THE RADIOSENSITIVITY OF NILE TILAPIA (*OREOCHROMIS NILOTICUS*) FINGERLINGS

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

Photographs

Graphs

LIST OF PLATES

PLATE

An undergraduate thesis of Michael Joseph T. Reyes and Pia Victoria V. Velasco, presented to the faculty of CAS, UPM, in partial fulfillment of the requirements for the degree of Bachelor of Science in Biology, entitled The Radiosensitivity of Nile Tilapia (*Oreochromis niloticus)* Fingerlings, under Dr. Florencio-Isagani S. Medina III, adviser.

ABSTRACT

The Nile Tilapia (*Oreochromis niloticus*), a very popular fish commercially in the Philippines, was studied to determine its radiosensitivity and to see its potential as a biological indicator in aquatic ecosystems.

Nile Tilapia was seen to be radiosensitive. The fish were exposed to gamma-irradiation and chromosomal aberrations were induced. The various types of aberrations seen were chromatid gaps, chromosome gaps, chromatid fragments, dicentric rings, fusions, despiralizations and translocations. Among the aberrations observed, dicentric rings, fusions and chromosome gaps were strongly correlated with dosage, with only the dicentric rings increasing steadily with increasing dosage.

In the course of the study, the lethal $dosage_{50}$ for Nile Tilapia within 18 days was determined and it was observed at 2.0 krad. The modal chromosome number was also established at 2n=44 with a karyotype exhibiting 22 pairs of acrocentric chromosomes with 2 pairs of marker chromosomes present.

THE RADIOSENSITIVITY OF NILE TILAPIA (*OREOCHROMIS NILOTICUS*) FINGERLINGS

I. INTRODUCTION

Background of the Study

The use of radiation offers many benefits to society such as energy production, military defense, communication industries, medicine and food production. However, this carries many risks to health and safety. In the last few decades, there has been a significant rise in the release of radioactive wastes into the environment.

In the case of nuclear power stations the heat ejected from the power generation cycle needs to be dissipated, and this is most easily achieved by using large quantities of water in a single-pass cooling system. Hence, the stations are usually situated on rivers, lakes and seacoasts. They also provide repositories for low-level radioactive wastes (IAEA. 1979).

Much of these radioactive wastes eventually reach aquatic ecosystems, adding to the background levels of natural radioactivity. Once present, these wastes have the potential for being concentrated in the higher trophic levels of the food chain and causing genetic damage (Kligerman, 1979). Because of this, more concern has been directed toward the control of radioactive waste disposal and the need to determine the limit when human populations are exposed.

Data that has been collected showing the effects of irradiation of aquatic organisms has two main uses. First, it can be used to evaluate the impact of radioactivity release

into the aquatic environment. Second, it can be used to assess the interaction of radiation with living tissue.

Many studies have been done in the past to determine the effects of ionizing radiation on the physiology, fertility and mortality. However, only a few studies have been done to evaluate the effects of such radiation on their genetic makeup (Kligerman, 1979). One way of evaluating the genetic effects of radiation on aquatic organisms is by observing the chromosome aberrations of irradiated cells. Cytogenetic methods are likely to find increasing application in aquatic radiobiology because chromosomal aberrations and mitotic disturbances are amongst the most sensitive indicators of radiation damage in an organism (UN Scientific Committee, Panel Summary Report, 1979).

Many studies have been conducted in the past to determine if there is any quantitative relation to the dose of irradiation with the number of induced chromosomal aberrations. The first cytological evaluations of chromosomal aberrations induced by radiation were done by Sax (1938,1939,1940,1941). Using *Tradescantia* microspores, he observed that terminal deletions and isochromatid aberrations increased approximately linearly with increasing dose. It was found that the yield of aberration was very dependent of dose rate. This classical theory of aberration was formulated by Sax (Wolff, 1963).

More recent studies done by H.J. Evans on chromatid breaks in *Vicia* and *Tradescantia*, and Bender's study on mammalian cells, support Sax's earlier conclusions. The frequency of this type of aberration was found to increase linearly with increasing dose (Wolff, 1963).

Among the aquatic organisms, fish are one of the most commercially important components and are cytogenetically well characterized (Kligerman, 1979). This makes

fish ideal for cytogenetic studies. They can be used to assess the impact of the amount of radiation or radioactive material in the environment and to determine the limit in the amount of radiation an organism can take.

Fish are the major vertebrate group in aquatic ecosystems, which are exposed to nuclear systems for cooling purposes. Therefore, they have the potential of serving as biological indicators of radiation contamination and radiation effects in a certain ecosystem. This study will be concerned with the radiosensitivity of Nile Tilapia *(Oreochromis niloticus)* fingerlings, a species of high commercial importance in the Philippines. Our study will not only look into the quantitative relationship between dose of irradiation with the number of induced chromosomal aberrations, but we will also determine which of these chromosomal aberrations are the most sensitive biological indicators.

Statement of the Problem

This study envisions to discover if Nile Tilapia is a potential candidate as a biological indicator organism to radiation exposure and effects.

Research Objectives

The main objective of this study is to determine the radiosensitivity of the karyotype of Nile Tilapia, *Oreochromis niloticus*. The specific objectives are:

1. To determine the lethal $dose_{50}$ of *Oreochromis niloticus* to ionizing radiation (gamma).
2. To determine the effect of ionizing radiation on the somatic chromosomes and karyotype of *Oreochromis niloticus*.

3. To determine if the radiation-induced chromosomal aberrations in Nile Tilapia can be used as a biological indicator.
4. To determine which of the chromosomal aberrations are the most reliable indicator of radiosensitivity.

Hypothesis

H_o

1. The frequency of chromosomal aberrations does not reflect the effect of induced radiation.
2. The frequency of chromosomal aberrations with increasing levels of radiation in *Oreochromis niloticus* cannot be used as an indicator of radiosensitivity.

H_A

1. The frequency of chromosomal aberrations does reflect the effect of induced radiation.
2. The frequency of chromosomal aberrations with increasing levels of radiation in *Oreochromis niloticus* can be used as an indicator of radiosensitivity.

Significance of the Study

In the future, we may have to consider using nuclear energy as an alternative form of energy. The opening of a nuclear power plant may impose some health and environmental effects. For us to have a better understanding of this, this study may reflect the existing conditions and its corresponding effect on organisms if a nuclear power plant were to operate.

In addition, this study will show how the induction of radiation can produce chromosomal aberrations. If it is found that chromosomal aberrations are a reliable biological indicator, this can serve as a method for determining the limit in the amount of ionizing radiation an aquatic organism, specifically *Oreochromis niloticus* can take. Data gathered from this study can be used as future reference especially since little cytogenetic studies have been done on *Oreochromis niloticus*.

Fish is commercially valuable to man. Neglect of their habitats such as the improper radioactive waste disposal can be harmful to the fish and other organisms living in that environment. More knowledge of the limit in the amount of ionizing radiation *Oreochromis niloticus* can take will significantly help radioecologists in understanding the limits of his environment as a whole. This study will serve as a reason and a need for man to protect himself and his environment.

Nile Tilapia, *Oreochromis niloticus,* are widely farmed in freshwater in the Philippines, the Philippines is in fact, the world's largest single producer of Tilapia (Rudd, 1992). For this reason, it would be appropriate that this species be used as the test organism for this study. To be able to establish Nile Tilapia as a good subject for experimentation would be of great help, especially in the Philippine context.

Scope and Limitations

1. This study is limited to Nile Tilapia, *Oreochromis niloticus,* fingerlings, in vivo.
2. The lethal dose of radiation for 50% of the experimental population will be determined in 18 days ($LD_{50/18}$).
3. The cytogenetic part of the study will involve only one replicate.
4. The study will only involve mitotic chromosomes.

5. Only chromosomal and chromatid aberrations will be used to measure the radiosensitivity of Nile Tilapia, *Oreochromis niloticus*.
6. This study is concerned with the relationship between the frequency of chromosomal aberrations and the different dosages of radiation and not the specific dose where an aberration will be initially observed.

Definition of Key Terms

Biological indicator. A biological system that undergoes measurable and reproducible changes when it is irradiated (Panlaque, 1982).

Chromatid. One of the longitudinal subunits of a replicated chromosome, joined to its sister chromatid at the centromere (Klug and Cummings, 1997).

Chromosome. In prokaryotes, an intact DNA molecule containing the genome. In eukaryotes, a DNA molecule complexed with RNA and proteins to form a threadlike structure containing genetic information arranged in a linear sequence (Klug and Cummings, 1997).

Chromatid aberration. They are modifications and rearrangements of the genetic material within or among chromatids.

Chromosomal aberration. Variations in the number of individual chromosomes as well as rearrangements of the genetic material within or among chromosomes (Klug and Cummings, 1997).

Colchicine. It is a chemical treatment that is used to prevent spindle fiber formation during cell division, which in turn suspends chromosomes at the equatorial plane. This process is done for easier viewing of the chromosomes.

