INNSPUB

International Journal of Biosciences | IJB |
ISSN: 2220-6655 (Print) 2222-5234 (Online)
http://www.innspub.net
Vol. 3, No. 3, p. 83-91, 2013

RESEARCH PAPER OPEN ACCESS

Cytological studies and karyotype analysis of *Oreochromis niloticus* L.

Lailatul Ferdaus, Md. Sarower-E-Mahfuj*, A.K. Shakur Ahammad, Maruf Hossain Minar and Md. Mukhlesur Rahman Khan

Department of Fisheries Biology and Genetics, Bangladesh Agricultural University, Mymensingh-2202, Bangladesh

Key words: Colchicine, metacentric, hydrolysis, metaphase and karyotype.

doi: http://dx.doi.org/10.12692/ijb/3.3.83-91 Article published on March 28, 2013

Abstract

The research reported herein consisted of chromosomal studies of *Orechromis niloticus*. Larvae of *O. niloticus* were collected from 'Reliance Fish Hatchery', Trisal, Mymensingh, Bangladesh. Colchicine (0.05%) treated (2 h, 2.5 h and 3 h) time intervals of day-old larvae were observed under research microscope after hydrolysis (10% HCl), mordanting (2% iron alum) and staining with haematoxoylen. Colchicine treatment 2 h gave satisfactory results in respect of degree of contraction of chromosomes. Photomicrographs were taken from selected plates and then enlarged. From enlarged prints chromosome number 2n = 44 was counted. In the late prophase stage chromosomes were condensed, thicker and shorter and the exclusively contracted chromosomes which were unsuitable for measurement. Chromosomes of metaphase stages were observed and clearly identified in three plates and finally one best plate was selected for karyotype analysis. The long and short arm detected only 8 chromosomes which consisted of 4 metacentric, 3 suubmetacentric and 1 subtelecntric. Finally the prepared karyotype consisted of 2n = 4m + 3sm + 1st + 36t. Length of the measured chromosome varied from 0.7 to 3.1 µ. This study may provide the first knowledge of chromosome analysis of *O. niloticus* and basic information for its ploidy manipulation and sex reversal.

* **Corresponding Author:** Md. Sarower-E-Mahfuj ✉ sarower_17@yahoo.com

Introduction

Bangladesh waters are rich in biological diversity with 260 fresh water fish, 475 marine fish, 24 fresh water prawn and 36 marine shrimp species (FSY, 2009). The total fish production of the country during the fiscal year 2009-2010 was about 28,99,198 metric tons in which 23,81,916 metric tons came from inland water which contributed 82.15% of the total fish and shrimp production. This sector contributes 3.74% of gross domestic products, 22.23% of agricultural products and 2.70% of total export earnings (DoF, 2011). More than 10% of total population (14.5 millions) of Bangladesh is directly or indirectly involved in fisheries sector for their livelihood. Fish is an excellent source of protein containing all the essential amino acids and fatty acids in desirable concentration for human beings (DoF, 2011). Fish plays the second important role next to agriculture food production. It contributes about 58% of the animal protein to our daily diet (DoF, 2011).

Tilapia is a generic term used to designate a group of commercially important fish species belonging to the family Cichlidae. Cichlidae family are classified three aquaculture genera- *Orechromis niloticus, Sarotherodon* and *Tilapia*, they inhabit the fresh and brackish waters (Sofy *et al.,* 2008).

Tilapia, *O. niloticus* was first introduced in Bangladesh in 1954 by the United Nations International Children Emergency Fund, and later by the Bangladesh Fisheries Research Institute (BFRI) from Thailand (Gupta et al., 1992). About 80 species of tilapia have been described out of which 10 species are reported to be used for culture (Macintosh and Little, 1995). Tilapias have distributed to so many different types of water, to so many different types of culture systems in the world that they have been even labeled as the "aquatic chicken" (Maclean, 1984). They have good resistance to poor water. It is a very popular food fish of the people of Bangladesh because of its delicious taste and high market value. The fish looks very attractive. It is commonly found in natural water bodies. In addition many other unique features make it perfect species for aquaculture. Nile tilapia is preferred by farmers because of its desirable features for aquaculture i.e. tasty flesh and ease of production (Ballarin and Haller, 1982). It has faster growth rate compared with any other short cycled fish species including other commonly used tilapia strains. Moreover like other culturable tilapias, this species is a hardy fish. It is good converter of organic wastes in to quality protein and resistance to disease (Stickney *et al.,* 1979; Pullin *et al.,* 1988 and Lowe-McConnel, 1982). Tilapia has been extensively genetically modified. One promising Genetically Improved Farmed Tilapia strain known as GIFT (Eknath, 1992) has been introduced in July 1994 from the Philippines. The GIFT (5^{th} generation) strain was developed by the International Center for Living Aquatic Resources Management (ICLARM) through several generations of selection from a base population involving eight different strains of Nile tilapia, *Oreochromis niloticus* (Eknath *et al.,* 1993). In spite of all of these advantages a lot of works has been done for the genetic improvement of this species but the cytogenetic works as well as the chromosomal and karyotypic data are inadequate.

