

A Seminar Paper on
PLOIDY MANIPULATION IN FISH: A PROMISING TECHNIQUE FOR
IMPROVING FISH PRODUCTION

Course Code: GFB 598

Term: Winter, 2022

Submitted to

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PLOIDY MANIPULATION IN FISH: A PROMISING TECHNIQUE FOR IMPROVING FISH PRODUCTION¹

By

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ABSTRACT

Ploidy manipulation or alteration in the number of chromosome in fish mainly includes production of haploids, androgens, gynogens, triploids and tetraploids commonly for the betterment of aquaculture industry mainly through increased fish production. This review paper summarized current state of research, benefits and drawbacks of ploidy manipulation in fish to increase production. Research on ploidy manipulation in fish has produced some promising result in enhancing fish growth and production throughout the world since the last century. Among ploidy manipulation technique androgenesis done by deactivating the egg genetic material through irradiation that results in broodstock for populations of all males and the most effective strategy for fish genome preservation through cryopreservation. Fertilizing the egg with UV irradiated sperm and diploidization by different shocking treatments results in Gynogen. In case of meiotic gynogens *Clarias macrocephalus* and *Paralichthys olivaceus* showed 18% and 35% quicker growth, respectively. Retention of second polar body due to shock treatment resulted in triploids and blocking the first cleavage produces tetraploids. Triploidy typically causes infertility, particularly in females and accelerate post maturity growth in species like *Labeo bata*, *Oreochromis mossambicus*, *Oncorhynchus mykiss* mainly due to the diversion of reproductive energy in somatic growth. Thermal shock ranging between 39-41°C for several minutes showed better result in different fish species. Hydrostatic pressure showed best result in survivability of triploid and tetraploid fish. Although low survival rate, deformities, and behavioral changes is seen in some of the ploidy manipulated fish, it still has a great potential to improve and enhance fish production.

Key Words: Ploidy manipulation, Haploidy, Androgenesis, Gynogenesis, Triploidy, Tetraploidy.

¹A seminar paper presented at seminar course GFB 598; Winter, 2022.

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Chapter 1

INTRODUCTION

Fisheries is a vital component of the global food system, contributing to the livelihoods of around 600 millions of people worldwide (FAO 2022). In 2020 the fisheries and aquaculture production made a record of 214 million tons among which 87.5 million aquatic animal came from aquaculture. Aquatic food consumption is anticipated to rise by 15% by 2030, reaching an average of 21.4 kg per person (FAO 2022) as a result of rising incomes, urbanization, better post-harvest procedures, and dietary patterns, necessitating the development of novel techniques to improve fish production. One such technique is ploidy manipulation, which involves altering the number of chromosomes in fish cells. It has the potential to increase growth rates, disease resistance, and overall productivity.

Ploidy manipulation is a promising approach to enhance fish production since it enables the production of haploid, androgens, gynogens, triploid or tetraploid fish, which grow faster and have improved flesh quality. haploid fish is produced to aid in studies of genetic control of polymorphic genetic loci though their survivability is very less. Androgenesis is a unique type sexual reproduction in which a male is the sole source of the nuclear genetic material in the embryo (Schwander and Oldroyd, 2016). Gynogenesis, on the other hand, involves the manipulation of the eggs of the female parent. In this technique, the eggs are stimulated to develop into embryos without fertilization, using the genetic material of a male donor as a stimulus. The resulting offspring are induced to become diploid through various means such as thermal or pressure shock, resulting in all-female progeny. In some gynogenetic species genetic homozygosity leads to growth suppression from 3 to 60%; however, meiotic gynogens of *Clarias macrocephalus* and *Paralichthys olivaceus* display 18 and 35% faster growth (Pandian and Koteeswaran, 1998). Polyploidy, or the duplication of the entire genome, can be induced in fish through a variety of methods, including exposure to heat shock, cold shock, pressure shock or chemicals that inhibit cell division. The resulting triploid or tetraploid fish have three or four sets of chromosomes, respectively, instead of the normal two sets found in diploid fish. Triploids is being successfully applied in improving aquaculture production and fisheries management over the last few decades (Rahi and Shah, 2012; Warner *et al.*, 2018). It induces sterile populations of fish as their

three chromosome sets that create difficulty in the cell division process during meiosis due to imbalanced chromosomal distribution (Janhunen *et al.*, 2019; Rahi and Shah, 2012). Theoretically, triploid fishes are expected to grow faster and more than the normal diploids, as triploid cells contain 33% more genetic material due to the presence of an extra chromosome set (Rahi and Shah, 2012; Warner *et al.*, 2018). Triploids are produced directly through the retention of the second polar body during the second meiotic division. Triploidy is often induced by heat shock, cold shock, pressure shock and chemical shock, but it may also be generated indirectly by the crossing of tetraploid and diploid individuals (Rahi and Shah, 2012). Moreover, sterility also results in faster growth of triploids over their diploid counterparts, as triploids divert their energy towards somatic growth rather than reproduction (Wasow *et al.*, 2004). The level of heterozygosity is also higher due to their extra chromosome set, which also result in better growth performance due to overdominance and reduced inbreeding depression (Coltman and Slate, 2003). Polyploid fish often exhibit larger cell size, increased growth rates, and improved disease resistance, which make them ideal for aquaculture. Despite the promising results of ploidy manipulation, there are still challenges that need to be addressed, such as the high mortality rates associated with some polyploid induction methods and the potential negative effects on genetic diversity. Additionally, there are still many unanswered questions about the long-term effects of polyploidy on fish performance and the ecological impacts of introducing polyploid fish into natural ecosystems.

Considering the conditions stated above, the review paper has been prepared with the following objectives:

- To provide a comprehensive assessment of the current state of research on ploidy manipulation in fish
- To evaluate the potential benefits and drawbacks of this technique for improving fish production

Chapter 2

MATERIALS AND METHODS

This is exclusively a review paper for seminar so all of the data, information has been collected from the indirect sources. During the preparation of the review paper, I went through various relevant books, journals, proceedings, reports, publications, internet etc. Findings related to my topic have been reviewed with the help of the library facilities of Bangabandhu Sheikh Mujibur Rahman Agricultural University. I got suggestion and valuable information from my major professor and my course instructors. After collecting all the available information, I myself compiled the collected information and prepared this seminar paper.

Chapter 3

REVIEW OF FINDINGS

3.1 Comprehensive assessment of ploidy manipulation in fish

The field of chromosome manipulation research in fish is relatively new when compared to its application in crops and animals. Beginning in 1913, early efforts were made to interfere with the metaphase spindle apparatus during cell division in fish eggs using various physical and chemical agents (Oppermann, 1913). Over time, several techniques have been developed to disrupt normal functioning, resulting in the production of individuals with diverse genomic statuses, such as polyploids (triploid and tetraploid), gynogenetics (both meiotic and mitotic gynogens), and androgenetics, within the fish population.