Fingerlings. Young fish usually no longer than a man's finger, weighing between 0.01 to 40 grams (Traballo, 1996).

Irradiation. The process of exposing radiation to a system (Panlaque, 1982).

Karyotype. It is a statement of the number and morphological description of the chromosomes (Elkind, 1967). It also refers to the arrangement of metaphase chromosomes in a sequence according to length and position of the centromere (Klug and Cummings, 1997).

LD_{50}. The number of days it will take to kill 50 % of the population of interest with a certain dose.

Metaphase. The stage of cell division in which the condensed chromosomes lie in a central plane between the two poles of the cell, and in which the chromosomes become attached to the spindle fibers (Klug and Cummings, 1997).

Mitosis. A form of cell division resulting in the production of two cells, each with the same chromosome and genetic complement as the parent cell (Klug and Cummings, 1997).

Radiosensitivity. It is the response of a biological system when it is exposed to radiation (Panlaque, 1982).

II. REVIEW OF RELATED LITERATURE

Radiation: Definition, Sources, and Effects

The biological effects of ionizing radiation represent the organism's ability to deal with energy left in them after interacting with an ionizing particle. Ultimately, radiation biology is the study of the sequence of events within organisms, which follows the absorption of energy from ionizing radiation. It is the study of the efforts of organisms to restore proper energy relationships within themselves and of the damage to the organisms, which may be produced by this excess of energy (Pizzarello and Witcofski, 1972).

Ionizing radiation naturally evolves from cosmic rays that have been released by terrestrial radionuclides in the environment and the earth. As of the UNSCEAR 1988 Report, the effective dose from natural radiation in areas of normal background has not changed from 2.4 mSv in 1983. One third of this is due to external exposure to cosmic rays and terrestrial radionuclides, and two-thirds from external exposure (Kaul, 1990).

Atmospheric testing of nuclear weapons has also released radionuclides to the environment. In 1963, a maximum annual dose occurred and was measured at 0.2 mSv on average for the world population. Higher values were recorded on the Northern hemisphere where more testing took place. Since 1980, no atmospheric testing have taken place, hence decreasing the annual doses to smaller fractions (Kaul, 1990).

Over the years, the use of electrical energy generation by nuclear reactors have been increasingly popular. Presently, nuclear energy contributes 20% of the world's electrical energy. The release of radionuclides, such as uranium has been found near these reactors. The annual effective doses found among individuals living near these nuclear

installations are very low at around 10 mSv. However, accidents such as what occurred in Chernobyl had more significant values from 0.3 to 0.8 mSv over eastern and central Europe (Kaul, 1990).

In the field of medicine, the use of X-rays and radiopharmaceuticals have been quite common. Most of these equipments are being used in industrialized countries. Diagnostic X-ray examinations consist of majority of the total medical radiation exposures because of its relatively high frequency. In these industrialized nations, the annual effective dose from all these diagnostic tests amounted to an average of 1.1 mSv. Comparatively, the world average is only 0.3 mSv (Kaul, 1990).

In the past 20 years, the average annual dose from occupational radiation exposures has significantly declined. The average annual effective dose were 3.5 mSv to workers in nuclear power plants, 0.9 mSv to workers in industries using radioactive materials, 0.7 mSv to workers in military and defense activities and 0.5 mSv to medical staff. For coal miners, a dose of 0.9 mSv was measured, 6 mSv for mineral miners and 3mSv for airline crew. Data from the evaluation of occupational radiation exposures are significant and must be considered to decrease the levels of radionuclides in our environment (Kaul, 1990).

The application of radiation and radioactive materials in medicine help in the diagnosis and treatment of illnesses. A special type of X-ray unit, the CAT scanner, is used to produce 3D images of the body. In nuclear medicine, radioactive materials are taken internally and are detected to produce images. For industrial purposes, radiation is used for quality control tests and inspections. Industrial X-ray machines are used to find foreign material in food products. In laboratories, high voltage X-ray machines and

particle accelerators are used in research facilities. Tracers use small amounts of radioactive material for extensive research as well. For military purposes, nuclear weapons are produced for national security. The radioactive materials in these weapons include tritium, and various isotopes such as plutonium and uranium (www.pantex.com/cs/pxradd2a.htm).

In recent times, studies have been conducted to study the frequency of harmful effects that had been induced by ionizing radiation. In the early stages, the physiologic effects of ionizing radiation are reversible. But in the long run, metabolic processes produce biological effects (www.via.org/viademo/pro/d8050.htm).

Biological effects depend on the total dose received, the type of body tissue receiving radiation, the type of radiation and the period the dose had been received. Some of these factors determine the type of radiation exposure, they include acute and long-term exposure (www.pantex.com/cs/pxrada1.htm).

Acute biological radiation damage occurs within days or weeks after exposure to extremely high doses of radiation over a short period of time. These effects may occur when one is exposed to doses hundreds of times higher than those received from environmental contamination, except from accidents or nuclear warfare (www.via.org/viademo/pro/d8050.htm).

On the other hand, long-term exposure to radiation produces continuing after-effects since biochemical and physiological reactions initiated with the absorption of radiant energy continues for a long time. These long-term biological effects may be genetic. They may damage sex chromosomes, induce gene mutations and pass it on to their

progeny. Somatic damage may appear several years after exposure and its effects consist mostly of cancers and decreased longevity (www.via.org/viademo/pro/d8050.htm).

Radiation induced chromosomal aberrations

The input of radiation on cells is largely exhibited by morphological changes in the chromosome. These modifications include variation in the number of chromosomes and the rearrangement of the genetic material either within or among chromosomes (Klug and Cummings, 1997). Collectively these mutations in the chromosome are called chromosomal aberrations.

In the stage of metaphase, the alignment of the contracted chromosomes along the equatorial plane makes it ideal for determining their overall dimensions and morphology. For this reason, one can determine the cell's karyotype, which is a visual representation of the number and morphological description of the chromosome. It is in the metaphase where the morphological changes of the chromosomes induced by radiation are clearly observable (Elkind, 1967).

The ability of radiation to produce chromosomal aberrations can be manifested by structural changes such as chromosomal breaks. Ideally, cells must be irradiated in interphase or early prophase such that by the metaphase and anaphase stages, these morphological changes can be seen. In such cases, colchicine can be used to prevent spindle formation and to allow easier observation (DeRobertis, 1975).

The induction of ionizing radiation to cellular DNA causes an array of lesions. Among them are single and double stranded breaks, base damage, mismatches, DNA-DNA crosslinks, and DNA-protein crosslinks. However among these lesions, it is the

double-stranded breaks that seem to be the most important in the production of chromosomal aberrations (Sinclair, 1987).

Chromatid aberrations are grouped into three types. There are chromatid breaks, which occur when either one or both chromatids (at nonadjacent positions) are broken. They produce a normal chromatid, a shortened chromatid and an acentric fragment. The second group consists of isochromatid breaks, which occur when both chromatids are broken at the same position and remain open at division. Following this type of break, there are four open ends, which may form several types of reunions. If there is a sister union or a union of fragments containing centromeres, an anaphase bridge will result and either one or two lagging fragments. The third group consists of chromatid exchanges. These arise when two or more chromatids are broken and the broken ends reassort themselves before rejoining. An intrachange occurs when the broken ends rejoin from the same chromosome, likewise an interchange occurs if they are from different chromosomes. Exchanges can be further subdivided into symmetrical and asymmetrical. If it is symmetrical, each daughter cell receives the same chromatid complement. In asymmetrical exchanges, fragments and rings are produced in intrachanges; and bridges and fragments or fragments alone in interchanges (Elkind, 1967). (Appendix B)

Chromosomal aberrations are grouped into four types. There are the chromosome breaks, which produce lagging fragments and bridge formations. The second group consists of symmetrical intrachanges, which have no acentric fragments or rings, therefore producing no deletions. The third group is the asymmetrical intrachanges, which produce rings. In the fourth group, the symmetrical interchanges produce daughter

cells, which receive a full but altered chromatin complement. Lastly the asymmetrical interchanges always exhibit lagging fragments and bridges (Elkind, 1967). (Appendix C)

Presently, many kinds of radiation have been applied to produce changes in the morphology of the chromosome. Among these are the corpuscular radiation such as α and β particles, and non-corpuscular radiation such as ultraviolet, X and γ rays in the electromagnetic spectrum (Pizzarello and Witcofski, 1972).

The effects of radiation on the biological level depend on the amount of energy they contain and its ability to transmit this energy to atoms and molecules in the cell. Radiation containing low energy levels excite the atoms and molecules, which only result to chemical rearrangements or by the release of heat. One example that exhibits this is ultraviolet radiation. However radiation with high energy levels can expel an electron out of its orbit, thus resulting to ionization. This can be exhibited by the action of X and γ rays. Generally radiation of a higher level will produce more changes in the orbital electrons and the resonance of molecules (DeRobertis, 1975).

