming years. The capture fisheries is no longer considered to be capable of sustaining the supply of fisheries products to fulfil the demand of the growing world population as the production has levelled off and a plateau has been attained. Aquaculture, on the other hand, is blooming with a tremendous potential to achieve the requirement of the fish eating population. India stands second in total aquaculture production after China, and around 95% of the production is contributed by freshwater aquaculture.

Aquaculture has been practised in extensive forms since time immemorial, but the intensive culture methods are new and are in use since last few decades. In intensive farming practice, the domestication efforts are required to raise the aquatic species. Sustainable aquaculture industry has a capacity to cope with reproduction processes of fish in control. To obtain quality seeds in future, the aquaculture production and development chiefly depend on the control of reproduction of aquatic species. In captivity, management of the technologies to increase gamete production is one of the primary steps that would ensure the growth and development of aquaculture sector (Bromage et al. 1992). Moreover, the reproductive potential could be modulated to enhance the fish production by reducing the excess energy used for breeding purposes like gonadal development in some prolific breeders like tilapia. There are several plants containing bioactive compounds with a potential to modulate the reproduction for improved performance and ultimate enhanced production. The plant extracts along with their bioactive compounds have been tested in many fish species for enhanced reproductive performance and gonadal development. The compounds have been shown to affect the brain or gonads directly via a well-connected axis called hypothalamic-pituitary-gonadal (HPG) axis. Well-presented information about the plant extracts on reproduction is not available yet and needs to be bought on a single document for future references. So, the aim of this chapter is to emphasize the HPG axis and reproduction in fish, effects of plant extracts on the reproductive processes and the different delivery approaches of phytochemicals to modulate the HPG axis.

HPG Axis in Reproduction of Fish

HPG axis is considered to be the main regulator of reproduction in all vertebrates, including fishes. The HPG axis of fish resembles with the higher animals, and all major components and functions found in the mammalian HPG axis are conserved in

the fish HPG axis (Golan et al. 2014; Rather et al. 2020). The reproductive pathway starts from the hypothalamus in which important peptide hormones like gonadotropin-releasing hormone (GnRH) and gonadotropin-inhibiting hormone (GnIH) are secreted. These hormones mainly control the secretion of gonadotropins from the anterior pituitary (Silverthorn 2010). In fish, another compound, dopamine, also plays a crucial role in inhibiting gonadotropin secretion. The main function of GnRH is to regulate the endocrine output of two important gonadotropins (FSH and LH) by the anterior pituitary via a G protein-coupled receptor. Other peptides like GnIH and dopamine also influence the production and secretion of gonadotropins in fish. The two peptides act directly and inhibit the production of GnRH and gonadotropin release in vertebrates (Lin et al. 1989). Kisspeptin is a neuropeptide with profound effect on HPG axis and is produced by the neurons located in the hypothalamus which stimulate the secretion of GnRH (Rather et al. 2017).

The hormones from hypothalamus trigger the pituitary to produce FSH and LH. The two hormones mainly control the secretion of steroid sex hormones produced by the fish gonads. The sex steroids in fish cannot be produced if there is any impairment in the production of gonadotropins. The receptors of the FSH and LH are mainly found in the gonads of fish, and upon interaction with ligand, the steroidogenesis pathway is initiated.

Steroidogenesis is the biological process by which the cholesterol molecule is converted into other steroids by the action of different enzymes (Hanukoglu 1992). The particular steroid production inside the cell depends on the regulation of expression of steroid genes such as cyp11a, cyp17, cyp19, 3βhsd2 and 17βhsd4 in that cell (Ma et al. 2011). The macromolecule cholesterol is transported into the mitochondria under the influence of steroidogenic acute regulatory protein (StAr). Then several steroid enzymes act and convert the molecule into the final androgen or oestrogen. The P_{450}-associated enzymes (such as hydroxylases and lyases) convert cholesterol into pregnenolone which is then transported from the mitochondria into the endoplasmic reticulum, where conversion to progesterone and androgens takes place (Bowen 2001). The androgenic hormones are converted into oestrogen with the help of aromatase by hydroxylation of the 19-methyl group of androgens, followed by elimination of this group and aromatization of the A-ring (C1, 2, 3, 4, 5, 10). The enzymes required in steroidogenic pathway are presented in Fig. 16.1.

The androgens and oestrogens are the male hormones responsible for the development of primary and secondary sexual characters of fish and the initiation of several behavioural changes related to spawning activities (Jobling 1995). The common androgen found in fish is testosterone, but one more important androgen present is 11-oxygenated androgens, especially 11-ketotestosterone (11-KT) (Borg 1994). The 11-KT is found in higher levels in the plasma of males than in females, whereas this is not the case for testosterone. The 11-oxygenated androgens are generally considered more efficient than testosterone in promoting the development of secondary sexual characters of males, reproductive behaviour and sperm production. During the breeding season, the plasma/serum levels of several hormones show significant changes in male teleosts like 11-KT, testosterone and 17α,

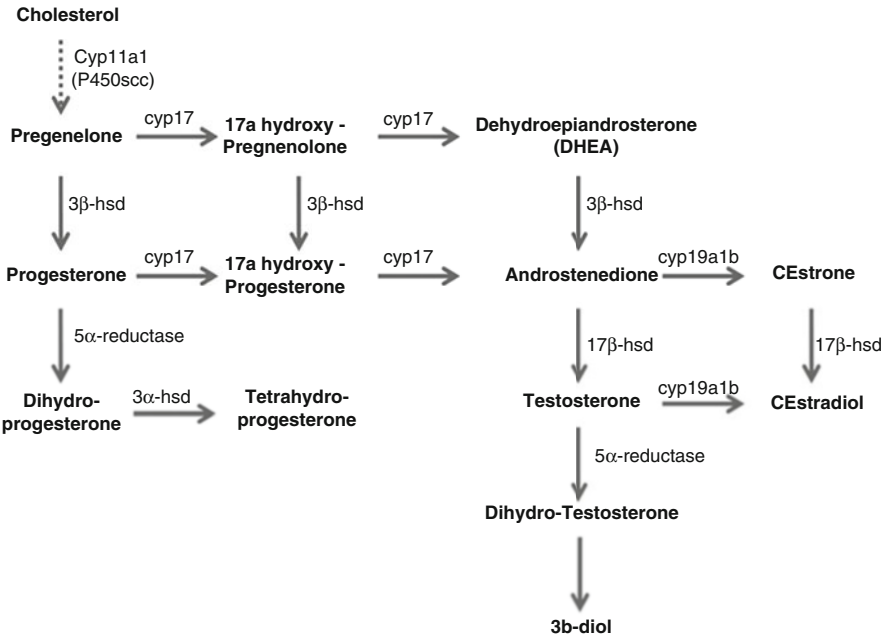

Fig. 16.1 Steroidogenesis pathway in fish

20β-dihydroxy-4-pregnen-3-one (17,20β-P) which subsequently seems to affect milt quality (Schultz and Miura 2002).

Phytochemicals and Reproductive Potential

The phytochemicals are the naturally occurring bioactive molecules or compounds derived from plants that could be bioactive or could be simply as secondary metabolites. The phytochemicals can be extracted from a variety of plants and principally can be categorized into flavonoids, alkaloids, phenolics, pigments, steroids, terpenoids and essential oils. Phytochemicals have been recognized to be having a great role in promoting a variety of biological activities such as immunomodulation, growth, feed consumption and antioxidant activity or can act as antiviral agents (Citarasu 2010; Chakraborty and Hancz 2011; Ponce et al. 2003; Lampe 2003; Citarasu et al. 2006). In addition to this, phytochemicals have been used for several hundred years as supplements to energize, vitalize and eventually to improve male and female reproductive functions. Till now, extracts from several plants have been used to improve the male and female fertility, spermatogenesis, reproductive output and other reproduction-related problems in animals. The common plants having such properties include *Eurycoma longifolia* Jack (Ang and Lee 2002), *Lepidium meyenii* (Cicero et al. 2001), *Terminalia catappa* (Ratnasooriya and

Dharmasiri 2000), *Tribulus terrestris* (Gauthaman et al. 2000) and *Fadogia agrestis* (Yakubu et al. 2005).

E. longifolia jack is a perennial herb found in South-east Asian countries and has been used to enhance the reproductive properties for many years. The compound eurycomanone is having such reproduction-related properties like it increases the testicular growth by increasing testosterone production, increases the spermatogenesis, reduces sperm motility and increases the sperm quality and sperm concentration (Noor et al. 2004; Low et al. 2013). In general, the compound has been found effective to enhance the sexual performance in animals (Zanoli et al. 2009). *Lepidium meyenii* (Maca) extracts have been reported to be potent modulators to enhance the fertility properties and sexual performance effectively (Cicero et al. 2002). Furthermore, extracts of Maca have been found effective in treating the sexual dysfunction primarily by protecting spermatogenesis and increasing the daily sperm count (Rubio et al. 2006). *Tribulus terrestris* popularly known as puncture vine with a worldwide distribution has been regarded as an aphrodisiac and claimed to improve the sexual functions by enhancing the levels of reproductive hormones (Koumanov et al. 1982; Adimoelja and Adaikan 1997; Gauthaman et al. 2000). *Withania somnifera* is also known as ashwagandha or Indian ginseng and is commonly used in Ayurvedic medicine for improving the sexual functions and decreases the infertility effects in animals like rats (Garg and Parasar 1965). The phytochemicals from the seeds of *Mucuna pruriens* were observed to enhance the spermatogenesis and weight of the testes in the albino rat (Saksena and Dixit 1987). In addition to this, there are several phytoextracts which have a tremendous potential of improving the sexual behaviour, functions and overall reproductive performance of animals, including humans, and very few reports are on fishes. Hence, there is a lot of scope to explore these phytoextracts in fishes through different administrative approaches.

Mechanism of Plant Extracts in Increasing the Reproductive Output

The mechanism of the plant extracts in enhancing the reproductive parameters may vary. Some directly affect the pituitary, some interact on gonads and some may have indirect effects. The detailed mechanism of one of the plant extract in enhancing the reproductive parameters and testicular development in fish has been studied in detail. The eurycomanone treatment increased the testosterone and enhanced the testicular development and sperm output in *Clarias magur* (Bhat et al. 2019). The plant extract was conjugated with chitosan nanoparticles in order to enhance its penetration and effect for a longer duration. Based on the results, it was found that eurycomanone acts on both FSH and LH of Sertoli cells and Lydig cells to enhance spermatogenesis and testosterone. Further, the increase in the expression of testicular genes like SOx9a and Dmrt was elevated due to increase in the production of testosterone that is transported to the testicular development genes through androgen receptors. The main effect of eurycomanone was found on aromatase gene (CYp19a). It

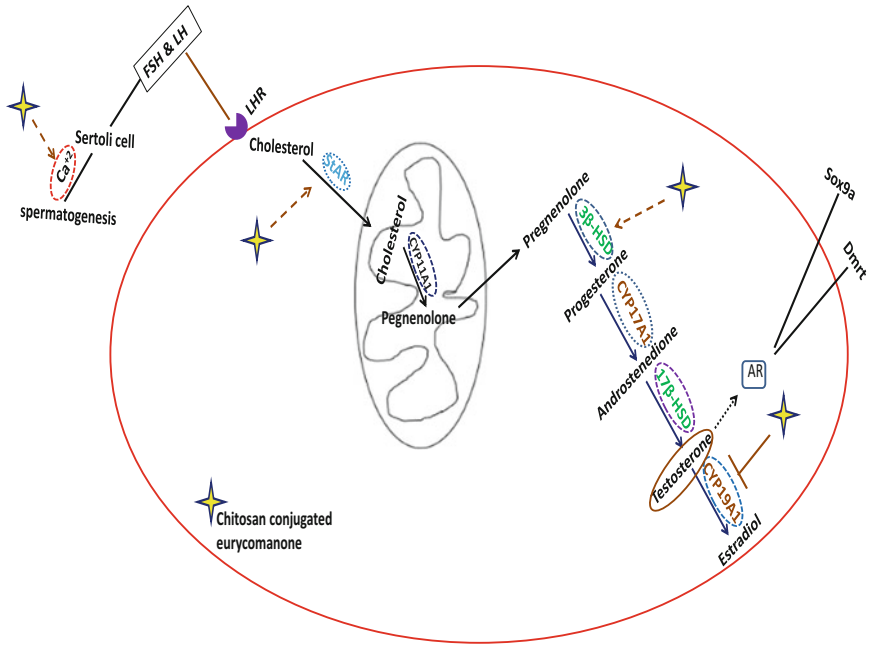

Fig. 16.2 Mechanism of the phytochemical eurycomanone to affect the steroidogenesis pathway and enhance the testosterone production of fish, which has been extracted from the study of Bhat et al. (2019) after the consent from main authors

blocked the expression of this transcript which is the main factor responsible for conversion of testosterone to oestradiol. The mechanism was diagrammatically shown in Fig. 16.2, which was extracted from the study of Bhat et al. (2019).

Administration of Phytochemicals

The way a phytochemical is administered has a great impact on the achievement of its optimum results. The phytochemicals like other drugs can be administered via different methods such as oral, immersion, intraperitoneal, intramuscular, etc.

Oral Administration Method

The oral administration method generally is considered the most convenient and safest effective method for the delivery of phytochemicals due to the reasons such as cost-effectiveness and less prone to problems like injection-site reactions. There are reports in which the dietary inclusion of phytoextracts results in the improved reproductive performances. Yeganeh et al. (2017) evaluated the effect of dietary *Tribulus terrestris* plant extract where the optimal levels enhanced the reproductive

performance in terms of belly diameter, fertilization rate and hatching rates. Prior to this study, the potential of *T. terrestris* extract was validated as a safe therapeutic alternative to synthetic modalities for the management of sexual problems in males (Elahi et al. 2013). On the other hand, Michael Babu (1999) examined the effect of herbal preparation in *Penaeus monodon* on reproductive functions where he observed an improvement in the spermatogenesis, gonadal maturation and high egg viability in male and female strains, respectively. This helps to achieve natural viable spawners especially out of season and improves egg quality and fecundity. Dhas et al. (2015) reported an increased fertilization and hatching rates of pearlspot broodstock when fed with herbal maturation diet prepared from *Mucuna pruriens*/*Withania somnifera*/*Moringa oleifera* (1:2:1). Besides the phytoextracts, even some leaf meals (mulberry) were also found to be promoting the hatchability and sperm count of African catfish (Olaniyi et al. 2016) when supplemented in the diets.

Immersion Method

This method is mostly followed in the disease management of fishes, where the stocks are given bath treatment for treatment. However, it is not often practised for enhancement of the fertility or overall reproductive performances of fish. There are few reports where the phytoextracts of some plants regulate the reproductive functions of fish. Sex reversal due to immersion treatment of *T. terrestris* extract (containing steroidal saponins as principle phytochemicals) was reported in newly hatched offspring of *Cichlasoma nigrofasciatum* (Cek et al. 2007). Similarly, an increase in the percentage of males was observed in a dose-dependent manner in African catfish after 1 month of immersion treatment (thrice weekly) with *T. terrestris* extracts (Turan and Cek 2007). Furthermore, it was reported that an immersion treatment of African catfish larvae with a commercial mixture of phytoestrogens yielded around 70% female population at the level of 1500 mg per 30 L.

Injection Method

This method is most commonly used during breeding time for the injection of gonadotropin-releasing hormones for spawning of fishes. However, fish should be prior anaesthetized to avoid any possibility of injury. The injection given to the fish can be intraperitoneal or intramuscular. Intraperitoneal is made into the mid-ventral side just cranial to the vent, whereas intramuscular injection is given into the epaxial musculature, usually middle side between dorsal line and the lateral line. There are reports where the injection of phytoextracts has resulted in the reproductive enhancement of animals. Ang and Sim (1997) evaluated the effects of different doses of *E. longifolia* root extracts containing eurycomanone on the sexual performance of male rats having a good sexual experience. From the obtained results, it was concluded that *E. longifolia* is a potent stimulator of sexual arousal in sexually

vigorous male rats with the absence of feedback from genital sensation. Ang and Lee (2002) could provide evidence pertaining to changes in sexual behaviour (orientation activities) among the middle-aged male rats after administering different fractions of the *E. longifolia* root extracts. They reported that male rats treated with a given dose of extract increased the orientation activities towards the receptive females (licking, anogenital sniffing and mounting) accompanied by the improved genital grooming towards themselves. Irshaid and Mansi (2009) revealed that the methanolic extracts of *Urtica pilulifera* after injection to diabetic rats prevented the development of diabetes along with the enhancement of reproductive performance. In our study on catfish, the injection of eurycomanone increased the reproductive parameters with the significant increase in reproductive output (Bhat et al. 2019)

Nanotechnology

The concept of nanotechnology and the possibility of manipulation of matter at the atomic level were first enlightened in a famous lecture of physicist Richard Feynman in 1959, "There's Plenty of Room at the Bottom". Feynman without realizing at that time planted the seeds of a new era in technology that has benefited almost all industries and areas of society. Nanotechnology nowadays is a fast-growing, interdisciplinary field of science, combining engineering with biology, chemistry, physics and medicine and expunges the traditional boundaries between them (Ray et al. 2009). Nanotechnology gives the ability to perceive and control individual atoms and molecules and pacts with structures in size range from approximately 1 to 100 nanometres, known as the nanoscale (Williams 2008). Scientists are making everyday material act in unimaginable ways by taking advantage of unique phenomena that naturally occur within this size range. The properties of particles at nanoscale such as colour, melting point, magnetic permeability, electrical conductivity and chemical reactivity change (Boverhof and David 2010). Various applications of nanotechnology were developed for aquaculture production (Rather et al. 2011). The fisheries and aquaculture has a strong history of adopting new methods and technologies (Aklakur et al. 2016). Hence, the fish farming industry may be among the best to incorporate and commercialize nanotech products.

Nanodelivery of Phytochemicals

Drugs used in aquaculture are mostly administered through three major delivery routes as bath or immersion, through feed or oral and injection method, as mentioned in the previous section. Immersion or bath route is more appropriate, but it requires the phytochemical or drug in more quantity, and the handling to fish causes unavoidable stress. The injection method leads to unavoidable stress and hence seems to be impractical for fishes. On the other hand, in-feed or oral method, where the drugs or phytochemicals are administered with normal feeding without stress and the extra cost, forms an easy method. Drug delivery is an area where

nanotechnology has already a substantial impact (Lavan et al. 2003). Certainly for this, aqua-medicines or phytochemicals have the major portion, and their delivery methods need several concerns like budget of the phytochemicals used in culture, less waste as feed cost is the major expenditure in aquaculture, efficacy and cost analysis of such medicines being used in aquaculture, environmental impact of these phytoextracts and additives used in aquaculture, monitoring the residues level and its implications in food chain and lastly toxicity of phytochemicals to fishes at higher dose.

Phytochemicals as mentioned previously have a plethora of biological properties, but the major constraints of their utilization are the low solubility, bioavailability, stability and target specificity in the body. Hence, the effective delivery to target tissues is more or less hampered by direct administration. The nanodelivery approach could be an excellent technology to accomplish the effective delivery to target tissues. Besides, nanodelivery has solved several of these concerns in aquaculture drugs or medicines like their safety, residue level in the flesh, aquatic environment and sustained release that ultimately will reduce the dose in an efficiency manner.

Chitosan has been widely used as a carrier for nano-conjugation in the field biomedical research and has been found to be extremely biocompatible. Furthermore, chitosan also possesses some other favourable bioactive properties like low toxicity, biodegradability, bacteriostatic, haemostatic, fungistatic, anti-carcinogenic properties, etc. (Hejazi and Amiji 2003). Nanoparticles of chitosan have been extensively examined for its potential in the development of the controlled release delivery system of peptides, protein antigens, genes, oligonucleotides, etc. (Ramya et al. 2014). Chitosan nanoparticles conjugated with vitamin C were delivered to rainbow trout (*Oncorhynchus mykiss*), and vitamin was released up to 48 h after oral administration. Prompt effect on the fish innate immune system was observed due to powerful synergism between chitosan and vitamin C (Alishahi et al. 2011a, b). Rather et al. (2013) used chitosan nanoparticles for hormone delivery in *Cyprinus carpio*. Luteinizing hormone-releasing hormone (LHRH) was conjugated to chitosan nanoparticles and was compared with naked LHRH group. Chitosan-LHRH-conjugated nanoparticle groups showed an increase in blood hormone levels with the sustained release of hormones, compared to the group injected with naked LHRH. Furthermore, Chitosan-LHRH-conjugated nanoparticles were administered in catfish, and similar results were obtained (Bhat et al. 2016). The chitosan-conjugated eurycomanone enhanced the drug effect for longer durations, the dosage of the compound was reduced, and the effect was significant compared to the naked drug (Bhat et al. 2019).

Phytoextracts and Sex Plasticity

Phenotypic sex manipulation becomes necessary sometimes in fisheries when one gender exhibits higher growth rate or have bright colouration, particularly in ornamental fishes (Uguz et al. 2003; Cogliati et al. 2010). Such type of modulation is

carried by synthetic steroids; however, due to their potential hazards, several alternatives were explored and among which the use of phytochemicals is promising one. Endocrine modulation with phytoextracts in different ways through sex reversal or fertility enhancers has been reported in several studies. Monosex population production in fishes like tilapia is beneficial to avoid the energetic losses involved in the reproduction of this prolific breeder. The prolific breeding behaviour and the precocious maturity ultimately affect the production and economic return of the culture. As mentioned previously, those several phytochemicals have the potential to modulate the sex ratio of fishes. Quillaja saponin is one of the important plant-based bioactive compounds that change the sex ratio of tilapia in favour of males (Francis et al. 2002). Saponin extracts of *Tribulus terrestris* also resulted in the significant male population when supplemented in the diets of Nile tilapia (Omitoyin et al. 2013). This change in sex ratio might be through the interference at hypophyseal level follicle-stimulating hormone (FSH) or gonadal level of androgens. It was also mentioned previously that the masculinization effect of saponin phytoextract could be explained due to its elevated production of testosterone (Ganzera et al. 2001). Similarly, there are plethora of reports which revealed the importance of phytoextracts in sex plasticity of fish, viz. dietary moringa and pawpaw crude extracts (Ampofo-Yeboah 2013), *Butea superb* (Kiriyakit 2014), *Mucuna pruriens* (Mukherjee et al. 2015) and dietary *Aloe vera* (Gabriel et al. 2017). Hence, phytoextracts have the potential to be used as alternatives for synthetic hormones and drugs for sex reversal phenomenon. Moreover, the phytoextracts are believed to have easy application long with their safety from the environment point of view.

Conclusion

It can be concluded that the phytoextracts/phytochemicals have a potential to modulate the HPG axis and control the reproduction. The phytochemicals could be fertility enhancers as well as the sex reversal agents with a great impact on the production of fishes. Moreover, the delivery approach is also essential to optimize the efficiency of phytochemicals, and in that case, nanodelivery method could be a feasible way for efficient administration. Although the phytoextracts have a plethora of beneficial effects in the aquaculture, the toxicity cannot be ignored. Toxicological studies should be conducted before utilizing a particular phytochemical as an alternative to synthetic drugs or hormones.

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Functional Role of Dietary Supplements on Reproductive Physiology of Fishes

17

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Abstract

Optimal nutrition and feeding influences the growth, reproduction, and health of fish. Feeding strategy, diet type, and additional supplements largely influence gonad maturation and spawning in fishes. Manipulations of feed type and source can invariably modify reproductive threshold of potential broodstock, and therefore precise knowledge on these focal areas is indispensible. Diet restriction during early phases of life cycle can delay age at first maturation, and a quantitatively restricted food supply at the time of oocyte differentiation reduces the number of egg, while reduction of food supply during later phases of oogenesis has minimal effect on egg size, composition, and hatchability. Also, at spawning time, mature female fishes can effectively utilize carbohydrate than immature fish as they valorize the energy from fats and, thus, have a low quantitative protein requirement. Addition of liposoluble vitamins and essential fatty acids in diets is necessary for normal reproduction, but fatty acids of the n-6 groups can play a more pronounced role in juveniles. Lipid and fatty acid profile of broodstock diet are regarded as major dietary factors detrimental for successful reproduction and survival of the offsprings. Also, it is observed that some species have capacity to incorporate dietary unsaturated fatty acids into eggs, even during the course of the spawning season. Alike the higher vertebrates, vitamin E deficiency affects reproductive performance, causing immature gonads and lower hatching rate and survival of offspring. Furthermore, the role of dietary supplements on quality of broods, offsprings, and even maturation time has been well documented. The

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available literature on various research outputs with regard to broodstock nutrition has been synthesized here. Thus, considering prominent role of the dietary factor in fishes, this chapter emphasizes the various nutritional strategies and role of various maturation and reproduction enhancing diets covered in finfishes.

Keywords

Broodstock · Energy · Egg quality · Protein · Fatty acids · Vitamin E

Introduction

Nutrition is one of the key aspects which decide the reproductive outcome in vertebrates, including fishes. Enhanced reproductive performance demands adequate nutrient inputs in order to supply and maintain the intensive energy demands for the developing gametes. A precise understanding about the sequential interactions between nutrition and reproductive process is imperative in formulating and developing designer feed to meet the increasing energy demands and hence redirecting the positive physiological aspects of fish reproduction. A good number of researches have been reported which examined the effects of different plant- and animal-based functional foods on early life stages and the broodstock reproductive performance in aquatic animals. In general, several studies have shown that decreased food availability/starvation causes gonad regression, with subsequent cease in spawning function as well as volume of eggs released, whereas abundant availability of food promotes growth and larger body sizes, resulting in timely maturation and increased fecundity in some species. It is well-known that the nutrient needs of fish groups differ in different life stages. Also, the complex morphological and physiological adaptations universally alter feeding and nutritional requirements of the species. In this midst, the function of few key nutrient components can improve the breeding performance of fish. For instance, inclusion of dietary lipids in broodstock feeds can improve the gamete quality, and so are the dietary protein sources and amino acids. The biological functionality of amino acids is manifold, as they take part in nutrient utilization, feed intake, and overall reproductive performance (Li et al. 2009; Wu 2009).

