

In Vitro Chromosomal Aberration Frequency by Electrofishing on *Poecilia latipinna* (Sailfin Molly) Fishes in Southern of Iraq

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Abstract

The present studies describe Chromosomal aberration effects of electrofishing, which were evaluated on *Poecilia latipinna*, located in Shat Al-Arab river in Al-gamma city (south of Iraq). The electrofishing derive used in work is simulated to that used in the commercial fishing. The apparatus generates voltage ranged from 40 to 280 volts. Nine bearers of *Poecilia latipinna* sailfin molly fish in chromosomal analysis were divided into three treatments. The first were a control, the fishes of the second were exposed to 110 volts (10 seconds), and final groups were exposed to 110 volts (15 seconds). Mitotic index of the electrofishing with a control for each group decreased with increasing exposed time in somatic cell kidney tissue of *Poecilia latipinna*. The chromosome aberration analysis revealed a significant increase in the most frequent aberration per 150 metaphase in analyzed groups (1.33 in T1 groups, 39.33 in T2 groups) was chromosome break, fragment, range chromosome, Sticky chromosome mean, were higher in comparison to non exposed electrical shock fishing groups (control groups T1). At the same time, it showed a higher positive correlation of total chromosome aberration frequencies between T1 and T2 groups, while, all fishes died in T3 groups. According to our results, we represented the first record in Iraq.

Keywords

Electrofishing, Chromosomal Aberration, *Poecilia latipinna* (Sailfin Molly)

1. Introduction

Electrofishing can be defined as a fish sampling technique using electric currents

and electric fields to control fish movement and/or immobilize fish, allowing the capture of fish [1]. Electrofishing has been used by fishery biologists since the 1950s [2]. Since then, there have been significant improvements innovations in the design of electrofishing equipment and its reliability and effectiveness for the capturing fish [3]. Electrofishing is a common tool used by fisheries biologists to monitor freshwater fish populations and communities [4] and is likely the most effective gear type for the sampling and assessment of stream fish assemblages [5]. *Poecilia latipinna*, the sailfin molly, belongs to the family Poeciliidae, and is a small species, seldom exceeding 12.5 cm in length [6] [7], located in Shat Al-Arab river in Al-gamma city within Basrah Province in the south of Iraq. Individuals have been found in shallow marsh area, because of *Poecilia latipinna* wide environmental tolerances fish [8] [9]. The body of *Poecilia latipinna* is oblong and the head is dorsally flattened with a small superior mouth. The caudal peduncle is deep, typically almost as deep as the body and the caudal fin is large and rounded. The dorsal fin is greatly enlarged in mature males but not all males display the enlarged dorsal fin [10]. The species has many rows of very fine teeth [11].

Chromosomal studies have received considerable attention in recent years. The most common abnormalities are categories as chromosome and chromatid break, a centric fragment, chromosome bridges, side arm bridges, fragment at anaphase, chromatid and sub-chromatid exchanges, chromatid gaps, heterochromatic regions. Chromosome break, fragments, chromatid exchanges and dicentric chromosome are generally considered as unstable aberration while deletion, inversions, duplications and translocations are considered as stable aberration. Such aberration is the result of unfinished repair or misrepair of DNA [12].

Many studies have examined effectiveness and utility of electrofishing, including the use of alternating current (AC), direct current (DC) and pulsed direct current (PDC) waveforms and on the effects of voltage, frequency, and other electrical field characteristics on the capture of fish [13]. There has been increasing concern among fishery biologists and managers regarding its potential for harming fish. Much of this increased concern began when [14] documented substantial injury to the spinal column and associated tissues of 44% to 67% of large rainbow trout [6]. The harmful influences of electrofishing include: hemorrhage, damages in nervous system, impact on growth and condition, disturbance in ionic regulation, mortality in fertilized eggs [10] [15] [16] [17] [18]. The objective of the present study is to know the impact of electroshock on the chromosomes aberrations in *Poecilia latipinna* sailfin molly fishes by using pulse direct current electroshock.

2. Materials and Method

2.1. Study Species

Poecilia latipinna, the sailfin molly, is a small popular ornamental fish that occurs as an introduced species in the aquatic habitats of at least 15 countries (ca-

bi). Generally occurs in the shallow, slow moving surface waters of marshes, ponds, streams, swamp, and estuaries, commonly associated with vegetation, widely tolerant of temperature, salinity, and low oxygen levels (fact sheet). After 28 days gestation, this species produces 10 to 100 young. The species feeds mainly on algae, also consumes animal material: rotifers, small crustaceans (such as copepods, and ostracods) and aquatic in sec.

2.2. Fish Collection and Experimental Condition

P. latipinna were collected by using a hand net of 40 cm diameter(10 mm mesh) from a farm of carp breeding cages(as a stranger fish non cultured) which located in Shat Al-Arab river in Al-gamma city within Basrah province south of Iraq (**Figure 1**). The fishes were held in 30 liter plastic water container and translated to the lab. Fishes have adapted before experiment started for seven days in an aerated tap water. The water temperature was 24°C, salinity 1.8% and pH 8.2.