Table 1. Major events in the history of ploidy induction in fish

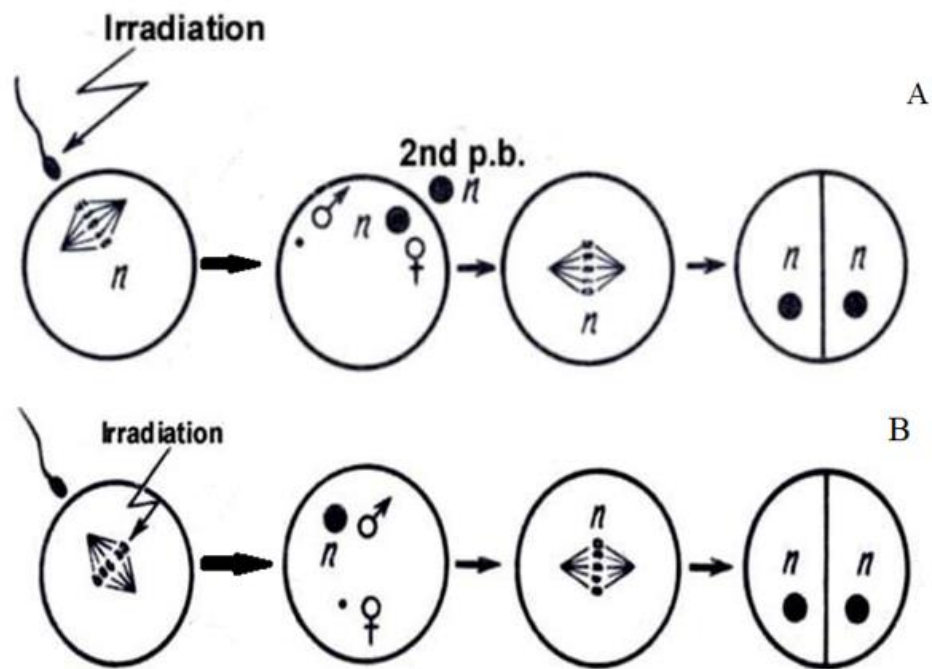
| Events | Author(s) |
|--|----------------------------------|
| Produced haploid brown trout using radiation | Oppermann (1913) |
| Production of autotriploid stickleback by heat and cold shocks | Swarup (1959) |
| Induction of mitotic gynogens in zebrafish | Streisinger <i>et al.</i> (1981) |
| Live, feeding mitotic tetraploid rainbow trout produced | Thorgaard <i>et al.</i> (1981) |
| Androgene produced using sperm from 4n rainbow trout | Thorgaard <i>et al.</i> (1990) |
| Applied electric shock to induce triploidy | Teskeredzic <i>et al.</i> (1993) |
| Produced feeding and growing gynogene haploid tilapia | Varadaraj (1993) |

Source: Pandian and Koteeswaran, 1998

3.1.1 Haploidy

Researchers have attempted to create a live haploid fish to aid in studies of genetic control of polymorphic genetic loci. Hence, efforts resulted in a live haploid fish (Uwa, 1965). While previous attempts only produced haploid embryos (Strelkov *et al.*, 1976) that were analyzed for synthetic ability in producing rRNA's and metabolic enzymes such as Lactate Dehydrogenase (Stanley, 1983), it was discovered that gene expression operates normally in haploid embryos of *Salmo salar* (Stanley, 1983), However, these

haploids experienced mass mortality at the time of hatching due to the expression of recessive lethal mutant genes.



Sources: Gomelsky (2011)

Figure 1. A schematic diagram of haploid fish production. A: Haploid gynogen, B: Haploid androgen.

The mechanism of haploid fish production is to fertilize an egg with irradiated sperm with no genetic material which will result in haploid gynogen fish.

A vice versa method of fertilizing an irradiated egg having no genetic material with normal sperm will result in haploid androgen fish.

In a notable exception, Varadaraj (1993) successfully induced haploidy in viable gynogenetic haploid tilapia (*Oreochromis mossambicus*) using 10 min UV-irradiated sperm at irradiation dose of 4.2 W/m^2 on the surface of the milt and confirmed it by karyological, nucleometric and flowcytometric techniques.

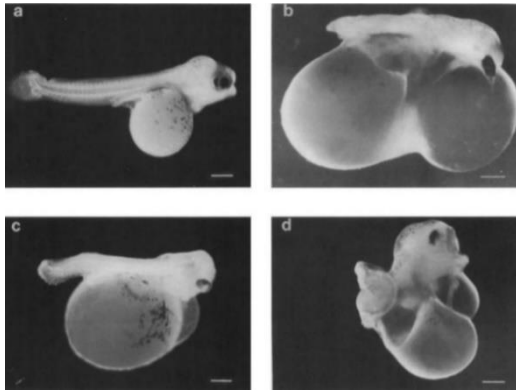
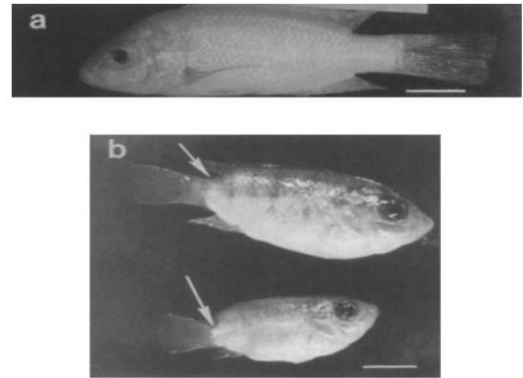


Figure 2. Deformities of haploid fry.

a: Normal *diploid O. mossambicus* fry b-d: Deformities in haploid fry. b) edematous and stunted body, c) poorly formed retina, and d) twisted body with curved tail.



Source: Varadaraj, 1993

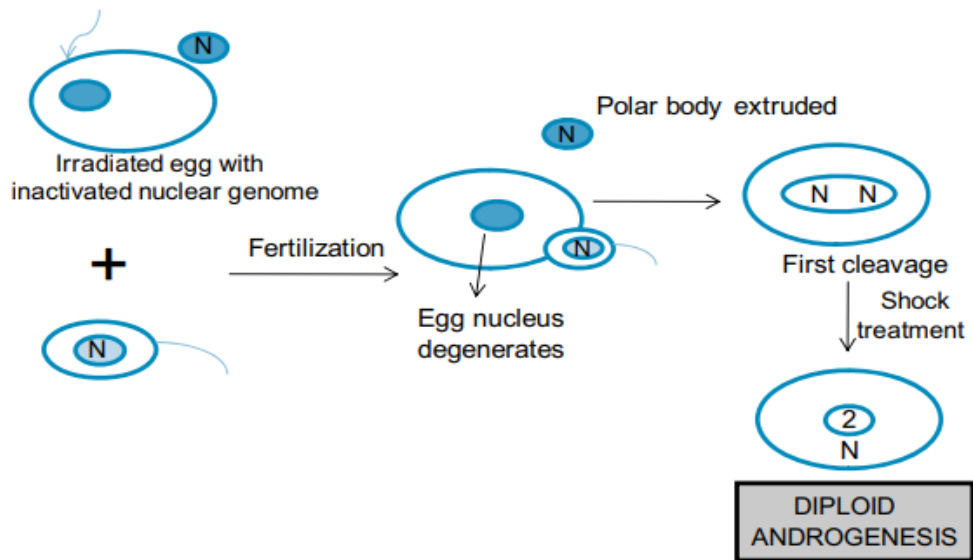
Figure 3. Reduced caudal region of haploid fish.

a: Diploid b: Haploid *O. mossambicus*. Arrows indicate the reduced caudal regions.