Life Stages in Fish Reproduction and Energy Demands

The reproductive cycle in fish includes a sequence of successive changes leading to progeny formation through fertilization of viable eggs by the spermatozoa as depicted in Fig. 17.1. Hence, the viability of the process is very much dependent on the successful advancement in each exercise involved in reproductive cycle. Here, broodstock nutrition is critical in many of these processes which decide the reproductive success. When immature juvenile fishes which can be said to be sexually quiescent reach a particular size, they will undergo puberty stage and finally

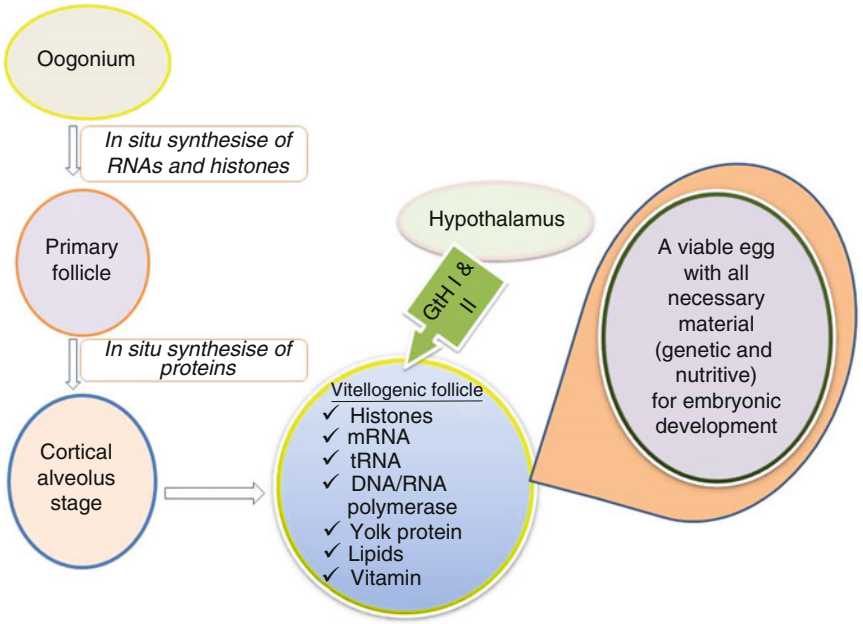

Fig. 17.1 A pictorial representation of reproductive process in fishes

on maturity start to release gametes and externally show sexual behavior. In general, the reproductive cycle comprises two distinct phases, i.e., gonadal growth and development collectively included as gametogenesis and the maturation phase, with a subsequent release of gamete and spawning commencement which follows gonadal recrudescence or the resting phase. In most cases, the mode of fertilization is external in water in majority of fish species documented.

Oviparous fishes release eggs into the environment to be fertilized externally, whereas in the case of viviparous fish species, fertilization is internal, and females produce living young ones, in which developing eggs/embryos are retained within the maternal reproductive tract. Once mature, most fish exhibit marked seasonality in their reproductive cycle, which is synchronized by annual variations in environmental factors like photoperiod, water temperature, and food availability. Spawning/mating usually occurs at specific periods of the year that coincide with optimal environmental conditions (high food availability) for growth of the offspring. Reproductive cycles are further influenced by nutritional status, besides release of gametes being controlled by the hormones. In the course of the maturing phase of the adult reproductive cycle, gonads develop until they reach a maximum size during the spawning season and produce gametes. During egg formation, females invest considerable energy to supply the egg with nutrient stores (yolk/vitellogenin). A variety of techniques is to evaluate reproductive situation in fishes, together with examination of the gonads, dimension of sex steroid level, and gonadal indices. The *gonadosomatic index*, abbreviated as GSI, is the calculation of the gonad mass as

a proportion of the total body mass. It is a tool for measuring the sexual maturity of animals in correlation to ovary development and testes development. It increases with the maturation of the fish, being maximum during the peak period of maturity and declining abruptly after spawning. The *number of eggs produced* by a *fish* differs in different species and depends on the size and age of the *fish*. It may *also* differ in different races of the same species. Thus, *fecundity* is a *measure of the reproductive capacity* of a *female fish* and is an adaptation to various conditions of the environment.

Endocrine Control of Reproduction

The endocrine system regulates the internal responses of the fish according to the cues received from changes in the external environmental factors. In fishes as well as many other vertebrates, reproductive process is coordinated and controlled by the hypothalamus-pituitary-gonadal (HPG) axis (Rather et al. 2017, 2020). First the hypothalamus produces gonadotropin-releasing hormone (GnRH), which regulates the synthesis and release of pituitary gonadotropins like luteinizing hormone (LH) and follicle-stimulating hormone (FSH) (Rather et al. 2016). These gonadotropins further will react on the gonads which stimulate gonadal development by secretion of sex steroid hormones. These steroids then relay the message back to the brain and the pituitary in a feedback mechanism.

It is seen that fishes often try to regulate their reproductive physiology as per the available energy reserves. For instance, food deprivation, leading to energy exhaustion, inhibits the HPG axis as a compensatory mechanism for energy saving to perform vital functions. A number of peptides are known to regulate both feeding and reproduction of both mammals and fish. Some significant peptides include brain factors (neuropeptide Y (NPY) and orexin), as part of few peripheral factors like leptin and ghrelin.

Effect of Nutrition on Fish Reproduction

In wild, fishes are often challenged with limited supply or deprivation in food during their life cycle, particularly during the seasonal cycles and spawning migrations. The sensory and endocrine systems sense these extrinsic and intrinsic circumstances like size, age, and storage of macromolecules (sugars, amino acids, and lipids) and react on prevailing conditions for reproduction to proceed further. Reproduction process can be completed only if conditions are optimal; however, it may also be delayed/terminated when nonexistence of the optimal conditions is figured out. In situations like malnutrition, female fishes tend to exhibit serious reproductive failure like inhibition of vitellogenesis, oocyte maturation, and spawning as compared to male counterpart which is particularly reflected in decreased sperm volume and diminished milt fluidity, which negatively affect the success of egg fertilization. However, the mechanisms involved in such complex process still remain unexplained.

Effects of Food Restriction

Most of the research outcomes show high variability with regard to effects of fasting on reproductive consequences in fishes. These accounts mostly due to the dissimilarities in age as well as size at puberty in fishes reported and, further, between different sexes of the same species. It is reported that growth in male groups typically slows when they are small in size and age, as the compared to females. In fishes, puberty is attained only after attaining an optimal age/size. Therefore, this is assured only in conditions where the individuals have stored sufficient energy reserves to overcome the nutritional and reproductive energy required for maturation. It is assumed that the start of puberty in fish is partially connected to the absolute levels/rates of lipid reserves stored. Besides, increased plasma levels of sex steroid too contribute to puberty initiation. The onset of puberty has positive effects on appetite and somatic growth, with relatively larger ones advancing much earlier than smaller ones in a population. However, in its advancement, further energy reallocation from body buildup process to other reproductive expenses like migration and/or sexual behavior continues. Also, it is documented that appetite is often low, just prior to onset and during extended part of the spawning process. Studies have shown that, in few species, feeding restriction lowers energy reserves and adiposity and can lower the population that accomplishes full maturation. In many species, reduction in rate of feeding during maturation decreases growth, GSI, gonadal maturation, spawning frequency, and duration during the reproductive peak periods. There are also cases of sex-specific reaction to the food restriction, with females found to be comparatively responsive. Food-restricted female fishes exhibit decreases in final oocyte maturation and egg quality and produce smaller eggs/hatched larvae, as related to those fed standard food quantity. Additionally, in some fishes, fasting decreases brain GnRH and plasma estradiol levels in female fishes. Therefore, it can be established that fasting negatively inhibits the HPG axis and there occurs a close relationship between food intake and reproduction in fishes.

Effect of Food Quality on Fish Reproduction

Obviously, nutritional requirement are dependent on the species and on their feeding habits, for example, carnivorous fish requiring higher protein level as compared to herbivorous/omnivorous species, and also the requirement variability among marine and freshwater fish species. Regardless of these, nutrition is quite linked to body growth and also interferes directly/indirectly in fish reproductive processes like gonadal development, reproductive performance, spawning rate, fecundity, and egg and sperm quantity and quality, vis-à-vis the quality of offspring. Among the most important nutritional factors, lipids (EFAs), proteins, and vitamins (A, E, and C) are inseparable when reproduction is concerned. Nutrient components like proteins, carbohydrates, and lipids are key nutrient required by the body to metabolize and generate energy needed for various physiological functions. There are considerable variations in the competence of the fish species to utilize these

energy-yielding constituents. This variation is much linked with the feeding habits in the natural environment. Thus, there is a direct connection between feeding habits in wild and the protein needed from diet. It is known that herbivorous and omnivorous fish need low dietary protein than some carnivorous fishes. On the other side, carnivorous species can efficiently utilize dietary protein and lipid for energy but less efficient towards carbohydrate uptake.

Lipids

Lipids and its related fatty acids components play an important part towards accomplishing various metabolic and reproductive functions of fish. Fish growth performance and metabolic efficiency will vary depending on the supplied carbohydrate and lipid levels in diets. Dietary lipids, which include triglycerides, provide energy, and EFA which are essential in maintaining structural and cellular function. However, fish cannot synthesize some of these EFAs, and therefore it is required to be delivered through diet. In fishes, lipids are mostly stored in the muscle tissue and liver, which are further used during period of gametogenesis, relayed to the ovaries and absorbed as nutritive material in the egg/yolk (utilized as the sole food for developing embryo in later stages). It is seen that low levels of lipids and fatty acids in diets have negative effects on reproduction and larval survival in several fish species such as carp, European sea bass, and flounder. Additionally, excess dietary lipid has been reported to cause inadequate protein intake and suppress growth.

Proteins

Dietary protein is relatively expensive, and nutritionists aim to formulate diet in such manner that the energy required by an animal is provided by nonprotein sources. Although lipids are the primary source of energy in fish, dietary proteins supply essential amino acids (EAAs), considered prime for growth and development of fish. A very well-balanced pretentious diet usually increases mean total weight of eggs/female and the number of eggs produced/released by females. Conversely, low-protein diets have been shown to increase maturation time and reduce reproductive performance, oocyte maturation and ovulation, number of eggs produced, and egg viability in some carnivorous fishes like seabream, sea bass, and catfish, often by altering the GnRH and gonadotropin release. Moreover, in some species (e.g., pacu, rohu), an excessive protein diets can additionally set off induced low reproductive performance, fertilization, and hatching rates. However, supplying excessive levels of dietary protein can also increase the excretion of nitrogenous waste with due production of excessive ammonia level, which might affect feed intake and growth and indirectly reproduction of fish. Therefore, there is an obvious need to optimize dietary protein level for better reproduction in fishes. For example, Watanabe et al. (1984a) found an interesting relationship between dietary protein quality and reproductive success. Efforts are also currently being made to replace

fish meal with cheaper, more readily available grain meals. Cumaranatunga and Thabrew (1989) substituted legume meal for fish meal and reported better ovarian growth and significantly larger oocytes, indicating that legume meal is an inadequate source of nutrients for egg production. They attributed this difference to higher level of vitellogenic proteins and or/lipids in fish meal.

Carbohydrates

Carbohydrates, also known as sugars or saccharides, are one of the most essential components of all living organisms, having roles as readily metabolized energy source, as molecules which facilitate transfer of energy throughout the organism and as structural component. The saccharides are divided into four chemical groups: monosaccharides, disaccharides, oligosaccharides, and polysaccharides. Some of the carbohydrate is deposited in the form of glycogen in the liver and muscle, where it is available as a ready source of energy in times of need. Additionally, some carbohydrate is converted to lipid which is deposited in the body as energy reserves. For example, omnivorous/herbivorous freshwater fish can metabolize excessive portions of carbohydrate in diets, whereas carnivorous species make use of it much less efficiently. Very low carbohydrate levels do not compromise reproductive performance. Tolerable levels for carbohydrate varies with the species as a function of feeding habits, with herbivores tolerating the highest levels, accompanied through omnivores and carnivores fishes.

Vitamins

Vitamins are organic substances necessary for health, growth, maintenance, and also spermatogenesis and oogenesis in aquatic animals. It is often not synthesized by fish but is necessary, though in small amounts, for their normal growth, metabolism, health, and also reproduction. Important vitamins include water-soluble vitamins like vitamins B and C (ascorbic acid) and fat-soluble vitamins (vitamins A, D, and E). Vitamin deficiency may result in less growth and impairments in coloration and reproduction. Among these, vitamins like A, E, and C are widely researched in fishes. Requirements of vitamin in different fish species depend on the diet and the structure of the gastrointestinal tract, and within a species, requirements can also vary according to age and the physiological state of the fish. Vitamin E is very crucial for fertility and reproduction in fish, and fishes do not have ability to synthesize vitamin E in their body, so the need for exogenous supply prior to oogenesis is an important determinant of reproductive fitness (Halver 2002; Mandal et al. 2013). Studies have shown that vitamin E influences the quality of gonads, fecundity, egg quality, embryonic development, percentage of fertilization, hatching, and survival of larvae in both herbivorous/omnivorous (e.g., carp, ayu) and carnivorous (e.g., seabream, salmon). Deficiency will result in immature gonads, low fecundity and fertility, and low hatching rates and fry survival. Conversely,

increased levels of dietary vitamin E in diets increase development of gonads and the GSI and gonadal maturation and improve egg quality and viability, hatching rates, and percentage of normal larvae. Vitamin E is a lipid-soluble antioxidant and also reported as important in fish reproduction. Subsequently, vitamin C has an effect on ovarian development, steroidogenesis, vitellogenesis, and embryogenesis. Low level of dietary vitamin C induces a decrease in the reproductive performance of females by reducing fecundity, restricting hatchability, and increasing both the number of deformed larvae and their mortality. It is reported that male fishes are less sensitive to low vitamin C supply as this does not affect sperm motility. Embryos and juvenile fish seem more affected by vitamin C deficiency than adults, due to the fact that these vitamins are necessary for the synthesis of collagen during embryonic development and growth.

Minerals

This nutrient group consists of inorganic elements the body requires for various purposes. Fish require the same minerals as terrestrial animals for tissue formation, osmoregulation, and other metabolic functions. However, dissolved minerals in the water might also satisfy some of the metabolic necessities of fish. Minerals are typically classified as either macro- or micro-minerals, based on the quantities required in the diet and stored in the body. Macro minerals are calcium, phosphorus, magnesium, chloride, sodium, potassium, and sulfur. Minerals like phosphates and calcium are required in the formation of embryos and are acquired through vitellogenin (Vtg) in yolk. In fish reproduction, phosphorus deficiency can induce low female fecundity, low hatchability rates, and high rates of deformities. Calcium is important for activation of eggs, which occurs when eggs come into contact with water for hardening. Micro-mineral deficiencies in diets apparently have no effect growth or reproductive processes however might also have negative impact if fish are exposed for prolonged intervals of time.

Broodstock Nutrition and Reproductive Interaction

In most cases on broodstock nutrition, it is well defined for high-value fishes raised in intensive systems; however, little effort is paid to significantly enhance reproductive success of fishes in low input level farming systems. These days, increased attention is laid towards functional role of different dietary components on broodstock performance. It is reported that EFAs, vitamins like A, E, and C, trace minerals, and carotenoids like β-carotene can affect fecundity, egg quality, hatchability, and larval quality (De Silva and Anderson 1995). It is observed that the dietary amino acid requirements of brood fishes are much like the body requirements of the fishes (De Silva and Anderson 1995). Further, greater variation of the nutritional requirement for fishes of species groups which have consequences on reproductive performances. Common carp, *Cyprinus carpio*, as a model species

has been studied in depth among carps in this regard; however, most other studies have been done for carnivorous fish species (De Silva and Anderson 1995; Izquierdo and Fernandez-Palacios 1997) of marine origin; therefore, reasonably little is known about its condition in freshwater counterparts. In this connection, there seems to be an urgent need to study about the nutritional role on broodstock performance and reproductive function for other commercial freshwater fish groups.

While, the nutritional needs of female broodstock is adequately researched, but the studies on male fishes have received minimal attention. The possibility of improving of sperm quality through dietary induction/supplementation calls for immediate action. Though, initial research has been done quite a long time ago, where Watanabe et al. (1984a) reported that EFA (n-3 PUFA)-deficient diets in red seabream produced eggs with significantly lower survival and high levels of larval deformity. Some other interesting short-term effects of dietary nutrients on males have also been described in this species. For example, it is found that specialized diets given immediately prior to, or during, spawning of red seabream affected the egg composition. Available pigments like carotene, canthaxanthin, or astaxanthin also have a role on improving buoyancy of eggs. Looking at these, the dietary role of functional nutrients needs to be evaluated for different steps of broodstock nutrition. Likewise, the nutritional requirements of brood fishes can also vary, which may depend on the reproductive phases. Shortly, these periods can be demarcated as:

- Time between commercial size to broodstock size
- Immediately prior to, or during, spawning
- Post-spawning period

Therefore, designing complete formulated diets should, therefore, be taken into consideration for life stage-specific, as well as species-specific nutritional requirements of the brood fishes. Thus, our research approach and commercial formulation strategies for broodstock diet formulation should be directed towards three specific groups of diets like:

- Conditioning diet
- Reproduction diet
- Recovery/maintenance diet

Firstly, the broodstock conditioning diet can be formulated as an optimized grow-out diet to meet the complete nutritional requirements of the fishes from commercial to broodstock size in maximal synergy with the environment. Further, the reproduction diet used before or during spawning should consider the maximal reproductive performance (spawning success and fecundity) needs, gamete quality, and vertical transfer of nutrients and other biologically active substances to offspring. Lastly, the recovery/maintenance diet should potentially help recover the fishes from reproductive stress and look forward for reconditioning for the next reproductive cycle.

Energy Partitioning for Reproduction

Initially, animal use energy for maintenance requirement of animal then extra energy is splited between growth and reproduction. The relative partitioning of energy between somatic growth and gonadal growth depends on different species or different strains of same species, and more generalizations are challenging to draw. The overall amount of energy on hand for utilization in the various physiological processes has been found to affect the size, quality, and number of eggs produced. In one of the earliest studies of its type, Scott (1962) described a relationship in which various starvation regimes caused regression of the gonads in rainbow trout, *Oncorhynchus mykiss*.

The second is that broodstock on reduced rations grow less throughout the experimental period (6 months) and the proportion of the body present as gonad is greater in the smaller fish. That study is confounded by the fact that the animals fed at 0.35% per day were smaller at spawning than those fed twice as much. However, there is evidence that total egg volume is significantly greater in fish fed a lower ration when the influence of fish size is removed (Bromage and Cumaranatunga 1988). The other effects of reducing rations for rainbow trout are modest reductions in the numbers of fish reaching maturity and a delay of 2–3 weeks in the time of onset of spawning (Bromage and Cumaranatunga 1988). Therefore it is apparent that reducing rations to rainbow trout, and possibly generally, results in reduced egg size but greater relative fecundity.

Effects of Nutrition on Fecundity of Brood Fish

Fecundity can be defined as the total number of ova produced by each fish during the spawning season. It is expressed in terms of either eggs/spawn in case of absolute or individual fecundity or eggs/body weight in case of relative fecundity. The dietary lipid levels from 12% to 18% in broodstock diets for rabbit fish (*Siganus guttatus*) resulted in an increase in fecundity and hatching (Duray et al. 1994). Lipid is one of the major nutritional factors that has been found to significantly affect have a bearing upon reproductive efficacy in fish is the dietary essential fatty acid content (Watanabe et al. 1984a, b). Fecundity in gilthead seabream (*Sparus aurata*) was chanced on to noticeably expand with a rise in dietary n-3 HUFA polyunsaturated fatty acids with 20 or more carbon atoms, essential for marine fish. In research on the reproductive efficacy of Nile tilapia (*Oreochromis niloticus*) as indicated by the number of females that spawn, spawning frequency, collection of fry per spawning and total number of fry production over a 24-week period, the efficacy was much greater in fish fed a basal diet supplemented with soybean oil rich in n-6 fatty acids, predominant for this fish species (Watanabe 1982) and comparatively low in fish fed a 5% cod liver oil-supplemented diet elevated in n-3 fatty acids (Santiago and Reyes 1993). In sparids, the fatty acid composition of the female gonad is greatly plagued by the dietary fatty acid content, which considerably influences egg quality within a short period of time (Harel et al. 1992). In gilthead seabream, the fatty acid

composition of eggs is directly influence by the n-3 HUFA content of the broodstock diet. In some fish species similar to cod *Gadus morhua*, a transparent impact of predominant fatty acid on fecundity was not noticed in fish fed commercial diets coated with different types of oils (Lie et al. 1993). However, dietary EFA deficiencies causing detrimental consequences in fish and their extra have been also reported to have a negative impact on reproductive efficacy of fish species. For example, higher levels of dietary n-3 HUFA decreased the total amount of eggs produced by gilthead seabream broodstock spited an increase in egg n-3 HUFA concentration (Fernandez-Palacios et al. 1995). Other nutrients which were proven to affect fecundity include vitamin E (Izquierdo and Fernandez-Palacios 1997; Fernandez-Palacios et al. 1998) and ascorbic mg/kg resulted in an enhancement in fecundity of gilthead seabream as expressed by the total number of eggs produced by female and egg viability. Vitamin C content of rainbow trout eggs manifested the content of this nutrient in the diet and was related to improved egg quality (Sandnes et al. 1984). Dietary tryptophan, a precursor of the neurotransmitter serotonin, may positively impact gonadal maturation of both males and females. Supplementation of 0.1% tryptophan in the diets of ayu (*Plecoglossus altivelis*) resulted in a significant increase in the serum testosterone levels thus advancing time of spermiation in males and induced maturation of females (Akiyama et al. 1996).

Impact of Diet Quality on Reproductive Output

There are very few studies pertaining to a particular nutrient requirement of aquatic animals for proper gonadal development, but few reported indicate a great variability on species experimented. Watanabe et al. (1984c, d) observed that low-protein, low-phosphorous, and EFA-deficient diets produced eggs significantly low in hatchability, with higher lot of the hatched larvae showing signs of deformity. They underline that the most significant nutrients comprise of n-3 PUFA, which are found to be high in eggs of broodstock-fed diets with high levels of n-3 PUFAs. However, they fail to define clearly the relationship between quality of eggs and their fatty acid distribution. Subsequently, Takeuchi et al. (1981) reported that fish fed on diets without supplemental trace elements produced significantly lower percentages of both eyed and viable eggs than fish fed a sufficient diet. The contents of manganese, zinc, and iron in the eggs of fish fed diet without supplemental trace elements were also found to be significantly lower. In other studies, feeding broodstock rainbow trout a diet deficient in EFA resulted in low growth rate, low rates of eyed eggs, and low hatchability (Watanabe et al. 1984a).

Feeding Specialized Diets prior to Spawning

Specialized diets provided just prior to or during spawning of can affect the composition of the eggs (Watanabe et al. 1984a). Pigments like β-carotene, cantha-xanthin, or astaxanthin can contribute towards improvement in the percentage of

buoyant eggs. On the other hand, feeding with corn oil can result in reduced viability of eggs. Similarly, fatty acids and vitamin E, but not cholesterol, fed immediately prior to or during spawning resulted in increased levels of these compounds in eggs. Apart from fatty acids and α-tocopherol, numerous other nutrients have also proven to impact reproductive efficacy of marine fishes. However, the consequences in regard to the impact of carotenoid egg content on egg quality in salmonids are contradictory. Limited number of studies has been conducted towards controlling the level of dietary carotenoid equipped in broodstock diets (Harris 1984; Watanabe and Kiron 1995). The addition of purified astaxanthin to broodstock diets for red seabream is known to improve the percentage of buoyant and hatched eggs, moreover because of proportion of normal larvae (Watanabe and Kiron 1995). By contrast, the inclusion of β-carotene has no impact on these parameters. This variability in results is possibly due to the lower intestinal absorption to those compounds. Alternative dietary nutrients which are found to have an impact on the reproductive efficacy of marine fish have included dietary protein and vitamin C. Ascorbic acid has been shown to play vital roles in salmonids reproduction (Eskelinen 1989; Blom and Dabrowski 1995). Rainbow trout (*Oncorhynchus mykiss*) broodstock needs for this vitamin seemed to be about eight times greater than those of juveniles (Blom and Dabrowski 1995). However, less demands for ascorbic acid are according in broodstock diets for cod (Mangor-Jensen et al. 1993). Supplemented dietary vitamin E up to 250 mg kg^{-1} can be helpful for improving the sperm and egg quality of Black Sea trout.

Supplementing Plant Extracts for Early Maturation

The functional role of several herbal products towards maturation of fishes is well studied, and several herbals are found to have aphrodisiac properties and have control on the reproductive success and larval quality. The herbal maturation diet triggers maturation by reducing stress and regulate the hormonal cycle. Commercially available herbal maturation diet such as *Nutra-Brood* (manufactured by Australian-based feed company) contains adaptogenic, hepato-protective, antioxidant and immune-modulating herbal extracts. These also enhance the hepato-pancreatic activity and thus contribute towards digestive function and nutrient assimilation. There are also reported products with high capacity to stimulate spermatogenesis in male fish and gonadal maturation and effective egg viability in females (Babu 1999). The key advantage of these extract diets links to achieving viable spawners from wild, especially outside the breeding period and a further improvement in egg quality and fecundity. Among many reports, combination of *Withania somnifera* and *Mucuna pruriens* with other herbals could improve maturation and offspring quality of the spent spawners of marine shrimp, *Penaeus monodon* (Citarasu et al. 2013; Babu 1999). Dhas et al. (2015) have reported an elevated level of GSI in the herbal diet (a combination of *Moringa pruriens* (prepared in methanol) *W. somnifera* (prepared in ethanol) and *M. oleifera* (resin)). They also reported an increase in fecundity, fertilization rate, and hatching rate and increased level in

striping response when compared with control (non-supplemented diet). Further, a decrease in the proportion of the deformed larvae and an increase in the populace of normal larvae in diets administered with this formula were reported. Thus, herbal diets can be used to overcome the problems created by the chemical.

Dietary Oils: Plant vs Marine Fish Oils

Dietary oils are considered as predominant nutritional components which modulate many reproductive assignments in fish. Studies with *Dicentrarchus labrax* revealed that PUFAs, particularly *n-3* HUFAs, are among the most important dietary oil components that facilitate a change in sex steroid hormones, which decides fate of fecundity success (Zhang et al. 2017). Effect of supplementation of different dietary oils are found to have effect on the *kisspeptin* system which responds to intrinsic factors like sex steroids and metabolic factors and extrinsic factors like environmental signals (Bogevik et al. 2014). Among different forms, *Kiss2* is known to have better potency than *Kiss1* in stimulating the levels of FSH and LH mRNA in the pituitary (Kitahashi et al. 2009). The expression of *Kiss2* mRNA was higher in the brain and gonad of the male *D. labrax* fed diets with fish oil, while, *Kiss1* expression was similar to those fed plant-/salmon oil-supplemented diets. Further, a delayed maturation in males fed plant/salmon oil, corresponding to higher expression of Cyp19b (a gene initiating gonadal maturation via GnRH stimulation of FSH synthesis) in the brain was observed by these authors. However, possible insight mechanisms revolving around the inhibition of early sexual maturation-associated hormonal gene expression from plant-based fatty acid are yet to be confirmed.

Effect of Food Restriction

Research towards role of feeding rate on reproductive performances of fishes is given little importance. Dietary restriction can be used as a strategy to minimize feed input and reduce production costs. Food restriction is indeed an important aspect from the economical viewpoint as in high-value fishes like rainbow trout. As the high feed cost in feeding carnivorous species like trout and salmon which contributes around 60%–80% of the cost of production. Therefore, the present focus in farming activity is towards feed restriction. Studies in salmonid fishes concealed that restriction of food to half of total ration can deplete the spawning success (Imsland and Gunnarsson 2011) and, in extreme cases, can lead to arrest of reproductive process (Cladwell et al. 2013). Additionally, food restriction can negatively affect egg size and quality of *O. mykiss* (Cleveland et al. 2017). Also, food restriction resulted to higher GSI, thus relating a positive side of the food restriction on reproductive process (Imsland and Gunnarsson 2011; Cladwell et al. 2013). Nevertheless, restriction did not affect hepato-somatic index, which is considered a reliable index of the body reserves which relates to the nutritional status of the species. A beneficial aspect of food restriction is the production of big size eggs with low intra-female size

variability as observed in *O. mykiss* (Cardona et al. 2019). These authors also reported that with no much difference observed in egg lipid content, feed restriction alters the egg's fatty acid composition, where it's composition in restricted female shifted towards higher content of n-6 PUFA and subsequent decrease in MUFA. An elevated level of PUFAs is very much linked to superior quality of the eggs. Among PUFAs, AA, ALA, and EPA in higher proportion in egg play a pivotal role in reproduction (Rønnestad et al. 1998; Torcher 2010). The n-3 PUFAs stored as reserves can potentially be used during developmental process or catabolized for energy release, post-hatching (Torcher 2010). On the other hand, ALA helps in optimum growth, egg development, and offspring survival. Thus, it is obvious that fish fed ad libitum utilize their energy mostly for growth and storage, while restricted fish show tendency for their gonadal development to ensure reproductive success.