The study of the effects of radiation on chromosomes have been supported by technical advances in recent years. One, there is the development of cell and tissue culture techniques for the growth of the cell *in vitro*. Then there is the development of new techniques which facilitate examination of chromosomes before and after radiation (Elkind, 1967).

It has been demonstrated in many tissues that the induction of radiation has been found to inhibit cell division. However, this effect occurs temporarily. Upon application of radiation, substances essential to mitosis are destroyed or inhibitory poisons are produced. For example, the accumulation of ribonucleic acid in the cytoplasm of

irradiated cells suggest that its metabolism may have been interfered with (DeRobertis, 1975).

It has also been demonstrated in many irradiated tissues that there is an increase in cellular or nuclear size. This is largely attributed and likewise a consequence of mitotic inhibition. Because of its inability to divide, the cells continue to grow in mass. For example, normal cells in the bean root system have a mean nuclear diameter of 8.5 μ. After irradiation, these non-dividing cells exhibit a mean nuclear diameter of about 11.5 μ (DeRobertis, 1975).

In other cases, the effects that have been previously mentioned may not be observed, however, the cell may die. A very high dose of radiation can induce death, which may occur immediately after nuclear division. It has been observed that these effects occur simultaneously, therefore death may be due to the inhibition of mitosis. It has been thought that cellular death occurs after division due to genetic changes, i.e. lethal mutations or chromosomal aberrations. It has also been found that even after a tissue had been irradiated and given time to recuperate, degenerating cells were observed and that their number being proportional to the dosage (DeRobertis, 1975).

Lastly, another observable cytologic effect due to radiation is the induction of the coalescence of the metaphase chromosome. Similarly to this is the "arrested metaphase" however they differ because this is produced by chemicals. Chromatin bridges formed in anaphase occur due to chromosome agglutination. This observation is considered to be a physiological effect and has been characterized by the depolymerization of nucleic acids since it has become more fluid (DeRobertis, 1975).

Importance of fish: As laboratory animals; As biological indicators

Although there are many species of experimental animals being used for testing the incidence of radiation-induced genetic damage, it is necessary for comparative purposes to have the vertebrates represented by more species than just the mice, *Mus musculus*. It has been demonstrated repeatedly by zoologists that fish possess behavior patterns, physiologies and diseases counterparts of which are found among warm-blooded animals which include man (Gordon, 1967).

Small, freshwater fish are well suited to the laboratory. They require little space, housing and equipment for their maintenance, which are not particularly costly. Those who have mastered the techniques of aquaculture are convinced that of all the vertebrate animals, the fish are the easiest and the cheapest to breed and maintain. Compared with mammals and birds, they are the cleanest and least odoriferous among them. Their behavior can easily be studied and their whole life cycle can be observed. The use of fish in these experiments is important in understanding other vertebrates, including man (Panlaque, 1982).

It has been found in aquatic organisms that the lower the phyla or group the greater the radiotolerance to ionizing radiation (Patel and Patel, 1979). The majority of research concerning radiation effects of chromosomes of aquatic organisms have dealt mainly with fish, which is likely due to the fact that fish are: the major aquatic vertebrate group, are highly important commercially, and to some extent, cytogenetically well characterized (Kligerman, 1979). It also has been said that the morphological structure and physiological functions in fish are similar to but simpler than those of mammals, thus it seems that fish are suitable for the analysis of radiation effects (Etoh, 1979). Being the

most important and vulnerable element of the aquatic ecosystem, fish have been the subject of an increasing number of cytogenetic investigations on the effects of irradiation (Tsytsugina, 1979). Fish have been used in mutational research for some years now, there are several detailed studies of their mutational response to irradiation; their sensitivity to X-irradiation mutation appears to lie between that of the mouse and *Drosophila* (Woodhead, 1979).

A number of reports have been published on the effects of whole-body irradiation with X and gamma rays on adult fish. Little consideration has been paid to the cause of radiation death. Lethal effect of radiation on individuals is represented by the $LD_{50/30}$, the dosage of whole-body irradiation required to kill 50% of the animals within 30 days (Etoh, 1979).

Genetic material is one of the primary sites of radiation damage, and mutations can exert effects on any stage or upon any process during the life cycle (Kligerman, 1979). It has been well established that ionizing radiation induce the breakage and rearrangements of chromosomes in the animal cell (Kligerman, 1979). Cytogenetic investigations should find wide application in radioecological research, since chromosomal aberrations and other nuclear disturbances are among the most sensitive criteria of radiation injury of an organism (Tsytsugina, 1979). The cytogenetic effects of irradiation are associated with the rearrangement of the chromosome structure following the breakage and exchange of sites and they can take place in somatic or germ cells (Tsytsugina, 1979).

Most of the studies concerning the clastogenic effects of radiation on fish genomes involve acute doses of relatively high doses of radiation. Researchers found that X-irradiation of the early embryonic stages of the loach (*Misgurnus fossilis*) and the salmon

(*Salmo salar*) caused dose-dependent increases in the number of cells with chromosome aberrations (bridges and fragments), pyknotic nuclei and multipolar mitosis (Kligerman, 1979). Anaphase and telophase were found to be the most radiosensitive stages of the cell cycle, whereas interphase was the most resistant stage (Belyaeva, 1959).

There have also been studies on the conservation of radiation damage in fish embryos that developed from irradiated gametes. It has been observed that chromosome damage was transmitted to the embryo and that the level of damage increased with increasing does of radiation up to the Hertwig effect (effect where very high doses of radiation can lead to the complete inactivation of the pronucleus of the irradiated gamete) was observed (Kligerman, 1979).

Kligerman et al. (1975) studied the effects of chromosome breaking on the fish chromosome in vivo using modern cytogenetic techniques. It showed that the central mudminnow (*Umbra limi*) can also be used as a model aquatic organism, with chromatid breaks and gaps found in the metaphase of the gills, kidney and intestines.

More recently, there have been studies of low-level chronic irradiation on fish chromosomes. Incubated salmon (*Salmo salar*) eggs in water were exposed to these levels of radiation and found that the embryos from the treatment had significantly higher numbers of cells with chromosome abnormalities than the control group (Migalovskaya, 1973). Tsytsugina (1972, 1973) obtained similar results with ruff (*Scorpanena porcus*) and turbot (*Scophtalmus maeoticus*) eggs respectively (Kligerman, 1979)

Test subject: Nile Tilapia

A member of the family Cichlidae, the fish *Oreochromis niloticus* is more commonly known as Nile Tilapia, or simply tilapia. Tilapia rank with the milkfish as the most extensively cultured finfish in the Philippines' inland waters. The fish is widely

distributed around the world and are now cultured for food in more than 30 countries (The Technological Committee for Tilapia, 1985). The tilapias are endemic to the Middle East and Africa but have been artificially distributed in East Asia and other tropical regions in the world. Their expansive geographical distribution reflects the species' intrinsic capacity for adaptation to various types of habitat and environmental conditions. It was first introduced in the Philippines in 1972 (Guerrero, 1983). Tilapia is chiefly used as a source of food in countries (Guerrero, 1983). Tilapia is characteristic of mild, soft, white fish fillets, with a slightly sweet taste, which is a culinary delight (http://www.aquanet.com/showcase/tilapia/tilapia.htm). However, tilapia also has other uses. In one study, *O. niloticus* was used to stimulate the population growth of phytoplankton through faecal fertilization. In other words, the organism can be used as an alternative for biofertilization (Benitezmandujano and Floresnava, 1997).

Tilapia are cultured into large-size fish, weighing as much as 3 to 4 kilograms (Guerrero, 1983). But on the average, its length is 25 – 30 cm and weighs from 0.8 – 1.5 kg. Body color ranges from grayish-blue to brown and black depending on the environmental condition (Conlu, 1986). The physical characteristics of tilapia are the following: 8-12 anal soft raps, 3 anal spines, 30-31 lateral line scales, and 22-28 gill rakers. Tilapia has large scales, a concave head shape, and red fringe fins (Mamaril, 1996). The presence of vertical, dark brown stripes on the caudal fin distinguish Nile Tilapia from other species (Guerrero, 1983).

Female Nile Tilapias are smaller than males and measure approximately 9 cm at 3 months. Female tilapias have 3 holes, the anus, the genital pore and the urinary pore and they also possess a yellow operculum. Male Nile Tilapias measure approximately 11 cm

at 3 months, and during the period of courtship their gular region turns noticeably red. Unlike the females, they only have 2 holes, the anus and the urogenital pore (Eguia *et al.*, 1996).