The chromosomes have been considered as the physical bases of heredity because they have a special organization, individuality, functions and are capable of self reproduction. The study of fish chromosome has become an active area of research (Thorgaard and Disney, 1993). Karyological studies of fishes can contribute significantly to the solution of many problems in areas of research ranging from taxonomy, systematic or genetics to phylogenetics, or environmental toxicology and aquaculture (Al-Sabti, 1991; Sofy *et al.,* 2008).

Chromosomal analysis is important for fish breeding from the viewpoint of genetic control, the rapid production of inbred lines, cytotaxonomy and evolutionary studies (Kirpichnikov, 1981). Cytogenetic markers are one of the authentic tools

for characterization of species as well to screen putative hybrids (Amemiya and Gold, 1988).

This species is a good candidate for genetic investigations such as Single sex populations and sex determination (McAndrew and Majumdar, 1988; Penman and McAndrew 2000; Griffin *et al.*, 2002; Harvey *et al.*, 2002), hybridization (Johnstone *et al.*, 1983; Goudie *et al.,* 1986) and sex reversal (Mair *et al.,* 1997), YY super male production, chromosome set manipulation (Karayücel *et al.,* 2002, 2004; Ezaz *et al.,* 2004), gene mapping (Ezaz *et al.,* 2004; Lee *et al.,* 2003; Boonphakdee, 2005). To the best of our knowledge, a lot of reports exist karyotype and all aspects of genetic data of tilapia species in the world, but is also necessary for cytology in Bangladesh, in order to help to future taxonomic and genetic studies.This research work is carried out with the aims of to study the general cytological conditions of *O. niloticus* and attempt to measure the metaphase stage.

Materials and methods

The research study reported here was carried out during the period from July 2008 to March 2009 in the laboratories of the department of Fisheries Biology and Genetics and Genetics and Plant Breeding, Bangladesh Agricultural University, Mymensingh. For chromosomal studies and karyotypic analysis the method of Ahmad *et al.* (1983) was followed with some modifications. The experimental procedures are described below.

Collection of sample and Fixation

One day-old alive, weight 0.005 ± 0.001 g and 300 number of *O. niloticus* larvae were collected from "Reliance Fish Hatchery", Trisal, Mymensingh, Bangladesh, (Latitude 24°30` to 24°50` North and Longitude 90°15` to 90°30` East).

For fixation the stock solution of colchicine was made by dissolving 0.05 g colchicine and 100 ml distilled water. Healthy and vigorously growing larvae were sampled and allowed to swim in 0.05% colchicine solution in small size petridish that was stirred gently. Then the larvae were kept in a glass petridish containing 0.7% NaCl for 15 min at 28°C room temperature. The head and yolk sac of the larvae were removed by using fine forceps and needle in the same solution. Then the larvae were transferred to another petridish containing distilled water for 15 min. The larvae were transferred into a vial containing freshly prepared fixative solution (acetic acid: ethanol, 1:3). After 1 - 2 h in the fixative solution the larvae tissues were prepared for slide preparation.

Hydrolysis of the sample

Hydrolysis of the fixed materials was done in 10% HCl solution to soften the tissue. 10% HCl was kept in an oven at 60°C for one hour in a sealed vial before actual hydrolysis of the tissues. Fixed tissues were than kept in the same preheated HCl solution in the oven for 15 min. After that the tissues were taken out and allowed to cool for few min. Then the tissues were washed in running water for 2 - 3 times.