Conclusion and Future Direction

Information regarding nutritional requirements of fishes is scarce and confined only to a few species. Key nutrients such as EFAs and vitamins are known to play an important role in successful reproductive program. Besides, minerals, such as phosphorous, and other nutritional aspects, such as protein quality, are known to be essential for fish reproduction. Recent reports on strategies of feeding at and before maturation are deciding factors of reproductive success. The importance of many other nutrients such as vitamin A, vitamin B6, and folic acid is yet to be established within broodstock feeds and deserves future research focus.

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Endocrine Disruption in Freshwater Fish from Contaminants of Emerging Concern

18

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Abstract

Emerging pollutants are a class of new chemicals that are not commonly monitored for their environmental presence but have the potential to enter the environment and cause adverse biological and ecological effects. The scientific community has awakened to the challenges posed by such pollutants in our environment, and there is a plethora of scientific literature to suggest that these contaminants are potent even at trace levels. Various environmental monitoring bodies, such as the EPA, REACH-ECHA and NORMAN, have also recognized the threat from these emerging contaminants. Contaminants of emerging concern are mostly the chemicals synthesized for human utility, for example, ingredients in pharmaceutical and personal care products and chemicals used in plant protection systems and fire prevention. Properties, such as increased bioavailability and bioreactivity of pharmaceutics and pesticides and increased persistence of halogenated aromatics, make them highly efficient at eliciting biological reactions even at low concentrations. Nanoparticles have emerged as one of the newest classes of contaminants in the twenty-first century, and they too have the ability to interact with biological pathways and disrupt them. The inventive and inquisitive nature of humans is expected to introduce more novel chemicals into the aquatic environment in future which will keep the scientists busy in investigating their biological effects. This chapter is expected to act as a primer for various budding

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ecotoxicologists to initiate their interest in the toxicology of contaminants of emerging concern.

Keywords

Aquatic pollution · Ecotoxicology · Organic pollutants · Aquatic toxicology · HPG-axis · HPT-axis · Sex hormones · Thyroid hormones

Introduction

The endocrine system comprises a network of ductless glands synthesizing and secreting endocrine messengers (hormones) into the bloodstream to regulate the function and behavior of distant organs. Hormones, at concentrations as low as ng/L of blood, can regulate a large variety of functions including ionic homeostasis, stress response, and reproduction in fish (Kime 1998). Sensitivity to low concentrations of hormones is one of the hallmarks of endocrine communication; however, the overall efficiency of hormonal communication depends primarily upon its concentration in the bloodstream and the numbers of receptors on the cells' surface. The concentration of the hormones in the bloodstream, on the other hand, is influenced by their rate of synthesis and secretion and the rate of their degradation. A large body of scientific literature already elucidates how common environmental pollutants disrupt normal endocrine functions in vertebrates (Ayobahan et al. 2020; Nelson et al. 2020; Singh and Chandra 2019). However, in addition to the toxicity from already known pollutants, the scientific community has more recently recognized adverse impacts from an emerging class of contaminants that are not commonly covered under the regulatory framework for environmental monitoring (Geissen et al. 2015). The Network of Reference Laboratories, Research Centers and Related Organizations for Monitoring of Emerging Environmental Substances (NORMAN) defines these contaminants of emerging concern (CEC) as "pollutants that are currently not included in routine monitoring programs at the European level and which may be candidates for future regulation, depending on research on their (eco)toxicity, potential health effects and public perception and on monitoring data regarding their occurrence in the various environmental compartments". Currently, the NORMAN list of CEC consists of 1036 substances that fall into the category of surfactants, flame retardants, pharmaceutics and personal care products (PPCPs), gasoline derivatives, biocides, polar pesticides and their derivatives, and various other suspected or proven endocrine-disrupting compounds (EDC) (NORMAN 2016). Such contaminants are increasingly being detected in the water bodies, and since most emerging pollutants, especially the PPCPs, are intentionally designed to be bioactive, they may have a profound impact on the aquatic fauna even at low concentrations (Balakrishna et al. 2017; Deblonde et al. 2011).

Sources of Contaminants of Emerging Concern in the Freshwater Environment

The contaminants of emerging concern that pollute aquatic habitats arise primarily from anthropogenic activities ranging from the combustion of fossil fuels, use of pesticides, manufacture and consumption of personal care products, and leaching of chemicals used in industrial and household items. The contaminants could reach water bodies from a point source, such as household or industrial effluent, or the source could be more diffused, such as agricultural runoff or leachates from landfills.

Pharmaceutics and personal care products (PPCPs) refer to thousands of pharmaceutical agents and cosmetic ingredients that are administered to humans or animals. The primary source of PPCP contamination is household or municipal discharge (Deblonde et al. 2011; Eggen et al. 2010). Wastewater from hospitals is also an obvious source of polluting pharmaceutics (Verlicchi et al. 2010). The PPCPs, after their administration, are partially metabolized and excreted through feces or urine—eventually finding their way into the water bodies. Certain pharmaceutical agents are intentionally manufactured to prevent their metabolism to non-bioactive metabolites; hence, they may resist wastewater treatment and persist in the water bodies (Deblonde et al. 2011; Rehman et al. 2015). Furthermore, certain pharmaceutics may appear persistent in water bodies because their rate of introduction exceeds their rate of degradation (Barceló 2003). Eventually, the persistent nature of bioactive PPCPs may result in their accumulation to toxic concentrations in water bodies and cause adverse effects due to chronic exposure (Wu and Shen 2018). It is obvious that a large population will result in larger drug consumption and eventually a higher contaminant load in the household and municipal discharge. Therefore, aquatic ecosystems in population-dense developing nations, such as Bangladesh, India, and Pakistan are, particularly at higher risk. However, a larger threat is from poor regulatory compliance by the pharmaceutical companies that have shifted to Asian countries because of cheap labor and attractive government policies (Lübbert et al. 2017; Mathew and Unnikrishnan 2012). Animal waste can also contain significant amounts of steroid hormones that either naturally synthesized in their bodies or administered to them for therapeutic purposes. Thus, farmyard manure is also a significant contributor to environmental levels of steroid hormones (Ying et al. 2002).

Many chemicals, commonly used for domestic or industrial hygiene and safety, may seem innocuous; however, recent literature suggests that some of them might have adverse ecological effects in the long term. For example, surfactants used in soaps and cleaning products for industrial and household applications can reach water bodies through municipal discharge and cause adverse effects in aquatic animals (Martínez et al. 2019; Shi et al. 2019; Wang et al. 2020). Surfactants, such as perfluoroalkyl carboxylic acids (PFCAs) and perfluoroalkane sulfonic acids (PFSA), are known to persist in aquatic habitat and bioaccumulate along the food chain to cause various aberrations in homeostasis (Martínez et al. 2019; Wang et al. 2020).

Flame retardants containing polybrominated biphenyl (PBBs) compounds are being increasingly used in compliance with stricter fire prevention policies and may have endocrine-disrupting effects under chronic exposure (Pittinger and Pecquet 2018; Stieger et al. 2014). Bromine moieties, which quench fire propagating free radicals generated during the process of burning, also make PBBs more persistent and lipophilic—thus increasing their concentration to toxic levels in the environment over time. The PBBs are mainly used in electric circuit boards and electronic fixtures which, at the end of their life cycle, end up in landfills and leach out PBBs into the soil and aquatic habitats.

The term pesticide is used to denote any chemical that is used to combat undesirable organisms. Pesticides could be regarded as plant protection pesticides if the intended usage of the chemical is the protection of crops (SCA 2020). Several plant protection pesticides have a long history of being known as endocrine disruptors as more than 100 chemicals from this class have already been identified as endocrine disruptors (Mnif et al. 2011). Most of these chemicals have been used historically for a long time in many parts of the world and, hence, may not be considered as emerging contaminants. However, many traditional pesticides are still the major tools for pest control (Costa 2018), and many novel pesticides are synthesized and introduced in the market regularly; hence, it is important to evaluate chemicals in this class for their potential endocrine-disrupting effects.

Plasticizers are additives that are mainly used to provide flexibility to polymers. However, plasticizers have the potential to leach out of the polymer which leads to environmental contamination (Mathieu-Denoncourt et al. 2015). Because of their globally widespread usage in polymers, plasticizers and their metabolites have been detected in many major environmental matrices such as tap water, surface water, melted snow, sediment, and landfill leachates (Horn et al. 2004). Such widespread contamination signifies the importance of studying the endocrine disruption potential of plasticizers more carefully. Two oldest known plasticizers are bisphenol A (BPA) and a group of chemicals called phthalates. The most well-known and the most widely used chemical among phthalates is di(2-ethylhexyl) phthalate (DEHP) (Rowdhwal and Chen 2018). BPA and DEHP have already been established as endocrine disruptors because their effects on endocrine systems are well documented, especially in mammalian species (Manikkam et al. 2013). However, many alternatives to BPA and DEHP have gained significant popularity in recent years because of the heavy restrictions on the use of BPA and DEHP in many countries. The endocrine activity of these alternate chemicals is not well characterized.

Similar to plastics, nanoparticles are also recent human invention which has applications in a wide range of industries including food, cosmetics, medicine, crop protection, and electronics (Katz et al. 2015; Schmid and Riediker 2008). The scientific literature suggests that nanoparticles have emerged as an unregulated class of aquatic pollutants due to their use in a wide range of products of industrial, agricultural, and domestic applications (Batley et al. 2013; Guo et al. 2019b). Nanoparticles in the aquatic environment can be classified into primary and secondary particles. The primary particles are purposefully manufactured and are also

called manufactured nanoparticles, whereas the secondary particles are formed by the disintegration of larger matter. Nanoparticles may enter aquatic ecosystems through household or municipal waste discharge, industrial effluents. Certain nanoparticles used in cosmetics [e.g. titanium dioxide (TiO_2) in sunscreens] may enter aquatic ecosystems through outdoor and recreational activities such as bathing and watersports (Gondikas et al. 2014).

Endocrine Disruption from the Contaminants of Emerging Concern

Hypothalamic-Pituitary-Gonadal Axis

The hypothalamic-pituitary-gonadal (HPG) axis controls gonadal development and reproduction through synchronization between hypothalamic gonadotropin-releasing hormone (GnRH), pituitary gonadotropins [luteinizing hormone (LH) and follicle-stimulating hormone (FSH)], and gonadal steroid hormones (estrogen and ketotestosterone) (Fig. 18.1). Environmental pollutants can disrupt the synchronized control of the HPG-axis over gonadal development by affecting the level of hormones in bloodstream through their inhibited synthesis and secretion or altered metabolic clearance from blood. The environmental pollutants may also interfere with the action of hormones at their site of action (Thomas 2008).

Hypothalamic-Pituitary-Thyroid Axis

Thyroid hormone plays an important in the maintenance of homeostasis and influences physiological processes involved in osmoregulation, gonadal development and reproduction, somatic growth, larval metamorphosis, and smolting (Janz 2000). Similar to HPG-axis, the neuroendocrine control of the hypothalamic-pituitary-thyroid axis (HPT-axis) is under the brain (see Blanton and Specker 2007 for more details). The brain perceives external stimuli and environmental cues through sensory receptors and relays the information to the hypothalamus after processing it (Fig. 18.2). In fishes, the neurosecretory cells of the hypothalamus secrete various peptides and neurotransmitters, including thyrotropin-releasing hormone (TRH), to regulate the secretion of thyroid-stimulating hormone (TSH) from the pituitary. Pituitary exercises regulatory control over secretion, and perhaps synthesis, of thyroid hormone via TSH (Blanton and Specker 2007; Chatterjee et al. 2001; Janz 2000).

The follicles in the thyroid gland synthesize L-thyroxine (T4) by incorporating free inorganic iodine from the blood into tyrosine (Fig. 18.2). L-thyroxine is a prohormone that is converted into biologically active 3,5,3′-triiodo-L-thyronine (T3) by deiodinase enzymes (DI) in the liver and various other tissues. Therefore, unlike the direct control of sex steroid synthesis by the central nervous system, the biological action of thyroid hormones is under the control of peripheral tissues that

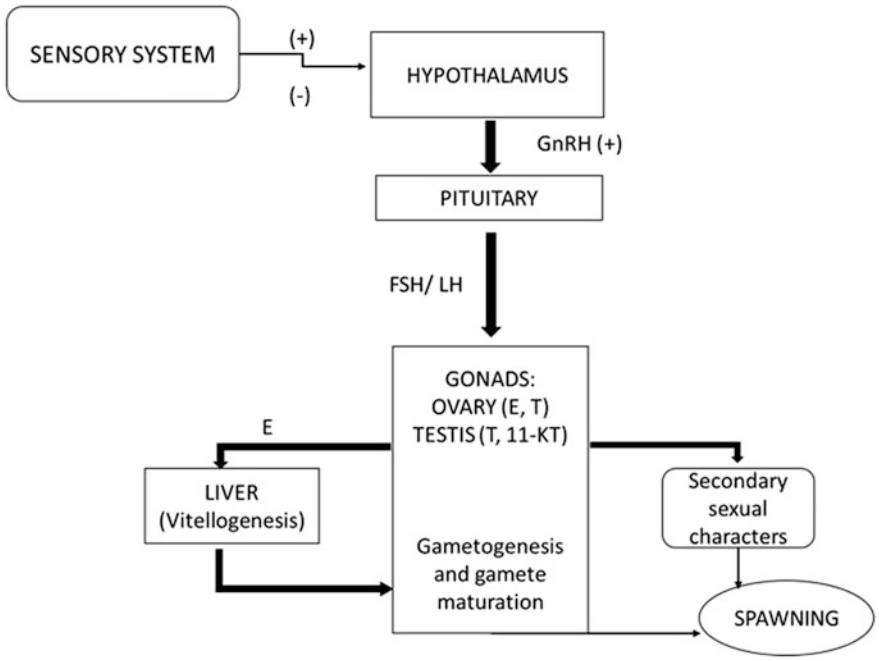

Fig. 18.1 Diagrammatic representation of the endocrinological pathway in the hypothalamic-pituitary-gonadal axis (HPG). The sensory system perceives environmental cues and relays them to the central nervous system where the hypothalamus initiates the process of gametogenesis that culminates in spawning and population recruitment. Environmental pollutants can interfere with HPG-axis by causing aberrations in the relay of hormones. The endocrine disruptors can interfere with the sensory perception of environmental cues or alter the synthesis of gonadotropin-releasing hormone (GnRH) which stimulates the pituitary gland. The contaminants can also interfere with the reception of GnRH at the pituitary and thus alter the synthesis and secretion of follicle-stimulating hormone (FSH). The endocrine disruptors can also alter the synthesis of sex steroid hormones [17 β-estradiol (E), testosterone (T), 11 α-ketotestosterone (11-KT)], thereby affecting vitellogenesis, gametogenesis, gamete maturation, and development of secondary sexual characters and courtship behavior. (Figure modified from Thomas 2008)

convert T4 into T3 (Blanton and Specker 2007; Eales and Brown 1993). Both T4 and T3 control thyroid hormone secretion through the feedback loop.

Since thyroid hormones control multiple functions in fish, the disruption of the HPT-axis can get cause aberrations in various metabolic, osmoregulatory, and growth functions. The inhibition of the HPT function can occur at the level of sensory perception of environmental cues by the brain, at the level of communication between CNS-hypothalamus-pituitary-thyroid route or at the conversion of T4 to T3 (Fig. 18.2).

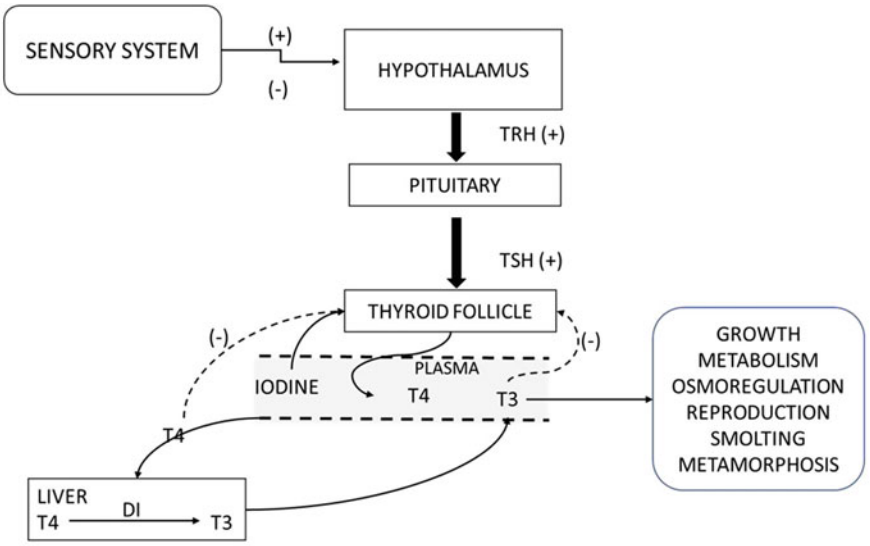

Fig. 18.2 Diagrammatic representation of the endocrine communication within the hypothalamic-pituitary-thyroid axis (HPT-axis) and its functions. The sensory system perceives environmental cues and initiates a cascade of endocrine communication that culminates with the modification of various metabolic processes, osmoregulation, reproduction, growth, metamorphosis, and smolting. The environmental pollutants can interfere with the sensory perception of environmental cues and relay of the message from the hypothalamus to thyroid follicles. The endocrine disruption can also interfere with deiodinase enzyme (DI)-mediated conversion of L-thyroxine (T4) to 3,5,3′-triiodo-L-thyronine (T3). (Figure adapted from Blanton and Specker 2007)

Disruption of HPG-Axis by Pharmaceutical Steroid Hormones and Cosmetics

The steroid hormone agonists/antagonists that are commonly used as birth control measures in humans are obvious pharmaceutical candidates that can cause hormone disruption in fish—leading to reduced fecundity and, in some cases, spawning failure (Nash et al. 2004). Synthetic hormone 17 α-ethinylestradiol (EE) is the primary component in birth control pills and is persistent when it gets partitioned into the sediments (de Mes et al. 2005). Exposure to environmentally relevant concentrations of EE can increase proportions of phenotypic females in fish and amphibians (Pettersson and Berg 2007). Although the exact mechanisms behind the endocrine-disrupting behavior of EE and other steroid contaminants are still unknown, mechanistic studies and focus on the adverse outcome pathways (AOP) have revealed various genomic and non-genomic candidates. Transcriptome analysis of live-bearer, guppy (*Poecilia reticulata*), revealed that exposure to EE can interfere with the gender-specific genes in the brains and induce changes in male fish that were more similar to that observed in a feminine fish brain (Saaristo et al. 2017). Thus, EE might disrupt endocrine functions at the level of the hypothalamus in the HPG-axis (Fig. 18.1). It was also demonstrated that a 96-h exposure to

environmentally relevant concentrations of EE in male fathead minnows can alter HPG-axis function by suppressing male hormone synthesis which, in turn, downregulated steroid metabolism pathway and *dmrt*1 gene which can cause feminization by reprogramming Sertoli cells into granulosa cells (Feswick et al. 2016). Similarly, EE was also shown to disrupt steroid homeostasis by adversely affecting energy homeostasis and adult proteomics in fish (Voisin et al. 2019; Zhou et al. 2019). Interestingly, early exposure to EE not only favors feminization and the development of intersex males, but it is also shown to reduce oogenesis in female killifish (*Heterandria formosa*) (Jackson et al. 2019). Exposure to waterborne EE is also known to affect reproductive and nonreproductive behavior in fish, which can have negative impacts on fish reproduction and population recruitment (Porseryd et al. 2019; Saaristo et al. 2019). Estrogen also increases hepatic vitellogenin synthesis, which is the most commonly used biomarker for endocrine disruption in male fish (Martyniuk et al. 2020). Although most studies are focused on the detection of estrogen-mimicking substances in wastewater, the androgens (both natural and synthetic) can also occur at toxic concentrations in household discharge (Chang et al. 2011), and their toxic effects need further investigation.

In addition to the therapeutic hormones, various other pharmaceutical compounds are also known to disrupt the endocrine system in aquatic organisms. Paracetamol (acetaminophen) and ibuprofen, commonly used analgesics, can disrupt HPG-axis by reducing testosterone and increasing estrogen levels in freshwater fish (Guiloski et al. 2017; Han et al. 2010). Similarly, diclofenac, a commonly used analgesic, also demonstrated estrogenic behavior in frogs, *Xenopus laevis* (Efosa et al. 2017). Metformin, used to treat type 2 diabetes, was demonstrated to induce intersex in female rice fish (*Oryzias latipes*), with a concomitant reduction in expression of vitellogenin and ERβ1 genes (Lee et al. 2019). In male fish, metformin can disrupt reproductive hormone signalling by upregulating the expression of estrogen receptor α (ERα) and vitellogenin genes (Lee et al. 2019; Niemuth et al. 2015). Similarly, antihypertensive β-blockers were also demonstrated to affect HPG-axis by disrupting steroidal hormonal balance, reduced fecundity, viability, and survival of eggs in fish (Huggett et al. 2002; Massarsky et al. 2011). There are reports of endocrine disruption in aquatic organisms by antimicrobial and antibiotics; however, their effects and underpinning mechanisms need further investigations (Kang et al. 2006; Witorsch and Thomas 2010).

Synthetic cosmetic products can also interfere with normal endocrine signalling to cause developmental and sexual abnormalities. For example, synthetic polycyclic musks used in fragrances were shown to act as an estrogen receptor (ER) antagonists and have antiestrogenic activities in zebrafish (Schreurs et al. 2004). Diethyl phthalate (DEP) used as binder and solvent in many cosmetics can also cause feminization in fish (Barse et al. 2007). Benzophenone-2 (UV filter in sunscreens) can inhibit spermatogenesis and cause feminization with increased increasing hepatic vitellogenin synthesis in fish (Nashev et al. 2010; Weisbrod et al. 2007).

Endocrine Disruption by Surfactants

The existing literature suggests that most commonly used surfactants and aqueous film-forming foams such as perfluorooctanesulfonate (PFOS) and perfluorooctanoic acid (PFOA) may adversely affect reproductive and somatic development in female fish through disruption of HPG-axis and metabolism of thyroid hormones (Ankley et al. 2005; Kim et al. 2010; Shi et al. 2019). Reproductive effects include reduced fecundity and delayed final maturation of ova in female fish (Ankley et al. 2005; Han and Fang 2010). PFOA can also result in increased serum vitellogenin levels in male fish with a recovery to normal levels within 1 week of removal of exposure (Kim et al. 2010). Furthermore, maternal exposure to PFOS can also cause reduced reproductive ability of the female offspring (Han and Fang 2010). Other surfactants such as novel perfluoropolyether carboxylic acids (PFECAs), synthesized as alternatives to PFOAs, have also demonstrated developmental abnormalities in zebrafish by disrupting thyroid hormone metabolism (Wang et al. 2020). Similarly, fluorotelomer surfactants can also cause developmental malformation and reduced female fecundity, fertility, and survival of offspring by disrupting HPG-axis and thyroid hormone metabolism (Shi et al. 2019).

Endocrine Disruption by Brominated Flame Retardants

Chronic exposure to polybrominated biphenyl (PBB) flame retardants can affect fish reproduction by disruption of the HPG-axis at various sites (refer to Sect. 2.1 and Fig. 2.1 for various sites at which environmental pollutants can interact with the HPG-axis) (Han et al. 2011, 2013). Chronic early-life exposure to hexabromocyclododecane (HBCD) was shown to disrupt olfactory function in Atlantic salmon which may interfere with the detection of environmental cues for successful migration and reproduction (Lower and Moore 2007). Polybrominated flame retardants may also upregulate mRNA expression of hypothalamic *GnRH* and gonadotropins (*GtH*; *FSHβ* and *LHβ*) in the pituitary, eventually resulting in aberrant concentrations of sex steroid hormones in serum (Han et al. 2011). Downregulated expression of *GnRH* and reduced serum concentrations of GtH have also been reported in response to dietary exposure of TBPH and TBB mixture in Japanese medaka (*Oryzias latipes*) (Saunders et al. 2015a).

In addition to the HPG-axis, the brominated flame retardants can also have detrimental effects on growth and development through disruption of the HPT-axis (Noyes and Stapleton 2014; Park et al. 2011). A suit of piscine studies has demonstrated that PBBs can disrupt thyroid metabolism by perturbing HPT-axis at multiple sites (Chen et al. 2010; Han et al. 2011; Saunders et al. 2015a, b). Depending upon the life stage of the fish or the exposure route (dietary, waterborne, or parental), the PBBs can either increase or decrease the concentration of T4 levels in fish serum. For example, dietary tetrabromoethylcyclohexane (TBECH) was shown to disrupt the HPT-axis and reduce total plasma T4 concentration in brown trout (*Salmo trutta*) (Park et al. 2011). A similar decrease in the T4 concentration was

also reported in during smoltification of Atlantic salmon (*Salmo salar*) (Lower and Moore 2007). In contrast, an increase in the plasma T4 levels was observed in zebrafish F0 generations that received PBB exposure through parental transfer (Yu et al. 2011). An increase in whole-body T4 levels was also recorded in zebrafish when exposed in the embryonic stage (Wang et al. 2019). These contrasting results highlight the importance of factoring the exposure route and the exposed life cycle of fish in aquatic toxicology. Furthermore, T3 is the metabolically active form of thyroid hormones, and the serum levels of T4 should always be studied in relation to the T3 levels to deduce the biological significance of PBB exposure. The significance of using T3/T4 ratio as a biomarker of HPT-axis can be emphasized by the fact that PBBs have a structural similarity with thyroid hormones and may influence synthesis of proteins responsible for thyroid transportation, T4 to T3 conversion, and T3 catabolic inactivation—causing functional hypothyroidism in fish (Guo et al. 2019a; Parsons et al. 2019; Ren et al. 2019). The piscine literature has conflicting reports on the effects of various PBB flame retardants on the expression of thyroid serum transport proteins. For example, Wang et al. (2019) reported an increased upregulation of thyroid transporter proteins mRNA in response to waterborne decabromodiphenyl ethane (DBDPE) in zebrafish larvae, whereas Wu et al. (2019) reported a decrease in mRNA expression of thyroid transporter proteins in zebrafish exposed to BDE-99 through parental route (Wang et al. 2019; Wu et al. 2019). The differences in the observation could be attributed to the type of PBB or even the exposure route. It has been demonstrated, using in vitro tools, that hydroxylated PBBs can mimic thyroid hormones and bind with thyroid hormone receptors and transporters (Ren and Guo 2012; Zhang et al. 2019). Thus various PBB may have agonistic/antagonistic interactions with thyroid hormone receptors and transporters. Polybrominated biphenyl flame retardant-mediated perturbation of HPT-axis by interfering with different classes of DI enzymes that convert T4 to T3 and also breakdown T3 have also been suggested (Parsons et al. 2019; Wang et al. 2019; Wu et al. 2019). Similarly, PBB-induced alterations in the expression of uridine diphosphate glucuronosyl transferase (*ugt*) enzyme, which metabolizes T3 for excretion, have also been reported in fish (Wang et al. 2019; Wu et al. 2019). Therefore, by causing alterations in the expression of DI and ugt enzymes, the PBBs may cause hypo- or hyperthyroidism and disrupt developmental homeostasis.