Unlike other fish, it is not difficult to maintain Nile Tilapia. Among locally available species, *O. niloticus* is the most preferred for culture. Essentially the organism is herbivorous but it can grow favorably even under a low protein diet. It will also feed a diversity of food types such as detritus, crustaceans, benthos, and various forms of supplemental feeds. Nile Tilapia is known to thrive in salt water. In fertilized ponds, *O. niloticus* grows to 80-100 grams in four months with at least 80% survival. The species grow sturdily. It tolerates crowding, is resistant to pests and diseases and can tolerate dissolved oxygen concentrations as low as 1 ppm (The Technological Committee for Tilapia, 1984). Taking these into consideration it is quite obvious that Nile Tilapia can be raised with relative ease. This is also why Nile Tilapia is the most important tilapia species culture in the Philippines. As of 1985, the Philippines had an annual yield of 50,000 tons of tilapia, over 90% of which were *O. niloticus* (Guerrero and Guerrero, 1988).

Nile Tilapia chromosomes were obtained by in vivo technique and the diploid number of this species is 2n=44 and the NF=62 (NF= number of chromosome arms). Karyotipic analysis done by banding and staining techniques showed that Nile Tilapia' s karyotype consisted of 1 metacentric, 8 submetacentrics and 13 subtelocentrics (Crosetti *et al.*, 1988). (Appendix D)

Aside from the fact that the generation time for *O. niloticus* is relatively short, which is about four months, and its capacity to breed year-round means that any breakthroughs

will be readily obtainable (Pullin and Capili, 1987). Tilapia has great commercial value to both its producers and consumers. For these reasons, Nile Tilapia have become a popular test organism in many studies. Breeders have gone into research to drastically improve both quantity and quality of their tilapia yield (http://www.aquanet.com/showcase/tilapia/tilapia.htm). Most experiments done on tilapia concern the factors that affect growth rate and concern the genetic methods of improvement of the species, both to produce better yields.

Research for genetic improvement of cultured fish has a short history compared to crops and domestic animals. There is now a broad consensus that applied genetics can have tremendous impact on aquaculture. However, most tilapia genetics research has been on hybridization (Pullin and Capili, 1987). On a comparative study evaluating sex reversed male tilapia (SRT) and genetically male tilapia (GMT), a Thai strain (EA-SRT) was 55.6 % and 61.9 % greater than both Philippine strains (ES-SRT and ES-GMT). Furthermore, the Thai strain (EA-SRT) and the inter-strain (EA x ES- SRT) grew significantly larger than the other crosses (Tuan *et al.*, 1998). The study reflects superior growth of the Thai strain as compared to the Philippine strain but nevertheless combination of both strains improved growth rate.

In the last two decades, tilapia genetics research has also revolved around monosex male fry production (Pullin and Capili, 1987). A major problem among tilapia culturists throughout the world is that because tilapia is very easy to breed, ponds usually result to overcrowding. Monosex culture is one way of controlling tilapia reproduction (Guerrero, 1983). This technique is done by manual separation of the sexes of tilapia. Males are generally preferred over females because of their higher growth rate.

Another obstacle is that good fish genetics research facilities are scarce throughout the tropics, where tilapia culture industries are the largest (Pullin and Capili, 1987). In some West African countries and the Philippines, the chance of possessing strains of more productive species like *O. niloticus* are oftentimes accidental, depending on the proximity of supply and acquisition of strains from countries where research has already been accomplished (Lazard, J. and X. Rognon, 1997). Apparently, enhancement of research with local species and monitoring of genetic characteristics appear to be essential, especially in the Philippines.

III. MATERIALS AND METHODS

The experiment wase divided into two parts:

I. Determination of the $LD_{50/18}$ of *Oreochromis niloticus*

II. Cytological Analysis of the Chromosomes of the *Oreochromis niloticus* exposed to different dose levels of gamma radiation

Experimental design and treatments

The Nile Tilapia fingerlings were divided into six groups in both the first and second parts. In both parts one and two, one control group and five experimental groups were used. The treatments varied in the dosage of radiation that the Nile Tilapia were exposed to. Part I had the doses of 0, 1, 2, 3, 4, and 5 krad, while the dosages in part II were determined from part one, that is 0, 0.5, 1, 1.5, 2, 3 krad. For part II of the study the Pearson's correlation coefficient will be used to determine the relationship between the specific aberrations and the dose.

Test subject

The *Oreochromis niloticus*, more commonly known as the Nile Tilapia, was the test organism chosen for this study. It is a popular freshwater fish in the Philippines, which is usually bred for consumer purposes as it is used for food. Nile Tilapia is about 25 – 30 cm in length and weighs from 0.8–1.5 kg. Its color ranges from grayish-blue to brown and black (Conlu, 1986). Tilapia have large scales, a concave head shape, and red fringe fins (Mamaril, 1996). And the presence of vertical, dark brown stripes on the caudal fin distinguish Nile Tilapia from other species (Guerrero, 1983).

Its importance in aquaculture is the primary reason for research into its characteristics and behavior. Many studies have been undertaken on Nile Tilapia, but majority of them are done primarily to study ways in which they can increase its yield and hence to determine the most suitable conditions in which the organism will achieve the greatest stocking capacity. In the field of cytogenetics it has been studied to improve its quality in the genetic level so as to breed new and advanced hybrids for greater yields. And due to Nile Tilapia's short generation time, their capacity to breed at any point in the year, their availability, and their economic importance, it has been noted that it can be used as a model test organism in aquacultural studies.

To accommodate the two parts of the study, 1500 Nile Tilapia fingerlings were obtained and were purchased in 2 separate batches (750 for each batch). The 1st batch needed 360 fish while the 2nd batch needed 480 fish. The surplus in acquiring the fish guaranteed that there was enough fish to begin the study in case that any mortalities are experienced. This is because they may experience stress in the conditions of transport and acclimatization before the experiment proper begins. The 1st batch was used for the LD_{50} determination, while the remaining batch was used for the cytogenetic analysis. All the Nile Tilapia obtained were randomly assigned to the varying treatments.

The Nile Tilapia were purchased from a local breeder of Tilapia, Amy Banasihan, who supplies Tilapia to many distributors in the metropolis. She works as a teacher at the School of Fisheries in Los Banos, Laguna.

Design and procedure

I. **LD_{50} determination**

A. Acquisition and Maintenance of Nile Tilapia

Seven hundred fifty (750) Nile Tilapia fingerlings were obtained from a breeder. Three hundred sixty (360) of them were used for the study and they were randomly divided into six groups (60 fish per group). The excess fish obtained ensured that there was to be an adequate number of fish left for the experiment proper in case there were mortalities to be experienced. Each group was placed in separate aquariums. Every aquarium had a volume of 5 gallons, with an installed aerator and filter. The water was simple tap water and temperatures were kept within 21 – 26 degrees Celsius. The fish were given one week to acclimatize to their new surroundings before the experiment proper. They were fed fish food twice a day, making sure that each fish would receive an adequate amount, until the whole observation period was over. Prior to irradiation the fish were also checked three times daily so that changes were accounted for.

B. Exposure to Radiation

The test organisms were exposed to varying doses of radiation, with each of the six groups being randomly assigned to specific dose levels. The dose levels were the following: 0 krad (control), 1, 2, 3, 4, and 5 krad. The exposure procedure was carried out using a Gamma-Cell 220 Irradiator, at the Cobalt-60 Facility of the Philippine Nuclear Research Institute (PNRI), as source of gamma-radiation. The fish, in groups of thirty, were placed in a 2000 mL beaker containing freshwater. After the irradiation procedure, the water used in the

irradiation was discarded and the fish were returned to their respective aquaria. Controls were sham-irradiated to ensure that they experienced the same conditions, irradiation exposure excluded, that the treatment groups experienced.

C. Observation and Data Collection

The fish were under close observation for the given 18 days. The determination of the LD_{50} was done by inspecting the fish three times daily, taking note of the number of deaths observed and the specific days they were observed. The data was then tabulated and the Lethal Dosage for 50% of the fish within 18 days was established and served as basis for the second part of the study.

Aside from the mortality time, fish behavior and external manifestations of radiation effects on the fish were also observed and recorded.

II. Cytological Analysis of the Chromosomes

The cytogenetic technique used is a modified version of Kligerman and Bloom's (1977) "in vivo piscine chromosome methodology."

A. Obtaining and Maintenance of Fish

Seven hundred fifty (750) Nile Tilapia fingerlings were obtained from a breeder and were randomly divided into 6 groups (60 fish per group). The Nile Tilapia were maintained in the same manner that they were maintained in part I of the study. This includes the time intervals and the feeding schedules.

B. Tissues to be Studied

Since fish chromosomes are best obtained from epithelium (Denton, 1973), the tissues that were observed were from the fin and gill epithelium both found on its

external body surface. Gill epithelia are constantly in an active state of division but in fins the number are generally few. To compensate for this, the fins of the fish will have to be snipped off and should be allowed to regenerate (Denton, 1973), the caudal fins were snipped off at the margins three days before irradiation giving it ample time to regenerate.