Mordanting of the sample

The samples were then treated with 2% aqueous solution in iron alum (Ferric ammonium sulphate) for about 6 min for mordanting. Mordanting was done to enhance the staining process.

Staining of the chromosomes

Iron alum treated tissues were then washed for two to three times with running water and stained in 0.5% haematoxylin for 8 min. The stained tissues were washed for several times until coloring of water stopped. The stained samples were kept in water. Slides were prepared within 2 - 3 h of staining.

Slide preparation and microscopic observation

At first a drop of 1% acetocarmine solution was placed in a clean glass slide and a small portion of the stained tissues were placed on that solution. Then the tissue was divided into two or three small portion. A clean cover glass was placed on the material avoiding any air bubble under the cover glass. The tissue was smeared by holding the cover

glass with a finger with a corner and gentle tapping on the cover glass with the back end of the needle. Gentle warming of the slide over a spirit lamp and further tapping on the smear until the smear spread and made a single layer were conducted without much overlapping in the cells, as well as any type of breakage was also avoided. Mitotic cells were observed using a microscope (Model Olympus CHD-64M061, Japan). To found out a perfect result without breaking of the cells and finding all the cells in a single layer the slide preparation was done very carefully.

Photomicrography and measurement of chromosome

Photomicrography of the selected plates was done from temporary slides using an Olympus research microscope (Model Olympus-BX40, Japan), under 100 X Plan Oil immersion objectives. Chromosome counting and measurements of some selected chromosomes were done from photo micrographic prints. Such measurements were taken into mm and converted into micrometer (μ) based on the magnification of the final prints. Arm ratios of the chromosomes were calculated by dividing the length of the long arm by that of the short arm (L/S). Finally chromosomes were arranged decently in pairs according to their length, where the longest pair at first and the shortest one at last. Classification of chromosomes was done by the conventions proposed by Levan *et al*. (1964). That is, ratio 1.0 to < 1.7 was "m" (metacentric) chromosome, 1.7 to < 3.0 "sm" (submetacentric) chromosome and 3.0 to < 7.0 "st" (subtelocentric) chromosome and > 7.0 "t" (telocentric) chromosome.

Results

Procedure related observations

Maximum number of dividing cells occurred when the samples were collected from day-old larvae of *O. niloticus*. Colchicine (0.05%) treatment for 2 h gave satisfactory results in respect of degree of contraction of the chromosomes and frequency of prometaphase plates. Variations in larvae age and colchicines treatment duration were investigated where clear chromosomes were not observed. The fixative (acetic acid: alcohol, 1:3) was found to be suitable for the present sample. Hydrolysis of the body tissues in 10% HCl for 15 min at 60°C was found adequate to soften the specimen. Mordanting of the hydrolysed tissues for 6 min in 2% iron alum solution gave satisfactory results. Staining for 8 min in 0.5% haematoxylin produced satisfactory degree of staining of the chromosomes.

General observations on the chromosome complement

Somatic chromosome number of *O. niloticus* was detected 2n = 44 in the present studies. No deviation in chromosome number was detected in each plate. Late prophase stage was observed; the nuclear envelope breaks up and the chromosomes were condensed and appeared as thicker and shorter to coil repeatedly to form overlap. This is called super coiling in Fig. 1. Exclusively contracted metaphase chromosomes were also observed in the body tissues which were unsuitable for measurement in Fig. 2. A plate representative of the four plates used in the karyotype analysis is shown in Fig. 3.

Of the 2n = 44 chromosomes the long arm and short arm were detected only in 8 chromosomes (10, 16, 20, 29, 32, 38, 42, 43) which consisted of 4 metacentric (10, 16, 38, 43), 3 submetacentric (29, 32, 42) and 1 subtelocentric (20). For easy reference, chromosomes in the photomicrography prints were numbered arbitrarily. Of the 44 chromosomes the longest one (chromosome 42) was 3.1μ long having arm ratio 2.9 and the shortest one (Chromosome 21) was 0.7μ long (Table 1).

Discussion

In the present study, one day-old *O. niloticus* larvae were allowed to swim 0.05% colchicine solution separately but 0.05% colchicine solution gave better chromosomal observation. The *O. niloticus* larvae kept in the colchicine solution for about 2 h, 2.5 h and 3 h. The slides, which were prepared with 2 h colchicine treated larvae, found the chromosomes well spread.

Table 1. Measurement of the chromosomes complement and their classification.

