(NSAZ-2022)

Science and Engineering Research Board (SERB) Sponsored
National Symposium
On
Applied Zoology, Profitable Animal Production, and Health: Current Status and Future Progress (NSAZ-2022) 23rd & 24th September- 2022

Recent Trends in Applied Zoology

Dr.D.S.Rathod
Editor

Associate Editors
Dr. K.S.Raut
Mr.Datta Nalle

National Edited Book

PRABHAKAR PUBLICATION

(ISBN: 978-93-92464-55-3)

Recent Trends in Applied Zoology

Edited by: Dr.D.S.Rathod

ISBN: 978-93-92464-55-3 (First printing edition, May, 2023)

Published by: **Prabhakar publication:**

Address: 304 Siddhivinayak apartments, sutmill road, Latur - 413512

Phone: +91 8600881127, +91 8149153018

Website: www.prabhakarpublication.com

Science and Engineering Research Board (SERB) Sponsored
National Symposium
On

Applied Zoology, Profitable Animal Production, and Health: Current Status and Future Progress (NSAZ-2022) 23rd & 24th September- 2022

Recent Trends in Applied Zoology

Editor
Dr.D.S.Rathod
Head
Department of Zoology and Fishery Science,
Rajarshi Shahu Mahavidyalaya (Autonomous),

Latur- 413531, Maharashtra

Associate Editors
Dr. K.S.Raut
Mr.Datta Nalle
Department of Zoology and Fishery Science, Rajarshi Shahu Mahavidyalaya (Autonomous),
Latur- 413531, Maharashtra

Index

Chapter 04

Analysis of chromosome by Karyotyping, and cryopreservation of gametes in fishes

Datta Ashok Nalle, Madhuri Y. Bhande

Department of Zoology and Fishery Science, Rajarshi Shahu Mahavidyalaya,
(Autonomous) Latur-413512 (MS)

Abstract: Cells may be linked to the flask in which they are cultivated or they may be grown on microscope slides in sterile chambers. Trypsin and EDTA are added if the liberation of the cells from the substrate is required. The culture medium, trypsin, and/or EDTA are then removed from the cells by centrifuging them. The cells are then swollen with a hypotonic solution, centrifuged, and fixed in a methanol:acetic acid solution. If the cells weren't grown on slides, they are placed onto them. The majority of banding techniques call prior preparation before staining. Giemsa and trypsin result in G-banded chromosomes (GTG). It is the process through which the germplasm of one species gradually infiltrates that of another as a result of hybridization and recurrent backcrossing. Pure mrigal in Bangladesh are gravely threatened by introgressive hybridization, The reasons for this species' declining quality include genetic drift, unintentional hybridization, and inbreeding depression. They believed that the easiest and least expensive way to conserve genomes that can be used to maintain future conservation options is through cryopreservation. Salmonids frequently experience the phenomenon of autochthonous populations mixing with introduced ones, which can lead to outbreeding depression and the replacement of potentially locally adapted populations by allochthonous ones.

Introduction

Chromatin refers to "colored" material in the nucleus. It is comparable to chromosomal material. Non-transcriptional active chromatin is referred to as heterochromatin. It can be constitutive heterochromatin, like the q arm of the Y chromosome or centromeric heterochromatin, which is never transcriptional active, or facultative heterochromatin, like the X chromosome, which can be active transcriptional but is not always active. Transcriptional active chromatin is referred to as euchromatin. Present tools given accurate information about new variation, and ultimately support for investigation of new genetic makeup of that organism. New genetic makeup surely has some new treats than that of previous one [1] Chromosomes are prepared for study by a process called kytotyping. Various tissues are employed; however the most often sampled ones include chorionic villi (CVS), bone marrow, skin fibroblasts, amniotic fluid cells, gonadal tissue, and lymphocytes (white blood cells). Bone marrow, lymphocytes, gonadal tissue, CVS, and tumours are directly prepared without the use of a mitogen. When cells are cultured, they are put in nutritional solutions with amino acids and other nutrients, a pH indicator, antibiotics, and either mitogens (PHA or pokeweed mitogen) or none at all. The cells are then grown at 37°C in a CO2 atmosphere. Three days are spent cultivating lymphocytes; ten days are spent cultivating amniotic cells, fibroblasts, CVS, and other foetal tissues. Before harvest, colcemid is added, which prevents the formation of microtubules in the spindle. cease mitosis and to gather enough prophase-prometaphase-chromosome-containing cells.