C. Exposure to Radiation

Based on the LD_{50} values from part I, six different dosage levels, including 0 krad (control), was determined. Six groups of Nile Tilapia, comprising of 60 tilapia each were used based on the $LD_{50/18}$ days. Each group was subjected to the different levels of radiation, once again using the Gamma-Cell 220 Irradiator. The dosages assigned were 0, 0.5, 1, 1.5, 2, and 3 krad. Assignment of the dose levels to groups were done randomly to ensure of equal probability for each group to receive any of the different levels of radiation. After irradiation the fish were returned to their respective aquaria.

D. Selection of Sample Representatives

In each group exposed to radiation, 10 out of the 60 irradiated fish were randomly selected for the extraction of chromosomes for the cytological analysis.

E. Pretreatment with a Mitotic Inhibitor

Chromosomes are most evident during the metaphase stage of cell division, thus it would be highly favorable to yield a high number of metaphase spreads for chromosome study. To do this the Nile Tilapia was subjected to colchicine which chemically destroys the spindle mechanism of a cell preventing the chromosome

migration to the poles, thus suspending the cells at metaphase. This makes metaphase figures accumulate.

After forty-eight hours from the time of exposure, the 15 randomly selected fish from each group were allowed to swim in a 0.01% solution of colchicine for six hours in separately labelled containers.

F. Hypotonic Treatment

The fish were then sacrificed by decapitation and the tissues from the gills and fins were obtained. Gill arches were dissected out and margins of the regenerated fins were snipped off. Immediately after obtaining the tissues, they were subjected to a hypotonic solution. The tissues were immersed in a 0.4% KCl solution for 45 minutes. This treatment ensured the adequate swelling of the cells.

G. Fixation

The tissues were then removed from the hypotonic solution and were placed in freshly prepared fixative, which were composed of 3:1 methanol and glacial acetic acid for 12-24 hrs. The tissues were stored in a refrigerator (4° Celsius) so as to prevent the risk of poor fixation. Great care was observed in handling the cells while they were swollen because they readily burst and the chromosomes would be lost. Fixation is carried out to kill the cells without distorting its components.

H. Slide Preparation and Staining

Before the actual slide preparation, clean glass slides (76x26 mm) were heated on a slide warmer at a constant temperature of 60° Celsius.

Next, the tissues were dabbed by absorbent paper to remove excess fixative. Then tissues were placed in a small Petri dish with approximately 1.5 ml of freshly prepared 60 % acetic acid. After this, the tissues were minced gently with forceps to form a cell suspension and the remaining unsuspended pieces of the tissue were discarded.

Using a micropipette, enough suspension was expelled onto a clean glass slide approximately 2.5 cm in diameter. Around 3 seconds later, using the micropipette again, the suspension on the heated slide was drawn back leaving behind a very thin layer of dried cellular material. This procedure was repeated until slide space was maximized.

After the slides were prepared, they were left on the slide warmer for 30 more minutes to allow the acetic acid solution to dry up. The prepared suspension was used for a maximum of 3 slides. The use of an old suspension would have allowed the acetic acid solution to dissolve the chromosomes.

The slides were then stained temporarily with Toluidine Blue. Using coverslips (24x50 mm), the slides were mounted and were ready for analysis.

I. Chromosomal Analysis

The chromosomal analysis of the cells was carried out using a Photomicroscope III (Carl Zeiss). A total of one hundred (100) cells were examined per dose leve (10 cells per fish) for chromosomal analysis. The cells

were first observed under low power magnification, selecting areas where there are a high number of metaphase figures. Close actual chromosomal analysis of the selected cells were done under high power magnification.

Before actual analysis, a determination of the chromosomal number was done to establish the modal chromosomal number. The total number of chromosomes per cell were counted in 150 cells and the frequency of each 2n was recorded. The 2n chromosome number with the greatest percentage out of the 150 cells scored will be the modal chromosome number for Nile Tilapia. (Crosetti, *et al.*, 1988)

The observed chromosomes in each cell were classified whether they normal or if they possess any chromosomal or chromatid aberrations. Also, the types of aberrations and the times these specific aberrations were encountered in each cell were taken note of. All the data gathered were recorded under their corresponding dose levels.

Clear and excellent metaphase figures were observed at oil immersion objectives and were photographed at each of the dose levels, including control. When possible, one of each type of chromosomal aberration seen were photographed and illustrated.

J. Karyotyping

The photographed cells were printed and enlarged for the karyotyping process. The enlarged photographs were cut out and were paired with its corresponding

homologous partner. The size, length and position of the centromere were used as the basis for the chromosome pairing. Only the control group was karyotyped.

K. Statistical Analysis

Statistical measures were used to determine which of the chromosomal aberrations are reliable and which chromosomal aberrations the most effective in indicating the effects of the different doses of radiation on the Nile Tilapia.

The Pearson's correlation coefficient was used for this purpose. This determined if there was a strong correlation between the aberrations and the levels of dosage.

L. Replicates

The cytogenetical analysis will only have 1 replicate.

III. RESULTS

The study aimed to look into the radiosensitivity of the Nile Tilapia. Part I determined the $LD_{50/18}$ days, the LD_{50} for 2 krad and 3 krad was 6 days, for 4 krad it was 5 days and for 5 krad it was 4 days (Table 1). No LD_{50} was recorded for the control group and 1 krad because mortality did not reach 50% (Table 2). It is also evident that at the higher doses of 3,4 and 5 krad, some fish did not die immediately.

Nile Tilapia was $LD_{18\ 33/18}$ at 0 krad, $LD_{36.67/18}$ at 1.0 krad, $LD_{85/18}$ at 2.0 krad and $LD_{100/18}$ at 3.0 krad, 4.0 krad and 5.0 krad (Table 2). Within the specified observation period of 18 days $LD_{50/18}$ would be seen to lie at 2 krad (Table II).

Before cytological analysis for radiation effects on chromosomes, the modal chromosome number was determined so as to assist in the screening for chromosomal aberrations in the latter procedures. Out of 150 cells randomly observed, the 2n number of 38, 40 and 42 had a percentage of 8.67, 17.33 and 23.33% respectively. The 2n number of 44 was seen to have the greatest incidence with (76 out of 150 cells) 47.60%, thus, making 2n=44 the modal chromosome number for Nile Tilapia (Table 3). A karyotype was also constructed and the results showed 22 pairs of acrocentric chromosomes in the modal karyotype with 2 pairs serving as possible chromosome markers (Figure 1).

In the cytological analysis proper, no aberrations were observed in the control group but in the treatment groups there were a number of types of chromosomal aberrations encountered. These were the following: (1) chromatid gaps, (2) chromosome gaps, (3) chromatid fragments, (4) dicentric rings, (5) fusions, (6) despiralizations and (7)

translocations. Among all the aberration types only the chromosome gaps, dicentric rings and fusions were observed to be present in all treatment doses. Only dicentric rings increased steadily as dosage increased (Table 4).

At 0.5 krad, 10 % of the observed metaphase spreads were found to have chromosomal aberrations, 1[illegible] % at 1.0 krad, 26 % at 1.5 krad, 28 % at 2 krad and 43 % at 3 krad (Table 4).

V. DISCUSSION

I. DETERMINATION OF LD_{50}

Based on Table 1, the lethal dose of Nile Tilapia wherein 50 % of the population died at 2.0 krad occurred within 6 days, 6 days at 3.0 krad, 5 days at 4.0 krad and 4 days at 5.0 krad. In short, the higher the dose, the earlier the specific day is met in which half of the population had died.

In the study, the observation period was limited to 18 days. This method of evaluation was used in order to minimize the influence of the Nile Tilapias that may live for extended periods after irradiation (Pizzarello and Witcofski, 1972).

Although the entire population died by the 18th day at doses of 3.0 krad, 4.0 krad and 5.0 krad, it was evident in that at these doses, there were fish that lived for extended periods (Table 1). This may be attributed to the fact that they may be older or may be of a more resistant individual or there is a species/strain resistance in any biologic species in terms of response variation.

Determination of LD_{50} was done by exposure of five groups, each comprising of 60 Nile Tilapias, *Oreochromis niloticus*, to gamma radiation. These five groups were irradiated in doses of 1.0 krad, 2.0 krad, 3.0 krad, 4.0 krad and 5.0 krad. Results of the lethal dose wherein 50 % of the population died are shown in Table 2. It shows that at higher doses, the percentage of mortality increases.

It should also be noted that although 18.33 % of the population of the control group died, this is relatively high considering it had not been subjected to radiation. Strictly speaking, if the control group had been grown in a condition closest to its natural habitat, it would have expected that 100 % of the population, or a percentage a little

below that would survive. Likewise, if the five treatment groups were kept in their natural habitat, rather than an artificial one, this would have also yielded a lower percentage of deaths for the populations.

Another factor which may have led to higher percentages of deaths was be due to transporting of the fish. Transporting often involves subjecting the fish to hazardous conditions for an undesignated period of time (Denton, 1973). Transportation from one region to another places stress on the fish.