Endocrine Disruption by Manufactured Nanoparticles

Detection of manufactured nanoparticles in the aquatic ecosystems is a recent phenomenon which is why the literature on their detrimental effects in aquatic animals, particularly on the endocrine system, is still scanty. Nonetheless, few studies have highlighted that various nanoparticles may have a direct impact on various endocrine axis of fish. For example, silver and cobalt ferrite nanoparticles were shown to affect HPG and HPT-axis, respectively (Ahmad et al. 2016; Degger et al. 2015). Gold nanoparticles were also reported to increase blood cortisol in gilthead sea bream (*Sparus aurata*) (Teles et al. 2017). However, most studies

suggest that the toxic effects of exposure to nanoparticles could be because of their increased ability to adsorb environmental pollutants and make them more bioavailable. Small size and increased surface area to volume ratio favor easier uptake and increased biological reactivity of nanoparticles (Gatoo et al. 2014). The TiO_2 nanoparticles were demonstrated to increase the bioavailability of water-soluble lead (Pb) in zebrafish and cause hypothyroidism (Miao et al. 2015). Similarly, TiO_2 nanoparticles also acted as carriers of BPA and BDE-209 to cause facilitated disruption of HPG- and HPT-axis, respectively, in zebrafish (Fang et al. 2016; Wang et al. 2014). Furthermore, the TiO_2 nanoparticles were shown to facilitate the deposition of BPA in the fish gonads thereby causing hypothyroidism in the F1 generation as well (Guo et al. 2019a).

Endocrine Disruption by Pesticides

Five major classes of plant pesticides are currently used: organophosphates, organocarbamates, organochlorides, pyrethroids, and neonicotinoids. As reviewed elsewhere, some of the traditional organophosphates such as malathion and diazinon have shown estrogenic and thyroid-related endocrine effects in fish (Kitamura et al. 2006). For example, exposure to diazinon caused a significant decrease in serum estradiol levels in bluegill fish (Maxwell and Dutta 2005). Similarly, malathion decreased serum T3 levels in freshwater catfish. In another study, commercial product cythion (50% formulation of malathion) caused adverse morphological effects on thyroid of freshwater fish *Channa punctatus* at 2–4 ppm exposure concentrations (Ram et al. 1989).

Similar to organophosphates, the estrogenic and thyroid-related effects of organocarbamates are well documented. For example, both carbaryl and carbofuran, two of the most notable organocarbamates, have shown an estrogenic effect in freshwater fish. Carbaryl was shown to have an adverse effect on the number and morphology of catfish oocytes (Kulshrestha and Arora 1984). Carbofuran, on the other hand, delayed the oocyte maturation in female catfish (Chatterjee et al. 1997). Acute carbaryl exposure in catfish and a sub-chronic 30-day exposure in *C. punctatus* have also been shown to reduce the serum T4 and increase the T3 levels (Ghosh et al. 1989; Sinha et al. 1991).

Majority of original organochlorine pesticides have been banned in the last few years mainly because of the ecological concerns; however, due to their extremely high persistence, exposure to this class of chemicals still persists to this day (Costa 2019). Several organochlorine pesticides have shown endocrine-disrupting effects in fish. For example, hexachlorocyclohexane (HCH) caused induction of vitellogenesis in male medaka fish (*Oryzias latipes*) at waterborne exposure concentration of 0.1–1 mg/L, which suggests that HCH has estrogenic effects in fish (Wester and Canton 1986). Endosulfan, another highly toxic organochlorine pesticide, has shown several indications of being a potent endocrine disruptor in both in vitro and in vivo studies in fish. For example, endosulfan was shown to decrease FSH and altered testes tissue structure in adult South American freshwater cichlid fish (*Cichlasoma*

dimerus) (Da Cuña et al. 2011). Moreover, in vitro culture of cichlid fish testes and ovaries, when exposed to endosulfan, showed a significant reduction of androgen and estradiol, respectively (Da Cuña et al. 2013). Widely used organochlorine pesticide dichlorodiphenyldichloroethylene (DDE) also demonstrated significant changes in genes responsible for vitellogenesis in largemouth bass fish (*Micropterus salmoides*) suggesting the endocrine disruption properties of this pesticide (Larkin et al. 2002).

Pyrethroids are the fourth most widely used group of pesticides in the world, and they are widely accepted as endocrine disruptors (Brander et al. 2016a). Bifenthrin is one of the most acutely toxic pyrethroid with significant endocrine-disrupting activities even at extremely low concentrations (Brander et al. 2016a). In a recent study, bifenthrin caused altered expression of estrogen-mediated proteins even at lower ng/L concentrations in inland silverside (*Menidia beryllina*) fish, suggesting that pyrethroid are extremely potent endocrine disruptors (Brander et al. 2016b). Permethrin, another highly toxic pyrethroid insecticide, is also considered to be an estrogenic compound (Brander et al. 2016a). Elevated levels of choriogenin protein were observed in silverside fish after an exposure with 1 μg/L permethrin (Brander et al. 2012). Similarly, in Japanese medaka (*Oryzias latipes*), an exposure concentration of 10 μg/L permethrin caused an induction in vitellogenin mRNA (Nillos et al. 2010). Based on these observations, it is apparent that pyrethroid insecticides cause endocrine disruption predominantly through sex steroid pathways; hence, other pyrethroids such as cyfluthrin should be studied in more detail because for this insecticide; currently sufficient evidences of endocrine disruption in fish are not available. Nonsex steroidal mechanisms of endocrine disruption have also been proposed for various pyrethroid insecticides. For example, a number of studies in fish and/or mammalian species have shown that fenvalerate, bifenthrin, and lambda-cyhalothrin can affect the thyroid function by reducing the T3 and T4 levels in serum (Akhtar et al. 1996; Giray et al. 2010; Kaul et al. 1996; Saravanan et al. 2009).

Neonicotinoids are relatively newer class of pesticides, which are chemically similar to nicotine, clothianidin, imidacloprid, thiamethoxam, acetamiprid, are the four most commonly used neonicotinoids (EC 2020; USEPA 2019). Available acute toxicity data suggests that neonicotinoids do not pose significant risk to freshwater fish (Sánchez-Bayo et al. 2016). For example, 96 h LC_{50} values of imidacloprid was in the range of 83–281 mg/L for rainbow trout (*Oncorhynchus mykiss*) and zebrafish (*Danio rerio*) (reviewed in (Sánchez-Bayo et al. 2016). Similarly, out of 34 freshwater and marine species tested, fish were among the least sensitive organisms, with reported LC_{50}/EC_{50} values significantly greater than the reported surface water exposure concentrations (Finnegan et al. 2017). As mentioned above, toxicity of neonicotinoids seems to be low in fish species; however, very little is known about the endocrine-disrupting potential of neonicotinoids in fish. Latest data, although limited, suggests that neonicotinoids could possess endocrine-disrupting properties. For example, although chronic exposure of up to 150 μg/L clothianidin did not reduce the survival of Sockeye salmon (*Oncorhynchus nerka*), there was an increase in 17β-estradiol levels at the exposure concentrations as low as 0.15 μg/L. These observations suggest that reproductive and endocrine effects of clothianidin should

be studied in more detail (Marlatt et al. 2019). In a recent study, chronic exposure to thiamethoxam caused reduced plasma T4 levels and a delayed gonadal development in both female and male Chinese rare minnow (*Gobiocypris rarus*). Moreover, vitellogenin (*VTG*), cytochrome P450 aromatase gene (*CYP19a*) in male liver, gonadotropin-releasing hormone (*GNRH*) and *CYP19b* in male brain, and *CYP17* and *CYP19a* in male gonads were upregulated, whereas estrogen receptor alpha (*ERA*) in female liver and *CYP17* in female gonads were significantly upregulated (Zhu et al. 2019). These experimental evidences are indicative of possible disruption of HPG and HPT axes by thiamethoxam. Overall, limited evidences suggest that endocrine-disrupting potential of neonicotinoids should be studied in more detail, especially given the fact that neonicotinoids have shown endocrinal effects in birds and bees, etc. (Baines et al. 2017; Pandey and Mohanty 2015).

Endocrine Disruption by Plasticizers

Two most common plasticizers are bisphenol A (BPA) and phthalates such as DEHP. BPA and DEHP are well-known endocrine disruptors because their effects on endocrine systems are well documented, especially in mammalian species (Manikkam et al. 2013). However, BPA and DEHP cannot be considered as emerging contaminants because these chemicals have been in commercial use since mid-twentieth century. In fact, because of their high toxicity and endocrine-disrupting potential, the use of both BPA and DEHP is heavily restricted in most countries. Because of restrictions, a lot of concerted effort is currently being put forth to find suitable alternatives for BPA and DEHP (Coltro et al. 2013; Harmon and Otter 2018). Many of these alternative chemicals can be aptly defined as contaminants of emerging concern because they are relatively new as compared to their predecessors and their toxicity and endocrine-disrupting potential are less well characterized. Recent studies are consistently demonstrating that newer alternatives may also be toxic and possibly endocrine-disrupting.

The most famous substitute for BPA is bisphenol S (BPS) (Liao et al. 2012). Emerging evidences suggest that BPS could cause disruption of endocrine system in fish via several mechanisms. For example, long-term BPS exposure to zebrafish embryos caused a decrease in sperm count, plasma thyroid hormones, and testosterone in males, whereas plasma 17β-estradiol and vitellogenin levels were increased in both male and females (Naderi et al. 2014). BPS has also been shown to possess antagonistic activity against human androgen receptor and an agonistic activity for human estrogen receptors and pregnant X receptor (Molina-Molina et al. 2013), which suggest that BPS could induce these effects in freshwater fish too. Finally, BPS has also been shown to have a potent neuroendocrine effect in developing zebrafish (Kinch et al. 2015).

DINP (diisononyl phthalate) and DIDP (diisodecyl phthalate) are some of the many phthalates whose exposures have been increasing in recent years in many parts of the world due to severe restrictions on traditional phthalates such DEHP and dibutyl phthalate (DBP) (Boberg et al. 2011). Results are conflicting in terms of

endocrine activity of DINP and DIDP. For traditional estrogenic endpoints, DINP and DIDP were shown to be inactive in estrogen receptor competitive ligand-binding and mammalian- and yeast-based gene expression assays (Zacharewski et al. 1998), which suggests that risk from estrogenic activity of DINP is expected to be extremely low to practically nonexistent, even for fish species. Lack of steroidal activity of DINP and DIDP in fish is further supported by data generated in a multigeneration toxicity tests with Japanese medaka, which showed no treatment-related effects on hatching success, post-hatch survival, gonad differentiation, fecundity, and histology in F0, F1, or F2 generations (Patyna et al. 2006). Moreover, there were no effects of both compounds on testosterone metabolites and ethoxyresorufin-O-deethylase (EROD) activity in both males and females. Despite no reproductive effects of DINP in Japanese medaka, a recent study showed that DINP caused a significant reduction in 3β- and 17β-hydroxysteroid dehydrogenase in ovary and testes of tilapia (*Oreochromis mossambicus*) (Revathy and Chitra 2018). Although reproductive endpoints were not measured in this study, the impact of DINP on ovarian and testicular steroidogenesis suggests that reproduction in fish may be compromised by DINP exposure. Using estrogen-responsive ChgH-EGFP transgenic medaka (*Oryzias melastigma*) eleutheroembryos, another recent study has shown that DINP could be estrogenic in fish species (Chen et al. 2014). However, in the same study, DIDP did not show any estrogenic endocrine-disrupting activity.

Effect of chemicals on endocrine organs may also include effects on adipose tissue because it is now accepted that adipose tissue is a metabolically active endocrine system (Kershaw and Flier 2004). A recent in vitro study in three T3-L1 cell lines showed that emerging plasticizers DINP, DIDP, DEGDB (diethylene glycol dibenzoate), and TMCP (tri-m-cresyl phosphate) all enhanced the lipid accumulation (Pomatto et al. 2018). This effect was at least partly mediated through direct binding to transcriptional factors peroxisome proliferator activated receptor gamma (PPARγ) and retinoid-X-receptor-alpha (RXRα) and, hence, causing changes in expression of several genes involved in adipogenesis. These evidences suggest that emerging novel plasticizers may not necessarily be safer than their predecessors. Moreover, available data also suggests that broader approach to assess endocrine disruption is required by also including less well studied metabolic pathways along with traditional endocrine pathways.

Conclusion and Future Direction

Household and industrial effluents contain trace quantities of a variety of chemicals that are not included in routine environmental monitoring protocols. A majority of these contaminants are products of human utility; however, lack of proper disposal protocols and their propensity to resist treatment make them a threat to the aquatic ecosystem. Some of these contaminants of emerging concern can be broadly classified into PPCPs, surfactants and cleaners, halogenated biphenyl flame retardants, chemicals used in plant protection, plasticizers, and manufactured nanoparticles.

Scientific evidence suggests that these contaminants may persist in water bodies and are potent toxicants at concentrations even in the range of ng/L—affecting a wide range of physiological processes. Adverse effects, especially on the endocrine system, are well documented for some emerging pollutants, whereas more research is required for pollutants such as the manufactured nanoparticles. Since the endocrine system is crucial for growth and reproductive functions, any detrimental effects on the hormonal communication channel can severely compromise the fitness of a species to proliferate. Therefore, it is important that the effects of emerging contaminants, from cellular to ecological level, are understood and incorporated in environmental impact assessment protocols.

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Hormonally Active Agents: A Menace for Oogenesis and Fertility in Teleosts

19

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Abstract

Oogenesis, an amalgamation of the balanced network of neuroendocrine, endocrine, and autocrine/paracrine factors, is inevitable for the production of fertilizable female gamete and sustenance of a progeny on earth. In today's up-to-the-minute world, aquatic organisms are exposed to a myriad of environmental anthropogenic contaminants that share structural similarity with natural hormones, putting fish fertility and aquaculture industries at stake. A subset of such endocrine disruptors is the "xenoestrogens" that carry the ability to mimic 17β-estradiol, a natural female hormone, leading to adverse outcomes such as early puberty, premature ovarian failure, and impaired fertility. The present review seeks to elucidate the voyage of a fish oocyte undertaking the endocrine as well as autocrine/paracrine inputs. The effects of EDCs on various ovarian processes have been summarized along with the diverse signaling cascades that might participate to induce significant alterations at the receptor level, steroidogenic potential, maturational competence, ovulatory response, or even epigenetics of the ovary. Since reproduction heavily relies on the metabolic state of an organism, the potential influence of endocrine disruptors on oxidative stress and energy homeostasis has also been taken into consideration.

Keywords

Fish oogenesis · Endocrines and autocrines/paracrines · Endocrine disruption and action · Energy homeostasis · Epigenetics · Infertility

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Introduction

In the present milieu of the twenty-first century encompassing profound urbanization and medications, a large list of chemicals termed as *endocrine disruptors* that possess the ability to interfere with the natural course of hormone action exists. The majority of information on the impact of endocrine disrupting chemicals (EDCs) on wildlife comes from the aquatic vertebrates, more specifically the fish. EDCs are known to (a) mimic the effects of endogenous hormones; (b) antagonize the effects of endogenous hormones; (c) alter the pattern of synthesis and metabolism of natural hormones; and (d) modify hormone receptor levels (Soto et al. 2006). Since a functional endocrine system relies heavily on the balanced network of hormones regulating and coordinating bodily functions, any alteration in the normal homeostatic events due to such chemicals might prove detrimental. The ovary is one of the primary targets for EDCs that mimic estrogen, the female steroid hormone, leading to a surfeit of female reproductive disorders such as early puberty, premature ovarian failure, and impaired fertility (Diamanti-Kandarakis et al. 2010). To acquire a clear and in-depth knowledge about the extent of damage inflicted by various EDCs, either alone or in combination, and the mechanisms of action of these potentially harmful agents/drugs, we need continuous research initiatives to unknot the complex dialogs between neuroendocrine, endocrine, and juxtacrine modulators, the basis of reproductive endocrinology.

The present chapter is focused on the ovarian physiology in fish encompassing its journey from primordial germ cells to ovulation followed by an introduction to EDCs and their probable interference with diverse intraovarian signaling events and ovarian functions. Besides, the present review seeks to provide an update on the multiple potential targets altered due to EDC action thereby adversely influencing the oocyte quality and fertility. Considering the immense requirement of energy in the entire voyage of a germ cell to give rise fertilizable female gamete, a newly emerging concept in the field of reproductive dysfunction and female infertility has been undertaken. How EDCs modulate oxidative stress, inflammation, and/or cell death signaling and can increase the risk factors for altered energy homeostasis remains an area of active discussion and investigation. Finally, the latest techniques related to omics in the research market have been mentioned that have boosted our limitations via updated initiatives giving the researchers a platform for a better understanding and alleviation of the negative impacts of endocrine disruptors in near future.

Oogenesis in Fish: A Saga of Neuroendocrine, Endocrine, and Autocrine/Paracrine Factors

Reproduction is a blend of spermatogenesis and oogenesis that gives rise to a new life. Oogenesis encompasses a string of complex yet highly organized and systematically designed cascades that define the journey of an egg from primordial germ cells (PGCs) to full-grown ovarian follicles ready for ovulation. Ovary in teleosts is

compartmentalized by numerous septa lined by germinal epithelium and contains nests of oogonia. Contrary to the situation in mammals, oogonia in teleosts keep on proliferating in the adult ovary thereby renewing the stocks of young oocytes and follicles (Jalabert 2005). The endodermally derived oogonia multiply mitotically to replenish its number and transform into non-yolky primary oocytes embarking the inception of meiosis. However, unlike mammals, fish primary oocytes remain arrested at the prophase of meiosis I (prophase I arrest), followed by a prolonged phase of massive growth (vitellogenesis) whereby it accumulates nutritional reserves and maternal determinants for developing embryo (Jalabert 2005). After the completion of the growth phase, oocytes become responsive to maturation inducing steroid (MIS), an event popularly known as maturational competence, and become ready for the next phase of oogenesis, i.e., the resumption of meiosis or final oocyte maturation (OM), marked by germinal vesicle breakdown (GVBD) and formation of first polar body, collectively termed as oocyte maturation. The so formed secondary oocyte remains arrested at metaphase of second meiotic division (MII arrest), undergoes ovulation, and if fertilized gives rise to the diploid zygote (Nagahama and Yamashita 2008).

Endocrine Regulation of Ovarian Dynamics

Ovarian development in teleosts is under the tight surveillance of neuroendocrine and endocrines (Reinecke 2010). Hypothalamic gonadotropin-releasing hormone (GnRH) acts on the pituitary (Fig. 19.1) to stimulate the secretion of gonadotropins (GTHs), namely, follicle-stimulating hormone (FSH) and luteinizing hormone (LH). Binding of FSH to its cognate receptor (FSHR) at follicular cell surface promotes follicular growth, while LH (LHR) drives the oocyte toward final maturation (Nagahama and Yamashita 2008). The endocrine regulation of follicular growth, OM, and formation of fertilizable female gamete are discussed briefly in this section.

Oogonial Proliferation

Oogonia, after transformation from PGCs, undergo repeated mitotic divisions to form oogonial nests that remain in association with pre-granulosa cells in teleost. This is followed by the transition of oogonia to primary oocytes wherein the oocytes are surrounded by granulosa cells, basement membrane, and theca cells and are further arrested at the diplotene stage of the first meiotic prophase (Le Menn et al. 2007). Although the hormonal mechanisms regulating oogonial proliferation and oocyte recruitment is not well dissected in vertebrates, participation of GTHs, steroids 17β-estradiol (E2) and 17,20β-dihydroxy-4-pregnen-3-one (17,20β-P), and growth factors during the proliferation phase of oogenesis in teleosts has been proposed earlier (Lubzens et al. 2010; Kagawa 2013). Primarily, the oogonial population proliferates mitotically under the stimulation of E2, while commencement of meiotic division is characterized by two important hallmark events, viz.,

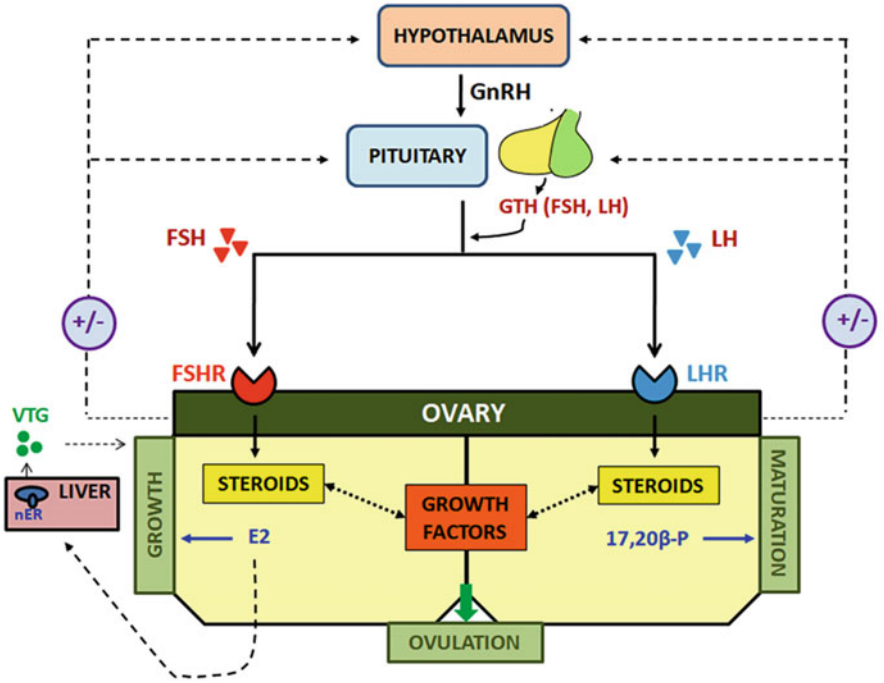

Fig. 19.1 An overview of hypothalamic-pituitary-gonadal-liver axis governing follicular growth and maturation in teleost ovary

formation of synaptonemal complexes (SCs), a marker of homologous chromosomal synapse and synthesis of Spo11, the protein involved in the generation of double-stranded DNA breaks at early stages of meiotic recombination (Fig. 19.2). Interestingly, increased abundance of SCs and Spo11 in 17,20β-P-treated ovarian fragments indicates a role of maturation inducing steroid (MIS) in inducing meiosis at early stages of oogenesis in teleosts (Yaron and Levavi-Sivan 2011).

Follicular Growth and Development

In teleost, ovarian growth is subdivided into the primary and secondary growth phases; while the major organelles to be used at later stages are being synthesized at the primary growth, synthesis and incorporation of hepatically derived yolk materials into growing oocytes are the major events associated with the secondary growth (Kagawa 2013). Although the primary growth phase is believed to be independent of GTHs, expression of FSH β and LH β mRNA and proteins in the pituitary of gilthead seabream at this phase cannot overrule the possible regulation by GTHs (Wong and Zohar 2004). After completion of primary growth, the ovarian development steps into the next segment, i.e., synthesis of vitellogenin (VTG) in the

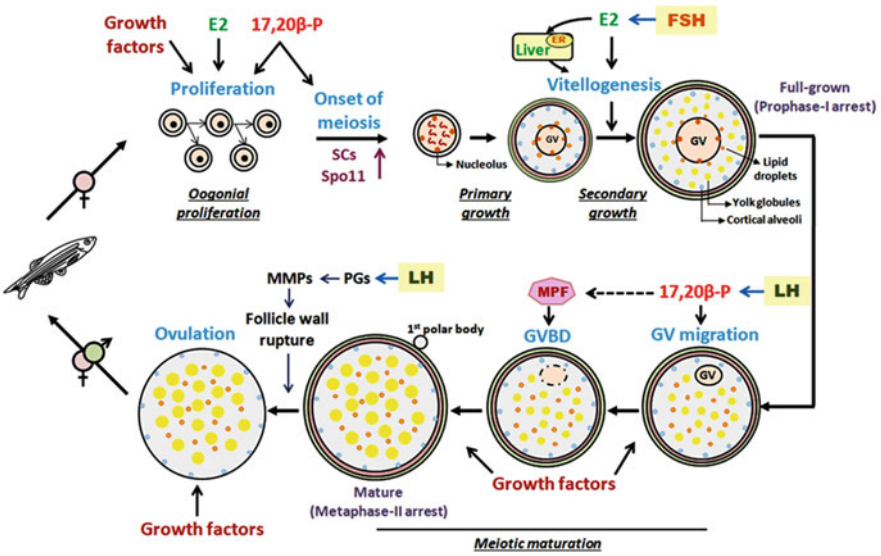

Fig. 19.2 The journey of a teleost egg: an amalgamation of neuroendocrines, endocrines, and autocrine/paracrine entities

liver and its sequestration by the growing oocytes (Fig. 19.2). FSH binding to its cognate G protein-coupled receptor at theca cell plasma membrane stimulates the biosynthesis of E2 in the granulosa cells. E2 from follicular cell layers traverses to the liver through circulation, enters into the hepatocytes, and binds to nuclear estrogen receptors (ER), predominantly to ERβ subtype (Nelson and Habibi 2013). This ligand-receptor complex dimerizes, translocates to the nuclear compartment, and attaches itself to estrogen-responsive elements (ERE) in the promoter region of VTG and other E2-responsive genes and initiates transcription (Okumura et al. 2002). Before their release into the bloodstream, VTG undergoes folding and extensive posttranslational modification such as lipidation, phosphorylation, and glycosylation. VTG in circulation is taken up by the growing oocytes through receptor-mediated endocytosis and undergoes molecular (proteolytic) cleavage by enzymes like cathepsin D to produce lipovitellins and phosvitin, essential components of egg yolk and deposited as yolk granules in the ooplasm (Hara et al. 2016).