Only four percent of the induced haploids survived and exhibited caudal defects (Figure 2, Figure 3), making them unable to swim, feed or escape predators. Nonetheless, one individual was successfully grown to maturity with great care.

3.1.1 Androgenesis

Androgenesis is a biological process that enables only the transmission of genetic information from the father. It necessarily requires the deactivation of the genetic material of the egg through the use of either dispermic activation or haploid or diploid gamete activation (Thorgaard *et al.*, 1990). Androgenesis has potential benefits in producing YY supermales in species with male heterogamety, creating inbred isogenic lines, and conserving germplasm.



Source: Padhi & Mandal, 2000

Figure 4. A schematic diagram of androgene induction in fish.

When UV irradiated deactivated egg with no genetic material is fertilized with normal sperm an androgen is produced. Shock treatment prevents the first cleavage and bring back diploid condition of the cell and a diploid androgen zygote is created which will develop in to an androgen fish.

Table 2. Technique of artificial induction of androgenesis in fishes

| Species | Inactivation of female genome | TAF | Treatment for diploidization | SR (% RC) | Affirmation method | Sources |
|---|--|-------------|---|---------------------|--------------------|-------------------------------------|
| Rainbow trout <i>Oncorhynchus mykiss</i> | ⁶⁰ Co γ -rays 36 kR | 320 min | Hydrostatic pressure 9000 psi 3 min | H: 3.8 Fd: 2.5 | Karyotyping g | Scheerer <i>et al.</i> (1991) |
| Tilapia <i>Oreochromis niloticus</i> | UV 450 J/m ² 5-8 min | 22.5-30 min | Heat shock 42.5°C 3-4 min | H: 5.3 | Karyotyping g | Myers <i>et al.</i> (1995) |
| Goldfish <i>Carassius auratus</i> | ⁶⁰ Co γ -rays 25 kR | 40 min | Heat shock 40°C 2 min | H: 40.6 Fd: 50.2 | RAPD assay | Bercsenyi <i>et al.</i> (1998) |
| Tiger barb <i>Puntius tetrazona</i> | UV 4.2 W/m ² 3.5 min | 24 min | Heat shock 41°C 2 min | H: 15 Fd: 7 | Karyotyping g | Santhakumar <i>et al.</i> (2003) |
| Mud loach <i>Misgurnus mizolepis</i> | UV 63.4 ergs/mm ² /s | 30 min | Heat shock 40°C 2 min | – | Flow cytometry | Nam <i>et al.</i> (2002) |

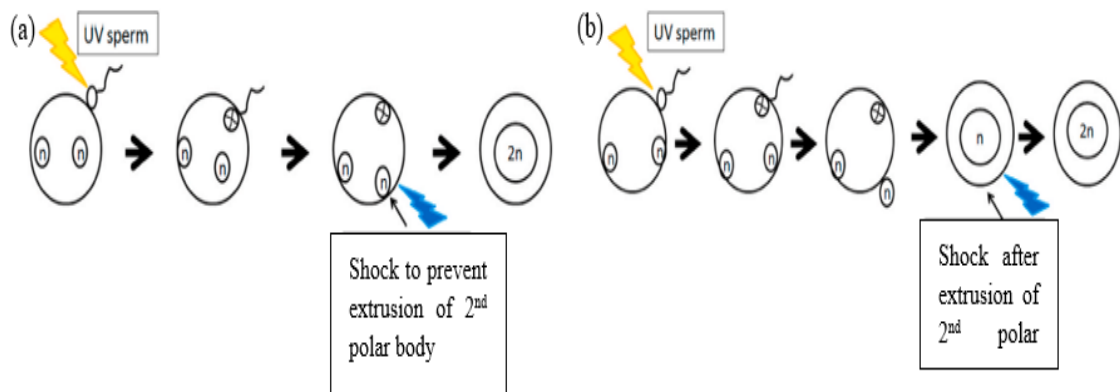
*TAF= Time after fertilization; *SR= Survival rate; *RC= Relative to control;

*H= Hatching; *Fd= Feeding

Table 2 summarizes the different techniques used in different fish species to produce androgenesis. To remove the female genetic materials strong irradiation like γ -rays or UV light of high intensity is used in case of androgenesis. For diploidization heat shock or hydrostatic pressure is very common. Karyotyping is the mostly used confirmation method for diploidization.

3.1.2 Gynogenesis

Gynogenesis, a well-known method for producing only female offspring, has been effectively used to produce diploid gynogenetic offspring in aquatic animals like fish and crustaceans. The practice of monosex culture, consisting entirely of female fish, is attributed to the superior size of female offspring compared to males, which can increase market size and profitability.



Source: Manan *et al.*, 2022

Figure 5. Gynogenesis process for production of diploid gynogens. a: Meiotic gynogenesis process, b: Mitotic gynogenesis process.

Figure shows the process of diploid gynogen production where UV irradiated sperm is used to fertilize the egg and treated with heat shock, cold shock or hydrostatic pressure shock to bring back diploid condition through retention of second polar body or blocking the first cleavage.