As the days proceed after fish are irradiated, the percentage of mortality increases (Fig. 8). In addition, the higher the dose the fish are irradiated in, the steeper the slope of the mortality curves. As a result, half the population of fish irradiated at higher doses are recorded dead within a shorter period of time. It was also seen that as doses of 3.0 krad, 4.0 krad and 5.0 krad, the slopes level off indicating that 100 % of the population died.

BEHAVIOURAL CHANGES

Immediately upon irradiation, the tilapia fingerlings were observed to be more hyperactive. To be specific, they were observed to swim at a faster speed as compared to unirradiated fish. This was most especially seen in fish irradiated at higher doses of 4.0 krad and 5.0 krad. These manifestations are characteristic of the Central Nervous System Syndrome, wherein upon irradiation, the organism becomes exceedingly active and irritable, experiencing tremor (Pizzarello and Witcofski, 1972).

Within a few days, these tilapia fingerlings demonstrated sluggishness. They were found to have lost their appetite and grown weak, to the point of lying on their sides. These manifestations are characteristic of the Gastrointestinal Syndrome (Pizzarello and Witcofski, 1972).

According to the Bone Marrow Syndrome, infections are associated with the terminal phases of radiation syndrome. This was evident due to the presence of mucus secretions, an obvious sign of bacterial infection in fish. There is an increased susceptibility to infection in totally irradiated animals compared to those not irradiated. Indeed infection plays a role in mortality. The precise cause of infection after total body irradiation is not known, but is correlated with the loss of circulating blood cells (Pizzarello and Witcofski, 1972).

II. CYTOGENETIC ANALYSIS

Selection of doses for the cytogenetic analysis was determined based on the results for determining LD_{50}. As mentioned earlier, doses at 3.0 krad, 4.0 krad and 5.0 krad led to death for the entire population. Therefore it was agreed upon that doses for the cytogenetic analysis would reach 3.0 krad to ensure that various types of abnormalities would be seen.

During the course of the study, the experimenters encountered the presence of fat chromosomes. It has already been emphasized that more metaphase figures are obtainable with colchicine treatment. This alkaloid is the most noted of all mitotic inhibitors. Essentially, colchicine destroys the spindle mechanism of a cell so that the chromosomes are suspended at metaphase, instead of migrating toward the anaphase poles. If the exposure is long, it can induce polyploidy and result to stunted and plump chromosomes (Denton, 1973). This explains why the chromosomes appeared fatter or bloated.

In the preparation of slides, a piece of tissue that had been snipped off, dabbed on absorbent paper and soaked in 60 % acetic acid was gently minced to form a cell

suspension. The cell suspension that had been prepared was used for a maximum of only three slides. This is because the use of a fairly old preparation will yield mitotic spreads with chromosomes digested by the 60% acetic acid.

In the cytogenetic analysis, approximately 10 cells with clear mitotic figures were used from one slide. Hence, approximately ten slides were used per dose in order to have a total count of 100 cells. This was done to ensure random selection of cells wherein all will have an equal chance of being selected.

MODAL CHROMOSOME NUMBER

Based on Table 3 showing the results of the modal chromosome number, it was found that the diploid number (2n)=44 of *O. niloticus* occurred most frequently at 47.60%. The Nile Tilapia karyotype of 44 chromosomes agreed with other previous studies such as Crosetti *et al.* (1988), Majumdar *et al.* (1986) as cited by Crosetti *et al.* (1988) and Arai *et al.* (1980) as cited by Crosetti *et al.* (1988). However, a study by Badr and El-Dib (1976) as cited by Crosetti *et al.* (1988) noted a modal chromosome number of 40.

According to a karyotypic study of *O. niloticus* by Crosetti *et al.* (1988), four of the chromosomes are large and readily classified, among the other forty, some have short arms, small and gradually decreasing in size. However it was difficult to distinguish submetacentric, submeta-subtelocentric borderline chromosomes and subtelocentric chromosomes. Indeed, the chromosomes were very small, in our study they were classified as 22 pairs of acrocentrics.

Variation in the number of chromosomes or rather cells with lower diploid counts can be attributed to chromosomal losses during slide preparation. Surprisingly, the study

by Crosetti *et al.* (1988) found that that these cells with lower diploid counts had similar frequencies as in the parental species.

Nevertheless, the diploid number of 44 chromosomes was officially characterized for this study of *O. niloticus*.

OBSERVED TYPES OF CHROMOSOMAL ABERRATIONS

When a karyotypic analysis was done on the control group, it was found that no chromosomal aberrations were present (Figures 1 and 2). Karyotypic analysis of the control group was done to show that the chromosomes are normal and that the presence of chromosomal aberrations could not have been inherent to the fish. Because the chromosomes did not exhibit any manifestations of damage due to radiation exposure, it can be assumed that their natural habitat had no contact with radiation sources such as radioactive materials or chemical mutagens (Kaul, 1990).

Based on the cytogenetic analysis of *O. niloticus*, it was found that exposure to gamma radiation will produce various forms of chromosomal aberrations, all of which occurring in various frequencies. The types of chromosomal aberrations and their frequencies have been summarized in Table 4. Indeed, ionizing radiation did affect the somatic chromosomes and thus, made a difference in the karyotype of *O. niloticus*.

Unexpectedly, other common structural aberrations that can be easily seen under a light microscope such as breaks and chromosome fragments, were not seen within the cytogenetic analysis proper. Neither exchanges and polycentric rings other than dicentric and rings were observed, but were found in other studies such as Medina and Wagan (1981) and Panlaque (1982).

In the study, it was found that dicentric rings were the most easily recognizable aberration because of its very distinct appearance, a ring with two centromeres (Panlaque, 1982). Gaps and breaks were found to be unreliable indicators of real damage to the genetic material since scoring them can only be extremely subjective, resulting in considerable observer differences, but also many gaps are caused by technical artifacts during slide preparation (Medina and Wagan, 1981).

In the cytogenetic analysis proper, the experimenters encountered difficulty in obtaining clear and excellent photographs of the chromosomal aberrations seen under the photomicroscope. As a result, another batch of tilapia was treated, sacrificed and prepared for viewing under the microscope in order to take photographs of the observed aberrations. In the process of doing so, other types of aberrations that were not encountered in the earlier cytogenetic analysis proper, were seen at this phase. Nonetheless, these chromosomal aberrations were photographed (Fig. 3-7).

However, there is another reason why more types of chromosomal aberrations were seen in the second phase. In the cytogenetic analysis, the fish were observed two days after irradiation. On the other hand, in preparation for the photomicroscope, fish were observed only one day after irradiation. As a result various types of aberrations were observed in the process of taking pictures, and not in the cytogenetic analysis.

This phenomenon can be explained by the fact that the time elapsing between irradiation and observation will determine the number and types of aberrations. Many more aberrations are observed immediately after treatment than after some time has passed. For example, it is apparent that most breaks reconstitute in the few minutes that elapse between irradiation and fixation (DeRobertis, 1975).

RATE OF ABERRATIONS

It was found that no chromosomal aberrations were inherent to the control group of Nile Tilapia fingerlings (Table 4). But although various types of chromosomal aberrations were observed in the treated groups, these structural types were not seen in all doses (0.5 krad, 1.0 krad, 1.5 krad, 2.0 krad and 3.0 krad). Nevertheless, the presence of chromosomal aberrations indicated damage due to ionizing radiation.

Based on Figure 12, there is an observed increase in the total number of chromosomal aberrations present when the fish were irradiated. Indeed, the frequency of chromosomal aberrations does reflect the effect of induced radiation.

STATISTICAL ANALYSIS

It is evident that not all types of chromosomal aberrations can be used as biological indicators. This is because their frequencies do not show a linear relationship and vary from dose to dose.

The most frequently observed chromosomal aberrations are namely: (1) dicentric rings at 10.67 %, (2) fusion at 5.0 %, and (3) chromosome gaps at 3.83 % (Table 4). Therefore, these 3 types of aberrations were selected for statistical analysis, not only because they were the most frequently observed, being sufficient in number, but also because they exhibited a general trend with regard to the dose.

Among the three most frequently observed chromosomal aberrations, dicentric rings were found to steadily increase in number as the dose increased (Fig. 11). In addition, Table 5 reveals that the computed Pearson's correlation coefficient for dicentric

rings is 0.9868. This indicates that dicentric rings have a very strong linear association and that the yield of dicentrics is directly proportional to the dose.

Although dicentrics were observed to steadily increase with increasing dose, it was also found that fusion and chromosome gaps exhibited a general increasing trend, but not as consistently as dicentrics (Fig. 9 and 10). The observations reveal a dip in the number of fusions at 4.0 krad, but increases once more at 5.0 krad (Fig. 9). On the other hand, chromosome gaps show a steady increase in the number but levels off at 3.0 krad and 4.0 krad (Fig. 10).

Although fusions occurred more frequently than gaps, the computed Pearson's correlation coefficient for fusions (0.8809) was lower than the chromosome gaps (0.9384). In other words, chromosome gaps had a stronger linear association to the dose than fusions.

