



## **BIOCHEMICAL STUDY OF DNA AND RNA IN OVARY OF TELEOST: A REVIEW**

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### **Abstract**

RNA/DNA ratios is a fast and reliable method which is used for assessment of fish condition, especially for the short-term study. This technique is based on the assumption that RNA content in cells varies according to the amount of protein synthesis in organisms, but the level of DNA content remains almost the same. Well-developed and healthy fishes demonstrate high RNA/DNA ratios while poorly developed and healthy ones show low RNA/DNA ratios. Non-coding RNAs (ncRNAs) were shown to play various regulatory roles, for example, in reproduction. Thus, an effective reproduction in teleost fishes was correlated with complete realization of the reproduction process, but there are not many publications concerning the role of ncRNAs presence and regulation in mature and immature gonads of fish. RNA content and RNA/DNA ratios proved themselves to be reliable parameters for estimation of growth rate and feeding conditions of fishes in their natural habitats. With the help of meta-analysis of scientific papers published it could be demonstrated that RNA/DNA ratios in larvae of fishes are influenced by starvation and the influence increases with starvation time

In teleost fishes, the ovaries constitute one of the most dynamic organs, which have nucleic acids acting as major components in their development, functioning, and proliferation. In this review, an attempt has been made to provide an insight into the biochemistry and biological importance of deoxyribonucleic acid (DNA) and ribonucleic acid (RNA) in the development of ovaries in teleost fishes. DNA content is quite constant and forms an accurate index of cell number, while RNA content depends upon the metabolic activity and protein synthesis. MicroRNAs

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(miRNAs) are short RNA molecules which act as gene expression regulators at the post-transcriptional level. There is growing recognition that miRNAs act as important regulators of many biological activities including reproductive function. By utilizing expression analysis of miRNAs from fish ovaries, numerous studies have demonstrated the presence of miRNAs in the fish ovary and their regulation during folliculogenesis and hormonal stimulation. Several recent studies using CRISPR/Cas9 gene knockout technique have identified the importance of miR-202 and miR-200 family members located on chromosome 23 for ovarian development, oogenesis, and ovulation in fish species.

**Keywords-** RNA: DNA ratio; Ovary; Tilapia fish; Reproductive biology.

### **Introduction:**

The concept of RNA:DNA ratio was initially introduced 38 years ago as a biochemical parameter for measuring the physiological condition of aquatic organisms in nature, and has since been extensively studied. These indices have been applied with success in marine ecology in microbial communities' and in invertebrates and fishes (Chícharo MA *et. al* 2008). The ratio of RNA:DNA, which could also be described as the measurement of nucleic acid ratio, is one of those techniques that can easily be utilized for providing a quick indication about the health status of the fish. The technique is based on the simple motion that within individual cells, DNA concentrations remain relatively constant, and it is RNA that varies which in turn increase the protein synthesis (Hussna *et. al* 2020).

The 5S rRNA was discovered as early as in 1963 and has been studied for RNA-protein interactions and used as a phylogenetic tool for several decades ([Zhi-Gang Shen](#), 2017). Any rise or fall in the value of the RNA/DNA and RNA/mg tissue ratio would be an indication of changes in the level of protein synthesis, growth rates, and the nutritional status of fishes in terms of their body weights. (G. K. SIVARAMAN *et. al* 2009).

Quantification of [nucleic acid](#) ratios (RNA:DNA) provides a short-term measure of condition and growth, and is based on the notion that DNA concentrations within individual cells remain fairly constant while RNA concentrations increase as [protein synthesis](#) increases ([Tomas O. Höök](#) *et. al* 2015). Substances that are toxic for aquatic life can affect the genetic makeup of fish reproduction systems. Studies have revealed that pesticides can lead to DNA damage and lower DNA levels in the ovary, thus interfering with the reproductive system of fish. (Van der Oost, *et. al* 2003). Studies based on DNA are useful for identifying the species, evolution, and understanding the effect of environmental stress, thus being vital in environmental conservation and management DNA studies for species identification, evolution & stress (Ward, R. D. *et. al* 2005).

Based on current scientific evidence, the use of chemicals such as industrial waste, pesticides, and heavy metals causes oxidation and DNA damage, which ultimately leads to a

decrease in species numbers and affects biodiversity (Lushchak, V. I. *et. al* 2011). Testing DNA integrity using biochemical methods helps determine the effects of genotoxins on aquatic organisms. Comet assay, used for this purpose, indicates DNA damage in fish tissue. Oxidative stress, it gives an idea about the pollution causing damage (Jha, A. N. *et. al* 2008).