Table 3. Technique of artificial induction of gynogenesis in fishes

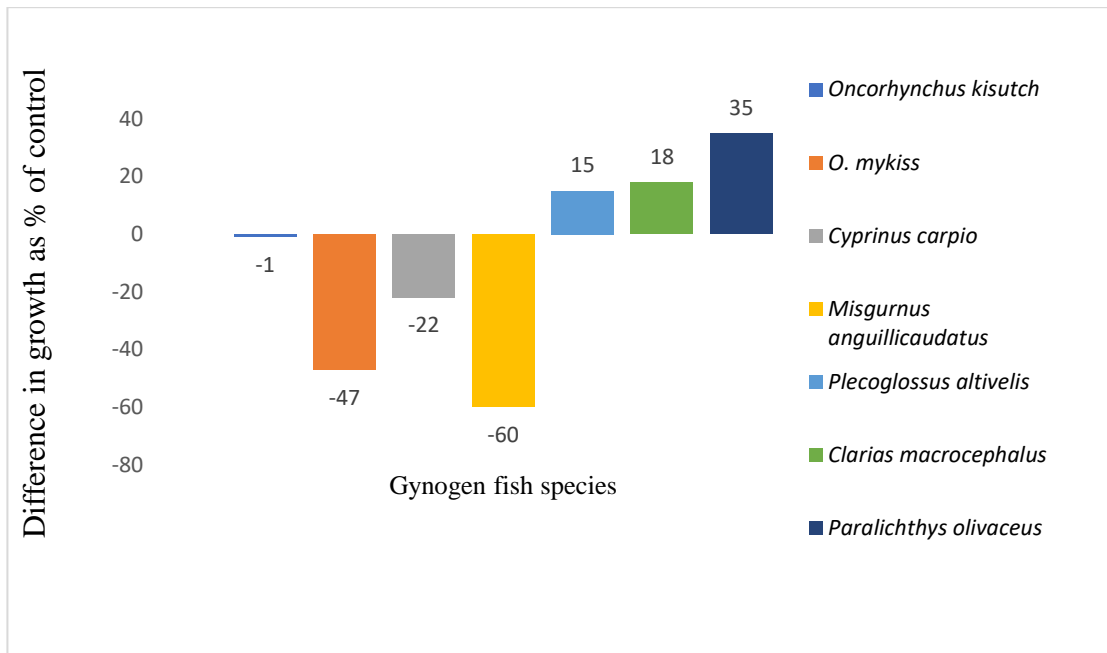
| Species | UV irradiation | TAF | Diploidization Technique | SR (%RC) | Affirmation method | Sources |
|---|--|---------|---|----------|--------------------|----------------------------------|
| Catfish (<i>Heteropneustes fossilis</i>) | 250 μ W/cm ² 2.5 min | 3-7 min | Cold shock 2 °C 10 min | 67.64 | Karyotyping | Gheyas <i>et al.</i> (2001) |
| Zebrafish (<i>Danio rerio</i>) | 254 nm 30 w 2 min | 13 min | Heat shock 41 °C 2 min | 30.35 | Karyotyping | Ozdemir & Aygul (2017) |
| Grass carp (<i>Ctenopharyngodon idella</i>) | 254 nm 30 w 10 min | 2 min | Cold Shock 4-6 °C 12 min | 4.58 | Karyotyping | Zheng <i>et al.</i> (2017) |
| African sharptooth catfish (<i>Claria gariepinus</i>) | 4.000 erg mm ⁻² 2 min | 3 min | Cold shock 1 °C 20 min | 15 | Karyotyping | Emefe & Sorhue (2014) |
| Olive barb (<i>Puntius sarana</i>) | 250 μ W cm ⁻² 2 min | 2-4 min | Heat shock 39 °C 4 min | 16 | Flow cytometry | Chakraborty <i>et al.</i> (2006) |
| Sea bass (<i>Dicentrarchus labrax</i>) | 32,000 erg mm ⁻² 2 min | 6 min | Hydrostatic pressure 8000 psi 2 min | 96 | Flow cytometry | Peruzzi <i>et al.</i> (1993) |

*TAF= Time after fertilization; *HR= Hatching rate; *SR= Survival rate; *RC= Relative to control

Source: Manan *et al.*, 2022 (modified)

Table 3 summarizes the different techniques used in different fish species to produce gynogenesis. Proper UV irradiation made sure 100% inactivation of sperm. Shock introduction resulted in diploid gynogenesis.

Few researchers have investigated the growth and reproductive processes in gynogens over an extended period of time. The percentage of growth seen in gynogens compared to typical diploids was calculated to compare the findings (Figure 6).



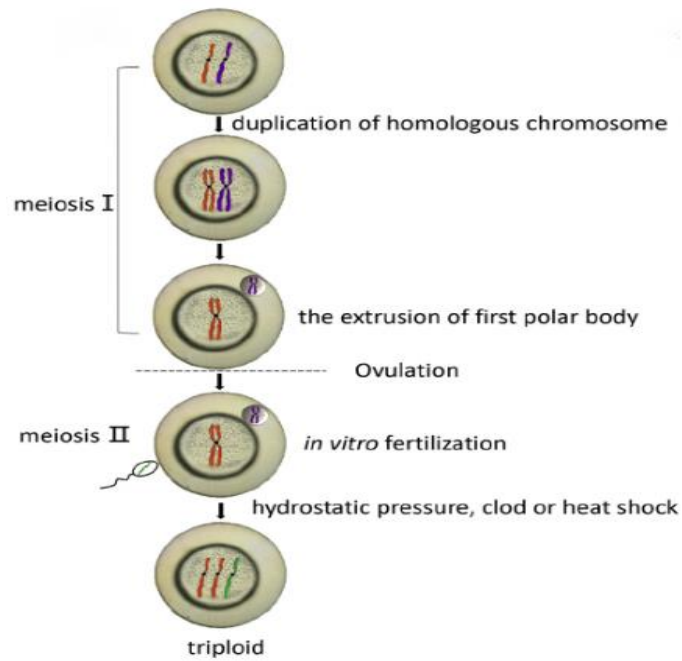
Source: Pandian and Koteeswaran, 1998

Figure 6. Growth performance of gynogens in selected fishes.

Throughout a three-year period, data were gathered on the development of the meiotic and mitotic gynogens in *Paralichthys olivaceus* and *Oncorhynchus mykiss*. Representative salmonid and cyprinid species showed severe growth depression regardless of meiotic or mitotic gynogenesis. Contrarily, Siluridae and Paralichthyidae expanded more quickly, suggesting that it could be advantageous to induce gynogenesis in these species.

3.1.3 Triploidy

The most prevalent type of polyploidy, which refers to animals or cells with three sets of homologous chromosomes, is triploids. The process of generating sterile fish for aquaculture and fisheries management via triploidy is widely accepted. Many species of salmon (Hussain, 1996), trout (Cassani and Caton, 1985) have been subjected to triploidy induction for aquaculture.



Source: Zhou & Gui (2017)

Figure 7. A schematic diagram of triploid induction in fish.

Fish that are triploid ($3n$) have 2 sets of chromosomes from the mother and single set of paternally inherited chromosomes. Triploidy induction, which is the process of creating organism with three sets of chromosomes, can be carried out in fish by shocking the eggs shortly after fertilization through the retention of second polar body or by preventing the second meiotic division and the extrusion of the second polar body (Peruzzi and Chatain, 2000). An alternate way to create hybrid triploid fish is by mating conventional diploid and tetraploid fish. Those who are triploid are anticipated to be endocrinologically and functionally sterile.