In addition, dicentric rings obtained a higher Pearson's correlation coefficient (0.9865) as compared to chromosome gaps because the number of gaps exhibited a leveling-off at doses of 3.0 krad and 4.0 krad (Fig. 10), whereas dicentric rings steadily increased (Fig. 11).

Based on the results, fusions and chromosome gaps were almost linearly increasing, but was most especially seen with dicentric rings.

Most studies reveal that not all aberrant chromosomes can be utilized as indicators of radiation effects because of their variability to dose. However, dicentric rings were found to linearly increase with the dose (Medina and Wagan, 1981 and Panlaque, 1982). These dicentric aberrations appear to be the most consistent index of radiation damage

(Dolphin, 1971 as cited by Medina, 1981), representing 10.67 % of all observed metaphase spreads.

Nonetheless, the use of Pearson's correlation coefficient proved that the yield of aberration is directly proportional to the dose (Table 4). The use of Pearson's correlation coefficient also proved that radiation does have an effect on the yield of aberrant somatic chromosomes.

VI. CONCLUSION

Nile Tilapia can be used as a biological indicator for radiation effects. Although it may not be the most ideal test subject for chromosomal analysis because of the inherent characteristics of its chromosomes, the results observed were sufficient enough to conclude that it may be used as a biological indicator. Taking also into consideration that it is commonly found in most fresh aquatic systems in the Philippines, then it makes it a suitable subject for our study.

The study showed that not all types of aberrations can be used to indicate radiation effects. Dicentric rings, chromosomal gaps and fusions showed strong positive correlation to dose, but only dicentric rings were observed to increase linearly with the dose. Thus, making dicentric rings the most reliable aberration indicator of radiation exposure.

It can also be concluded that $LD_{50/18}$ for Nile Tilapia is 2.0 krad and its modal chromosome number is at 2n=44, with 22 pairs of acrocentric chromosomes and 2 marker chromosomes present.

VII. RECOMMENDATIONS

The authors would like to recommend that similar studies should be conducted using other mutagens on Nile Tilapia instead of radiation. These effects may then be compared with the effects of radiation on Nile Tilapia chromosomes.

Another prospect would be doing a similar study on radiosensitivity with another organism. Ideally, an organism that has chromosomes more easily distinguishable from one another should be chosen.

The study can also be repeated but now using statistical analysis, such as ANOVA, to conclude whether differences between the frequency of the aberrations types and the doses given are significant or not.

The authors would also like to recommend that before one embarks on a similar study, one should first be very familiar with the proper cytogenetic techniques involved and be very proficient in preparing metaphase spreads.

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IX. TABLE

Table 1. Specific days certain number of fish died at assigned doses

DAYS \ DOSE	0	1.0	2.0	3.0	4.0	5.0
1			2	2	1	
2		2	2	6	3	3
3	2	2	6	4	5	7
4	1	4	4	5	4	14
5		3	7	6	15	13
6		3	14	9	11	9
7			4	7	8	3
8		1		9	7	4
9		3	1	6	5	6
10	1		3	5		
11	1		1			
12	2		3			
13			2	1		
14	3		1			
15	1	1				
16		2				1
17		1			1	
18			1			

Table 2. LD_{50} determination results

DOSE (KRAD)	NUMBER OF EXPOSED FISH	TIME OBSERVED	MORTALITY (%)	SURVIVAL (%)
0	60	18 days	18.33	81.67
1.0	60	18 days	36.67	63.33
2.0	60	18 days	85	15
3.0	60	18 days	100	0
4.0	60	18 days	100	0
5.0	60	18 days	100	0

Table 3. Modal chromosome number determination results

Chromosome Number	Frequency	Percentage Out of 150 Cells
38	13	8.67
40	26	17.33
42	35	23.33
44	76	47.60

Table 4. Cytogenetic analysis of the chromosomes of the Nile Tilapia

DOSE	No. of cells analyzed	ABNORMAL CELLS											
		CHROMATID		CHROMOSOME		FRAGMENTS		Dicentric ring	FUSION	DESP.	TRANS.	EXCH.	TOTAL
		Gap	Break	Gap	Break	Chr'td	Chr'me						
0 kr	100												0
0.5 kr	100			1		1		6	2				10
1.0 kr	100	1		3				8	2	1	3		18
1.5 kr	100			6				11	9				26
2.0 kr	100			6				14	8				28
3.0 kr	100	1		7				25	9		1		43
Total cells scored	600	2		23		1		64	30	1	4		125
Ave/ 100 cells		0.33 %		3.83 %		0.17 %		10.67%	5.0 %	0.17 %	0.67 %		20.83%

Table 5. Pearson's correlation coefficient for chosen chromosomal aberrations

ABERRATION TYPE	PEARSON'S COEFFICIENT
Chromosome Gap	0.9384
Fusion	0.8809
Dicentric Ring	0.9865