Cells may be linked to the flask in which they are cultivated or they may be grown on microscope slides in sterile chambers. Trypsin and EDTA are added if the liberation of the cells from the substrate is required. The culture medium, trypsin, and/or EDTA are then removed from the cells by centrifuging them. The cells are then swollen with a hypotonic solution, centrifuged, and fixed in a methanol:acetic acid solution. If the cells weren't grown on slides, they are placed onto them. The majority of banding techniques call prior preparation before staining. Giemsa and trypsin result in G-banded chromosomes (GTG).

A cytogeneticist often looks for metaphase spreads on the slides and examines 10 to 30 cells under the microscope. When a decent spread is discovered (minimal number of chromosomes overlaps), a picture is taken or the analysis is carried out by a computer. The chromosomes are organized from longest to shortest according to a common presenting format. Chromosome 21 really has a lesser size than Chromosome 22.

The chromosomes are grouped in a conventional karyotype according to their size and centromere position. 7 groups are present: A, 1–3 long metacentrics; B, 4–5 long sub-metacentrics; C, 6–12 sub-metacentrics; D, 13–15 acrocentrics that may have satellites (normal chromosome polymorphisms) on the p arm; E, 16–18 sub-metacentrics; F, 19–20 short metacentrics; G, 21–22 acrocentrics with or without satellites (normal polymorphism) on In a karyotype, the longer arm is on the bottom and is referred to as the p arm. bottom and is called the q arm.

The bands on banded chromosomes can be identified in a conventional manner. With the exception of some chromosome polymorphisms like the satellite of the D and G group chromosomes and some heterochromatic regions near the centromeres of 1, 9, 16, and Yq, the karyotypes of normal individuals are mostly identical. With a few chromosome rearrangements, the chromosomes of the great apes are very identical to ours. Instead of 46, gorillas, orangutans, and chimpanzees have 48. Two chromosomes found in great apes were translocated to create the human chromosome #2. A chimpanzee with Down syndrome and our 21 extra-small chromosomal-like chromosomes has been documented.

Banding techniques

G (trypsin pretreatment followed by Giemsa stain); Q (quinicrine, fluorescent bands); R (reverse banding), which calls for heating in a saline buffer before staining with Giemsa or Quinicrine and also reveals telomeres; SCE (sister chromatid exchanges) requires replication in 5 BudR, an analogue of thymidine, followed by Höescht stain. C (Centromere or Constitutive heterochromatin) stains the heterochromatin in the centromeres, especially of chromosomes 1, 9, and 16; NOR (nucleolar organising regions) selectively stains the satellite stalks of the acrocentric chromosomes. Starting from the centromere, the primary dark and light regions have numbers. At the centromere, each band has a number that starts with 11 or 11.1 on it. The area, band, and sub-band indicated by the allocated numbers are, respectively, the first, second, and third numbers. For instance, the notation 31.2 denotes the region, the band, and the sub-band. About 550 light and dark bands are produced using standard banding. Cell culture must be somewhat synchronised in order to use high resolution banding (HRB) to detect microdeletions before the cells compress too much. It generates almost 800 bands.

Fluorescence in situ hybridization

The DNA of interphase or metaphase chromosomes that have been fixed on a slide, it is said to be done "in situ." FISH employs whole-chromosome painting (spectral painting), repetitive DNA such as centromeric alpha satellite DNA, and locus-specific DNA probes. Using probes that are unique to each chromosome (painting probes), it can be used to identify marker chromosomes, rings, and other structural defects. You observed an overhead of a 3p+ in which chromosome 13 was identified as the source of the "+"; Fluorescence in situ hybridization, also known as FISH, is a staining technique that is frequently used to identify known DNA sequences. Examples of such DNA sequences include fluorescan iso 5q, which was actually a 5 with a bit of 6, an 8 p+ where the + was a piece of 21, and an 8 p+ where the + was a piece of 22. FISH is now almost always used to diagnose microdeletion syndrome, which we shall cover later. Painting probes can be made to paint the p or q arm, for example, or to uniformly decorate the entire chromosome. The chromosome or arm drawn is specific to the DNA sequences used. When structural rearrangements such translocations, rings, isochromosomes, etc. occur, they can be used to pinpoint the chromosomal origin. The process of "whole" chromosome specific painting is referred to as "spectral karyotyping".