Maintenance of Prophase Arrest

The involvement of cyclic nucleotides in the maintenance of meiotic prophase arrest is a long-standing verity in vertebrate (mammalian or piscine) oocytes. In this section we summarize the role of two essential cyclic nucleotides, namely, 3′,5′-cyclic adenosine monophosphate (cAMP) and 3′,5′-cyclic guanosine

monophosphate (cGMP), in regulation of follicular events with special emphasis on fish ovary.

cAMP—A Prime Regulator of Cell Cycle Progression

Immature oocytes at prophase I possess high cAMP, and forced elevation of this second messenger within the oocyte has been shown to attenuate spontaneous as well as 17,20β-P-induced OM (Conti et al. 2002; Nagahama and Yamashita 2008). Oocyte may produce its own pool of cAMP or may receive it from follicular cells through gap junctions. Disruption of gap junctions can initiate resumption of meiosis in follicle-enclosed oocytes indicating functional relevance of the communication between follicular cell layer and the oocyte to ensure prophase I arrest (Cerda et al., 1993). The endocrine connection behind the maintenance of elevated intra-oocyte cAMP and meiotic arrest has been explored extensively in the recent past. E2 maintains cell cycle arrest at meiotic G2-M1 transition and prevents precocious oocyte maturation in several teleosts. Discovered first in cancer cells (Xu et al. 2019), E2 binding to its novel G protein-coupled estrogen receptor, GPER1 (or GPR30), at oocyte surface promotes intra-oocyte adenylate cyclase (Ac) activity and cAMP synthesis through $G_{\alpha s}$ to prevent meiotic G2-M1 transition (Mehlmann 2005; Pang and Thomas 2010; Pan and Li 2019). Additionally, studies in teleosts implicate E2 in transactivation of EGFR and subsequent phosphorylation of ERK1/2 in maintaining meiotic arrest reportedly in a cAMP-independent manner (Peyton and Thomas 2011). Available information indicates that E2/GPER/$G_{\beta\gamma}$-mediated activation of Src family kinases can promote the upregulation of matrix metalloproteinase (MMP) which in turn releases the heparan-bound EGF (HB-EGF). HB-EGF binding to EGFR at the oocyte surface promotes MAPK3/1 phosphorylation, possibly through Ras/Raf/MEK signaling cascade, and prevents spontaneous maturation in zebrafish (Peyton and Thomas 2011). A more recent study has established the crucial role of progesterone receptor membrane component 1 (PGMRC1) in regulating the expression of EGFR, which in turn, influences the inhibitory effect of estrogens on the resumption of meiosis through GPER in this species (Aizen and Thomas 2015).

In search of the primary transducer of cAMP-mediated signaling, PKA (a serine-threonine kinase) was implicated to be the major candidate in ensuring cell cycle arrest in the majority of fish oocytes. Binding of two cAMP molecules to each regulatory subunit of PKA leads to dissociation of the catalytic subunits and subsequent phosphorylation of substrate proteins (Francis and Corbin 1994). Negative correlation between PKA activation and attenuated maturation promoting factor (MPF) activation to promote OM has been reported extensively (Nagahama and Yamashita 2008; Das et al. 2017). Importantly, one proposed mechanism of PKA action in *Xenopus* oocytes is inhibitory phosphorylation of Cdc25, a dual-specific phosphatase that removes the inhibitory phosphorylation of cdc2 at Thr14 and Tyr15 thereby activating cyclin B-cdc2 complex (Duckworth et al. 2002). On the contrary, elevated PKA activity can activate Wee1, maintaining MPF in an inactive state in mammalian oocytes (Han and Conti 2006). Moreover, active PKA negatively regulates polyadenylation of maternally stored *c-mos* mRNA and synthesis of Mos

protein and ensures cell cycle arrest in mammalian oocytes (Lazar et al. 2002). Although the potential targets of PKA are less clearly understood in fish oocytes, available information consists of some valuable additions in this arena of investigation (Haider and Baqri 2002; Mishra and Joy 2006; Khan and Maitra 2013; Maitra et al. 2014). Interestingly, the potential involvement of the cAMP/PKA signaling pathway in the regulation of cdc25 in the teleost ovary is evident from the fact that forced elevation of intra-oocyte cAMP attenuates MIS-induced OM and maintains Cdc25 in a hyper-phosphorylated inactive state in perch oocytes. Moreover, pharmacological inhibition of endogenous PKA activity by either H89 or PKI-(6–22)-amide is sufficient to promote meiosis resumption in this species (Khan and Maitra 2013). Apart from PKA inhibition of direct regulators of MPF activation, the interaction between PKA and MAPK3/1 has also been studied in piscine oocytes. The requirement of MAPKs during steroid or growth factor (insulin/IGFs) stimulation of meiotic maturation has been reported earlier (Liang et al. 2007). High cAMP/PKA has been shown to prevent MAPK phosphorylation in perch, *Xenopus*, and mammalian oocytes (Matfen et al. 1994; Lu et al. 2001; Khan and Maitra 2013). In perch oocytes, while PKA inhibition triggered MAPK activation, inhibition of MAPK through MEK inhibitor U0126 could successfully reverse H89 action on OM in this particular species (Khan and Maitra 2013). Further, experiments in zebrafish oocytes revealed that although MAPK activation is not a prerequisite for initiation of meiotic maturation in this species, priming with cell-permeable cAMP or cAMP modulators attenuates insulin action on GVBD response and MAPK phosphorylation. Collectively, these data suggest a potential cross talk between cAMP/PKA and MAPK activation in insulin-stimulated zebrafish oocytes in vitro (Maitra et al. 2014).

cGMP Modulation of Follicular Events

In addition to cAMP, the role of cGMP in the regulation of oocyte maturation has received considerable attention in the past few years. Nitric oxide (NO), a free radical synthesized in biological systems, binds to its cognate receptor the soluble guanylate cyclase (sGC) and catalyzes the formation of the second messenger (cGMP) from guanosine triphosphate (GTP). The existence of locally produced NO/cGMP in the mammalian and teleost ovary has been depicted over the years of advancing research. Physiologically, NO is produced by the oxidation of L-arginine in an NADPH-dependent mechanism catalyzed by a heme-containing enzyme nitric oxide synthase (NOS) (Palmer and Monconda 1989). Although differential expression of different NOS isoforms, namely, neuronal (nNOS) or NOS1, inducible (iNOS) or NOS2, and endothelial (eNOS) or NOS3, is well studied in mammalian ovary (Tamanini et al. 2003; Basini and Grasselli 2015), information from teleost ovary is also coming forth. Differential expression of all three NOS isoforms at various stages of ovarian folliculogenesis has been shown to correlate well with annual breeding cycle in catfish, *Heteropneustes fossilis* (Tripathi and Krishna 2003) and *Clarias batrachus* (Singh and Lal 2014). These observations

indicate the physiological importance of the NO/NOS network within the teleost ovary. cGMP attenuation of oocyte-specific PDE3 resulting in accumulation of cAMP has significant influence in maintaining prophase-I arrest in mammalian oocytes (Conti et al. 2002; Jaffe and Egbert 2017). Conversely, identification of specific NO targets and potential involvement of NO/cGMP signaling in regulation of meiosis resumption in fish oocytes is from recent past (Nath et al. 2017; Li et al. 2018b). Our recent data demonstrate that elevated NO inhibition of meiotic G2-M1 transition in perch oocytes involves activation of PKA congruent with attenuation of cdc25 and Mos-MAPK signaling events (Nath et al. 2017). More recently the functional significance of NOS/NO/sGC/cGMP cascade in regulating follicular cAMP level to modulate OM in this seasonal breeder teleost species has been demonstrated by us (Nath et al. 2019). Importantly, NO donors like SNP prevent OM potentially through elevated cGMP synthesis and show a positive relation with PDE3 inhibition, cAMP accumulation, and PKA activation. Besides, expression of all three NOS enzymes and four soluble sGC isoforms at mRNA level and a dual regulatory nature of NO/sGC/cGMP pathway in either activation or inhibition of meiotic maturation in zebrafish ovary has been reported more recently (Li et al. 2018b).

Importantly, granulosa cell-derived peptide hormone ligands (natriuretic peptide type C, NPPC) can interact with membrane-bound or particulate guanylate cyclase receptor (natriuretic peptide receptor 2, NPR2) to elevate cGMP accumulation in ovarian follicles (Vaccari et al. 2009; Zhang et al. 2010; Robinson et al. 2012). Preovulatory LH surge or hCG treatment has a negative influence on NPPC/NPR2/cGMP cascade allowing meiosis resumption in murine follicle-enclosed oocytes (Kawamura et al. 2011; Conti et al. 2012). Recently, the presence of NPPC and NPR2, at both mRNA and protein levels, as well as elevated cGMP synthesis in exogenous NPCC-treated follicle-enclosed oocytes has been demonstrated in zebrafish (Pang and Thomas 2018). Taken together, these findings indicate the importance of cGMP in intercellular communication within ovarian follicle including modulation of cyclic nucleotide-mediated signaling events, phosphodiesterases (PDEs), and protein kinases.

Resumption of Meiotic Maturation

A radical shift in follicular steroidogenesis before the onset of OM primarily occurs under the influence of LH to produce more of MIS over E2 in the fish ovary (Senthilkumaran et al. 2004; Rajakumar and Senthilkumaran 2020). While, in the majority of teleosts studied so far, 17,20β-P has been shown to act as a potent MIS and stimulate the heterodimeric cyclin B-cdc2 complex (the histone H1 kinase activation) to promote GVBD response, 17α,20β,21-trihydroxy-4-pregnen-3-one (20β-S) has been identified as naturally occurring MIS in sciaenids and marine perciform species (Nagahama and Yamashita 2008). MIS binding to membrane progestin receptors (mPRs), more specifically to mPRα, stimulates a pertussis toxin-sensitive inhibitory G protein (G_i), resulting in decreased intracellular cAMP

level, inactivation of protein kinase A (PKA) triggering a robust increase in histone kinase activity or maturation promoting factor (MPF) activation and resumption of meiotic G2-M1 transition and GVBD response (Zhu et al. 2003; Nagahama and Yamashita 2008; Das et al. 2017). In *Xenopus* and also in mammalian oocytes, downregulation of PKA promotes translational activation of masked cyclin B mRNA and its binding to preexisting cdc2, which undergoes Thr161 phosphorylation (activation) by a CDK-activating kinase (CAK) forming active MPF to promote G2-M1 transition (Jessus and Ozon 2004; Mehlmann 2005). However, more extensive studies in G2 arrested perch (*Anabas testudineus*) and rainbow trout oocytes revealed the existence of pre-MPF comprising of inactive cdc2 phosphorylated at T14/Y15 (inhibitory phosphorylation) bound to cyclin B. Contrary to the situation in goldfish oocytes (Kajiura-Kobayashi et al. 2000), MIS-induced meiosis resumption in perch oocytes involves decreased PKA activity congruent with cdc25 activation allowing dephosphorylation of cdc2 at T14/Y15. Besides, de novo synthesis of Mos (c-Mos proto-oncogene product) protein due to MIS stimulation promotes Myt1 kinase (homolog of Wee1) inhibition potentially through elevated MAPK (ERK1/2) activation (Basu et al. 2004; Priyadarshini et al. 2009; Khan and Maitra 2013). So formed active MPF resumes meiosis through histone H1 kinase activation, chromosome condensation, spindle formation, GVBD, and first polar body exclusion (Fig. 19.2).

Ovulation

Release of mature ova from surrounding theca-granulosa cell layer during ovulation involves mechanical retraction of microvilli congruent with contraction and localized enzymatic rupture of the follicle envelope (Lubzens et al. 2010). A tightly coordinated cascade of events, principally under the aegis of preovulatory LH surge, plays a conserved regulatory role before initiation of ovulatory process across species. Extensive studies in mammalian and nonmammalian models have established the functional relevance of the prostaglandin synthesizing enzyme (PTGS) catalyzing the rate-limiting step of prostaglandin (PG) synthesis prior to ovulation. Importantly, LH promotes prostaglandin-endoperoxide synthase 2a (*ptgs2a*) and nuclear progestin receptor (*n*PR) expression in a manner sensitive to cAMP/PKA signaling in teleost ovary (Tang et al. 2017). While nPR is required to synthesize *ptger4b* (the cognate receptor of PGE2), binding of PGE2 to this receptor at follicular cells promotes ovulation potentially through up regulation of matrix metalloproteinases (MMPs) and other ovulatory genes (Takahashi et al. 2017; Tang et al. 2017). Before ovulation, series of proteinases are required to induce the rupture of the follicular cell layer, and five different MMPs, namely, adam8b (a disintegrin and metalloproteinase domain 8b), adamts8a (a disintegrin and metalloproteinase with thrombospondin motif 8a), adamts9, mmp2 (matrix metalloproteinase 2), and mmp9, have been shown to trigger ovulation in zebrafish (Liu et al. 2018). While nPR knockdown facilitates sharp decline in expression of MMP genes in zebrafish ovary, MIS action through nPR could upregulate multiple MMPs in preovulatory

follicles (Liu et al. 2018). However, elucidation of downstream factors and their underlying mechanisms mediating the action of LH, MIS, and/or PG in the regulation of ovulation in teleost is still lacking.

Autocrine/Paracrine Aspects Underlying Ovarian Functions

Though it is well-known that follicular growth and oocyte maturation are dependent primarily on the two pituitary-derived gonadotropins, FSH and LH, pictures emerging from recent studies have put forward the potential involvement of local autocrine/paracrine factors, more specifically growth factors, to integrate with endocrine inputs and control ovarian and follicle development (Albertini et al. 2001; Gilchrist et al. 2004; Biswas and Maitra 2017). These factors include (but not limited to) mainly insulin-like growth factors (IGFs) (Xiai et al. 1994; Reinecke 2010; Li et al. 2015), epidermal growth factors (EGFs) (Su et al. 2010; Park et al. 2004), and members of transforming growth factor-β (TGF-β) superfamily (Peng 2003; Knight and Glister 2006). Though much of information on regulation of ovarian development and function by various local factors released from both the somatic components of the follicle and oocytes are from studies in mammals, (Hsueh et al. 2000; Hillier 2001), relatively limited research has been done in nonmammalian vertebrates. The current section provides a brief update on the participation of these nonsteroidal factors in the ovarian processes in teleost (Fig. 19.3).

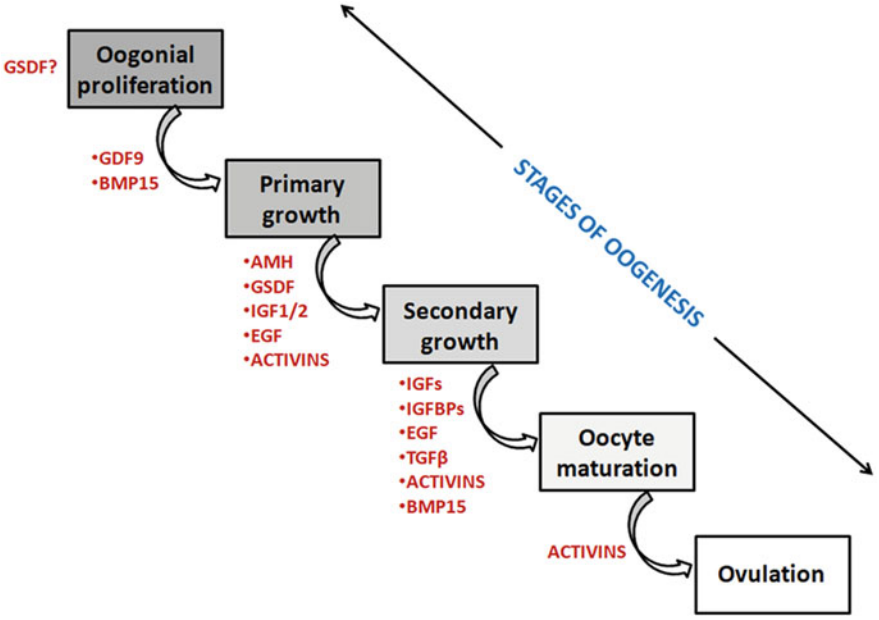

Fig. 19.3 Participation of intraovarian autocrine/paracrine factors at various stages of oogenesis

Several growth factors have been reported to participate in the regulation of oocyte growth. Gonadal soma-derived factor (GSDF), a novel member of TGF family, is a teleost-specific growth factor that has been cloned from rainbow trout recently. Expression of GSDF in the genital ridge somatic cells having direct contact with PGCs of rainbow trout embryos and in gonadal tissues of adults suggests its potential role in regulation of oogonial proliferation (Lubzens et al. 2010). Two other members of the TGF-β family, viz., growth and differentiation factor 9 (GDF9) and bone morphogenetic factor 15 (BMP15), are expressed in oocytes and regulate primary ovarian follicle development (Liu and Ge 2007). Interestingly, anti-Mullerian hormone (AMH), GSDF, IGF1, and IGF2 have been implicated in the transition of oocytes into secondary growth in teleost ovary (Lubzens et al. 2010; Lokman et al. 2006). In addition to its mitogenic activity, IGF1 (systemic or granulosa cell-derived) has been shown to regulate P450 aromatase expression or steroid production in ovarian follicles at the onset of vitellogenesis (Nakamura et al. 2004; Kagawa 2013), whereas activins and EGFs appear to be crucial in promoting follicular growth (Calp et al. 2003; Wang and Ge 2003, 2004a). Besides, acquisition of maturational competence relies heavily on nonsteroidal factors; more specifically the participation of IGFs and IGFBPs in the acquisition of maturational competence and oocyte maturation has been demonstrated earlier (Kamangar et al. 2006). Interestingly, the gonad-specific follicular IGF ligand, IGF3, can serve as a crucial mediator in gonadotropin-induced meiosis resumption in zebrafish oocyte (Li et al. 2015). Moreover, ovarian factors, specifically the EGFs and TGF-β superfamily members, could significantly enhance GTH-induced meiotic maturation in the teleost ovary (Pang and Ge 1999; Wang and Ge 2004a, b; Ge, 2005). Our recent data demonstrate potential synergism between IGF1 and MIS to overcome the E2 inhibition of OM in zebrafish full-grown oocytes in vitro (Das et al. 2016). Furthermore, intraovarian factors are also reported to participate in the culminating physiological event of fish eggs, i.e., ovulation. Recent updates support the notion of IGF3 serving as a mediator of hCG-induced ovulation in zebrafish (Li et al. 2018a).

Endocrine Disruption: What, Where, and How?

In today's urbanized world, endocrine disruptors are enlightened as a topic of paramount importance because of their multidimensional hazardous effects from higher to lower trophic levels. Industrial effluents, particulate matters, and household chemicals are some of the critical anthropogenic sources of EDCs (Vega-Morales et al. 2013). An exogenous agent that can interfere with synthesis, secretion, transport, metabolism, binding action, or elimination of natural blood-borne hormones that play important role for maintenance of homeostasis, reproduction, and developmental process is classified as endocrine disruptor (Diamanti-Kandarakis et al. 2009). US Environmental Protection Agency or EPA has defined EDCs as environmental contaminants that can potently interfere with the endocrine homeostasis leading to reproductive anomalies, developmental deformities with augmented cancer risk, and immune and nervous system dysfunction.

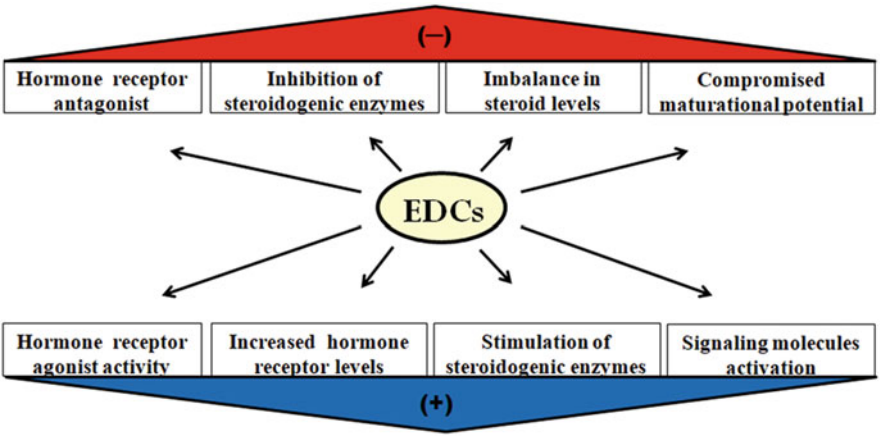

Fig. 19.4 Diverse mechanisms through which endocrine disruptors can alter hormonal regulation of female reproduction

For better understanding, before perturbing into mechanistic regulation, it is important to get an overall idea about the classifications of EDCs that can help us to visualize their ubiquitous distributions as well as devastating effects on humans and wildlife. EDCs are primarily categorized into heavy metals (Cd, Hg, etc.), pesticides (DDT, methoxychlor, atrazine, tributyltin, etc.), fungicide (vinclozolin), persistent organic pollutants (halogenated aromatic hydrocarbons and polybrominated diphenyl ethers), pharmaceuticals and sewage effluents containing synthetic steroidal estrogens, plasticizers (bisphenol A), nonionic surfactants (alkylphenol ethoxylates), paper mill effluents, and so on. Relative abundance of EDCs has been shown to vary widely, from nanograms to milligrams level, in river and wastewaters, landfill leachates, sewage effluents, and also in bottled water samples (Kudłak et al. 2015). Nonetheless, water bodies are the most vulnerable component of the environment for direct exposure to EDCs.

Majority of endocrine disruptors can activate or antagonize hormone receptors, modulate their expression pattern, and alter signaling cascades in hormone-responsive cells (Fig. 19.4). Besides, EDC modulation of hormone biosynthesis, transport, distribution, and metabolism or clearance and epigenetic regulation has been reported earlier (Diamanti-Kandarakis et al. 2009). EDC binding, albeit with much lower affinity than endogenous hormones, to classical nuclear estrogen receptors (ERα and ERβ), nonclassical estrogen receptor GPR30, progestin receptor (PR), androgen receptor (AR) or even glucocorticoid and mineralocorticoid receptors (GR and MR), peroxisome proliferator-activated receptors (PPARs), retinoid X receptor (RXR), and aryl hydrocarbon receptor (AhR), has been reported earlier (Denslow and Sepúlveda 2007; Swedenborg et al. 2009; Sakkiah et al. 2018). Given that these receptors play important role in the regulation of reproduction, metabolism, immune function, and cancer in a tissue-specific manner (Huang et al. 2010), for comprehensive exploration upon the pernicious role of EDCs and their

metabolites, a detailed study on the regulation of receptors and downstream signaling events has been an area of intense research initiatives.

EDCS at the Crossroads of Diverse Signaling Cascades

As EDCs can interfere with processes regulated by endogenous hormones, it can lead to disruption to ovarian functions leading to adverse outcomes such as anovulation, infertility, estrogen deficiency, and premature ovarian failure (Craig et al., 2011). A major subset of EDCs capable of eliciting estrogenic effects is the "xenoestrogens" (XEs) that has received a global concern for putting female reproduction at stake. XEs can exert their influence at both genomic and non-genomic level thereby activating a wide variety of signaling pathways and downstream kinases (Zhang et al. 2014b). The current section summarizes the multiple layers of interference by EDCs, more precisely XEs, under genomic and non-genomic behavior.

Modulation of Genomic Pathway

The classical genomic pathway is mediated via estrogen receptors (ERs). Typically, two subtypes of ER are prevalent, ERα (ESR1) and ERβ (ESR2), each of which is encoded by two different genes on distinct chromosomes. A typical ER contains four functional domains, viz., activation function 1 (AF-1), DNA-binding domain (DBD), ligand-binding domain (LBD), and activation function 2 (AF-2) (Ascenzi et al. 2006). Before ligand binding, ER is maintained in a competitive inactive state with chaperone proteins that prevent its ubiquitination-induced degradation. Upon ligand binding, ER forms homodimers or heterodimers (Fig. 19.5a) which then bind to estrogen response element (ERE) and recruit different cofactors ultimately altering target gene expression (Stender et al. 2010; Smith and Toft 2015). XEs have the full scope to cast a negative impact on the genomic pathway by interfering with unliganded ER-cochaperone machinery, liganded ER conformation, ER-DNA interaction, or cofactor recruitment.

ER-Cochaperone Machinery

Hsp90, a well-known chaperone protein, interacts with the ER to regulate its receptor activity and target gene expression. It aids in refolding the LBD to a loose conformation to make it available for ligand binding and also facilitates ER translocation to the nucleus with the assistance of additional cochaperones. Posttranslational modification and the expression pattern of cochaperones are also known to influence steroid receptor activity (Smith and Toft 2015). Blocking the action of Hsp90 has been shown to result in the reduction of steroid receptors (Liu and Defranco 1999). Interestingly, researchers have proved that XEs can also regulate

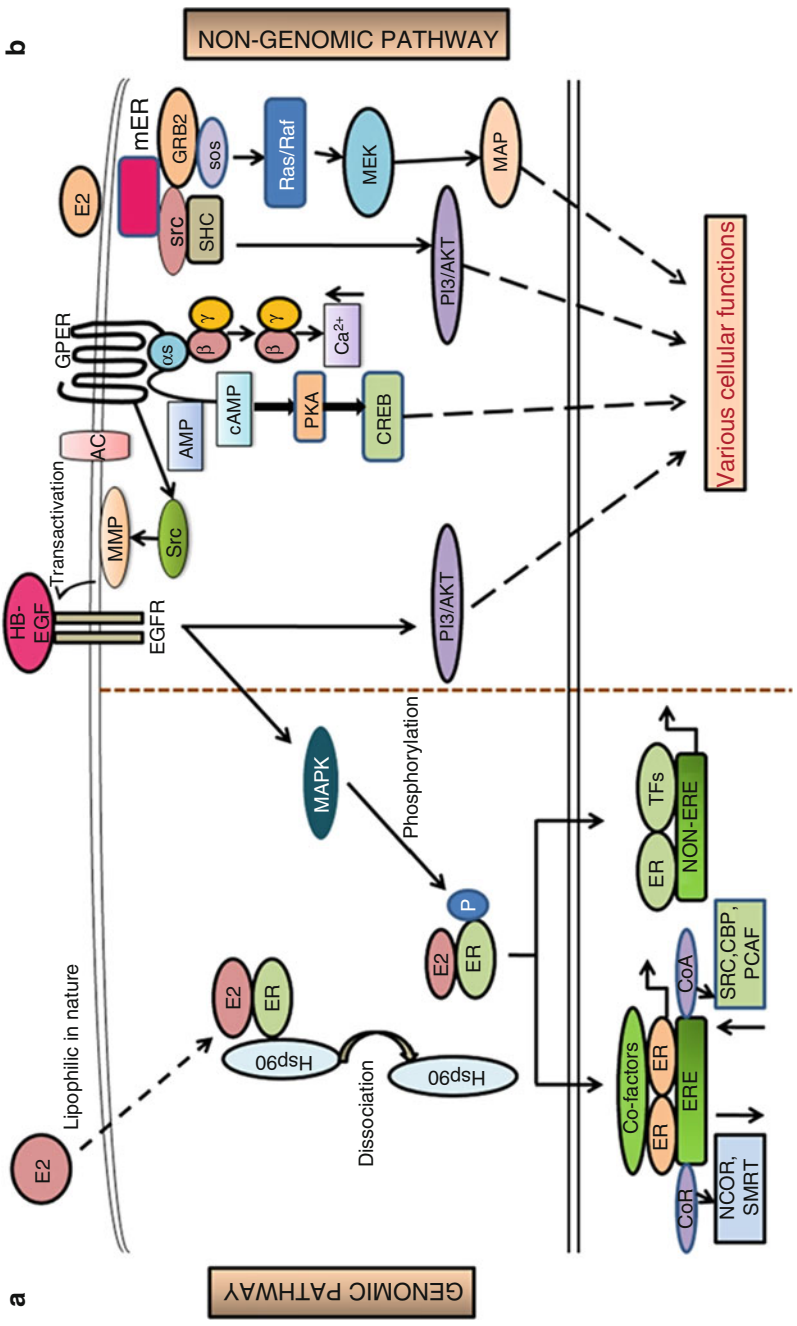

Fig. 19.5 Cellular mechanism of estrogen action through genomic (a) and non-genomic (b) pathways. The classical mode of steroid action through genomic pathway envisages direct binding of ligand-ER complex to response elements upstream to transcription start sites to promote expression of E2-responsive genes. Conversely, non-genomic action does not involve direct binding to DNA and is mediated by GPER1 (GPR30) and mER. GRB2: growth factor receptor-bound protein 2; SHC, (Src homology 2 domain containing) transforming protein; SOS, Son of sevenless

the expression of chaperones. Both natural estrogen (E2) and synthetic estrogen (DES and BPA) have been shown to upregulate Hsp90. Noteworthily, while E2 does so via regulating heat shock factor 1, BPA upregulates Hsp mRNA via modulating protein kinase C activation (Papaconstantinou et al. 2001; Kekatpure et al. 2009).

