Karyotypic Analysis of Walking Catfish *Clarias batrachus* (Linnaeus, 1758)

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ABSTRACT: Karyotyping is one of the useful tools for species identification, taxonomy, evolutionary and breeding section. The present study represents the cytogenetic investigation of African catfish *Clarias batrachus* (Linn), inhabiting in Upper Lake Bhopal, using C-banding and Nor-banding techniques. *Clarias batrachus* was found to have standard karotype and diploid chromosome number of 2n=54, which comprised of 6 pairs of metacentric chromosome, 9 pairs of sub-metacentric chromosomes and 7 pairs of telocentric chromosomes. The study documented the karyotypic polymorphism of *Clarias batrachus* resident in the upper lake Bhopal.
[Shabeena Yaqoob, Tahir Mohi-uddin, Swaleha. **Karyotypic Analysis of Walking Catfish *Clarias batrachus* (Linnaeus, 1758).** Researcher 2012;4(5):46-48]. (ISSN: 1553-9865). http://www.sciencepub.net/researcher. 9

Key words: - Chromosome, Karyotype, *Clarias*, walking catfish, cytogenetic

INTRODUCTION:-

The walking catfish, *Clarias batrachus* is a species of fresh water air breathing catfish found primarily in south east Asia including Malaysia, Thailand, Eastern India, Sri Lanka, Bangladesh, Myanmar, Indonesia, Singapore and Borneo. The fish normally lives in slow-moving and often

stagnant waters in ponds swamps, streams and rivers(Mekong and chao Phraya basins). The *Clarias batrachus* is called walking catfish, so named for the ability to "walk" across dry land, to find food or suitable environments. It is named for its prominent barbells, a cylindrical body, flattened bony head and a broad transverse mouth with four pairs of long barbells around it. It also has an accessory air-breathing organ allowing it to survive in oxygen poor water or even out of water (Kottelat *et al*., 1993).

Walking catfish are around 30 cm (a foot or so) in length. Body often covered laterally with small white spots, the body is mainly colored a grey or grayish brown (Fig. 1).

The natural diet of this creature is omnivorous; it feeds on small fish, mollusks and other invertebrates as well as detritus and aquatic weeds.

Cytogenetic studies on fish have been useful in providing information conserving evolutionary and taxonomic studies, as well as for the genetic improvement of commercial fish stocks (God), 1979. Karyological and cellular DNA content data are important in understanding the genetic and systematic of fishes (Gold *et al*., 1980). The although the application of fish karyotype has received a considerable attention in recent years in many parts of the world, there is still a very limited data on karyotype of the endemic fish. In view of the above context, the present study entitled "Karyotypic Analysis of walking catfish C*larias batrachus* (Linn)" is proposed which will be investigated in detail for cytogenetic characterization.

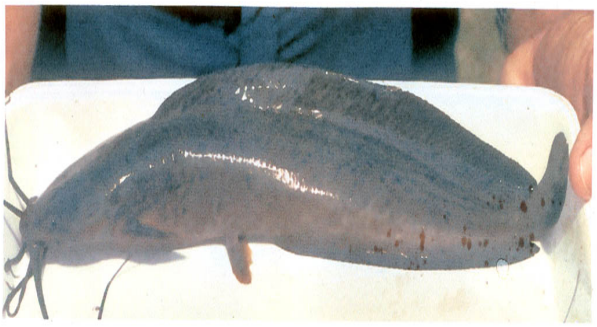

Fig. 1 Clarias batrachus.

Material and Methods:-

Live *Carias batrachus* specimens, were collected from Upper Lake Bhopal with the help of dip net by fisherman, weight ranging from 20 to 100 g and standard length ranging from 16cm to 40cm. The samples were brought to the laboratory in air tight containers. 0.5% colchicine was injected intramuscularly into the fish sample to disrupt spinde formation at mitosis and prevent the replicated chromosomes from migrating to their respective poles (Hartwell *et al*., 2000) .Two hours later, the fish specimens were anasthelized with ethylene glycerol and dissected. Kidney tissues were extracted and crushed to obtain an epithelial cell suspension. Tissue was homogenized with 6-8 ml hypotonic solution (0.56% KCl) in glass tissue grinder to prepare cell suspension. The cell suspension was poured in 15ml centrifuge tube and incubated for 20-25 minutes at room temperature for swelling the hypotonic action