Chromosome no.	Length of long arm (µ)	Length of short arm (µ)	Total length (µ)	Arm ratio (L/S)	Classification
1	1.2	0.00	1.2	∞	t
2	1.2	0.00	1.2	∞	t
3	1	0.00	1	∞	t
4	1.2	0.00	1.2	∞	t
5	1.1	0.00	1.1	∞	t
6	0.8	0.00	0.8	∞	t
7	1.1	0.00	1.1	∞	t
8	1.2	0.00	1.2	∞	t
9	1.2	0.00	1.2	∞	t
10	0.5	0.45	0.95	1.3	m
11	1.2	0.00	1.2	∞	t
12	0.9	0.00	0.9	∞	t
13	1.1	0.00	1.1	∞	t
14	1.1	0.00	1.1	∞	t
15	0.8	0.00	0.8	∞	t
16	0.9	0.8	1.7	1	m
17	0.8	0.00	0.8	∞	t
18	1.2	0.00	1.2	∞	t
19	1.1	0.00	1.1	∞	t
20	2.1	0.6	2.7	3.5	st
21	0.7	0.00	0.7	∞	t
22	1.1	0.00	1.1	∞	t
23	1.2	0.00	1.2	∞	t
24	1.2	0.00	1.2	∞	t
25	1.5	0.00	1.5	∞	t
26	1.2	0.00	1.2	∞	t
27	1.2	0.00	1.2	∞	t
28	1.2	0.00	1.2	∞	t
29	1.2	0.5	1.7	2.4	sm
30	0.8	0.00	0.8	∞	t
31	1.1	0.00	1.1	∞	t
32	1.5	0.8	2.3	1.9	sm
33	1.1	0.00	1.1	∞	t
34	1.2	0.00	1.2	∞	t
35	0.8	0.00	0.8	∞	t
36	1.1	0.00	1.1	∞	t
37	1.1	0.00	1.1	∞	t
38	0.8	0.5	1.3	1.6	m
39	1	0.00	1	∞	t
40	0.8	0.00	0.8	∞	t
41	1.1	0.00	1.1	∞	t
42	2.3	0.8	3.1	2.9	sm
43	1.1	1	2.1	1.1	m
44	1.5	0.00	1.5	∞	t

[M = metacentric, sm = submetacentric, st = subtelocentric, t = telocentric]

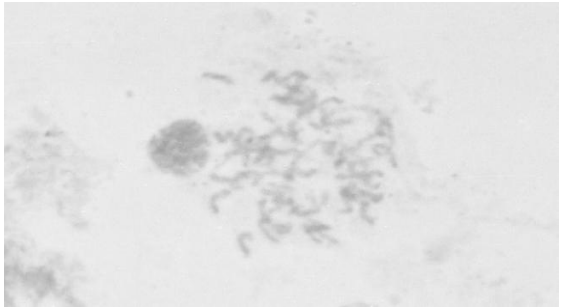

Fig.1. Complement of one day-old larvae of *O. niloticus* at late prophase stage, showing thickly stained chromosome segments. Chromosomes at stages not used in karyotypic analysis. X 100.

The present finding of 2n = 44 chromosomes in *O. niloticus* is in agreement with the earlier reports of Kornfield *et al.* (1979) and Sherwood and Patton (1982). In this experiment, the length of chromosome ranged between 0.7 to 2.9 µ and the arm ratio of chromosome ranged between 1 to infinity (∞). The long arm, short arm and arm ratio

were detected for few chromosomes. Four types of chromosomes were found. They were metacentric, submetacentric, subtelocentric and telocentric which consisted of 2n = 4m + 3sm + 1st + 36t chromosomes. On the other hand Sofy *et al.* (2008) in their experiment found that 2n = 2sm + 24st + 18t in *O. niloticus,* 2n = 2m + 12sm + 14st + 16st in *Sarotherodon galilaeus* and 2n = 20sm + 8st + 14t respectively.

The cytogenetic study of three fish species in Egypt, *O. niloticus*, *Sarotherodon galilaeus* and *Tilapia zilli* to cichlids was done by Sofy et al. (2008). They injected 0.01% of freshly prepared colchicine solution intraperitionaly per gram of body weight of fish. The specimens were then placed in to a holding tank for 2 - 4 h but in case of larger fish more than 20 cm were held for at least 6 h after injection. Here hydrolysis of the sample was done for 15 min.