Cryopreservation of Gametes

The storage method of cryopreservation has virtually no time restrictions. This method has been tested for fish gametes preservation.Sperm cryopreservation is a well-known practise in animal husbandry. Cryopreserved sperms are now successfully employed in the insemination process for breeding cattle, horses, pigs, sheep, and poultry. It's interesting to see how much work has gone into adapting this technique for the preservation of fish sperm, ova, and embryos.

According to FAO predictions for the next 15 years, food production in developing nations can rise by 2% annually if agricultural innovation proceeds at a fair rate. According to a different World Bank assessment, the global food supply must increase by at least 50% by the year 2050 if 9 billion people are to be fed. Supporting agricultural research can have a significant positive impact on lowering food poverty and malnutrition if the natural resources have strong potential for agricultural growth [1]. Fish can be a key component of the solutions to the aforementioned problems, in addition to grains and vegetables.

One third of the world's population gets at least 20% of their protein from fish and other aquatic products, and developing nations are highly dependent on fish .Because they provide more than half of the protein and minerals for more than 400 million people in the food-deficit nations of Africa and South Asia, small-scale fisheries are seen to be particularly crucial for food security [2].

Although fish is not an energy food, it is a necessary sustenance for humans. In many developing nations, it is a crucial source of protein, minerals, and oils. In the Micronesian and Polynesian diets, fish protein makes up about 30% and 15%, respectively [2]. With its high quality animal protein, vital fatty acids, and vitamin content, fish is more nutrient-dense than other common foods. Utilising fish as a complementary food to improve children's nutritional status, promoting nutrient-dense fish consumption among women and children through community-level nutrition education, and increasing production of

more sought-after fish species through efficient technology dissemination are a few interventions related to fish consumption and aquaculture production.

Since fish is a staple diet for people, every technique of producing it has been investigated. The development of culture by organised means deserves current attention in addition to the use of natural resources like the sea and natural water impoundments. The availability of fish through fishing has been influenced by this shift in population in the natural waters, which has prompted people to develop aquaculture as a means of producing fish. The effects of climate change on aquaculture, however, likewise could not be delayed.

According to studies conducted in Asia, low-income households consume less fish than wealthy households [5], but they nevertheless rely heavily on fish as a source of animal protein [6]. This generally implies that, in order to keep the low-income group of people from suffering from nutritional illnesses, there should be an adequate supply of fish to offset their deficiency.

At this point, anthropologists from all over the world insist that in addition to focusing on boosting agriculture output, organisations like FAO and the World Bank need to set up aquaculture projects using species that are both positively impacted by climate change and high in nutrients to eradicate malnutrition. Carps, catfish, murrels, tilapia, and prawns are a few of the fish that are high in nutrition [7].

Over 3.3 billion individuals worldwide get 20% of their average daily intake of animal proteins from fish. Fish makes up at least 50% of the animal protein in various nations, including Bangladesh, Cambodia, the Gambia, Ghana, and Indonesia. Global fish consumption increased by around 1.5% year, from 9.0 kg (live weight equivalent) in 1961 to 20.5 kg in 2018 [8].

These benefits of using this method for fish breeders include:

(1) If sperm or ova are kept in storage, the issue of non-coincidence in the maturation of male and female can be resolved.

(2) A system of selective breeding can be implemented to enhance the local stock and assist the developing globe in breeding its native species and creating unique strains of superior stalk.

(3) As many native Indian fish cannot compete with foreign fish, the method of cryopreserving gametes will play a significant role in the protection of indigenous germplasm. This method involves storing gametes from vulnerable species as a protection against the genetic variability loss brought on by environmental perturbations.

(4) It might aid in the development of mono-sex cultures.

(5) If When gametes are effectively stored, we are able to grow fish all year long to meet our needs, contribute to the establishment of gene banks to protect population genetic diversity, and keep the fish ready for reintroduction whenever conditions are more conducive to survival.

The ability to cryopreserve sperms, eggs, and embryos exists, but the preservation of eggs and embryos is challenging due to the cryoprotectant's inability to be effectively absorbed in these cells due to their larger size compared to spermatozoa.

Fish spermatozoa can be successfully frozen for human use; species These species include Oncorhynchus, Salmo, Salvilinus fontinalis, Hucho, Hucho, Thymallus, Esox, Cyprinus, Cyprinus carpio, Serothodon, Catla, and Cirrhinus.