X. FIGURES

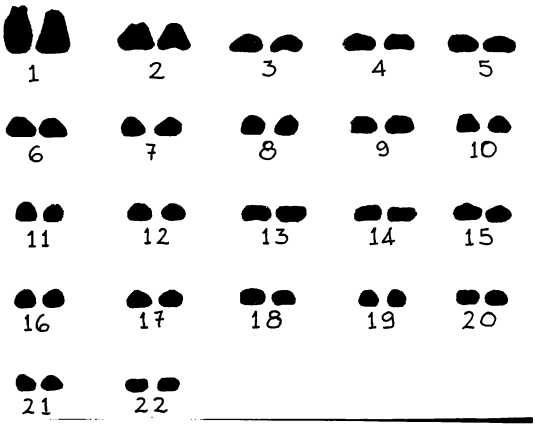


Figure1. Karyotype of Nile Tilapia

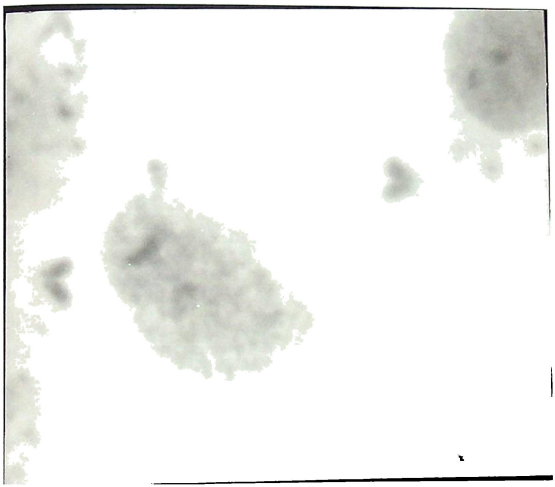


Figure 2. Metaphase spread of normal Nile Tilapia chromosomes

Figure 3. Complex: Dicentric and ring

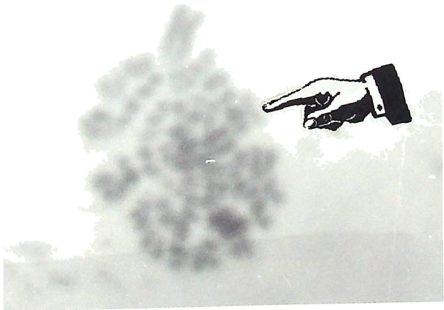


Figure 4. Fusion

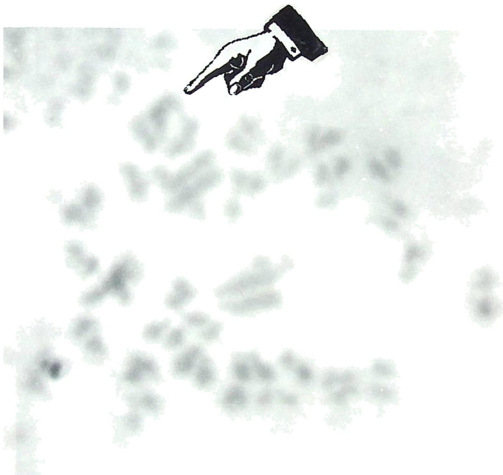


Figure 5. Translocation

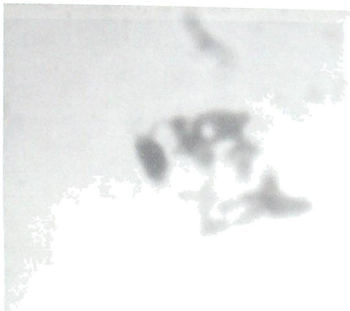


Figure 6. Sticky chromosomes

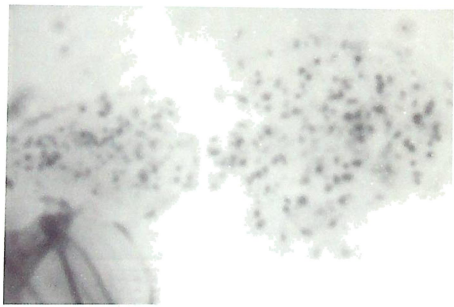


Figure 7. Despiralization

Graphs

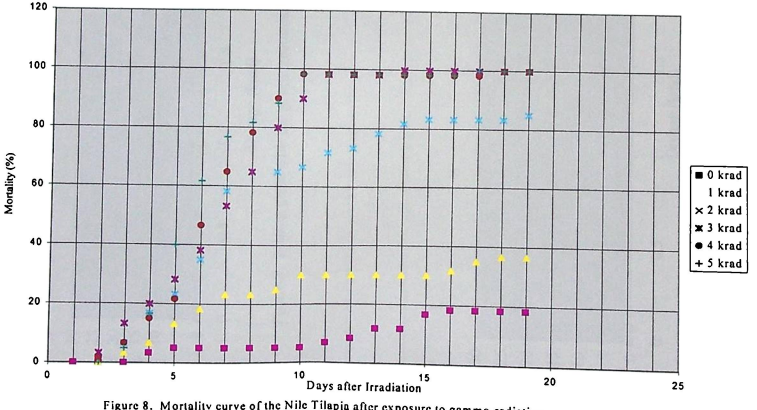


Figure 8. Mortality curve of the Nile Tilapia after exposure to gamma-radiation

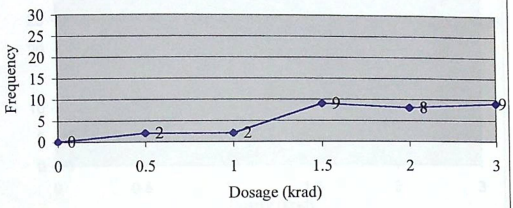


Figure 9. Fusion yield with the given treatment doses

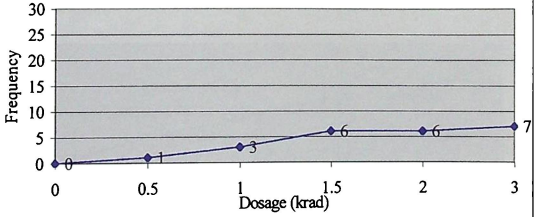


Figure 10. Gap yield with the given treatment doses

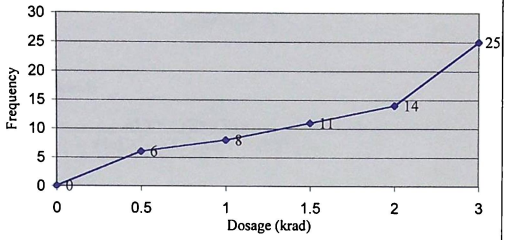


Figure 11. Dicentric ring yield with the given treatment doses

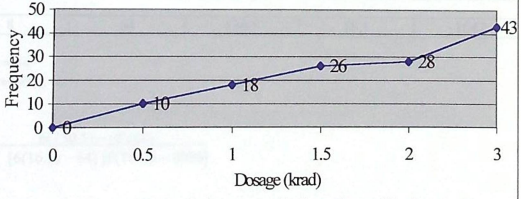


Figure 12. Total chromosomal aberrations with the given treatment doses

XI. APPENDICES

Appendix A

Pearson's Coefficient:

$$r = \frac{n\Sigma XY - (\Sigma X)(\Sigma Y)}{\sqrt{[n\Sigma X^2 - (\Sigma X)^2][n\Sigma Y^2 - (\Sigma Y)^2]}}$$

X= dosage
Y= frequency of aberration
n= number of groups

Sample Computations:

For Dicentrics rings.

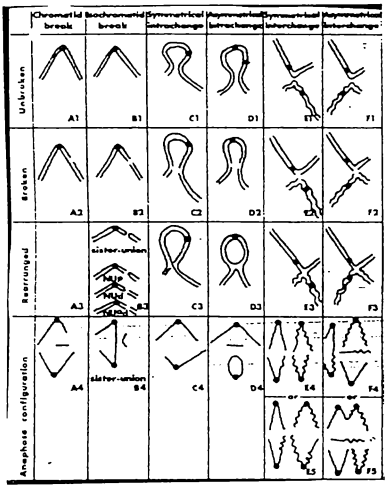
X	Y	XY	X^2	Y^2
0	0	0	0	0
0.5	6	3	0.25	36
1	8	8	1	64
1.5	11	16.5	2.25	121
2	14	28	4	196
3	25	75	9	625
Σ				
8	64	130.5	16.5	1042

$(\Sigma X)^2 = 64$
$(\Sigma Y)^2 = 4096$

$$r = \frac{6(130.5) - (8)(64)}{\sqrt{[6(16.5) - 64][6(1042) - 4096]}}$$

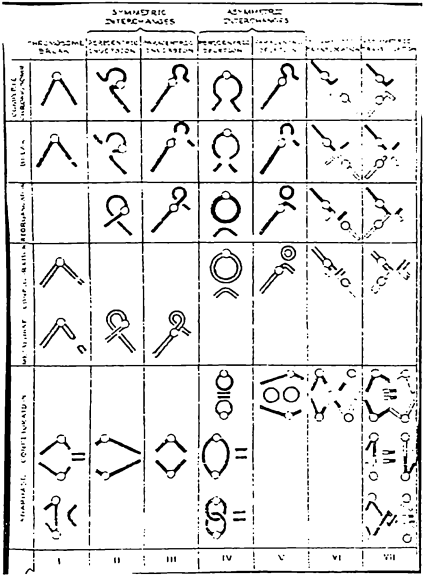
$= 0.9865 =$ strongly correlated, directly proportional

Appendix B



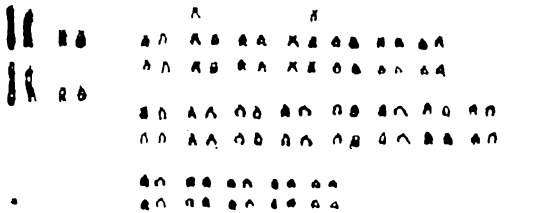
(Elkind, 1967) Chromatid Aberrations

Appendix C



(Wolff, 1963) Chromosomal Aberrations

Appendix D



(Crosetti, 1998). Geimsa stained (upper rows) and C-banded (lower rows) karyotypes of Nile Tilapia

XII. PLATES

PLATE 1. Obtaining of the test organism: Nile Tilapia

PLATE 2. Acclimatization the Nile Tilapia to new surroundings

PLATE 3. PNRI: the workplace

PLATE 4. Irradiation of Nile Tilapia

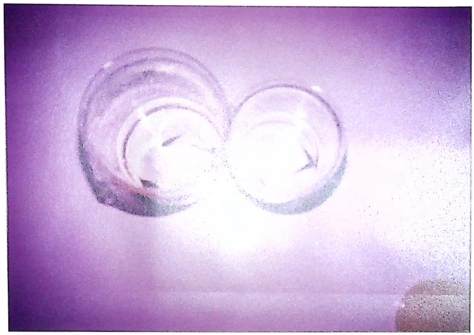


PLATE 5. Colchicine treatment of Nile Tilapia

PLATE 6. Obtaining the tissues from the gills and fins of Nile Tilapia

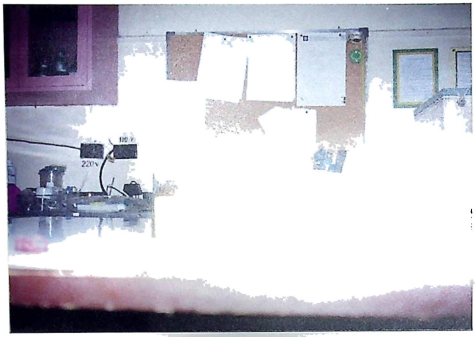


PLATE 7. Storing Nile Tilapia tissues in fixative (3:1 - methanol:glacial acetic)

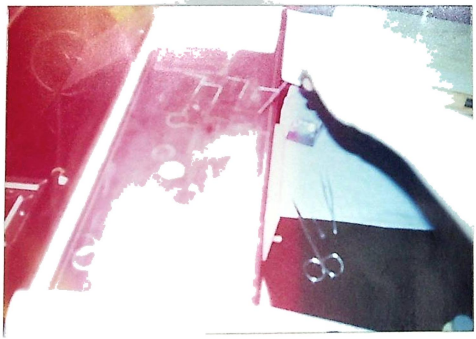


PLATE 8. Slide preparation

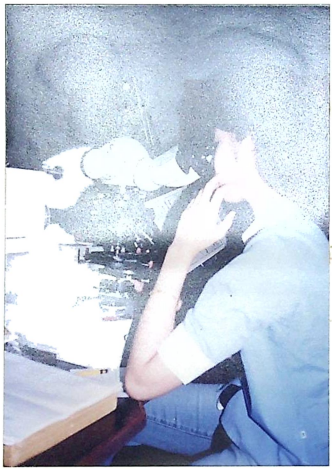


PLATE 9. Viewing cells under photomicroscope for analysis