Conformational Change in Ligand-Bound ER

The LBD region of ER comprises a large ligand-binding cavity which allows a diverse set of chemicals to be captured by the ER (Shanle and Xu 2011). Functional domains H12 and AF-2 in LBD play a key role in ligand-dependent activation of ER. E2 binding promotes H12 refolding, thereby revealing the interacting surface in AF-2. In addition to its effect on the ER-chaperone complex, XEs can also modulate LBD folding. A wide spectrum of XEs hinders proper folding of H12 thereby inducing different degrees of agonist, antagonist, and partial agonist effects in stimulating ER (Ascenzi et al. 2006; Zhang et al. 2014b). The role of coactivators and corepressors in ligand-dependent transcriptional activation by ER is also of particular importance in the induction of diverse gene expression (Xu et al. 1999). While agonist binding induces target gene expression by recruiting coactivators, antagonists on the other hand recruit corepressors to repress the target gene (Hall et al. 2002). Steroid receptor coactivator (SRC) family, CREB-binding protein (CBP) family, CBP-associated factor (PCAF), etc. comprise the coactivators, whereas nuclear receptor corepressor (NCOR), silencing mediator for retinoic acid receptor and thyroid hormone receptor (SMRT), etc., falls under the classical corepressors. Intriguingly, aforementioned cofactors are reportedly susceptible to XE stimulation suggesting modulation of transcriptional regulation by ER under potential threat to EDCs (Inoshita et al. 1995; Xu et al. 2007; Salian et al. 2009).

ER-DNA Interaction

Under the stimulation of XEs and their binding to ER, dysregulated transcriptional regulation can occur by recruiting ER to the promoter region of target genes through direct (through EREs) or indirect (through activator protein 1 (AP1), SP1 transcription factor) ER-DNA interaction (Stender et al. 2010).

Modulation of Non-genomic Pathway

Membrane-initiated non-genomic effects came to the forefront due to the estrogen-induced rapid signaling that occurs within seconds to minutes. Current research paved a new way from genomic to the non-genomic mode of signaling and their cross talk. Multiple signaling molecules participate under non-genomic pathways such as several kinases, transcription factors, and adaptor proteins (Fig. 19.5b) and are mediated through membrane receptors, which include membrane-anchored ER,

orphan receptor GPER, receptor tyrosine kinase EGFR, and IGF1R (Kelly and Levin 2001).

The discovery of mER raised a question on its localization and translocation as ER is not an intrinsic membrane protein. Later researchers revealed that lipid modifications and interaction with scaffold and adaptor proteins localize ER in the membrane (Lu et al. 2004; Song et al. 2004). At the membrane, liganded ER associates with a chain of diverse signaling molecules to mediate downstream signaling. These include, but not limited to, G protein, Src kinase, Ras, CSK, IGF1R, EGFR, insulin receptor, and B-Raf (Razandi et al. 2003). Liganded ERs dimerize and associate with Gα and Gβγ to induce calcium influx and cAMP generation leading to activation of proximal (Src, PI3K) and distal (ERK) kinases (Levin 2009). Similarly, GPER-induced Src kinase activation promotes metalloprotease-mediated shedding of heparin-binding EGF (HB-EGF) thereby transactivating the EGFR pathway (Maggiolini and Picard 2010). This further induces intracellular responses, such as PI3K and ERK activation. Cassettes of XEs can cross talk with either of the membrane receptors, GPER or mER, and activate downstream kinases or transcription factors to elicit physiological functions in a deregulated manner (Zhang et al. 2014b).

Additionally, few other hormone receptors, including PGR, FSHR, and LHR, have a significant contribution in female reproduction and might undergo alteration under EDC-induced ovarian toxicity (Craig et al. 2011). Though extensive studies have indicated the detrimental effect of EDCs on the hormone receptors expression and downstream signaling pathways, elaborative research is still required to unveil the physiological relevance of EDCs modulation of receptor function in the vertebrate ovary.

EDCS: Potential Threat to Fish Eggs and Fertility

A growing number of endocrine disruptors have been shown to alter ovarian follicular events either by altering the availability of ovarian hormones or activating major hormone receptors in the ovary, casting a negative influence altogether on folliculogenesis, oocyte maturation, and ovulation. Though studies in piscine models are also very promising, they are still limited in number and hold lacunae. In this section, we will expand our understanding of the influences of some popular EDCs on the crucial events associated with fish egg fertility (Table 19.1).

EDC Modulation of Ovarian Receptors and Hormone Synthesis

Beginning with GTH receptors (FSHR and LHR) that are the direct mediators of GTH action on the ovarian follicular layers, numerous studies in mammalian models have demonstrated that exposure to EDCs can suppress the transcript abundance of GTH receptors and alter downstream signaling cascades. To name a few, HPTE (bis-hydroxymethoxychlor) which is an active metabolite of the well-known

Table 19.1 Potential influence of selected EDCs on various ovarian physiological processes

Physiological process	EDCs	Mode of actions/potential impacts	Target sites	Organism	References
Receptor modulation	BPA	*Alteration in fshr and lhcgr transcript abundance*	Ovary	Zebrafish	Santangeli et al. (2016), Biswas et al. (2020)
	DiNP	*fshr*↓, *lhcgr*↓, reduced GSI and fecundity	Ovary	Zebrafish	Santangeli et al. (2017)
	BPA	*esr1*↓, *esr2a*↓, *disrupted ERα/ERβ homeostasis*	Ovary	Zebrafish	Santangeli et al. (2016), Biswas et al. (2020)
	2,4-DCP	*esr1*↑, *esr2a*↑	Female liver	Zebrafish	Ma et al. (2012)
	BPA, 4-nonylphenol, tetrachlorobisphenol A, tetrabromobisphenol A	Inhibition of meiotic maturation through GPER	Full-grown oocyte	Zebrafish	Fitzgerald et al. (2015)
Growth factor modulation	DEHP	*bmp15*↑	Ovary	Zebrafish	Carnevali et al. (2010)
	BPA	Suppressed expression of Igfs (*igf1* and *igf2*) and its receptor	Ovary	Rainbow trout	Aluru et al. (2010)
	BPA, 4-nonylphenol, tetrachlorobisphenol A, tetrabromobisphenol A	Inhibition of meiotic maturation through EGFR transactivation	Full-grown oocyte	Zebrafish	Fitzgerald et al. (2015)
Steroidogenesis	BPA	*StAR*↑, *cyp11a1*↑	Ovary	Zebrafish	Santangeli et al. (2016)
	BPA	Inhibitory effect on *cyp19a1a*, *StAR*, *cyp11a1*, *cyp17a1*	Ovary	Rare minnow, Zebrafish	Liu et al. (2012), Biswas et al. (2020)
	EE2	*StAR*↓, *3β-hsd*↓, *cyp17a1*↓	Ovary	Rare minnow	Liu et al. (2012)

(continued)

Table 19.1 (continued)

Physiological process	EDCs	Mode of actions/potential impacts	Target sites	Organism	References
	EE2, DES	Altered expression of aromatase, *StAR*, *P450scc*, *cyp17*, *3β-hsd*, *17β-hsd1*	Ovary	Catfish	Sridevi et al. (2015)
	2,4-DCP	*Cyp19a1*↓	Ovary	Zebrafish	Ma et al. (2012)
Oocyte maturation	Endosulfan and malathion	Inhibits LH-induced OM in vitro	Ovary	Common carp	Haider and Inbaraj (1988)
	Genistein, endosulfan, malathion, iprodione, carbaryl, glyphosate, BPA	Blocked hCG-induced G2-M1 transition in vitro	Full-grown oocyte	Zebrafish	Maskey et al. (2019), Biswas et al. (2020)
	DES	Stimulate OM through binding to mPRα	Full-grown oocyte	Zebrafish and goldfish	Tokumoto et al. (2004, 2011)
Ovulation	DEHP	*Ptgs2*↓	Ovary	Zebrafish	Carnevali et al. (2010)
	MPA, DDG	Egg production↓	Ovary	Zebrafish	Zhao et al. (2015)
	DES	Impedes ovulation	Ovary	Zebrafish	Tokumoto et al. (2011)
	DDG	PGF2α↓, blocks spawning leading to oocyte over-ripening and increased atretic follicles	Ovary	Zebrafish	Jiang et al. (2019)

organochlorine pesticide methoxychlor (MXC), has been shown to affect FSH signaling pathway and suppresses the expression of PGR and LHR in rat granulosa cells (Harvey et al. 2009). Among another class of EDCs are dioxins like TCDD (2,3,7,8-tetrachlorodibenzo-p-dioxin) that can reduce the expression and mRNA stability of FSH-induced LH receptors in cultured immature rat granulosa cells (Minegishi et al. 2003). Although limited, EDC modulation of ovarian GTH receptors in fish models has also received commendable interest in recent past. Considering the need of genome similarity with humans, for proper understanding of adverse impact of EDCs on human welfare, research has been initiated taking zebrafish as a model organism. Interestingly, an in vitro assay using zebrafish ovarian follicles depicted *lhcgr* as an estrogen-responsive gene to categorize and characterize the estrogenicity of a wide range of EDCs (Liu et al. 2013). A more recent study on bisphenol A (BPA) showed that this plasticizer could markedly reduce *lhcgr* transcript abundance in zebrafish ovary at a concentration as low as 5μg/L (Santangeli et al. 2016). Moreover, diisononyl phthalate (DiNP), another well-known plasticizer, could also negatively impact *fshr* and *lhcgr* expression in this species. In addition to reduced gonadosomatic index (GSI) and fecundity, DiNP profoundly impacted other ovarian parameters such as a sharp reduction in number of vitellogenic and mature oocytes, invagination and breakdown of zona radiata proteins, resorption of yolk, and follicular atresia (Santangeli et al. 2017).

Since estrogens play an indispensable role in vertebrate reproduction and are known to mediate its action either by nuclear ERs or GPER, the negative influence of EDCs on such receptors has received intense research interest. Different EDCs have been reported to show varied effects depending on their binding affinity to the ESRs in mammals. Several lines of evidences suggest that environmental chemicals like DES, genistein, MXC, and BPA have binding affinities for different ESRs causing ovarian dysfunction and abnormalities in female reproduction (Craig et al. 2011). Binding of xenoestrogens to nERs and even alteration in its transcript profile have been well documented in multitude of tissues in teleosts (Loomis and Thomas 1999; Xia et al. 2000; Mukherjee et al. 2020; Biswas et al. 2020) . Low concentration of BPA has been shown to downregulate *esr1* and *esr2a* expression but not *esr2b* in zebrafish ovary (Santangeli et al. 2016). In addition to this, low concentrations of BPA and related alkylphenols exert non-genomic estrogenic actions through a G protein-coupled estrogen receptor 1 (Gper)/epidermal growth factor receptor (Egfr) pathway to inhibit meiotic maturation in zebrafish oocytes (Fitzgerald et al. 2015).

Sustenance and development of ovarian follicles in teleosts greatly rely on steroid biosynthesis. Steroidogenesis starts with two rate-limiting steps, firstly the transport of cholesterol into mitochondria by steroidogenic acute regulatory protein (StAR) and secondly the conversion of cholesterol into pregnenolone by the enzyme Cyp11a1. Several steroidogenic enzymes (Cyp17, Hsd3b, Hsd17b, Cyp19a1a, Hsd20b) then act sequentially to convert pregnenolone to active steroids like 17,20β-P, testosterone (T), and E2. Studies on the regulation of steroidogenic enzyme genes in teleosts using diversified techniques like site-directed mutagenesis, luciferase assay, electrophoretic mobility shift assay, and chromatin immunoprecipitation have revealed Ff1b (homolog of SF1), Ad4BP/Sf-1, and CREB as

transcriptional activators of *cyp11a1*, *cyp19a1a*, and *hsd20b*, respectively (Rajakumar and Senthilkumaran 2020). Several EDCs have been reported to target these crucial steps of steroidogenic pathway. Upon BPA exposure, upregulated expressions of *StAR* and *cyp11a1* genes in zebrafish (Santangeli et al. 2016) and significant alterations in *cyp19a1a*, *StAR*, *cyp11a1*, and *cyp17a1* transcripts in rare minnow (Liu et al. 2012) ovary were reported. Other than BPA, 17-α ethinylestradiol (EE2), another xenoestrogenic EDC, has also been shown to suppress *StAR*, *cyp11a1*, *hsd3b*, and *cyp17a1* in *Gobiocypris rarus* (Liu et al. 2012). Transcript levels of aromatase (the rate-limiting enzyme of estrogen biosynthesis) along with *StAR*, *P450scc*, *cyp17*, *hsd3b*, and *hsd17b* have been significantly altered upon 17α-ethynylestradiol (EE2) and diethylstilbestrol (DES) exposure in catfish ovary (Sridevi et al. 2015). Interestingly, the presence of ERα, ERβ, and AR-binding elements in 5′-flanking regions of *StAR*, *hsd3b*, *cyp17a*, and h*sd11b2* as well as *cyp19a1a* hints toward the action of BPA on steroidogenic gene expressions probably via ESR1, ESR2, and AR in the ovary of adult *G. rarus* (Wang et al. 2010). Nonetheless, the molecular cascades involved in ovarian toxicity upon EDC exposure needs surely more enrichment in fish species.

EDCs on Oocyte Maturation and Ovulation

The drive from oogonial stem cells to mature fertilizable female gametes (ovum) is chiefly controlled by gonadotropins, steroids, and multiple (multitude of intraovarian) growth factors, oocyte maturation being the principal episode of this journey for consequent positive successful fertilization. In the past few years, OM has proved to be a target for endocrine disruption by environmental chemicals capable of acting as hormone mimics, receptor blockers, and/or enzyme inhibitors. The inhibitory nature of organochlorine pesticides (methoxychlor, lindane, and dieldrin) and natural isoflavones (genistein) on OM is well documented in mammals (Picard et al. 2003; Chan 2009). Not limited to mammals, the impact of diverse endocrine disruptors on aquatic species, specifically the fish, has also become a global concern. An organochlorine pesticide and organophosphorus insecticide (endosulfan and malathion, respectively) have been shown to inhibit LH-induced OM in vitro in the common carp (Haider and Inbaraj 1988). More recently, an in vitro incubation assay using zebrafish full-grown oocytes demonstrated the inhibitory action of six environmental chemicals (genistein, endosulfan, malathion, iprodione, carbaryl, and glyphosate) on G2-M1 transition (Maskey et al. 2019). Further, expression of *bmp15* gene (a member of TGFβ family), which prevents precocious OM in zebrafish by inhibiting the expression of *lhcgr* and *mPRβ* (Clelland et al. 2007), is significantly increased due to DEHP (Di-(2-ethylhexyl)-phthalate) exposure, one of the most commonly used plasticizer in PVC formulation (Carnevali et al. 2010). On the contrary, some EDCs may mimic the actions of progestins and stimulate OM. The fungicide prochloraz is known to induce OM in both mammals and fish (Kan et al. 1985; Blystone et al. 2007). Moreover, EDC like DES (diethylstilbestrol), a nonsteroidal estrogen that was previously prescribed to

pregnant women to prevent abortion, preeclampsia, and other complications of pregnancy, has been shown to synergize the 17,20β-P-induced meiosis resumption in goldfish and zebrafish oocytes via its interaction with the MIS receptor, mPRα (Tokumoto et al. 2004, 2011). Interestingly, several lines of research have proved the potential impact of EDCs on ovarian growth factor expression and function too. To cite a few, environmental chemicals like the dioxin (TCDD), at an environmentally relevant concentration (10 ppm), can negatively influence the synergy between IGF1 and FSH thereby decreasing LH receptor mRNA expression in human granulosa cells (Minegishi et al. 2003). Moreover, 1,1-dichloro-2,2-bis(p-chlorophenyl)ethylene (DDE), a metabolite of DDT, has also been shown to upregulate the expression of VEGF and IGF1 in human luteinized granulosa cells eventually leading to impairment of ovarian steroidogenesis and infertility (Holloway et al. 2007). A more recent update on BPA reflects its downregulatory effect on FSH-induced aromatase expression by activation of peroxisome proliferator-activated receptor-gamma (PPARγ) and also dramatically decreases expression of IGF1 and IGF receptor (IGFR) in a human granulosa-like tumor cell line (Kwintkiewicz et al. 2010). Recently, a group of researchers has demonstrated the altered mRNA abundances of IGF-1/IGFR upon BPA exposure in juvenile rainbow trout (Aluru et al. 2010). Additionally, BPA has recently been shown to suppress the transcript abundance of Igf and Egf family ligands with a probable involvement of the transcription factor, CREB in the zebrafish ovary (Biswas et al. 2020). However, existing literature reflects that the modulatory effect of EDCs, either alone or in combination, on ovarian growth factors is much limited and requires more research initiatives to unveil the molecular mechanism underlying EDC modulation of ovarian functions.

Beyond its critical and persistent impact on OM, the influence of EDCs on the ovulatory response has become an emerging concern for modern biologists. Although the effects of anthropogenic contaminants on humans and mammalian species have largely been investigated, few data are available on their influence on the ovulatory response, the ultimate step of oogenesis. Due to structural and functional similarities of many zebrafish genes with their human homologs, zebrafish has been considered in recent years as a starting point for molecular studies associated with environmental risk assessment (Carnevali et al. 2010). DEHP, a ubiquitous environmental contaminant, has been demonstrated to strongly impair fecundity in female zebrafish through downregulation of *ptgs2* expression, the final trigger of ovulation. Further, exposure of adult zebrafish to the widely used herbicide 2,4-dichlorophenol (2,4-DCP) disrupted steroidogenesis and resulted in significant reduction in the average number of eggs spawned (Ma et al. 2012). The negative influence of synthetic progestins used in a wide range of gynecological conditions, including hormone replacement therapy or even recurrent miscarriage, is also coming forth. A group of researchers have suggested alterations in multiple transcriptional responses due to two different synthetic progestins, medroxyprogesterone acetate (MPA) and dydrogesterone (DDG), and their mixtures leading to impaired reproductive capacities in terms of egg production (Zhao et al. 2015). Interestingly, a more recent study has elucidated on the mechanisms of the

adverse effect of DDG on ovulatory response in this particular species. During the process of ovulation, the ovulated eggs stay in the ovarian lumen until spawning. However, if spawning is blocked, these eggs undergo degeneration and progressive resorption, a phenomenon termed as over-ripening. Using ovarian metabolomics analysis, DDG was shown to increase the concentrations of a range of metabolites catabolized from proteins and lipids, suggesting the existence of ovulated oocytes over-ripening. Moreover, suppression of PGF2α production upon DDG exposure could block spawning and damage follicular tissue digestion resulting in oocytes over-ripening and increased atretic follicles in the treated ovary (Jiang et al. 2019). Additionally, DES has also been shown to impede ovulation in zebrafish (Tokumoto et al. 2011). Surely, it would be interesting to get a more in-depth insight into the molecular cascade involving the modulatory effects of EDCs on ovulatory response in teleosts in near future.

EDC-Induced Oxidative Stress, Impaired Energy Homeostasis, and Female Infertility

Endocrine disruption could be among important reasons for fish breeding inefficiency/failure and the consequent decline in fish production. Superoxide anion (O_2^-), hydrogen peroxide (H_2O_2), and the hydroxyl radical (OH^-), generated by the sequential addition of electrons to oxygen, can induce oxidative stress and alteration in diverse signaling cascades principally mitochondrial energy-sensing network, inflammatory response, apoptosis, and insulin signaling affecting ovarian functions. However, potential impact of impaired metabolic performance on reproductive fitness in EDC-exposed female fish has not been investigated in great detail yet and may well become a phenomenal central theme of research in future.

O_2^- is the initiator reactive oxygen species (ROS) (Fridovich 1997) and is formed due to NADPH oxidase action (NOXs) or from molecular oxygen or by the mitochondrial leakage during aerobic respiration (Brand 2010). Though it seldom affects second-line redox system, O_2^- has irreversible damaging effect on cellular macromolecules. Besides, O_2^- interaction with nitric oxide (NO) promotes rapid generation of peroxynitrite radical with widespread negative influence on cellular functions. Superoxide dismutase (SOD) transforms superoxide anion into hydrogen peroxide (H_2O_2), which even in nanomolar concentration may lead to oxidative stress and results in permanent protein damage. Conversely, the antioxidant defense system comprising catalase (CAT), glutathione peroxidase (GPx), and peroxiredoxins (PRx) readily converts H_2O_2 into H_2O (Schieber and Chandel 2014). Besides, reduced and oxidized glutathione (GSH and GSSG) in presence of GPx and glutathione reductase (GR) helps to control the level of H_2O_2, either by the salvage pathway or by de novo synthesis of GSH (Liu et al. 2014). Some other second-line antioxidants such as glutathione S-transferase (GST) help in the detoxification system by promoting the conjugation of GSH and xenobiotic compounds (Wu et al. 2011). However, increased level of H_2O_2 further reacts with ferrous ion in the cell by Fenton reaction and generates hydroxyl radical (OH·) and causes genomic

instability (Dizdaroglu and Jaruga 2012). Interestingly, EDC-induced oxidative stress has been well documented over a multitude of tissues including ovary. Our recent data demonstrate that in a freshwater teleost, *Labeo bata*, BPA attenuation of inflammatory response and insulin signaling might have serious implications on metabolic homeostasis (Mukherjee et al. 2020). Moreover, EDC-mediated oxidative stress induction and altered female fertility in the ovary of *Oreochromis mossambicus* (Revathy and Chitra 2018) have been reported earlier. However, such research initiatives in the ovary are far from adequate and requires thorough investigation.

Importantly, ROS has important physiological role in reproduction and fertility, from oocyte maturation to ovulation, fertilization, and embryonic development. Elevated follicular ROS synthesis, principally by the corpus luteum and leukocytes, has been associated with onset of ovulation as well as natural and prostaglandin-induced luteal regression. Conversely, ROS-mediated disruption of normal ovarian functions and pathogenesis of female infertility has been an area of active research initiative in recent past. Uncoupling of LH receptor from adenylate cyclase prevents trans-mitochondrial cholesterol transport and inhibits ovarian steroidogenesis under elevated ROS synthesis (Behrman et al. 2001). Intriguingly, a recent demonstration has reported BPA modulation of GTH receptors, markers associated with steroidogenesis and growth factor expression as a consequence of disrupted redox balance, elevated inflammatory and apoptotic modulators in the zebrafish ovary (Biswas et al. 2020). Besides, knockout of Cu/Zn-SOD results in reduced fertility (Matzuk et al. 1998). Elevated H_2O_2 in luteal cells, in a manner sensitive to OH^- synthesis, promotes DNA damage, abrogates de novo synthesis of proteins, and depletes ATP. Further, H_2O_2 attenuation of GTH action and progesterone secretion in human granulosa and luteal cells has been reported earlier (Behrman et al. 2001; Agarwal et al. 2005). Elevated ROS has been implicated as one of the early markers of apoptosis. More specifically, inhibition of GSH synthesis promotes follicular atresia in the rat ovaries (Lopez and Luderer 2004).

As shown in Fig. 19.6, oxidative stress has profound negative influence on energy-sensing network, which includes inter alia downregulation of the potent energy-sensing marker SIRT1 (silent mating type information regulation 2 homolog) as well as PGC1α (peroxisome proliferator-activated receptor gamma coactivator 1-alpha), a major player of mitochondrial biogenesis (Cantó et al. 2009; Chen et al. 2013). Impaired SIRT1 action has multidimensional negative influence on antioxidant defense system, inflammatory response, and insulin signaling. SIRT1 downregulation leads to the induction of oxidative stress by inhibiting Nrf2 (nuclear factor erythroid-2-related factor 2)-mediated activation of antioxidant enzymes (CAT, SOD, GSH) and activation of NF-κB (nuclear factor kappa-light-chain-enhancer of activated B cell) and its downstream pro-inflammatory cytokines (Huang et al. 2010; Kauppinen et al. 2013). It is also well documented that SIRT1 downregulation is associated with impaired insulin signaling and development of insulin resistance (Zabolotny and Kim 2007). SIRTs also play a significant role in female reproduction; folliculogenesis in rat ovary and steroidogenesis in FSH-induced granulosa cells is mediated by SIRT1 through FOXO3a (forkhead

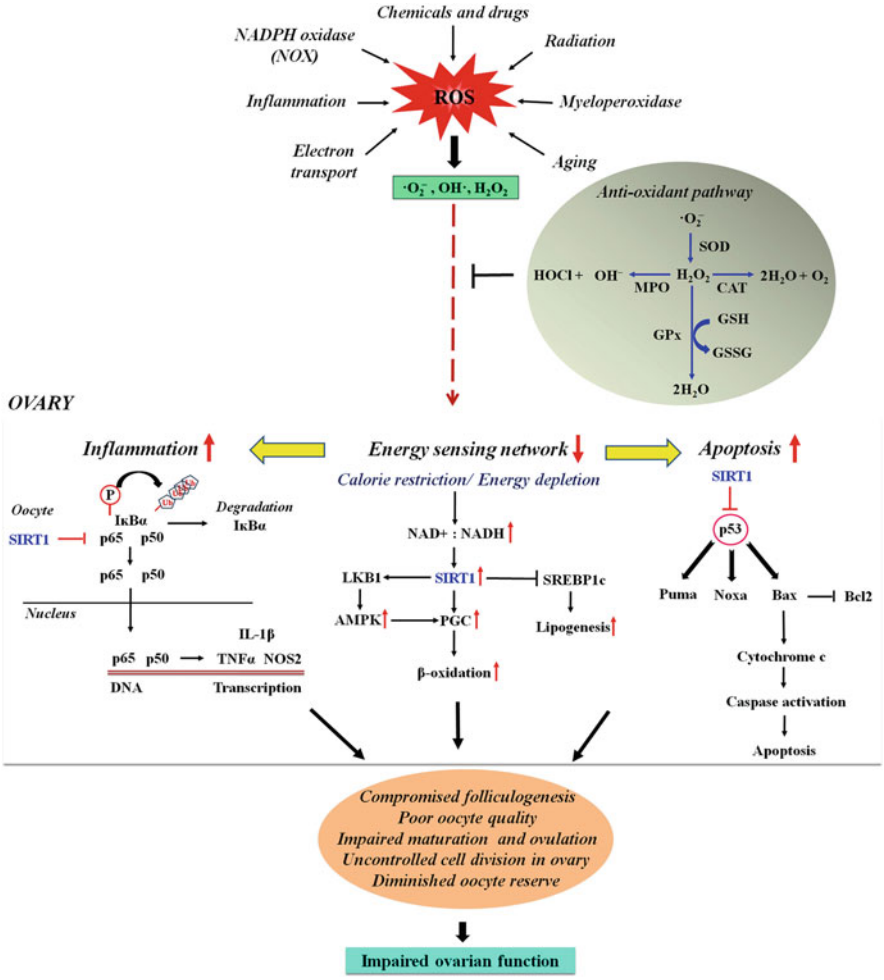

Fig. 19.6 Elevated ROS synthesis and its impact on ovarian homeostasis: relative importance of altered physiological functions, which include inter alia, cellular energy-sensing network, inflammation, insulin signaling, and cell death mechanisms

box protein) and StAR, respectively (Morita et al. 2012; Wang et al. 2014). Moreover, proliferation and secretory activity of porcine granulosa cells are regulated by SIRT1 mediated NF-κB and p53 modulation (Sirotkin et al. 2014). Moreover, SIRT2 activates histone H4K16 and α-tubulin and helps in metaphase II spindle assembly, chromosome alignment, and the aging process in mouse oocytes (Zhang et al., 2014a), whereas, folliculogenesis, luteinization, progesterone secretion, and oxidative stress response are controlled by SIRT3 modulation of GDH, SOD1, CAT, 17βHSD1, StAR, and P450arom in human GC and cumulus cells (Tatone et al. 2015).