Table 4. Triploidy induction techniques in fishes

| Species | TAF | Induction technique | TR (%) | SR (%) | Affirmation method | Sources |
|--|-------------|---|--------------|--------------|--------------------|------------------------------|
| Rainbow Trout <i>Oncorhynchus mykiss</i> | 375 CTMs | Hydrostatic pressure 9500 psi 5 min | 88.9 | 100 | Flow-cytometry | Loopstra & Hansen (2008) |
| Rainbow Trout <i>O. mykiss</i> | 20 min | Heat Shock 28°C 10 min | 100 | 60.5 | Karyotyping | Dillon (1988) |
| Tilapia <i>Oreochromis mossambicus</i> | 4 min | Heat shock 41°C 5 min | 89.7 | 67 | Karyotyping | Pradeep <i>et al.</i> (2012) |
| European sea bass (<i>Dicentrarchus labrax</i>) | 6 min | Hydrostatic pressure 8500 psi 2 min | 100 | 41 | Flow-cytometry | Peruzzi & Chatain (2000) |
| Atlantic salmon <i>Salmo salar</i> | 20 min | Hydrostatic pressure 10150 psi 3 or 6 min | 100 | 70-90 | Flow-cytometry | Benfey & Sutterlin (1984) |
| South American catfish <i>Rhamdia quelen</i> | 3 min | Cold shock 4 °C 20 min | 97.9 ±1.1 | 65.4 ±5.3 | Karyotyping | Silva <i>et al.</i> (2007) |

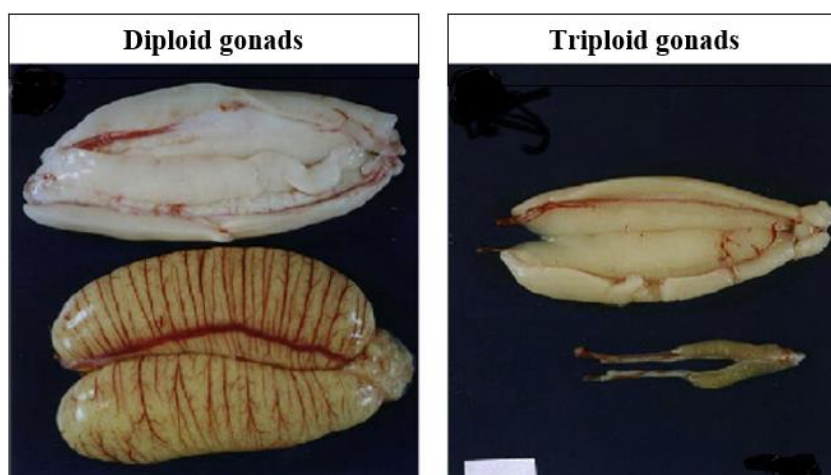
*TAF= Time after fertilization; *SR= Survival rate; *TR= Triploidization rate

Table 4 summarizes the shocking method like heat shock, hydrostatic pressure that prevents the extension of second polar body and introduces triploidy. Survivability is higher among the triploids that are produced through use of hydrostatic pressure due to less deformities. For triploidy confirmation Karyotyping and Flow-cytometry is mainly used.

Table 5. Positive effects on growth performance and gonadosomatic index (GSI) of induced triploidy in some commercially important fishes

| Species | Growth increasement | GSI decreasement | Sources |
|---|---------------------|------------------|------------------------------|
| Tilapia <i>O. mossambicus</i> | 9.69% in 120 days | 62% | Pradeep <i>et al.</i> (2012) |
| Chinese catfish <i>Clarias fuscus</i> | 10% in 175 days | 86.65% | Qin <i>et al.</i> (1998) |
| Asian catfish <i>Clarias macrocephalus</i> | 50.76% at 8 months | 45.25% | Fast <i>et al.</i> (1995) |
| Turbot <i>Scophthalmus maximus</i> | 10.3% at 47 months | 99% in female | Cal <i>et al.</i> (2006) |
| Bata <i>Labeo bata</i> | 71.03% in 12 weeks | – | Afroza <i>et al.</i> (2021) |

Table 4 summerizes growth increasement and GSI decreasement data in several triploid fishes. Their culture will result in better profitability due to faster growth and lower feed conversion ratio.



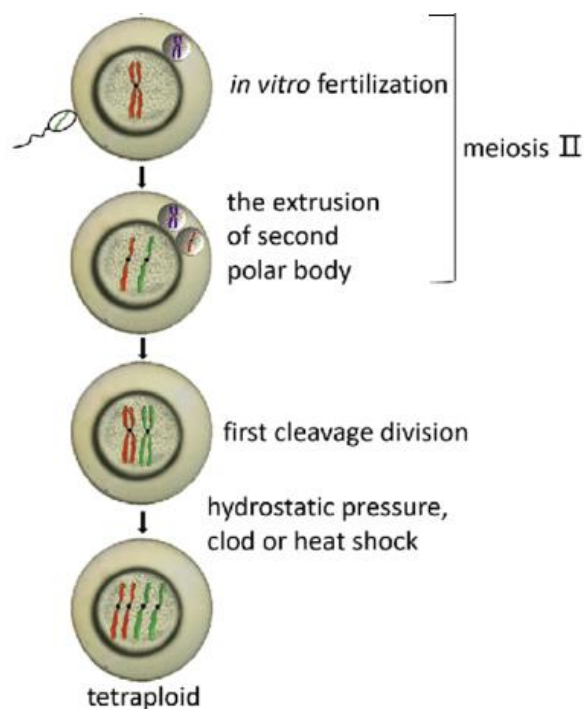
Sources: Felip *et al.* (2001)

Figure 8. Photographs of gonads of adult diploid and triploid European sea bass, *Dicentrarchus labrax* (testis is in the top and the ovary at the bottom).

The reduction of European sea bass gonads size and underdeveloped condition in figure 8 suggest their nonviable condition for reproduction and indicate the retention of energy for somatic growth rather than reproduction.

3.1.4 Tetraploidy

Early 1980s saw the start of tetraploidy efforts; they offer a substitute for mass producing triploid and the initial step to induce penta, hexa, and heptaploids; and secondly, tetraploids may react differently to selective breeding than diploids (Chourrout & Nakayama, 1987).



Source: Zhou & Gui (2017)

Figure 9. A schematic diagram of tetraploid induction in fish.

Although it is theoretically possible to suppress the first cleavage to artificially tetraploidize a diploid species according to figure 9, this has proven challenging in practice for many fish species. As a result, this method has only been used to produce viable tetraploids in some fishes (Table 5).