Modern techniques like single cell RNA sequencing allowed for a huge progress in the field of ovarian development, with identification of diverse cell types and expression of genes involved in the process being described Gong, X., *et. al* (2022). Single-nucleus RNA sequencing allowed researchers to create an accurate cellular landscape of the adult Nile tilapia ovary, highlighting specific cell types and pathways active during oogenesis (Yao, Z. L., *et. al* 2024). Research on mitochondrial DNA of Nile tilapia population, including cytochrome b analysis, demonstrated a high level of haplotype variability and low levels of nucleotide variation (Popoola, O. M. *et. al* 2024).

Moreover, environmental conditions like day lengths affect ovarian development, where studies involving RNA sequencing have demonstrated that light exposure at a longer period or continuous exposure leads to fast maturation and increased activity of reproductive genes (Tang, Z., *et. al* 2019). Different RNA types (miRNAs, lncRNAs, circRNAs) interact with mRNAs to regulate gene expression. These RNAs form complex regulatory networks controlling follicular development and oogenesis. Non-coding RNAs regulate key biological processes like gene transcription, translation, and mRNA degradation (Liang, Y. *et. al* 2021). MicroRNAs are involved in regulating gene expression post-transcriptionally. They are essential in the regulation of ovarian development and reproduction. miRNAs are expressed in a hormonal and stage-dependent manner in fish ovaries (Kim, J. *et. al* 2021). RNA estimation in fish ovaries is essential for understanding oocyte development and reproductive physiology. RNA levels increase during vitellogenesis to support yolk protein synthesis. Changes in ovarian RNA content also serve as biomarkers of reproductive toxicity caused by pollutants (Arockiaraj *et al.*, 2010).

### **Review of literature:**

**Al-Thuwaini *et. al* (2023)** In conclusion, the success of molecular experiments will depend highly on the quality of DNA isolation. Efficient methods for isolation are very important because quality of the DNA is directly connected with procedures such as PCR and sequencing. In addition, there are great differences between fish species; therefore, each time the method used needs to be adapted accordingly.

**Alvi, S. M. *et. al* (2021)** Studies using microscopy have shown that there is a differential expression of genes in Nile tilapia depending on the sex of the organism. Some microRNAs are abundantly found in the ovaries and participate in the development of the oocytes and hormone secretion, whereas other microRNAs are more common in male reproductive glands and play a vital role in sperm formation. Such studies have shown that microRNAs are important regulators of fish reproductive activities.

**Hussna et., al (2020).** Investigated because DNA content is fairly stable in cells. RNA content can fluctuate depending on protein synthesis activity. So, RNA:DNA ratio is an indicator of metabolic rate and growth potential. RNA:DNA ratio is determined to evaluate short-term growth and condition. Fish with high RNA:DNA ratio had better growth and protein synthesis. Lower RNA:DNA ratio is associated with poor nutrition state, disease stresses, or unfavorable environment

**Foley, C. J. et., al (2016)** The RNA:DNA ratio is a valuable measure of fish cellular growth and feeding state since DNA is a constant entity in the cell throughout its life cycle but RNA fluctuates according to protein metabolism. Thus, fluctuations in RNA:DNA ratios are associated with changes in growth rates. Originally established under experimental settings, this tool is widely applied in ichthyology for studying the growth and reproduction of fish, which are dependent on genetic and environmental determinants, including water temperatures.

**Zhu, X. et., al (2025)** flow cytometry method has proven to be extremely useful in studying the genetics of fish species. It enables scientists to determine the amount of DNA in individual cells rapidly. Flow cytometry can be extremely valuable for determining whether an organism has the appropriate chromosome number and whether it was affected by some form of modification. It can also be used to artificially breed organisms which cannot reproduce sexually or possess genetic material from two separate species.

**Baroiller, J. F. et., al (2009)** It is also necessary to discuss the impact of nutrition. Poor nourishment can affect fish's nucleic acid metabolism negatively, as fish will not receive adequate amounts of nutrients required for proper nucleic acid metabolism. For example, if you do not give shiners any food, the proportion of RNA to DNA will be low because protein synthesis will be reduced. However, if you begin feeding them, the ratio of RNA to DNA becomes higher very fast.

**Connell, M. L. et., al (2024)** Environmental contaminants also substantially affect the condition of fish nucleic acids. As an illustration, studies have demonstrated that exposing *Channa striata* to pesticides such as cypermethrin lowers DNA and RNA concentrations in gill tissues and diminishes RNA: DNA ratios, suggesting metabolic disruption and hindered protein production.

**Zhu, Z. et., al (2026)** DNA replication and RNA transcription are very active in Nile tilapia during the early stages of egg development, which helps to drive cell division and control gene expression. Nevertheless, environmental factors like light and temperature can affect these processes.