Besides, possible correlation between SIRT1 downregulation and AMPKα (5'adenosine-monophosphate activated protein kinase) has been documented earlier (Potenza et al. 2019). Interestingly, transcriptional activation of FoxO by AMPK signaling has important role in the maintenance of normal reproductive activities during nutrient-depleted conditions (Templeman and Murphy 2018). SIRT1 induces cell cycle arrest during oxidative stress by upregulating p27 (cyclin-dependent kinase inhibitor), MnSOD (manganese superoxide dismutase), Bim (pro-apoptotic Bcl2-interacting mediator of cell death), and Gadd45α (growth arrest- and DNA damage-inducible gene 45 α) by activating FoxO factors that controls apoptotic regulation (Gu et al. 2016), important to restore oocyte reserve in the ovary, whereas, EDC-mediated apoptosis induction is a well-documented theory that might have some correlation with abrogated expression of SIRTs (Xu et al. 2002; Yang et al. 2017). Undoubtedly, it would be worth investigating in future the relative importance of EDCs on modulation of cellular energy-sensing network and its impact on ovarian function and breeding efficiency in fish models.

Importantly, elevated ROS synthesis has been implicated in the activation of NF-κB, AP1, and HIF-1 (hypoxia inducing factor) and upregulation of as IL-1β (interleukin 1-beta), TNFα (tumor necrosis factor-alpha), and IFNβ (interferon beta), the major pro-inflammatory cytokines (Rains and Jain 2011). Interaction between IL-1β and NOS promotes the accumulation of nitrite in the rat ovary. Moreover, cytokines secreted by the leukocytes, ROS, and vascular endothelial growth factor (VEGF) participate in ovulation or follicular rupture in mammals suggesting cross talk between oxidative stress and cytokines may contribute to intra- and intercellular communication in the ovary (Agarwal et al. 2005). Collectively, oxidative stress-mediated altered energy homeostasis and its negative impact on female fertility would be worthy of investigation in future.

EDC-Induced Epigenetic Changes: A Tale Beyond Genetics

Epigenetics is a new arena that is seeking worldwide attention among geneticists, endocrinologists, and reproductive biologists. In a broader sense, "epigenesis" describes the study of mitotically and meiotically heritable changes in the gene function without changing the DNA sequence (Wu and Morris 2001). The major types of epigenetic modifications include DNA methylation, histone modification, and changes associated with noncoding RNAs (ncRNAs) (Tammen et al. 2013). DNA methylation occurs at cytosine residues in CpG dinucleotides and is essential for processes such as genomic imprinting, suppression of retrotransposons, and X chromosome inactivation (Ooi and Bestor 2008). It is well conceptualized that while methylation of DNA causes interference in transcription factor binding and thus downregulating gene expression, demethylation allows access of transcription factors and upregulation of gene expression. Many instances reflect that increased DNA methylation is associated with aging and gene silencing that in turn can increase the risk for cancer because of the silencing of tumor suppressor genes (Bird 2002; Mohn and Schubeler 2009). Moreover, DNA methyltransferases

(DNMTs) are required for the establishment (DNMT3A and 3B) and maintenance (DNMT1) of DNA methylation.

Numerable evidence suggests that exposure to EDCs can also induce such pathologies by changing methylation properties and turning on/off critical genes in various tissues (Zama and Uzumcu 2010). Another category of epigenetic modification is histone modifications. In contrast to acetylation of lysine residues and methylation of lysine 4 of histone 3 (H3K4) and H3K79 that allows relaxation of chromatin and access to transcription factors, deacetylation along with various methylations (H3K9, H3K27, and H3K20) leads to compaction of chromatin and silencing of loci (Sharma et al. 2005; Turner 2005). More recently, the functional relevance of noncoding RNAs in biological processes is coming forth in terms of gene silencing like X chromosome inactivation, chromatin structure regulation, and genomic imprinting (Chang et al. 2006). Although the effect of EDCs on these important epigenetic regulators is a topic of particular interest globally (Zama and Uzumcu 2010; Santangeli et al. 2016), more research initiatives are required for the proper understanding of the mechanism of action of such endocrine disruptors.

Conclusion and Future Directions

Considering the pervasive nature of EDCs discussed so far, it is clear that aquatic species, in particular, fish, are considered to be one of the primary risk-associated organisms. Achieving gametes of the utmost quality that can undergo successful fertilization is an absolute requirement for any aquaculture industry and is under constant threat of deterioration due to the prevalence of diverse endocrine modulators in the environment. Additionally, fish are no doubt major protein sources for human consumption indicating its high economic value for the aquaculture industries. Miserable is the fact that we have altered the world in ways that have unintended consequences on aquaculture industries as well as human welfare. Populations that are particularly at risk for EDC exposure include people living near hazardous wastewater and also those having high fish consumption, importantly, pregnant women and nursing mothers who are unknowingly transferring the risk factors to the new life. The “endocrine disruptor” concept and the interest for specific studies on their adverse effect of hormonal regulation and the link between human health and environmental exposure flashed for the first time at the European Workshop on the Impact of Endocrine Disruptors on Human Health and Wildlife (Weybridge, UK) in 1996. In today’s urbanized world, the fact that exposure of EDCs is associated with a multitude of ovarian diseases (obesity, PCOS, endometriosis, ovarian cancer, etc.) has ignited the need for new tools to monitor the global effects of EDCs at genome, transcriptome, or proteome level. In response to these concerns, diverse research is being carried out in various fish models that might prove integral in comprehending the risks posed to human health. Whenever the choice for the appropriate fish model system to study the effects of EDCs has arisen, zebrafish has been considered the state of emerging art because of its short life span, transparent embryos, conserved similarities with humans in many developmental

pathways, well-characterized reference genome, and accelerated genetic studies by gene knockdown or overexpression. Further, in comparison to the human reference genome reveals that 70% of human genes have at least one obvious zebrafish ortholog (Dooley and Zon 2000). Coming to the ongoing research advances in understanding and developing epigenetic classifiers and biomarkers, researchers have come a long way for a better understanding of the effects of exposure of EDCs. A major technological shift in toxicogenomics in the past few years has been the adoption of massively parallel sequencing technologies to facilitate the whole genome and transcriptome sequencing (Ten Bosch and Grody 2008; Tucker et al. 2009). Toxicogenomics is a field that emerged from the combination of conventional toxicology and functional genomics. In the past decade, the delineation of the transcriptome of teleost fish has been evaluated by the help of large-scale expressed sequence tag (EST) sequencing of cDNA libraries of zebrafish (Zeng and Gong 2002; Li et al. 2004) and Atlantic salmon (Davey et al. 2001), substractive hybridization of cDNA libraries of medaka (Kanamori 2000), and microarray-based analyses of rainbow trout gonads (Schalburg et al. 2005). In recent years, parallel sequencing of the RNA content of the cell, tissue, or organism (RNAseq) and high-throughput genome sequencing (HTS) can provide an unbiased platform that allows RNA quantification from very small amounts of cellular materials (Maher et al. 2009; Wang et al. 2009; Ozsolak and Milos 2010). Currently, omic technologies provide valuable tools that enable researchers to assess thousands of genes, proteins, or metabolites in a single sample offering the potential to investigate responses to chemical stressor by investigating the changes in genomes (genomics, epigenomics), global gene expression (transcriptomics), protein levels (proteomics), and biochemical molecules involved in metabolism (metabolomics). Many studies have already applied omic approaches to identified genes involved in the regulation of cell cycle, ubiquitin system, and glutathione peroxidase to be affected and associated with the changes observed in gametes quality (Santos et al. 2007). By applying gonadal transcriptome analysis, scientists can identify the disruption of oocyte development and spermatogenesis in adult rare minnow due to the exposure of EDCs (Gao et al. 2017). However, more comprehensive testing methods are still required to identify other possible endocrine disruptors, their sources, routes of exposure, mechanism of action, and negative outcomes on female fertility because existing literature on EDCs is only the "tip of the iceberg." Based on which major amendments in existing policies, long-term planning and implementation of child and women healthcare measures at the grassroot level may help in empowering women of this country to gain access to the globe of sound reproductive health.

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Anthropogenic Exposure and Its Impact on Reproductive System of Fishes

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Abstract

The term aquaculture is defined as a controlled cultivation and harvest of aquatic animals such as fishes, crustaceans, and molluscs along with some aquatic plants as a source of food. Fishes are often a rich source of nutritional supplement for humans. Industrial revolution has deteriorated the quality and diversity of aquatic life for a long duration. Industrial wastes consisting of by-products such as heavy metals, pesticides, herbicides, weedicides, antifouling compounds, nanoparticles, and microplastics are often known as xenobiotics, foreign substance or chemical to the body or to an ecological system. The aquatic environment has become dumping place as environmental pollution increases due to anthropogenic activities and leads to developmental and functional abnormalities of the reproductive system of fishes and often even to mortalities of aquatic organisms. Thus, the present chapter aims to review various issues related with aquatic pollution, anthropogenic activities, effects of sublethal pollutants on fish reproductive system and mechanism. The study also urges on safe elimination of household sewage and manufacturing effluents as well as laws execution legislated in order to protect the aquatic environment.

Keywords

Aquatic ecosystem · Anthropogenic activities · Water pollution · Heavy metals reproductive abnormalities · Gonadal growth

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Introduction

The land-based aquaculture is vital for global for fish (28.8 million tons), mollusc (13.1 million tons), and crustacean (5.0 million tons) production (John 2007; FAO 2010). International fish production in 2016 reached about 171 million tons, with percentage of 47%aquaculture performing of the total and 53%, if nonfood uses (including fish oil and fishmeal) are eliminated. With relatively stable capture fishery production since late of 1980s, aquaculture has been responsible for keeping the fundamental growth to provide humans with fishes for consumption (SWFA 2018). Recently the fish consumption has increased quickly especially with the consciousness of its therapeutic and nutritional benefits. Fishes are considered to be the source of vitamins, protein, and unsaturated fatty acids (omega3) and rich with essential minerals (El-Moselhy 2000).

Fatima and Usmani (2013) opined that the fishes are continually exposed to heavy metals and waterborne particulate concentration due to continued stream of water over food sources and gills. This leads to bioaccumulation of heavy metals in various tissues following various types of bioaccumulation factors. Detrimental materials such as paper mill waste, heavy metals, pesticides, crude oil, and polychlorinated biphenyl are usually emitted into the aquatic environment. When large amounts of these pollutants are emitted, there perhaps a severe effect as measured by large-scale unexpected mortalities of fish kills. In fish's metabolism decreasing, gills damaged and epithelia occurs and immune suppression. Diseases related to pollution include gill disease, ulceration, hepatic damage, and fin/tail rot have been observed (Bukola et al. 2015). Gallo and Tost (2019) have reported that chemical pollution conflicts with hormone function get rises and cause endocrine disruption. Due to susceptibility of hormone receptor systems, it causes endocrine disruptors (EDs) which affect the normal reproductive functions as well as embryo evolution. For a long time, this leads to reproductive defeats and aberrations in the reproductive organs of fish, reptiles, mammals, and birds. Some evidence have been found that hormonally active substance have adverse impacts on fish populations. Field and laboratory studies reveal that a number of chemical substances hamper the reproductive performance of adult fishes, in courtship behavior and parental care. Further it results in mutilation of impaired quality of eggs and sperm (Segner 2011). However we have observed several anthropogenic activities which are accountable in the developmental abnormalities in fish health. The prime purpose of this present chapter is to estimate the factors responsible for poor reproductive development in fishes and their endocrinology.

Significance of Nutritional Value of Fish in Human Diet

Alimentary fish is an essential and opulent protein source. Fish consuming has been reported to share almost 50% of the animal protein consumed in numerous Asian countries (William and Dennis 1988). According to (FAO) report, fish is a premium nutritional importance having protein with high quality and a broad variety of

minerals and vitamins, including magnesium, phosphorus, vitamins A and D, selenium, and iodine in marine fish. Fish oils are the richest source of a sort of fat that is necessary to the development of normal brain in fetuses and infants. Shellfish is a major part of worldwide seafood production. It provides bioactive peptides, digestible proteins, long-chain polyunsaturated fatty acids, essential amino acids, vitamin B_{12} and other vitamins, astaxanthin and other carotenoids, and minerals, including sodium, selenium, zinc, inorganic phosphate, potassium, iodine, and copper (Venugopal and Gopakumar 2017). Nutritional structure study of ordinarily found fishes in Agatti Island water of Lakshadweep Sea in India was carried out. Proximate analysis revealed that the carbohydrate and protein, as hand lipid contents, were high in *Thunnus albacares* (13.69%), *Hyporhamphus dussumieri* (6.97%), *Parupeneus bifasciatus* (6.12%), and *T. albacares* (1.65%). Major amino acids were methionine, leucine, and lysine registering 2.64–3.91%, 2.67–4.18%, and 2.84–4.56%, respectively. Fatty acid structures ranged from 31.63% to 38.97% saturated (SFA), 21.99–26.30% monounsaturated (MUFAs), 30.32–35.11% polyunsaturated acids (PUFAs), and 2.86–7.79% branched fatty acids of the total fatty acids (Dhaneesh et al. 2012). Similarly Sandhya and Smita (2013) found that *R. daniconius* fish has the highest protein content. Previous results reveal this fish possesses a good amount of protein so they can be used safely in food to supplement protein.

Heavy Metals Exposure and Its Impacts

Nowadays modern human society is facing environmental pollution as a major challenge (Ali and Khan 2017). Pollution caused due to heavy metals is a serious menace and key concerned to the environment (Ali et al. 2013; Hashem et al. 2017). The toxicity of heavy metals has proven to be a prime menace and causing several health risks. Toxicity of metals depends upon the route of exposure, duration of exposure, and the absorbed dose, i.e., chronic or acute (Jaishankar et al. 2014). Naturally weathering of metal-containing volcanic eruptions and rocks are the sources of its occurrence, while human sources are mining, industrial emissions, and smelting, along with agricultural activities such as use of phosphate fertilizers and pesticides. Also, fossil fuel burning adds heavy metals like cadmium (Cd) in the environment (Spiegel 2002). Heavy metal remains permanent in the environment which enters in food chain through bioaccumulation and causes various health issues due to its toxicity. Their chronic exposure in the environment now became real menace to living organisms (Wieczorek-Dąbrowska et al. 2013). Mercury is a potent heavy metal neurotoxin for living organisms. Effects and exposure of sublethal mercury on fish have been investigated. Studies reveal and suggest that the restrained effects of mercury in reproduction occur at numerous sites within the genital axis, including the gonads, hypothalamus, and pituitary. Further, ill effects including circulating reproductive steroids and reducing in gonad size in fishes have been reported (Kate and Vance 2009). In aquatic environment mercury is present in various forms, including elemental, ionic, and organic (Morel et al. 1998). Due to industrial activities highly polluted local fish population, with mercury

concentrations, ranged from 8.4 to 24 μg/g wet weight in Minamata Bay, Japan (Kitamura 1968), and from 6.3 to 16 μg/g wet weight in Clay Lake, Ontario, Canada (Lockhart et al. 1972), observed. Methylmercury, one of the most lethal forms, bioaccumulates in fish mostly by dietary uptake (Spry and Wiener 1991; Hall et al. 1997). Watras and Bloom (1992) reported that the specific bioaccumulation of methyl mercury in the aqueous environment ranges to 15% in phytoplankton, 30% in zooplankton, and more than 90% in fish. Changes in reproduction, behavior, growth, biochemistry, survival, and development in fishes can occur due to exposure of methylmercury (Weiner and Spry 1996; Sorensen 1991). Rao (1989) opined that mercury may obstruct in proper functioning of sperm mitochondria, which results in decrease in energy production for sperm mobility.

Gonadotropin-releasing hormone regulates the synthesis and release of the gonadotropins (follicle-stimulating hormone [FSH] and luteinizing hormone [LH]), making it a decisive neuroendocrine arbiter of genital function. The evidence from histological analysis of neurons in the hypothalamic preoptic nuclei of the murrel (*Channa punctatus*) reveals that mercury as a potent inhibitory factor for neurosecretion (Ram and Joy 1988). A number of authors have reported that gonadotropic hormones (LH and FSH) released in fish from pituitary manage their yearly cycle of gonadal growth, sperm release in males, ovulation in females, and production of sex steroids in both genders (Breton et al. 1998; Weltzien et al. 2004; Kamei et al. 2005). Hence, distraction in gonadotropin excretion plays a great role on fertility. An investigation reports the decreased in atrophied seminiferous tubules, and spermatogenesis were observed in male Nile tilapia (*Oreochromis niloticus*) after the exposure to methylmercury within 7 months (Arnold 2000). The gonadotropic regulation of spermatogenesis and spermiogenesis in fish is mediated by androgens secreted by the interstitial cells (Yaron 1995). Exposure to methylmercury in male guppies has resulted in fibrosis and inflammation of the interstitium (Wester 1991; Wester and Canton 1992). These cytotoxic effects involve potential reverse effects on the steroidogenic potential of the interstitial cells. Androgen helps in sexual behavior, gonadal development, and secondary sexual characteristics in male fish (Kime 1998). Similarly Kime (1993) found that the secretion of testosterone and/or 11-ketotestosterone is high during gonadal recrudescence, which is rejected before spermiation. The levels of plasma testosterone drastically decline in male fathead minnows fed diets including 0.87 to 3.93 μg/g of methylmercury for 250 days (Drevnick and Sandheinrich 2003). The decreased level of 11-ketotestosterone is also observed after a 6-month exposure of methylmercury in male Nile tilapia (*O. niloticus*) (Arnold 2000). Now it became obvious that anthropogenic activities have great impacts on fish reproductive health.

Exposure and Impacts of Microplastics

Plastic pollution is a global issue for aquatic animals in almost marine and ocean on the earth and threat to marine life, and great economic loss for aquaculture (Eriksen et al. 2014; Derraik 2002; Thompson et al. 2009). Plastic can demean the coastal

benthic habitats via smothering; this is due to formation of plastic sheet layers over the benthos and also through alterations in sediment, permeability occurs by buried plastics (Carson et al. 2011). Engulfed plastics cause gastrointestinal (GI) tract. For example, it can cause abrasions and lesions or physical disruption of the GI tract, as plastics compress in the gut (Di Bello et al. 2013). Various authors suggested that PCBs are capable to change the proper regulation and function of foremost hormones such as estrogen, testosterone, and thyroxine (Goncharov et al. 2009; Colborn et al. 1993). Moreover microplastics (MPs) are smaller pieces of plastic debris which are not visible by the naked eye termed as microplastics in aquatic medium which is a matter of concerned. Microplastics are derived from marine debris and have potential ecological impacts on marine creatures (Anthony 2011). Due to its small size and ever present microplastics found to various organisms in both pelagic and benthic aquatic habitats (Foekema et al. 2013; Mathalon and Hill 2014). During experimental study it was found that small fragments of microplastics are transferred into aquatic animals through food web, in small amounts (Lehtiniemi et al. 2018). A study carried by Critchell and Hoogenboom (2018) explains the exposure and effects of microplastics on juveniles of a planktivorous fish (*Acanthochromis polyacanthus*), which is prevalent and abundant on Indo-Pacific coral reefs. During study it was observed that when individual fish was exposed of plastic after a 1-week the increased numbers of plastics present in GI tract greatly get increased on decreasing the size of plastics to approximately one-fourth the size of the food particles, having maximum of 2102 small (<300 μm diameter) particles present in the gut of that fish. The result reveals negative effects on the growth of fish.

Impacts of Oil Spills

Oil spills draw a great concern, and its effects on marine biological systems cannot be ignored, due to rich biological production of harvested resources (Langangen et al. 2017).

Fish larvae and eggs are normally susceptible to toxic materials present in oil components, due to its small size, poorly developed membranes, and their positions in water column. Various experimental studies found that presence of toxic materials in oil components (such as polycyclic aromatic hydrocarbons, PAHs) at very low concentrations can cause a sublethal damage to fish larvae and eggs (Carls et al. 1999; Hicken et al. 2011; Meier et al. 2010; Scott and Sloman 2004; Sørhus et al. 2015). The oil spills in the aquatic medium cause a sublethal damage to fish eggs as well as their larval stage; for example, morphological deformities, reduced feeding, and growth rates increase susceptibility to predators, and starvation occurs (Sørhus et al. 2016; Hicken et al. 2011). It spoil their habitat, loss of hatching ability of eggs, impaired reproduction, growth, development, fouling of gill structures, feeding, respiration (Blackburn et al. 2014). Mostly various species of fishes utilized most of their time living in groups, along with group coordination which plays an important role in the emergent benefits of group-living. But these group cohesions

can be faint due to several factors in which exposure to toxic environmental contaminants is one of them. Experimental study reveals that oil spill components and its exposure have negative effects on fish behavior which may lead to reduce their ecological success (Armstrong et al. 2019). Osuagwu and Olaifa (2018) suggested that presence of crude oil spills over coastal water increases the risk of contamination to fish hatcheries of valuable fishes. Their findings show that oil spills have negatively affected fish production. However another study reveals that oil spills contain polycyclic aromatic hydrocarbons (PAHs) which have endocrine-disrupting properties. This shows a negative effect on the polar cod (*Boreogadus saida*), an Arctic keystone species which possesses an extensive and energy-intensive reproductive development systems. Study reveals the considerable alterations in sperm mobility due to crude oil exposure in males, and data were compared to the controls. During investigation somatic indices (gonad and hepatic cells), germ cell development along with plasma steroid levels (estradiol-17 [females], testosterone [males and females], and 11-ketotestosterone [males]) was found not drastically distorted by constant dietary exposure to crude oil (Bender et al. 2016).

Impacts of Pesticides and Insecticides

Pesticides are broadly used in the advanced agricultural practices, in diverse quantities, over the world. However it is very helpful for crop yielding, there are some severe issues in the environment, such as health- and safety-related concerns for aquatic as well as terrestrial living organisms which include humans, animals, and plants (Sana Ullah et al. 2018). Pollution due to pesticides is a major menace to freshwater ecosystems throughout the world (Madeleine et al. 2015). Freshwater ecosystems are essential collections of almost all ecosystems, like potable water, irrigation water for agriculture, industrial water, water storage, water recreation, and an environment for organisms which helps to grow fish and other important foods. An invertebrate which is part of food chains provides transfer of energy and nutrients from primary producers to higher tropic levels (Madeleine et al. 2015).

Fishes get exposed to pesticides and their residues through various routes such as runoffs or spray coming directly from agricultural fields and gardens. In aquatic ecosystem fish are predictably one of most affected organisms by pollutant present in aquatic medium. Pesticides present in aquatic bodies deteriorate its quality through alteration in physicochemical parameters and threat aquatic organisms (Sarwar et al. 2007; Sabae et al. 2014). A number of insecticides have been documented by the UK-based Pesticides Action Network (UK PAN 2009). Globally, pesticides are found in various compositions which consist of herbicides (15%), fungicides (1.46%), and insecticides (80%) (Marigoudar 2012). Cypermethrin (CYP), a pyrethroid, derived from pyrethrin, extracted from *Chrysanthemum cinerariaefolium*, is a broadly used, capable, and readily available insecticide. It is almost widely used insecticide found in various kinds of agricultural practices, gardens, lawn, buildings, and forestry in order to protect from insects and cotton and soya beans from pests as

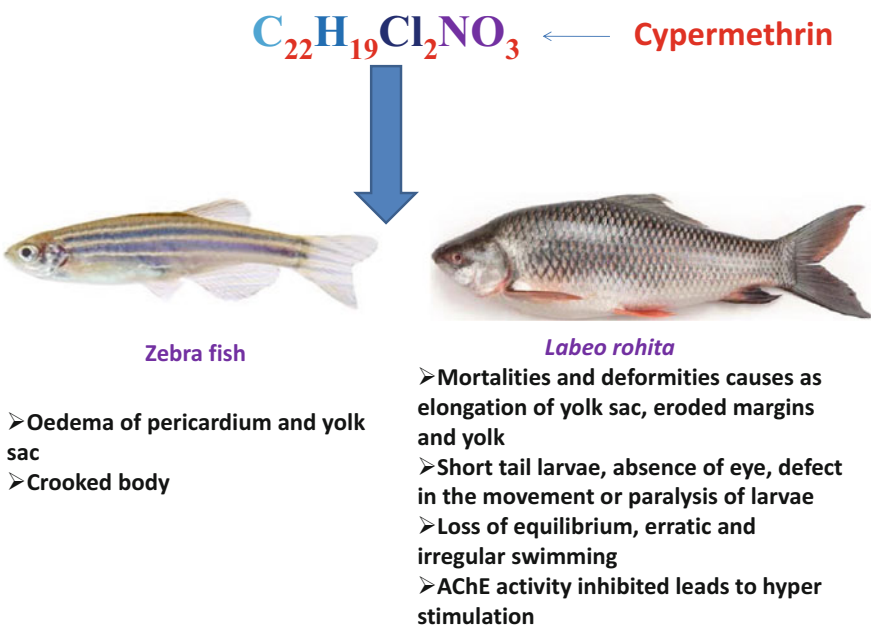

Fig. 20.1 The toxic effects through cypermethrin induced in fish (adapted from Sana Ullah et al., 2018; image of *Labeo rohita* uploaded by S M Majharul Islam and Zebra fish (https://www.google.com)

well as repel and control mosquitoes which are malaria parasite carriers. Furthermore it is the expansively used pesticide in agriculture and households, and also its concentration in aquatic ecosystem has been reported. Its existence in our environment is posing a brutal threat to humans as well as other nontarget terrestrial and aquatic organisms (Sana Ullah et al. 2018). The negative effects of cypermethrin and toxicity in fish have been illustrated in Fig. 20.1. Similarly the developmental abnormalities and effect of cypermethrin (CYP) in various fish breed have been tabulated in Table 20.1.