was stopped by adding 1.0ml freshly prepared chilled fixative (methanol: glacial acetic acid, 3:1) slowly .Then the suspension was centrifuged at 1200 -15-00 rpm (revolution per minute) for 10 minutes at room temperature to get cell pellet at the bottom. The supernatant was removed with pipette and slowly overlaid with 6-8ml freshly prepared chilled fixation. The solution was allowed to stand for 30 sec and the fixative changed by here successive centrifugations, till clear transparent cell suspension was obtained . Small quantity of cellsuspension was taken in a pasture pipette and drop it onto greese free,pre-cleaned glass slide from a height of 1-1.5 ft .The slide was allowedto air flame dry and stained with 60% Giemsa in phosphate buffer(pH=6.8) for 45 minutes. After air dry the slides were stored in slide box.Observe metaphase spreads in Trinocular microscope with CCD camera. The slideswere made permanent by mounting in synthetic neutral mountant e.g;DPX. The slides were screened for good spreadsand photographs of metaphase spreads were taken under oil emmersion objective (100x).(Fig 2 to Fig 5). Classification and Karyogram of the chromosomes were performed according to the techniques described by Levan *et al.,*(1964) and Ergene *et al.,*(1998,a,b). The final karyogramwas scanned and printed .

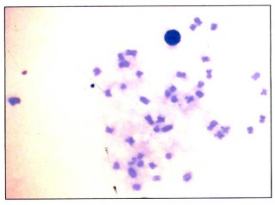
Fig. 4

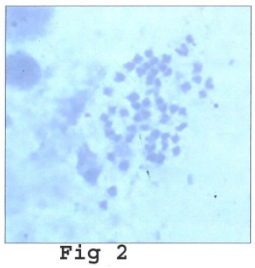
Fig 2

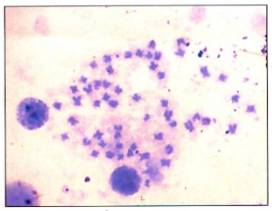
Fig. 5

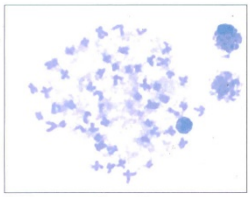
Fig 3

Figures 1 to 4; Metaphase spread of *Clarias batrachus* (100X magnification) From Kidney Cells]

Result and Discussion:-

The cytogenetic profile of *Clarias batrachus* was found to have a total of 54 chromosomes Karyotyping of the metaphase prepared from kidney cells was performed. Ten well spread metaphases were selected to prepare the karyograms of the fish .Chromosomes were grouped on the basis of their size and the position of centromere. The chromosomes of *Clarias batrachus* were grouped into four groups. Group-I comprised of xis pairs of metacentric chromosomes, Group II comprised of nine pairs of submetacentric chromosomes, group III comprised of five pairs of subtelocentric chromosomes and Group IV comprised of seven pairs of telocentric comprised of seven pairs of telocentric chromosomes (Fig 6).In *Clarias batrachus* the diploid number of chromosomes was found to be 2n=54. These findings are in agreement with earlier reports By Manna and Prasad (1971); Rishi, 1978; Panday and Lukra, 1996 and Nagpure *et al*., 2000 reported Jainxun *et al*., (1991) and Vasil, Yev (1980) reported that chromosome number of *Clarias batrachus* 56 and 52 respectively. The chromosome formulae (CF) in *Clarias batrachus* was established to be;

CF= 12M+18SM+10ST+10T

However, Manna and Prasad(1971) reported

CF=18M+ 2-0SM+12T and

CF=16M+8SM+14ST+12T respectively in the same species.Pandey and Lakra (1997) reported female heterogamety in *clarias batrachus* with:

CF= 16M+ 10SM+4ST+20T in male and

CF=16M+ 11SM+ 4ST+19T in females

According to Ozouf-Costaz *et al*., (1990) the diploid chromosome numbers are not uniformly distributed in the same species in different continent.

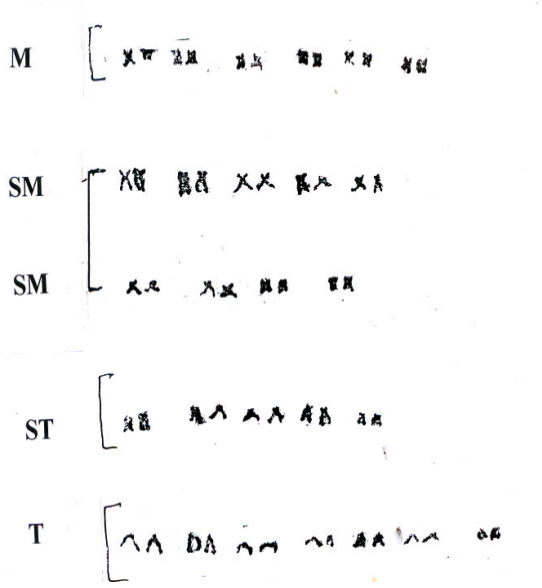

Fig. 6 .The Karyotype of *Clarias batrichus*, 2n=54

Acknowledgement:-

I am thankful to honourable DR. N.C. Sharma Professor and Head of department of Biochemistry and Genetics B.U. Bhopal M.P. for providing me the necessary facilities to carry out this work.

4/19/2012

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