**Tesfaye, G. et., al (2021)** Sequencing of mitochondrial DNA, especially genes like cytochrome b, is widely used when studying genetic diversity and population structures in Nile tilapia. These data are vital and useful for the purposes of conservation and breeding.

**Yao, Z. L. et., al (2024)** Likewise, single-nucleus transcriptomics have enabled scientists to identify major ovarian cells, which include oogonia, oocytes, granulosa cells, and thecal cells, each of which has unique transcriptomic profiles. Thus, there is an elaborate understanding of ovarian cellular

biology. Differences in DNA content across tissues and taxa unequivocally indicate that both genetics and the environment play critical roles in the development and survival of fish.

**Shen, Z. G. et., al (2017)** Furthermore, sequencing ribosomal RNA can act as a biomarker for sex determination and ovarian maturation because fully developed ovaries show increased quantities of 18S and 28S rRNA compared to developing gonads and testes.

**Al-Thuwaini et., al (2023)** In conclusion, the success of molecular experiments will depend highly on the quality of DNA isolation. Efficient methods for isolation are very important because quality of the DNA is directly connected with procedures such as PCR and sequencing. In addition, there are great differences between fish species; therefore, each time the method used needs to be adapted accordingly.

**Buckley, L. J. et., al (1984)** The correlation between RNA and DNA levels is especially relevant to predict success of reproduction in juvenile fish. It was found out in studies of various fish species that individuals with high RNA/DNA ratios usually have more developed metabolism, faster growth and higher chances of survival, whereas low RNA/DNA ratio results in reduced growth rate and mortality. Other factors can also influence the findings. Thus, for instance, increasing water temperatures resulted in high RNA/DNA ratios in larvae of North Sea plaice and fast development, implying growth success.

**Sumaira Rashid et., al (2020).** Fish growth has traditionally been assessed using methods such as length–weight relationships and radiocarbon dating, though these approaches are often limited by cost, time, and potential inaccuracies. The RNA:DNA ratio offers a reliable biochemical index based on constant DNA levels and variable RNA associated with metabolic activity. Higher ratios indicate active growth and good nutritional status, whereas lower ratios reflect reduced metabolism and poor condition

It identified thousands of sex-biased differentially expressed genes, with ovary-enriched genes linked to oogenesis and estrogen pathways, and testis-enriched genes linked to spermatogenesis, steroidogenesis, and gonadal development. Observed the RNA-Seq study on Pearlscale angelfish (*Centropyge vrolikii*) compared ovaries and testes using Illumina sequencing and transcriptome analysis **Yonghua Jiang et., al (2021).**

**Jun Xiao et al., (2014).** Reviewed that Solexa/Illumina sequencing to profile small RNAs in Nile tilapia ovaries and testes. Millions of clean reads were obtained, revealing both conserved and novel miRNAs. Several miRNAs showed sex-biased expression, with some enriched in ovaries (linked to oocyte development) and others in testes (linked to spermatogenesis). qRT-PCR confirmed these patterns, and target prediction suggested roles in gonadal development, hormone signaling, and gametogenesis. Overall, the results highlight miRNAs as key regulators of reproduction and sexual differentiation in tilapia

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Researchers conducted RNA sequencing on Nile tilapia ovaries and testes, uncovering more than 21,000 circular RNAs (circRNAs), many of which were specific to individual tissues. Distinct expression profiles were observed between the two gonads, with several circRNAs associated with key reproductive pathways, including oocyte meiosis, steroid biosynthesis, and spermatogenesis. Additionally, some circRNAs were predicted to function as microRNA sponges, indicating a potential role in regulating gene expression during gonadal development and sexual differentiation [Yongju Luo et., al \(2022\)](#).