Table 6. A selection of key studies on tetraploid production in fish

| Species | TAF | Induction method | Results % | SR (%RC) | Conformation method | Sources |
|--|-----------|--------------------------------------|-----------|----------|---------------------|---------------------------------|
| Stinging catfish, <i>Heteropneustes fossilis</i> | 30 min | Heat shock 40°C 4min | 40±8 | 26±5 | Karyotyping | Haniffa <i>et al.</i> (2004) |
| Indian carp rohu, <i>Labeo rohita</i> | 20 min | Heat shock 39°C 2 min | 70 | 36 | Karyotyping | Reddy <i>et al.</i> (1990) |
| Catla, <i>Catla catla</i> | 25 min | Heat shock 40°C 2 min | 65 | 25 | Karyotyping | Reddy <i>et al.</i> (1990) |
| Masu salmon, <i>Oncorhynchus masou</i> | 30 min | Pressure shock 10000 psi 7 min | 90.8 | 90 | Flow cytometry | Sakao <i>et al.</i> (2006) |
| European sea bass, <i>Dicentrarchus labrax</i> | 70–90 min | Pressure shock 13200 psi 4 min | 94 | 25 | Flow cytometer | Francescon <i>et al.</i> (2004) |
| Turbot, <i>Scophthalmus maximus</i> | 15 min | Pressure shock 9790 psi 6 min | 100 | 85 | Flow cytometry | Wu <i>et al.</i> (2019) |

*TAF= Time after fertilization; *SR= Survival rate

Fish mosaicism, aneuploidy, decreased cell surface, atypical cytological occurrences, and/or high homozygosity, which often result in short survival, high levels of abnormalities, and low reproductive potential, are probable explanations for failure to induce and produce live tetraploid stock (Diter *et al.*, 1988; Pandian and Koteeswaran, 1998; Lou, 1999).

3.2 Constrains in ploidy manipulation of fish

3.2.1 Low survival rate and deformities

Sugama *et al.* (1992) believed that a large number of malformations in triploid red sea bream led to their poor survival. Lower jaw abnormalities in triploid Atlantic salmon were thought by Sadler *et al.* (2001) to be caused by the triploid condition itself rather than by the induction shock given to eggs. Nevertheless, Sadler *et al.* (2001) also pointed out that triploids had smaller gill surfaces and were more likely than diploids to have skeletal, opercular, and gill filament abnormalities. Additional investigations (Oppedal *et al.*, 2002) revealed a generally low incidence of external vertebral axis abnormalities, however they were greater in triploids than in diploids early in development but lower in triploids at slaughter. Varadaraj (1990) produced repeated generations of *Oreochromis mossambicus* clones. G₀ mitotic progeny expectedly had a lower survival rate (22%) due to the homozygous diploid expression of recessive harmful genes.

3.2.2 Behavioral change

Triploid fish frequently exhibit altered behavior. The swimming and feeding habits of triploid rainbow trout larvae were unusual (Myers and Hershberger, 1991). Atlantic salmon, however, While Carter *et al.* (1994) demonstrated that triploid had more severe fin damage than diploids, indicating of aberrant swimming behavior, McGeachy *et al.* (1995) found triploid larvae in a condition of prostration.

3.2.3 Genetic contamination

In Androgenesis, since the egg contains significant amounts of mitochondrial DNA and messenger RNA, Carter *et al.* (1991) questioned whether the genome of an egg could be completely eliminated. The mitochondrial DNA in *Oreochromis niloticus* eggs was shielded by the mitochondrial membrane, preventing any damage from UV irradiation (Myers *et al.*, 1995).

When gynogenesis is induced in male heterogametic species, 100% of the progeny should be female. Yet, several articles have noted variances of 2 to 100% from the

anticipated all-female gynogens (Piferrer *et al.*, 2009) that may be due to- (i) paternal genetic admixture and (ii) the presumed presence of minor sex genes.

3.2.4 Reduced growth performance

Triploid fish must have individual cells that are 1 and 1/2 times larger than diploid counterparts as ploidy status increases. Therefore, it is anticipated that triploid fish will eventually reach a final body weight that is roughly 1 and 1/2 times greater than that of its diploid counterpart. But in some cases the expected final body weight of a triploid is not greater than that of a diploid because the triploid's cell number is controlled (Small & Benfey, 1987).

3.2.5 Impact on biodiversity

As it has been shown that synthetically created allo- and autotetraploid fish are reproductive in lab or hatchery settings, their release into the environment presents a serious threat to ecological stability and biodiversity. Tetraploid fish or shellfish broodstocks must be kept in quarantine whether they are being utilized for commercial purposes or experimental research since there is such a high danger of possible genetic and environmental effects following the escape of tetraploids. This will stop tetraploid larvae, juveniles, or adults from escaping into the environment or from accidentally releasing gametes.

Chapter 4

CONCLUSION

Ploidy manipulation has the potential to be utilized in the field of aquaculture. In case of androgenesis inactivation of egg and in gynogenesis sperm's genetic contents is destroyed through UV or γ -rays irradiation respectively. After fertilization diploid state is bring back through different shocking treatments like thermal or hydrostatic pressure shock to prevent the extrusion of second polar body or blocking the first cleavage. After fertilization of egg with normal sperm, triploidy is introduced through retention of second polar body and tetraploidy is through blocking the first cleavage. Hydrostatic pressure shock showed better survival rate than thermal shock as a treatment. It has been used as an aid to study genetic control of polymorphic loci through the assessment of haploid fish. YY supermale production, conservation of germplasm, creation of isogenic line, culture of monosex population through the application of androgenesis and gynogenesis results in better yield in aquaculture industry. Conservation of reproductive energy through production of sterile population that results in lower feed conversion ratio is the main theme of triploidy induced better yield. Increased cell size due to presence tetraploid nucleus also result in faster and better growth of fish and increased final body weight than the diploid counterparts.

Low survival of hatchling and early mortality of fry, detection of proper treatment, shocking method, time and duration for diploidization to tetraploidization and overall development of species-specific protocol with high rate of success is the main challenges. Risk of tetraploid fish escape in the nature possess a great threat to our natural aquatic biodiversity. It can result in population decline in a great extent for a specific species. Proper husbandry and secure and safe culture practice can bring a solve to this problem.

By introducing the ploidy manipulation technique we can make aquaculture more efficient and ensure low-cost protein supply to the world population. Though more research should be done to improve survivability and to examine growth performance, behavioral condition, environmental impact of ploidy manipulated fish in normal culture condition in large number. Overall there is still great scope of research in solving the problem associated with ploidy manipulation induction in aquaculture and bring this technique in field level.