Conclusion and Future Direction

Anthropogenic activities result in accumulations of heavy metals, oil spills, pesticides, insecticides, and microplastics in aquatic ecosystem which renders several reproductive abnormalities in fish. Contaminations of pesticide toxicity in natural resources may impose significant risks on environment and untargeted organisms of different verities from helpful soil microorganisms to insects, aquatic animals, birds, humans, and plants. Metabolism due to such toxicity of heavy metals and other noxious constituents mostly affects the rate of survival and growth of fishes and alters their various body functions inducing neurotoxicity, hematotoxicity,

Table 20.1 Some toxic effects of cypermethrin in fish

Fish species	Effects observed	References
Labeo rohita	Deformities occurs in developmental systems, changes of antioxidant enzymes at developmental stage and survival	Dawar et al. (2016)
Oncorhynchus mykiss	Induction in oxidative stress reduced quality of spermatozoa	Kutluyer et al. (2016)
Odontesthes bonariensis	Decreased observed in growth as well as in survival rates	Carriquiriborde et al. (2009)
Heteropneustes fossilis	Interruptions occurs in the action of spermatogenic cells and follicular wall	
Danio rerio	Edema observed in pericardium and yolk sac	Xu et al. (2010)
Danio rerio	Apoptosis in embryos and Immunotoxicity	Jin et al. (2011)
Catla catla	Changes in biochemical and hematological parameters	Kannan et al. (2014)
Heteropneustes fossilis	Alterations in the histoarchitecture of ovary	Monir et al. (2016)
Prochilodus lineatus	Changes occurs in hepatic enzymes' activities	Loteste et al. (2013)
Cyprinus carpio	Alterations in hematological parameters	Masud and Singh (2013)
Prochilodus lineatus	DNA damage occurs in gill cells	Poletta et al. (2013)
Clarias batrachus	Alterations in functioning of ATPase and glycogen phosphorylation	Begum (2009)

toxic histopathological effects, immunotoxicity, genotoxicity, and disruptions of endocrine systems. It also distorts the neuroendocrine system, which plays a vital role in reproductive function of LH and FSH hormones by its mechanisms through hypothalamus which maintains homeostasis, proper functioning of reproductive system, metabolic process, energy utilization, osmoregulations, and blood pressure. Therefore the present study concludes that unsystematic disposal of any kind of pollutants without proper pretreatment should not be allowed, in order to minimize the negative effects of water pollution on fish health. Safe and sound disposals of any domestic sewage and industrial effluents should be practiced through the enforcement of enacted laws in order to protect aquatic environment.

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Effect of Climate Change on Endocrine Regulation of Fish Reproduction

21

Abhilipsa Biswal, P. P. Srivastava, and Tapas Paul

Abstract

Climate change is a serious concern for aquatic environment which alters physical and chemical properties of the water causing negative impacts on the aquatic organisms including fish. Temperature alteration, ocean acidification, and hypoxia are the major factors associated with climate change, which affects the endocrine regulation of fish reproduction profoundly. Fish being poikilothermic animals, the change in environmental temperature directly affects their body temperature. Seasonal change in temperature has either fastened the spawning process or delayed the process depending upon the species and their spawning window. Ocean acidification and hypoxia had caused threat to larval survival by impairing larval behavior and sensory capacity. Often climate change shows extreme effect of the demography of fishes by leading to a non-spawning season in some species. Depending upon species, geographic location, and spawning ground, exogenous factors possess significant threat on fish reproduction. The present chapter will provide baseline information on effect of different factors of climate change such as temperature, ocean acidification, and hypoxia on fish reproduction and early ontogenesis phase of fish.

Keywords

Temperature · Hypoxia · Ocean acidification · Reproduction · Ontogenesis

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Introduction

Over the years, anthropogenic activity due to the industrial revolution has played a vital role in contributing to the major contemporary issues such as climate change and global warming. Climate change not only alters the physical and chemical properties of the water but also has negative impacts on the biological characteristics of the aquatic environment (Hartmann et al. 2013). Physical changes in a water body such as elevation of mean water temperature, the sporadic prevalence of extreme temperature events (Kaushal et al. 2010), and chemical changes such as alteration in dissolved oxygen content (Ito and Momii 2015) and change of salinity (Bonte and Zwolsman 2010) have contributed to various kinds of biological changes in the water body. It has been reported that environmental perturbation has caused the habitat change (Beldade et al. 2017) and altered the physiological (Sapolsky et al. 2000), morphological (Boonstra 2004), and behavioral patterns of fish (Wingfield and Sapolsky 2003). In the context of urgency for conservation and management of fishery sectors, reproductive success plays a major role. But climate change leads to the alteration of demography, due to the modification in reproductive physiology (Whitney et al. 2016). In various cases the climate-induced change leads to the frequent occurrence of "skipped spawning," eventually leading fishery sectors into trouble (Rideout et al. 2005). It has been considered that among the various adverse effects of climate change, disruption of reproductive physiology and reproductive behavior holds a very sensitive position (Beldade et al. 2017). Keeping the sensitivity of this topic in view, in a holistic manner, we have tried to synthesize a report on the effect of different kinds of climate-induced stressors on the reproductive physiology of fishes.

Endocrine Regulation in Fish Reproduction

The mechanism of reproduction in fishes is controlled by the regulation of the BPG (brain-pituitary-gonadal) axis (Fig. 21.1). The central nervous system plays a vital role in the regulation of fish reproduction by converting the external environmental factors (such as photoperiod, temperature, rainfall, social stimuli) into hormonal signals (Miranda et al. 2013; Rather et al. 2017, 2020). It's already known that hypothalamus and hypophysis are the organs which are primarily involved in the neuroendocrine control of reproduction. In cases of teleosts, instead of the presence of the hypothalamic-pituitary portal system, it is observed that pituitary directly innervated with neuronal axons of the hypothalamus. Either by positive stimulatory or negative inhibitory effects, many neuropeptides such as dopamine, neuropeptide y, serotonin, gamma-aminobutyric acid, and kisspeptin control reproductive actions of the hypothalamus; for example, dopamine, exerts as inhibitory effect on GnRH hormone (Breton et al. 1971; Zohar et al. 2010).

GnRH hormone is a decapeptide, which is reported to exist in three different forms (GnRH-I, GnRH-II, GnRH-III) in teleost and is differentiated mainly based on molecular characteristics. Among all these three variants, GnRH-I is believed to be

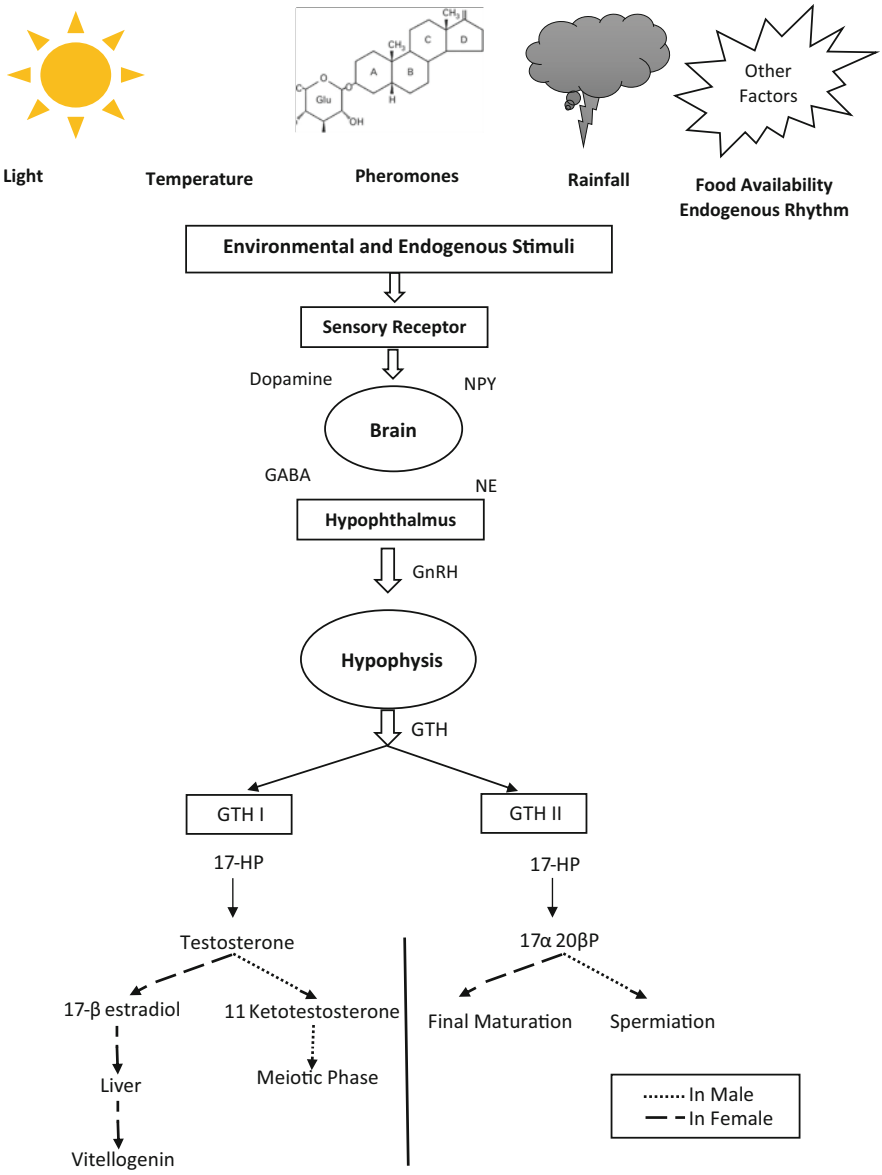

Fig. 21.1 Diagrammatic representation of endocrine regulation of fish reproduction through brain-pituitary-gonadal axis. GABA gamma-aminobutyric acid, GnRH gonadotropin-releasing hormone, NPY neuropeptide Y, NE norepinephrine, GTH gonadotropin, GTH I gonadotropin I, GTH II gonadotropin II, 17HP 17 α hydroxyprogesterone, 17α 20β BP 17 α 20β-dihydroxy-4-pregnen-3-one

having a profound effect in reproduction for a large group of teleost fishes, and its function is the induction of gonadotropins (GtHs) synthesis in the pituitary (Levavi-Sivan et al. 2010). The two gonadotropins such as GtH-I and GtH-II are otherwise known as follicle-stimulating hormone (FSH) and luteinizing hormone (LH), which play a critical role in stimulating the synthesis of gonadal steroids. However, in many species, the release of FSH and LH is dependent upon the integrative action of the stimulatory effect of GnRH and inhibitory effect of dopamine (Dufour et al. 2010). Interestingly, the above described two GtHs are the glycoprotein hormones which are composed of two different subunits called α and β subunit, and they vary among each other through the β subunit, whereas the α subunit remains conserved for both the hormones. Through stimulation of specific enzymes, these GtHs assist in the formation of sex steroids (Lubzens et al. 2010). These gonadotropins exert their effect on the fish gonad by binding to the ligand-binding site of trans membrane receptor GPCR (G protein-coupled receptor), and thereafter through the involvement of intracellular second messenger cAMP and further activation of PKA (protein kinase A), there will be the gonadal synthesis of sex steroids from a sequential breakdown of base molecule cholesterol (Planas et al. 2008). The function of sex steroids (androgen, estrogen, and progestin) is related to gonadal development. Progestin is involved with final maturation and spawning of both male and female sexes (Nagahama 1997). In general, androgens (testosterone and 11-ketotestosterone) regulate the development of spermatogenesis, and estrogens (estradiol) regulate the process of oogenesis (Lubzens et al. 2010).

During the gonadal development, a principal mechanism involved with the female gonad is the hepatic production of vitellogenin (Vtg) by the regulation of estradiol (E2). This vitellogenin is thereafter released into the portal system and further taken up by the developing oocyte which acts there as yolk precursor. The function of estradiol is not only limited up to yolk development, but also it stimulates the production of hepatic and ovarian synthesis of zona pellucida proteins (ZP), which will eventually lead to the formation of a chorionic layer (eggshell) of an ovulated ovum (Tyler et al. 2000; Modig et al. 2007).

Effect of Temperature on Fish Reproduction

Fish being an ectothermic animal, its body temperature depends on the environmental temperature. So any variation in environmental temperature will directly affect its body physiology to many extents (Ficke et al. 2007). Thus, the temperature is considered to be one of the most important physical factors which can modulate the overall physiology of this aquatic organism (Jeppesen et al. 2010; Mooij et al. 2009). Similar to other physiological processes, reproductive process in fishes only occurs in specific environmental condition, i.e., in the specific temperature ranges (Bromage 2001; Portner and Farrell 2008). Thus, in this context, any alteration in ambient temperature will cause a profound effect on the process of fish reproduction (Zucchetta et al. 2012) (Table 21.1).

Table 21.1 Effect of increased temperature on various fish species having different biology

Species	Biology	Results	References
Atlantic cod	Spring spawner Marine habitat	Low fertility	Hutchings and Myers (1994)
Atlantic halibut	Spring spawner Marine habitat	Reduced quantity and quality of eggs, delayed spawning	Brown et al. (2006)
Roach	Spring spawner Freshwater habitat	Advancement of spawning	Gillet and QueTin (2006)
Rainbow trout	Delayed spawning, delayed steroidogenesis, lower embryo survival	Fall spawner Freshwater habitat	Pankhurst et al. (1996), Pankhurst and Thomas (1998)
Atlantic salmon	Delayed spawning, lower estradiol level Reduced egg size and embryo survival	Fall spawner Marine habitat	King et al. (2007)

The alteration in temperature affects the fish reproductive axis (brain-pituitary-gonadal axis) at multiple points and exerts abnormality in reproductive functions (Pankhurst and Munday 2011). It is evidenced by the occurrence of the conformational changes of glycoprotein hormone (FSH, LH), their receptors, steroid synthesizing enzymes, and the formation of water-soluble abnormal steroid available for excretion through the kidney (Pankhurst 1997).

High temperature-induced gonadal regression and reproduction impairment are associated with alteration in expression of specific genes that are associated with the reproductive axis. In the case of male fish, although no alteration was observed in the expression of FSH and LH receptors, a significant reduction was observed in the level of plasma testosterone and 11-keto testosterone suggesting the inhibitory effect of high temperature on steroid genesis of males (Clark et al. 2005; Soria et al. 2008). The gene expression of digestive enzymes during ontogenic development (Mir et al. 2018, 2019a, b) may also be affected due to climate change on the elevation of temperature. The downregulation in the expression of 11β-hydroxylase enzyme (which converts the testosterone to 11-keto testosterone) in high temperature-induced *P. major* fish can indicate the reason for the decreased number of spermatocyte count the male fishes (Lim et al. 2003; David and Degani 2011). However, in the case of female fishes, temperature-induced reduction in the level of plasma estradiol is one of the most frequently observed responses in almost all the studied species (Pankhurst and Munday 2011). It may be due to the thermal sensitivity of P450arom enzyme (responsible for androgen to estrogen production), due to which estradiol synthesis is suppressed. Aromatase plays an important role in sex determination and differentiation, and its sensitivity toward temperature has been well described in earlier reports (Guiguen et al. 2010). High temperature-induced female species showed low P450arom activity along with a simultaneous downregulation of cyp19α1α, which indicates the lower activity of the enzyme is mainly due to the difficulty in the synthesis of the enzyme rather than the posttranslational modification of enzyme (Elisio et al. 2012). However, in some other high temperature-

induced species, lower production of P450arom along with lower testosterone level was reported which may suggest that high temperature also affected the androgen synthesis in females as well as males (Tveiten and Johnsen 2001; Elisio et al. 2012). In the context of glycoprotein hormones, a reduction was observed in the secretion of LH hormones in *S. alpinus* fish, while an elevation in the level of only FSH was observed in *S. salar* fish with a nonsignificant change in the LH levels. Not only that, but induction of high temperature during the period of final maturation has also led to the inhibition in the production of final maturational inducing steroid 17,20-β-dihydroxy-4-pregnen-3-one (17, 20 bp), and thus it creates impendence in final oocyte maturation (Gillet et al. 2011; Pankhurst and King 2010).

However, not only temperature, but there are several other associated factors that influence the reproduction in fishes in an integrated manner such as phenology, nutritional status, and broodstock age class of fish. Global warming can possess dissimilar impact among species existing within the same geographical region depending on the phenology of those species. Based on of phenology, the fishes can be broadly divided into two categories: (a) "autumn spawner" (fall spawner), these are the species where the maturation takes place during the spring-summer period, and the spawning takes place during the autumn, and (b) "spring spawner," species where vitellogenesis occur during the autumn-winter and spawning occurs during the spring (Shuter et al. 2012). The effect of elevated temperature will not only be dependent on absolute but also strongly dependent on the cyclic or seasonal pattern of reproductive strategy. It has been suggested that in the case of fall spawner, the elevation of temperature during the maturation period will delay the breeding season and in the case of spring spawner, it will provide a cue for the reproductive activity (Miranda et al. 2013).

Another factor considered to be controlling the reproduction in fishes is the nutritional status. Under a certain level of nutritional condition, there will be no occurrence of gonadal maturation, and overall energy will be spent for the maintenance physiology of fishes (Lambert et al. 2000). Also, there exists a well-established connection between the nutritional profile and endocrine status. Poor availability of nutrition can result in reduced thyroid hormone and insulin-like growth factors in several fishes (Cerdá-Reverter et al. 1996). It has been demonstrated that triiodothyronine induces the production of a thyroid hormone-induced protein which in turn stimulates the activity of the steroid-converting enzyme 3b-hydroxysteroid dehydrogenase (Datta et al. 1999), and IGF1 increases pituitary synthesis and release of follicle-stimulating hormone in *Oncorhynchus kisutch* (Baker et al. 2000) along with the direct stimulatory effect on final oocyte maturation procedure (Weber and Sullivan 2000). For example, in the case of spiny damselfish, high nutritional status has shown some degree of protection against inhibitory effects of exposure to high temperature due to feeding-regulated endocrine control of reproduction (Donelson et al. 2010). Therefore, along with temperature, nutritional status also possesses an impact on fish reproduction.

However, the effect of thermal stress may also be different with the same population. For example, it was observed that although the elevation of temperature showed decrement of plasma estradiol and vitellogenin level, expression of ZP gene,

and delayed ovulation in both first spawning season (maiden) and second spawning season (repeat) in Atlantic salmon females, yet repeat females exhibited higher survival and fertility than maidens (Pankhurst 2011). However, the reason behind this was unclear, but it can be suggested that it was due to maternal endowment in the second spawning season females.

Effect of Ocean Acidification on Fish Reproduction

Ocean acidification is considered the "evil twin of global warming." It has been reported that from 1775 to 1996, the surface ocean pH has been reduced from approximately 8.25 to 8.14 which is potent enough for disrupting the functioning of the marine ecosystem at various levels by the end of the year 2100. Along with the increase of industrialization, consequences such as the atmospheric increase of carbon dioxide and decrease in oceanic pH have contributed to the decreased carbonate ion concentration in ocean water, hampering the growth of exoskeleton of invertebrate and coral bleaching (Beldade et al. 2017). The consequences of this anthropogenic nuisance are not only limited to the alteration in other physiological functions such as respiration, circulation, and metabolism but also affect the demography and population size due to reproductive failure (Ishimatsu et al. 2005).

In this context, several researches were conducted to understand the effect of higher CO_2 on the reproductive pattern of the fishes. It was reported that a higher concentration of carbon dioxide can impede sperm motility and thus, by the reduction in intracellular pH, will hinder the fertilizing ability of sperms (Bencic et al. 2000). This response may vary from species to species, for example, mild increase in pCO_2 alters the sperm motility of *Limanda yokohamae,* but there was no such observation of the arresting of sperm motility found in ten other species from different families (Inaba et al. 2003). But in some species, such as *Amphiprion percula*, the elevation of CO_2 up to 1000 ppm was found to show no adverse effect on survival and embryonic duration of eggs (Munday et al. 2009). Effects of CO_2 on the fish eggs are less understood, and it may vary depending upon the species and different stage of development (Ishimatsu et al. 2005). Further, limited scope for aerobic performance in adult fishes could be one major causes of the impact of carbon dioxide on their reproduction (Portner and Farrell 2008). However, although several pieces of researches were conducted with regard to understanding the effect of CO_2 on the reproductive system of fishes, there still exists a huge lacuna in understanding the effect of carbon dioxide on the endocrine regulation of the reproduction axis.

Effect of Hypoxia on Fish Reproduction

The condition in which the dissolved oxygen content is found to be less than 2.8 mg O_2/L (equivalent to 2 mL O_2/L) can be considered as hypoxic condition (Diaz and Rosenberg 1995). In the present scenario, hypoxia is also considered as a global issue for the aquatic environment, because natural phenomenon such as vertical stratification of formation of haloclines and thermoclines and anthropogenic activities by excessive anthropogenic input of organic matters has led to a serious threat of oxygen depletion which affects the biology of the aquatic environment into various extents (Hoback and Barnhart 1996; Wu and Lam 1997; Aarnio et al. 1998). The vast portion of a marine water body such as marine waters surrounding North and South America, Africa, Europe, India, Southeast Asia, Australia, Japan, and China and freshwater body such as over 77% freshwater ecosystem of china have been reported to be affected by the hypoxic condition, and with the increasing time, this situation is predicted to be worse (Wu 1999; Ma and Li 2002).

Along with various other physiological phenomena, hypoxia has also affected the reproductive physiology of the fishes. But it has been observed that the effect of hypoxia on the reproductive process occurs by affecting the BPG axis at various points rather than altering the common pathway of downregulation of metabolism and reproductive function (Wu 2009). It was reported that on the induction of hypoxic condition (0.6 mg O_2/L) for 3 weeks, female zebrafish showed significant downregulation of FSHβ mRNA.

Similarly, chronic hypoxia condition (1.8 mg O_2/L) for 3 months induces a dramatic suppression of brain tryptophan hydroxylase mRNA and ovary FSH receptor mRNA, yet no significant change was observed in the expression of GnRH, GnRH receptor, FSH, and LH of male fish (Wu 2009). In terms of sex steroid, it was observed that the steroidogenic enzymes are regulated at the transcriptional level (Omura and Morohashi 1995). As oxygen is needed for the production of sex steroids, the induction hypoxic conditions hinders the production of sex steroids (Raff and Bruder 2006). It has been reported that by chronic exposure of hypoxic condition, a significant downregulation of sex steroid hormones such as estradiol, testosterone, and 11-keto testosterone was observed in Atlantic croaker (*Micropogonias undulatus*). Further, simultaneous reductions of plasma vitellogenin and hepatic estrogen receptor along with a decrease in the gonadal somatic index (GSI) and fecundity were also reported due to hypoxia-induced stress (Thomas et al. 2006). Similarly, a 50% reduction in E2 and 11-KT was observed in both sexes of hypoxia-induced Gulf killifish (*Fundulus grandis*); however, there was no significant alteration in the level of testosterone and vitellogenin (Landry et al. 2007). Hypoxia is also potent enough to cause alteration in reproductive behavior. For example, male marine gobies have shown increased effort and time of ventilating the eggs and also have shown to reduce the time for selection of opposite sex partner partners (Jones and Reynolds 1999). In the context of reproductive output, the GSI of hypoxia-exposed adult carp was found to be decreased by 40% in male fishes and 33% in female fishes by the exposure of hypoxic condition (1 mg O_2/L) for 8 weeks (Wu et al. 2003).

Effect of Climate Change on Early Ontogenesis Phase of Fish

Among all the stages of fish, eggs and larvae are generally more sensitive to the thermal alteration and are having the lowest tolerance (Rombough 1997). However, it has been observed that both in temperate and tropical areas, the larval growth rate increases with the higher temperature (Blaxter 1991; Green and Fisher 2004). The larval growth rate increases in warm water. In the case of *Thalassoma bifasciatum*, although the recruitment increases, it also possesses a high risk of variability at high temperatures (Sponaugle and Cowen 1996), possibly because of the increased risk of starvation for cohorts dwelling in warmer water. The possible reason behind this might be the increasing requirement of food for the growing larvae in a high-temperature environment, which might increase the risk of starvation within the cohort and thus be responsible for failed recruitment, imbalanced demography, and population dynamics (Pankhurst and Munday 2011). Further, according to Cushing's match-mismatch hypothesis, climate-induced elevated temperature conditions might alter the reproductive timing, so that the recruitment period will not match the timing of the highest plankton availability leading to reduced larval survival (Cushing 1990).

In comparison to the adults, the degree of vulnerability of the egg and larval stage is generally higher. Fish eggs and larvae are very sensitive to the lower pH and hypercapnic exposure (Brown and Sadler 1989). It has been observed that in the case of red seabream (*P. major*), the cultured larvae showed more mortality in lower pH induced by carbon dioxide rather than lower pH induced by mineral acid HCl (Kikkawa et al. 2004). This is due to the higher biomembrane permeability of CO_2 gas than that of H^+ (Morris et al. 1989). According to Munday et al. (2009), a higher concentration of CO_2 exposure can damage the olfactory system in the fish larvae hindering them to differentiate between various types of ecologically important chemical signals. For example, due to CO_2 exposure of 1000 ppm in clownfish, larvae became tempted toward the smell of inappropriate habitats and allured toward the smell of predators (Munday et al. 2009). In another study, Munday et al. (2010) demonstrated that exposure of 700–850 ppm CO_2 alters the olfactory impairment and causes behavioral change of clownfish and damselfish (*Pomacentrus wardi*) larvae in natural coral reef habitat resulting in five- to ninefold higher mortality because of increased predation. But the exact physiological mechanism behind this phenomenon is still not clear.

Hypoxia also has a profound effect on the larval development of fishes. In several reports, it was described that the embryonic development phase particularly gastrula and blastula is extremely vulnerable to any kind of stress in the environment (Johnson and Landahl 1994; Cameron and von Westernhagen 1997). For example, lower O_2 concentration in water retarded the brown trout alevin ontogenesis in a side stream of the Adour river in Southwest France (Dumas et al. 2007). Wu et al. (2003) demonstrated that post-24 h of hatching the larval survival percentage was dramatically reduced to 46.4% in the hypoxic group in comparison to the normoxic group which was as high as 93.7%. Moreover, it was observed that for several species the parental history is also important to some extent. In this context, the larvae of parents

who are reared under high temperature showed greater thermal tolerance under high temperature (Rombough 1997).

Conclusion

As in the present scenario, the climate change has led to the creation of a multiple stressor environment for the fishes, and all the physiological aspects including the reproductive physiology of the fishes are being affected to various extent. The various reproductive processes including reproductive behavior, hatching, larval survival, and quality of new offspring are being affected in a negative manner, which is putting threat to the future population for upcoming undesirable yet unavoidable events such as imbalanced population dynamics, imbalanced demography, and ultimately species extinction. Therefore, there is an urgent need for a clear understanding of climate change and its consequence in a biological system. Although assessing the impact of this climate change on the reproductive physiology of the fish is a quite arduous job, but by knowing the impact of the stressor on different species and various life stages, we can give special emphasis on the endangered species and protect the most vulnerable life stages of fish.

Future Research Prospects

Firstly, there exist a lot of research gaps in this sector as the research data is available for only a few species, so in the future utmost importance should be given for understanding the impact on various other species, along with the rest of the economically important species. Secondly, as maximum research is available on the fishes of the temperate zone, there still exists a lacuna for the tropical zone fishes. Researchers must take this fact under consideration for future researches.

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