Sr. No	Model Organism	Inference	Biochemical ratio of DNA	Biochemical ratio of RNA	References
1	Fish (teleost)	MicroRNAs regulate ovarian development by controlling gene expression in fish.	Constant	Increased	(Alvi et al. 2021)
2.	Fish (teleost)	RNA:DNA ratio reflects recent growth rate and metabolic condition in fish.	Increased	Increased	(Bulow, F. J. et., al 1970)
3.	Fish (teleost)	Non-coding RNAs show stage-specific expression, regulating gene activity during teleost gonad maturation.	Constant	Increased	( <a href="#">Maria Papadaki et., al 2020</a> )
4.	Golden Mahseer ( <i>Tor putitora</i> )	RNA:DNA ratio increases with fish growth, indicating higher protein synthesis and metabolic activity in <i>Tor putitora</i> .	constant	Decreased	(G. K. SIVARAMAN et., al 2013)
5.	Fish (teleost)	rRNA profiling provides a quantitative tool for reproductive stage identification.	Constant	Increased	( <a href="#">Zhi-Gang Shen et., al 2017</a> )
6.	fish larvae	A review and assessment of the potential use of RNA:DNA ratios to assess the condition of entrained fish larvae overlay panel	Constant	Increased	( <a href="#">Tomas O. Höök<sup>a b</sup> et., al 2015.</a> )
7.	<i>Oreochromis niloticus</i> (Nile tilapia ovary)	DNA barcoding effectively identifies fish species using genetic variation in mitochondrial DNA.	Constant	Constant	(Ward et., al 2005)
8.	Fish	DNA and biochemical biomarkers help assess pollution-induced stress and bioaccumulation in fish.	Constant	Increased	(Van der Oost et., al 2003).
9.	Fish (teleost)	Higher RNA:DNA ratios reflect better growth and nutritional condition in fish.	Constant	Increased	(Hussna, et., al 2020)
10.	Fish (general)	Flow cytometry accurately measures DNA content for fish breeding and genetics.	Increase	No change	(Zhu, X., et., al 2025)
11.	<i>Oreochromis niloticus</i> (Nile tilapia ovary)	Oxidative stress alters cellular macromolecules including DNA, impacting fish physiology.	Decreased	Increased	(Lushchak, V. I. et., al 2011).
12.	Fish (genetic breeding studies)	Application of flow cytometry to determine cell DNA content in the genetic breeding of fish.	Increase	No change	(Zhu, X. et., al 2025)
13.	Tilapia ( <i>Oreochromis spp.</i> )	Sex determination in tilapia is controlled by both genetic and environmental (temperature) factors.	No change	No change	(Baroiller et., al 2009)

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14.	Zebrafish ( <i>Danio rerio</i> )	Single-cell RNA sequencing enhances understanding of reproductive toxicology in fish.	No change	Increase	(Connell, M. L. et., al 2024)
15	<i>Oreochromis niloticus</i> (Nile tilapia)	Genetic variation in tilapia populations is revealed through DNA analysis.	Increase	No change	(Popoola, O. M. 2024).
16.	<i>Oreochromis niloticus</i> (Nile tilapia)	Photoperiod changes influence ovarian gene expression and reproductive development.	No change	Increase	(Tang, Z. et., al 2019).
17.	Teleost fish (ovary)	RNA expression and its role in ovarian development and reproductive physiology in teleost fish.	No change	Increase	(Arockiaraj et., al 2010)
18.	<i>Nile tilapia (Oreochromis niloticus)</i>	DNA markers reveal genetic diversity important for conservation of tilapia.	Increase	No change	(Tesfaye, G. et., al 2021).
19.	Larval fish	RNA:DNA ratio is a strong indicator of larval fish growth in natural environments.	Stable	Increase	(Buckley et., al 1984)
20.	Fish larvae	Photoperiod changes influence ovarian gene expression and reproductive development.	Stable	Increase	(Foley et., al 2016)
21.	<i>Danio rerio</i> (Zebrafish)	Status of single-cell RNA sequencing for reproductive toxicology in zebrafish and the transcriptomic trade-off.	No change	Increase	(Connell et., al 2024)
22.	<i>Oreochromis niloticus</i> (Nile tilapia)	Regulatory mechanisms of temperature on the gonadal development of fish	No change	Increase	(Zhu et., al 2026)
23.	<i>Oreochromis niloticus</i> (Nile tilapia ovary)	Distinct ovarian cell types show unique RNA expression patterns in Nile tilapia.	No change	Increase	(Yao et., al 2024)
24.	Fish (ovary studies)	rRNA levels rise during ovarian development and can be used as a biomarker for sex identification and maturation stages.	No change	Increase	(Shen et., al 2017)
25.	Fish tissue	Efficient DNA extraction methods improve molecular studies in fish.	Increase	No change	(Al-Thuwaini et., al 2023)
26.	(Nile tilapia ovary)	Single-cell RNA sequencing reveals gene expression dynamics during ovarian development.	No change	Increase	(Gong et., al 2022)
27.	<i>Oreochromis niloticus</i> (Nile tilapia ovary)	Non-coding RNAs play crucial regulatory roles in reproductive processes.	No change	Increase	(He. M. et., al 2021)

## **Conclusion:**

In relation to the biochemical analysis of DNA and RNA within the teleost ovaries, there is an observation that DNA concentration is fairly stable and serves as a genetic blueprint, whereas RNA concentrations vary due to gene expression. Differential expression of RNA types like mRNA, rRNA, and noncoding RNA is involved in crucial biological processes such as follicle development, steroid hormone synthesis, and gamete production. The ratio of RNA/DNA can thus be used as a reliable biochemical parameter in evaluating ovarian development, nutrition, and reproduction capacity. It should be noted that external and internal conditions such as temperature, nutrition, and stress affect nucleic acid biosynthesis.

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