Genetic problems that impact seed quality

Many nations rely on a sufficient supply of high-quality seeds for their aquaculture industries. The lack of effective population size (Ne), inadequate brood stock management, inbreeding depression, genetic drift, introgressive hybridization, unconscious selection, and genetic erosion of domesticated stock, among other factors, have all been linked to stock deterioration, according to numerous hatcheries.

Cryopreservation can be used to stop the harmful effects of inbreeding and genetic drift.

Fish's survival, growth, and developmental stability are all negatively impacted by inbreeding and genetic drift, which also increases developmental instability [9]. Inbreeding depression, which results in lower growth rate, low fecundity, and poor survival, is frequently brought on by unplanned and unregulated breeding and is typically accompanied with allele loss due to genetic drift [10]. Uncontrolled In closed hatchery populations, inbreeding and genetic drift coexist, and these effects are influenced by the population's Ne. In order to avoid negative effects on productivity and profit, maintain the desired Ne [10].

Inbreeding is more common in aquaculture than in other domesticated animals due to the high fecundity of fish. This applies especially to highly fecund species like Indian Major Carps (IMC) (catla, Catla catla, rohu, Labeo rohita, mrigal, Cirrhinus mrigala) and Chinese carps (silver carp, Hypophthalmicthys molitrix, grass carp, Ctenopharyngodon idella, common carp, Cyprinus carpio) where few broodstock are necessary to meet demands for fry and broodstock replacement. Inbreeding has been shown to have negative impacts, including a 30% or more reduction in growth, survival, and reproduction [11].

By producing more fish than necessary, it is possible to lessen the issue of inbreeding and genetic drift. One or two females and males can be bred to produce the necessary number of fingerlings because some species have high fertility. However, if inbreeding and genetic drift are to be managed, the capacity to produce relatively few fish needs to be moderated. By spawning a more equal sex ratio, you can also boost Ne and lower the rate of inbreeding and genetic drift. When they spawn their fish, the majority of farmers and hatchery managers employ skewed sex ratios. One male can typically fertilise the eggs of numerous females, therefore this is done. Farmers may use and care for fewer males as a result, which cuts production expenses. The rarer sex has a disproportionate impact on Ne size when a skewed sex ratio is present. By producing a greater number of fish, cryopreservation can assist sustain Ne by keeping fewer males in the hatchery [10].

Cryopreservation and intrusive hybridization

It is the process through which the germplasm of one species gradually infiltrates that of another [12] as a result of hybridization and recurrent backcrossing. Pure mrigal in Bangladesh are gravely threatened by introgressive hybridization, according to Sarder et al. [13]. The reasons for this species' declining quality include genetic drift, unintentional hybridization, and inbreeding depression. They believed that the easiest and least expensive way to conserve genomes that can be used to maintain future conservation options is through cryopreservation. Salmonids frequently experience the phenomenon of autochthonous populations mixing with introduced ones, which can lead to outbreeding depression and the replacement of potentially locally adapted populations by allochthonous ones [14].

In an effort to conserve two salmonid species, including the marble trout (Salmo marmoratus) and the Adriatic lineage of the grayling (Thymallus thymallus), which are native to the drainage of the Soa river in Slovenia, Horvath et al. [15] used cryopreservation. The hybridization and introgression of these species with allochthonous species like brown trout (Salmo trutta m. fario) and the Danubian lineage of grayling, which were introduced to the Soa drainage throughout the 20th century, had a significant negative impact on their populations. Sperm from marble trout and adriatic grayling have been cryopreserved as a crucial component of conservation efforts. Since there was no pure population of graylings, the percentage of Adriatic genotype in the broodstock was raised. genetic studies Sperm was cryopreserved and kept until each sample's genetic study was finished. Sperm that had been cryopreserved and came from individuals with more Adriatic genotypes than a set threshold was thawed and used to fertilise eggs from Adriatic females. The offspring were raised as broodstock, and between 70 and 80 percent of the local grayling broodstock was created from cryopreserved sperm. Cryopreservation was utilised to establish "sanctuary" streams for marble trout. Prior to the spawning season (early November), wild males of a particular pure population have their sperm harvested and cryopreserved. Until the spawning season (December–January), sperm is kept in liquid nitrogen (LN2) and used to fertilise the eggs of females from the same population. Then, eyed eggs are placed in nests made of fake materials. in the ready-made "sanctuary" flow. As a result, a large proportion of the given pure population's males take part in the development of the new population.