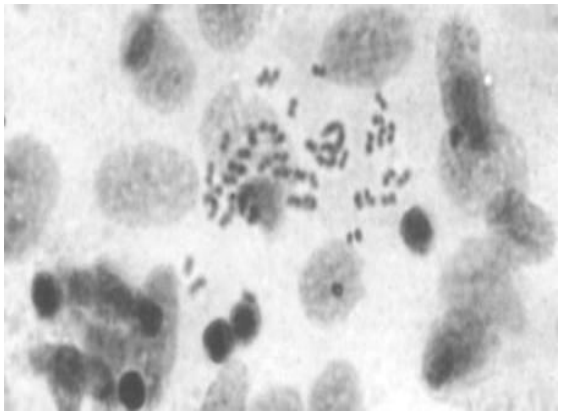

Fig. 2. Chromosomes highly contracted. Chromosomes at stages not used in karyotypic analysis. X 100.

Datta *et al.* (2003) conducted an experiment to study chromosomes of *Ompok pabda*, where the hydrolysis of the larvae was done for 10 min. Haematoxylene treatment for staining was done for 5 min, 6 min, 8 min but only 8 min treatment of haematoxylene gave satisfactory results.

The study of chromosomes receives the interest for classification of species and understanding of evolution. In spite of being used extensively in taxonomic research of invertebrates and even in vertebrates, fishes from the group for which cytological data are mostly lacking (Alves, 2000; Artoni and Bertollo, 2001), in this concept the present research work is very much useful to know the cytogenetic and karyotypic arrangement of *O. niloticus.*

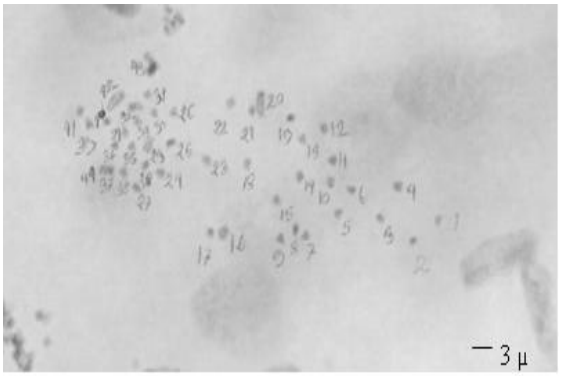

Fig. 3. Representative complement of larvae tissue included for karyotype analysis. X 100.

Karyological study of teleost fish presents technical difficulties which are not encountered in the study of vertebrates and these difficulties are due to the small size and high number of chromosomes (Cucchi and Baruffaldi, 1990). In the present study chromosomal constitution of *O. niloticus* could be difficulties encountered in staining and slide preparation using appropriate fish larval tissues. Cells tend to suffer breakage easily causing unavailability of intact chromosome complements in higher numbers. The major difficulty encountered is the morphological variation existing even between homologous chromosomes in the same nucleus (Al-Sabti, 1991; Levan et al., 1964). Fish karyotypes are not identical as in human being or other animal species, but polymorphism often occurs within the same fish species (Al-Sabti, 1991).

The karyotype analysis is a key step towards the stock improvement by polyploidy manipulation, detection of hybridization and related genetic engineering (Tan et al., 2004). Although chromosome of this compliment could be measured in the present study, it is worthwhile to perform the work to gain information in this complete chromosome complement of *O. niloticus*.

Acknowledgement

The authors express their sincere thanks and respect to Professor Dr. Qazi Nasiruddin Ahmad, Department of Genetics and Plant Breeding, BAU, Mymensingh for allowing using all chemicals, instruments and measurement of chromosomes in Cytogenetics Laboratory of the Department of Genetics and Plant Breeding, Bangladesh Agricultural University, Mymensingh.

References

Ahmad QN, Britten EJ, Byth, DE. 1983. A quantitative method of karyotype analysis applied to the soybean, *Glycine max*. Cytologia **48,** 879-892.

Al-Sabti K. 1991. Handbook of Genotoxic effects and fish chromosome. Ljubljana p. 97.

Alves AL. 2000. Analise da evolucao dos generos da subfamilia Hemipsilichthiinae (Ostariophysi, Siluriformes, Loricarridae) com base em caracteres cromossomicos e de DNA mitocondrial. MSc Thesis, Universidade de sao Paulo, faculdade demedicina de Ribeivao preto, 164.