REFERENCES

- Afroza, K. B., Shah, M. S., Salin, K. R., & Rahi, M. L. (2021). Growth and survival of diploid and triploid bata, *Labeo bata* (Hamilton, 1822). *Aquaculture, Fish and Fisheries*, 1(1), 42-50.
- Benfey, T. J., & Sutterlin, A. M. (1984). Triploidy induced by heat shock and hydrostatic pressure in landlocked Atlantic salmon (*Salmo salar* L.). *Aquaculture*, 36(4), 359-367.
- Bercsényi, M., Magyary, I., Urbányi, B., Orbán, L., & Horváth, L. (1998). Hatching out goldfish from common carp eggs: interspecific androgenesis between two cyprinid species. *Genome*, 41(4), 573-579.
- Bongers, A. B. J. inft Veld EPC, Abo-Hashema K., Bremmer IM, Eding EH, Komen J. & Richter CJJ 1994. Androgenesis in common carp (*Cyprinus carpio* L.) using UV-irradiation in a synthetic ovarian fluid and heat shocks. *Aquaculture*, 122, 119-132.
- Cal, R. M., Vidal, S., Gómez, C., Álvarez-Blázquez, B., Martínez, P., & Piferrer, F. (2006). Growth and gonadal development in diploid and triploid turbot (*Scophthalmus maximus*). *Aquaculture*, 251(1), 99-108.
- Carter, C. G., McCarthy, I. D., Houlihan, D. F., Johnstone, R., Walsingham, M. V., & Mitchell, A. I. (1994). Food consumption, feeding behaviour, and growth of triploid and diploid Atlantic salmon, *Salmo salar* L., parr. *Canadian Journal of Zoology*, 72(4), 609-617.
- Carter, R. E., Mair, G. C., Skibinski, D. O. F., Parkin, D. T., & Beardmore, J. A. (1991). The application of DNA fingerprinting in the analysis of gynogenesis in tilapia. *Aquaculture*, 95(1-2), 41-52.
- Cassani, J. R., & Caton, W. E. (1985). Induced triploidy in grass carp, *Ctenopharyngodon idella* Val. *Aquaculture*, 46(1), 37-44.
- Chakraborty, B. K., Miah, M. I., Mirja, M. J. A., & Habib, M. A. B. (2006). Induction of gynogenesis in endangered sarpunti, *Puntius sarana* (Hamilton) and evidence for female homogamety. *Aquaculture*, 258(1-4), 312-320pp.

- Chourrout, D., & Nakayama, I. (1987). Chromosome studies of progenies of tetraploid female rainbow trout. *Theoretical and Applied Genetics*, 74, 687-692.
- Coltman, D.W. & Slate, J. (2003) Microsatellite measures of inbreeding: a meta-analysis. *Evolution: Internation Journal of Organic Evolution*, 57, 971– 983.
- da Silva, F. S. D., Moreira, R. G., Orozco-Zapata, C. R., & Hilsdorf, A. W. S. (2007). Triploidy induction by cold shock in the South American catfish, *Rhamdia quelen* (Siluriformes)(Quoy & Gaimard, 1824). *Aquaculture*, 272, S110-S114.
- Dillon, J. C. (1988). Production of triploid rainbow trout for evaluation in South Dakota waters.
- Diter, A., Guyomard, R., & Chourrout, D. (1988). Gene segregation in induced tetraploid rainbow trout: genetic evidence of preferential pairing of homologous chromosomes. *Genome*, 30(4), 547-553.
- Emefe, O., & Sorhue, U. G. (2014). *Gynogenesis: An effective way of controlling fish population and increasing profit in aquaculture*. Conference paper/38th Anual GSN conference/Genetics Public Awareness and National Transformation (pp. 78–82pp).
- FAO. (2022). The State of World Fisheries and Aquaculture 2022. Towards Blue Transformation. *FAO Rome*.
- Fast, A. W., Pewnim, T., Keawtabtim, R., Saijit, R., Te, F. T., & Vejaratpimol, R. (1995). Comparative growth of diploid and triploid Asian catfish *Clarias macrocephalus* in Thailand. *Journal of the World Aquaculture Society*, 26(4), 390-395.
- Felip, A., Piferrer, F., Carrillo, M., & Zanuy, S. (2001). Comparison of the gonadal development and plasma levels of sex steroid hormones in diploid and triploid sea bass, *Dicentrarchus labrax* L. *Journal of Experimental Zoology*, 290(4), 384-395.
- Francescon, A., Libertini, A., Bertotto, D., Barbaro, A., 2004. Shock timing in mitogynogenesis and tetraploidization of the European sea bass, *Dicentrarchus labrax*. *Aquaculture* 236, 201–209.
- Gheyas, A. A., Mollah, M. F. A., Islam, M. S., & Hussain, M. G. (2001). Cold-shock induction of diploid gynogenesis in stinging catfish, *Heteropneustes fossilis*. *Journal of applied Aquaculture*, 11(4), 27-40.

- Gomelsky, Boris. (2011). *Fish Genetics: Theory and Practice*.
- Haniffa, M. A., Sridhar, S., & Nagarajan, M. (2004). Induction of triploidy and tetraploidy in stinging catfish, *Heteropneustes fossilis* (Bloch), using heat shock. *Aquaculture research*, 35(10), 937-942.
- Hussain, M. G. (1996). Effects of Triploidy on Sexual Maturation and Reproduction in Nile Tilapia, *Oreochromis niloticus* L. In *The Third International Symposium on Tilapia in Aquaculture* (Vol. 41, p. 320). WorldFish.
- Janhunen, M., Vehvilainen, H., Koskela, J., Forsman, A. & Kankainen, M. (2019) Added value from an added chromosome: Potential of producing large fillet fish from autumn to spring with triploid rainbow trout, *Oncorhynchus mykiss*. *Aquaculture Research*, 50, 1–8. <https://doi.org/10.1111/are.13952>
- Kirankumar, S., & Pandian, T. J. (2003). Production of androgenetic tiger barb, *Puntius tetrazona*. *Aquaculture*, 228(1-4), 37-51.
- Loopstra, D. P., & Hansen, P. A. (2008). Induction of triploidy in rainbow trout (*Oncorhynchus mykiss*) using hydrostatic pressure. Alaska Department of Fish and Game, Division of Sport Fish, Research and Technical Services.
- Lou, Y. (1999). *Fish Breeding*. China Agriculture Press, Beijing, pp. 136 – 137.
- Manan, H., Hidayati, A. N., Lyana, N. A., Amin-Safwan, A., Ma, H., Kasan, N. A., & Ikhwanuddin, M. (2022). A review of gynogenesis manipulation in aquatic animals. *Aquaculture and Fisheries*, 7(1), 1-6.
- McGeachy, S. A., Benfey, T. J., & Friars, G. W. (1995). Freshwater performance of triploid Atlantic salmon (*Salmo salar*) in New Brunswick aquaculture. *Aquaculture*, 137(1-4), 333-341.
- Myers, J. M., & Hershberger, W. K. (1991). Early growth and survival of heat-shocked and tetraploid-derived triploid rainbow trout (*Oncorhynchus mykiss*). *Aquaculture*, 96(2), 97-107.
- Myers, J. M., Penman, D. J., Basavaraju, Y., Powell, S. F., Baoprasertkul, P., Rana, K. J., & McAndrew, B. J. (1995). Induction of diploid androgenetic and mitotic gynogenetic Nile tilapia (*Oreochromis niloticus* L.). *Theoretical and Applied Genetics*, 90, 205-210.