Introgressive hybridization as a response to environmental change may or may not be desirable from a management standpoint, depending on the situation. It is beneficial when the ensuing adaptation, such as one to the abrupt temperature swings that might become more frequent or intense with climate change, has the potential to save a native species from extinction. In these situations, management measures to

prevent hybridization, like the protection of hybrid zones, may improve the capacity of species to adapt to environmental change [16].

References

1. Datta A. Nalle ,Divya D Nagapure, Madhuri Y. Bhande. 2021. Molecular Techniques for Identification of Microorganisms Obtained From Different Sources Review Based Research upon Lactic Acid Bacteria, international Research Journal of Modernization in Engineering Technology and Science, Volume:03/Issue:12/ p: 270-273 . https://www.researchgate.net/publication/357251061.
2. Beare D. Fisheries and aquaculture: The importance of fish for food and nutritional security. Beare, D., 2012. Fisheries and aquaculture: The importance of fish for food and nutritional security. In: Thornton, P. and Cramer, L. (Eds.),
3. Roos N, Wahab MA, Chamnan C, Thilsted SH. 2007 The role of fish in food-based strategies to combat vitamin A and mineral deficiencies in developing countries. The journal of Nutrition.;137(4):1106-9.
4. Thilsted SH, Roos N, Hassan N. 1997,The role of small indigenous fish species in food and nutrition security in Bangladesh. Naga, the ICLARM quarterly.;20(3-4):82-4.
5. Hossain M, Naher F, Shahabuddin Q. 2005.Food security and nutrition in Bangladesh: progress and determinants. eJADE: electronic Journal of Agricultural and Development Economics.;2(853-2016-56126):103-32. DOI: 10.22004/ag.econ.110131
6. Islam FU. 2007.Self-recruiting species (SRS) in aquaculture: their role in rural livelihoods in two areas of Bangladesh [thesis]. University of Stirling;
7. Kawarazuka N. 2010. The contribution of fish intake, aquaculture, and small-scale fisheries to improving nutrition security: a literature review.: p. 51
8. FAO. 2020.The State of World Fisheries and Aquaculture. Food and Agriculture Organization of the United Nations, Rome.: p. 206.
9. FAO. 2008. Aquaculture development. 3. Genetic resource management. FAO Technical Guidelines for Responsible Fisheries. No. 5, Suppl. 3. Rome, FAO.: p. 125.
10. Tave D. 1999. Inbreeding and brood stock management. Fisheries Technical Paper. No. 392. Rome, Food & Agriculture Org.: p. 122.
11. Dunham RA, Majumdar K, Hallerman E, Bartley D, Mair G, Hulata G, Liu Z, Pongthana N, Bakos J, Penman D, Gupta M. 2000. Review of the status of aquaculture genetics. In Aquaculture in the Third Millennium. Technical Proceedings of the Conference on Aquaculture in the Third Millennium, Bangkok, Thailand: pp. 137-166.
12. Anderson E. 1953. Introgressive hybridization. Biological Reviews. 1949:28(3),pp.280-307. doi:10.1111/j.1469-185x..tb01379.x
13. Sarder MR, Rafiquzzaman SM, Sultana R, Islam MF. 2009. Cryopreservation of spermatozoa of Mrigal, Cirrhinus cirrhosus with a view to minimize inbreeding and hybridization. Journal of the Bangladesh Agricultural University.;7(1):211-218. DOI: 10.3329/jbau.v7i1.4986

14. Horváth Á, Jesenšek D, Csorbai B, Bokor Z, Raboczki É, Kaczkó D, Bernáth G, Hoitsy G, Urbányi B, Bajec SS, Snoj A. 2012. Application of sperm cryopreservation to hatchery practice and species conservation: A case of the Adriatic grayling (Thymallus thymallus). Aquaculture. 2012 Aug 15;358:213-215. doi:10.1016/j.aquaculture..07.012
15. Horvath A, Bokor Z, Bernath G, Urbanyi B, Snoj A, Susnik Bajec S, Jesensek D. 2015. Application Of Sperm Cryopreservation To The Culture And Conservation Of Salmonid Species: A Slovenian-Hungarian Collaboration. Serbia.