Amemiya CT, Gold JR. 1988. Chromosomal NORs as taxonomic and Systematic characters in North American cyprinid fishes. Genetica **76,** 81-90. DOI: 10.1007/BF00058806.

Artoni RF, Bertollo LAC. 2001. Trends in the karyotype evolution of Loricarridae fish (Siluriformes). Heriditas **134,** 201-210. DOI: 10.1007/BF00058806.

Ballarin JD, Haller RD. 1982. The intensive culture of tilapia in tanks raceways and cages. Croon Heln Ltd. London, p. 265-266.

Boonphakdee C. 2005. Isolation, analysis and chromosomal mapping of genes related to sex determination in tilapia. PhD thesis, Stirling University, Scotland.

Cucchi C, Baruffaldi A. 1990. A new method for karyological studies in teleost fishes. Journal of Fish Biology **37,** 71-75. DOI: 10.1111/j.1095-8649.1990.tb05928.

Datta S, Rahman MM, Haque MA, Islam MM, Mollah MFA. 2003. Chromosomal studies on *Ompok pabda* (Hamilton). Online Journal of Biological science **3(5),** 460-465.

DoF. 2011. Jatiyo Matshapakha Sankalon 20 - 26th July. Department of Fisheries, Ministry of Fisheries and Livestock, Government of People`s Republic of Bangladesh, Matshya Bhabon, Dhaka, p. 136.

Eknath AE. 1992. Genetic Improvement of Farmed Tilapia-GIFT Phase 1 Final Report. International Center for Living Aquatic Resources Management (ICLARM). Metro Manila.

Eknath AE, Tayamen MM, Palada-de Vera MS, Danting JC, Reyes RA, Dionisio E, Capili JB, Bolivar HL, Abella TA, Circa AV, Bentsen HB, Gjerde B, Gjedrem T, Pullin RSV. 1993. Genetic improvement of farmed tilapias: the growth performance of eight strains of *Oreochromis niloticus* tested in different farm environments. Aquaculture **111,** 171-188. DOI: 10.1016/0044-8486(93)90035-W.

Ezaz, MT, Harvey SC, Boonphakdee C, Teale AJ, McAndrew BJ, Penman DJ. 2004. Sex-linked AFLP markers in the Nile tilapia *Oreochromis niloticus* L. Marine Biotechnology **6(5),** 435-445. DOI: 10.1007/s10126-004-3004-6.

FSY, 2009. Fisheries Statistical Yearbook of Bangladesh (25th edn). Department of Fisheries, Ministry of Fisheries and livestock, Government of the People's Republic of Bangladesh, p. 12-53.

Griffin DK, Harvey SC, Campos-Ramos R, Ayling L-J, Bromage NR, Masabanda JR, Penman DJ. 2002. Early origins of the X and Y chromosomes: lessons from tilapia. Cytogenetics and

Genome Research **99,** 157-163. DOI: 10.1159/000341887.

Gupta MV, Ahamed M, Bimbao AA, Lightfoot C. 1992. Socioeconomic impact and farmers' assessment of Nile tilapia, *Oreochromis niloticus* culture in Bangladesh. International Center forLiving Aquatic Resources Management, Manila, Philippines. ICLARM Technical Report 35.Kirpichnikov, V.S. 1981. Genetic Bases of Fish Selection. Springer Verlag, Berlin, Heidelberg, New York, p. 342.

Goudie CA, Shelton WL, Parker NC. 1986. Tissue distribution and elimination of radiolabelled methyl testosterone fed to sexually undifferentiated blue tilapia. Aquaculture **58,** 215-226 http://dx.doi.org/10.1016/0044-8486(93)90035-W

Harvey SC, Boonphakdee C, Campos-Ramos R, Ezaz MT, Griffin DK, Bromage NR, Penman DJ. 2002. Analysis of repetitive DNA sequences in the sex chromosomes of *Oreochromis niloticus*. Cytogenetics and Genome Research (special issue on Sex Determination in Vertebrates) **101,** 314-319.