- Myers, J. M., Penman, D. J., Rana, K. J., Bromage, N., Powell, S. F., & McAndrew, B. J. (1995). Applications of induced androgenesis with tilapia. *Aquaculture*, 137(1-4), 150-150.
- Nam, Y. K., Cho, Y. S., Cho, H. J., & Kim, D. S. (2002). Accelerated growth performance and stable germ-line transmission in androgenetically derived homozygous transgenic mud loach, *Misgurnus mizolepis*. *Aquaculture*, 209(1-4), 257-270.
- Oppedal, F., Taranger, G. L., & Hansen, T. (2003). Growth performance and sexual maturation in diploid and triploid Atlantic salmon (*Salmo salar* L.) in seawater tanks exposed to continuous light or simulated natural photoperiod. *Aquaculture*, 215(1-4), 145-162.
- Oppermann, K. (1913). Die Entwicklung von Forelleneiern nach Befruchtung mit radiumbestrahlten Samenfaden. *Arch. mikrosk. Anat.* 83: 141–189.
- Ozdemir, R. C., & Aygul, E. (2017). Different heat shock application effect on gynogenetic production of zebrafish (*Danio rerio*). *Fisheries and Aquaculture Journal*, 8, 196.
- Padhi, B. K., & Mandal, R. K. (2000). *Applied fish genetics*. Fishing Chimes.
- Pandian, T. A., & Koteeswaran, R. (1998). Ploidy induction and sex control in fish. *Hydrobiologia*, 384, 167-243.
- Peruzzi, S., & Chatain, B. (2000). Pressure and cold shock induction of meiotic gynogenesis and triploidy in the European sea bass, *Dicentrarchus labrax* L.: Relative efficiency of methods and parental variability. *Aquaculture*, 189, 23–37pp.
- Pradeep, P. J., Sriyaya, T. C., Papini, A., & Chatterji, A. K. (2012). Effects of triploidy induction on growth and masculinization of red tilapia [*Oreochromis mossambicus* (Peters, 1852) × *Oreochromis niloticus*]. *Aquaculture*, 344, 181-187.
- Qin, J. G., Fast, A. W., & Ako, H. (1998). Growout performance of diploid and triploid Chinese catfish *Clarias fuscus*. *Aquaculture*, 166(3-4), 247-258.
- Rahi, M.L. & Shah, M.S. (2012). Production of inbred lines of *Labeo rohita* and *Cirrhinus mrigala* by gynogenetic technique. *Advance Fisheries Research, Bangladesh*, 1, 45–57.

- Reddy, P. V. G. K., Kowtal, G. V., & Tantia, M. S. (1990). Preliminary observations on induced polyploidy in Indian major carps, *Labeo rohita* (Ham.) and *Catla catla* (Ham.). *Aquaculture*, 87(3-4), 279-287.
- Sadler, J., Pankhurst, P. M., & King, H. R. (2001). High prevalence of skeletal deformity and reduced gill surface area in triploid Atlantic salmon (*Salmo salar* L.). *Aquaculture*, 198(3-4), 369-386.
- Sakao, S., Fujimoto, T., Kimura, S., Yamaha, E., & Arai, K. (2006). Drastic mortality in tetraploid induction results from the elevation of ploidy in masu salmon *Oncorhynchus masou*. *Aquaculture*, 252(2-4), 147-160.
- Scheerer, P. D., Thorgaard, G. H., & Allendorf, F. W. (1991). Genetic analysis of androgenetic rainbow trout. *Journal of Experimental Zoology*, 260(3), 382-390.
- Schwander, T., & Oldroyd, B. P. (2016). Androgenesis: where males hijack eggs to clone themselves. *Philosophical Transactions of the Royal Society B: Biological Sciences*, 371(1706), 20150534.
- Setoguchi, T., Santhakumar, S., Takao, M., Kim, T. H., & Kaneko, K. (2003). A modified Wells turbine for wave energy conversion. *Renewable Energy*, 28(1), 79-91.
- Small, S. A., & Benfey, T. J. (1987). Cell size in triploid salmon. *Journal of Experimental Zoology*, 241(3), 339-342.
- Streisinger, G., Walker, C., Dower, N., Knauber, D., & Singer, F. (1981). Production of clones of homozygous diploid zebra fish (*Brachydanio rerio*). *Nature*, 291(5813), 293-296.
- Sugama, K., Taniguchi, N., Seki, S., & Nabeshima, H. (1992). Survival, growth and gonad development of triploid red sea bream, *Pagrus major* (Temminck et Schlegel): use of allozyme markers for ploidy and family identification. *Aquaculture Research*, 23(2), 149-159.
- Swarup, H. (1959). Production of triploidy in *Gasterosteus aculeatus* (L.). *Journal of Genetics*, 56(2), 129-142.

- Teskeredžić, E., Teskeredžić, Z., Donaldson, E. M., McLean, E., & Solar, I. (1993). Triploidization of coho salmon following application of heat and electric shocks. *Aquaculture*, 116(2-3), 287-294.
- Thorgaard, G. H., Jazwin, M. E., & Stier, A. R. (1981). Polyploidy induced by heat shock in rainbow trout. *Transactions of the American Fisheries Society*, 110(4), 546-550.
- Thorgaard, G. H., Scheerer, P. D., Hershberger, W. K., & Myers, J. M. (1990). Androgenetic rainbow trout produced using sperm from tetraploid males show improved survival. *Aquaculture*, 85(1-4), 215-221.
- Varadaraj, K. (1993). Production of viable haploid *Oreochromis mossambicus* gynogens using UV-irradiated sperm. *Journal of Experimental Zoology*, 267(4), 460-467.
- Varadaraj, K., & Pandian, T. J. (1990). Production of all-female sterile-triploid *Oreochromis mossambicus*. *Aquaculture*, 84(2), 117-123.
- Warner, J.L., Gomelsky, B., Delomas, T.A., Kramer, A.G. & Novelo, N.D. (2018). Reproductive ability of second-generation ornamental (koi) carp (*Cyprinus carpio* L.) x goldfish (*Carassius auratus* L.) hybrids and characteristics of their offspring. *Aquaculture Research*, 49(6), 2317–2321.
- Wasow, T., Kuzminski, H., Woznicki, P., Ziomek E. (2004) Blood cell alteration in triploid brook trout *Salvelinus fontinalis* (Mitchill). *Reviews in Fisheries Science*, 73, 115–118.
- Wu, Z., Wang, L., Lu, Y., Zhu, X., Yue, X., & You, F. (2019). Artificial induction and genetic structure analysis of tetraploid turbot *Scophthalmus maximus*. *Frontiers in Marine Science*, 6, 637.
- Zheng, G., Wang, C., Guo, D., Jiang, X., & Zou, S. (2017). Ploidy level and performance in meiotic gynogenetic offsprings of grass carp using UV-irradiated blunt snout bream sperm. *Aquaculture and Fisheries*, 2(5), 213–219pp.
- Zhou, L., & Gui, J. (2017). Natural and artificial polyploids in aquaculture. *Aquaculture and Fisheries*, 2(3), 103-111.