Johnstone R, Macintosh DJ, Wright RS. 1983. Elimination of orally-administered 17-alpha methyltestosterone by *Oreochromis mossambicus* (tilapia) and *Salmo gairdneri* (rainbow trout) juveniles. Aquaculture **35,** 249-257 http://dx.doi.org/10.1016/0044-8486(93)90035-W

Karayücel S, Karayücel I, Penman D, McAndrew, BJ. 2002**.** Production of androgenetic Nile tilapia, *Oreochromis niloticus* L.: Optimization of heat shock duration and application time to induce diploidy. Israeli Journal of Aquaculture-Bamidgeh **54(4),** 145-156.

Karayücel I, Tariq ME, Karayücel S, McAndrew BJ, Penman DJ. 2004. Evidence for two unlinked "sex reversal" loci in the Nile tilapia, *Oreochromis niloticus*, and for linkage of one of these to the autosomal red body colour gene. Aquaculture **234,** 51-63.

Kirpichnikov VS. 1981. Genetic Bases of Fish Selection. Springer Verlag, Berlin, Heidelberg, New York, p. 342.

Kornfield IL, Rette U, Richler C, Wahrman J. 1979. Biochemical difference among Cichlid fishes of the sea Galilae. Evolution **3,** 1-14.

Lee W-J, Penman DJ, Kocher TD. 2003. Identification of a sex-determining region in Nile Tilapia (*Oreochromis niloticus*) using bulk segregant analysis. Animal Genetics **34,** 379-383**.**

Levan A, Fredga K, Sandberg AA. 1964. Nomenclature for centromeric position on chromosomes. Heredities **52,** 201-220. DOI: 10.1111/j.1601-5223.1964. tb01953.x.

Lowe-McConnell RH. 1982. Tilapias in fish communities. In The biology and culture of tilapias, (R. S. V. Pullin and R. H. Lowe-McConnell, eds.), p. 83 - 113. ICLARM (International Center for Living Aquatic Resources Management) Conference Proceedings, vol. 7, Manila.

Macintosh DJ, Little DC. 1995. Brood stock management and fry production of the Nile tilapia (*Oreochromis niloticus*). In: N.R. Bromage and R. J. Roberts, (eds.). Brood fish Management and Egg and Larval Quality. Blackwell Science, Oxford, p. 277 - 320.

Maclean JL. 1984. Tilapia- the aquatic chicken. Environments ICLARM Newsletter, p. 12-17.

Mair GC, Abucay JS, Skibinski DOF, Abella TA, Beardmore JA. 1997. Genetic manipulation of sex ratio for the large-scale production of all-male tilapia, *Oreochromis niloticus*. Canadian Journal of Fisheries and Aquatic Science **54,** 396-404.

McAndrew BJ, Majumdar KC. 1988. Growth studies in juvenile tilapia using pure species, hormone treated and nine interspecific hybrids. Aquaculture and

Fisheries Management **20,** 35-47. DOI: 10.1111/j.1365-2109.1989.tb00439.x.

Penman DJ, McAndrew BJ. 2000. Genetics for the Management and Improvement of Cultured Tilapias p. 227-266. In Tilapia: Biology and Exploitation. Beveridge and McAndrew (Eds) Kluwer Academic Publishers.

Pullin RSV, Bhukaswan T, Tonguthai K, MacLean JL. 1988. The second international symposium on tilapia in aquaculture. ICLARM Conference Proceedings 15, Bangkok and Manila, 623 p.

Sherwood SW and Patton JI. 1982. Genome evolution in pocket gophers (Genus, Thompmys). 11 variation in cellular DNA content. Chromosoma (Beri) **85,** 111-114.

Sofy HI, Layla AM, Iman MKA. 2008. Karyotypic diversity of three tilapia species from Egypt. Nature and Science **6(1),** 19-37. ISSN: 0740 - 1545.

Stickney PR, Hesby JH, McGeachinm RB, Isbell WA. 1979. Growth of Tilapia nilotica in ponds with differing histories of organic fertilization. Aquaculture **17,** 189-194. DOI: 10.1016/0044-8486(79)90122-4.

Tan X, Jian GQ, Chen B, Chen L, Li X. 2004. Karyological analyses on redclaw crayfish *Cherax quadricarinatus* (Decapoda: Parastacidae). Aquaculture **234,** 65-76, http://dx.doi.org/10.1016/j.aquaculture.2003.12.020

Thorgaard GH, Disney JE. 1993. Chromosome preparation and analysis. Chapter, 6. p. 171-186.