2.3. Electrofishing Device and Chromosomes Preparation

The electrofishing device which used in this work is simulated to that which used in the commercial fishing (**Figure 2**). It converts the alternative current (AC) into pulse direct current (PDC). The apparatus generates voltage ranged between 40 - 280 volts (0 HZ).The device consists of glass aquarium (100 × 30 × 30 cm) with tow electrodes placed in a facing sides of aquarium. The device was connected to the main source of the electricity (220 V, 50 HZ). Nine bearers of *P. latipinna* were used in chromosomal analysis. The experiment was divided into three treatments. The first was a control. In the second, the fish was exposed

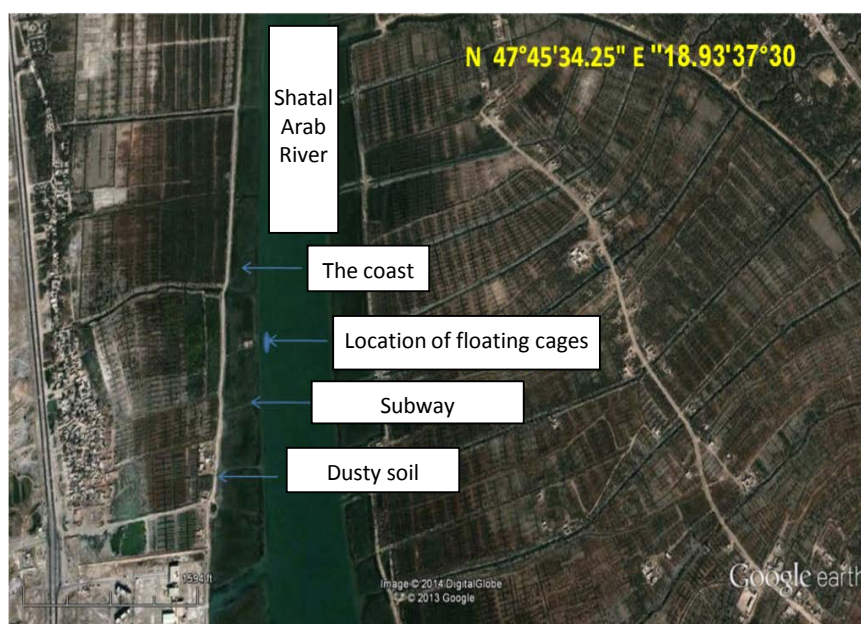


Figure 1. Which located in Shatt Al-Arab River north of Basra city belongs to the Department of Fisheries and Marine Resources/University of Basrah.



Figure 2. The electrofishing device which is used in this work is simulated to that which is used in the electricity commercial fishing.

to 110 volts (10 seconds), and in the third treatment was used 110 volts (15 seconds). After exposure to electroshock, chromosomal aberration was measured according to [19]. Take healthy fish (3.33 - 4.49 g). Inject 0.05% Colchicine intramuscularly at 1 ml per 100 g of body weight. Kept, fish alive for 1 - 2 hours after Injection of Colchicines, than dissect out the kidney tissue in a Petri dish, and cut into small pieces. Tissue homogenize in 8 ml hypotonic solution in homogenizer. Pour the cell suspension in 15 ml centrifuge tube and incubate for 20 - 25 minutes at room temperature. Stop the hypotonic action by adding 1 ml freshly prepared Conroy's fixative and left for 20 - 30 minutes. Mix it gently with pasture pipette. Centrifuged cell suspension at 1500 rpm for 10 minutes at room temperature. Removed supernatant with a pipette and slowly over layer 6 - 8 ml freshly prepared chilled fixative. Keep the tube in refrigerator for 15 - 30 minutes. Mix contents for 10 - 15 minutes at room temperature. Remove supernatant without disturbing cell pellet at the bottom, add freshly prepared Conroy's fixative, and keep the tube in refrigerator for half an hour. Repeat this step 3 - 5 times until transparent cell suspension is obtained. Take cell suspension in pipette and dropped it into grease free, per cleaned slide. Allow the slide to flame dry. Keep the slides for ageing (1 - 3 days). Stain with 4% - 5% Giemsa in phosphate buffer (pH 6.8) for 15 - 20 minutes. Washed with DDW and air-dried. Screen in oil immersion objective (100×) and observed chromosomal aberration under light microscope (OLYMPUS CX21) with Sony camera 8 MP.

3. Result

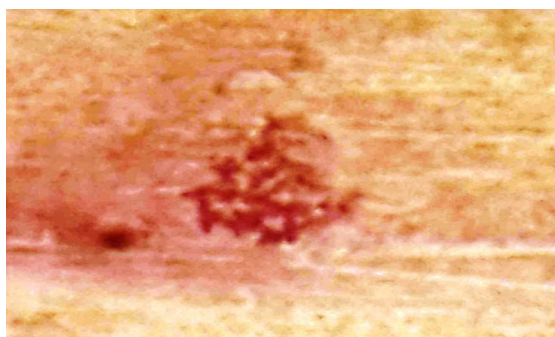
The results of chromosomal aberrations and mitotic index in somatic cell kidney tissue of *Poecilia latipinna* exposed to range of electrical shock fishing (10.15

second) by electricity (220 V, 50 HZ), were shown in (Table 1, Figure 3, Figure 4) chromosome aberration analysis revealed a significant increase in the most frequent aberration per 150 metaphase in analyzed groups (1.33% in group T1, were 39.33 in group T2), were chromosome break, fragment, range chromosome Stiky chromosomes mean and were higher in comparison to non exposed to electrical shock fishing group (control group T1) (Table 1, Figure 3, Figure 4).

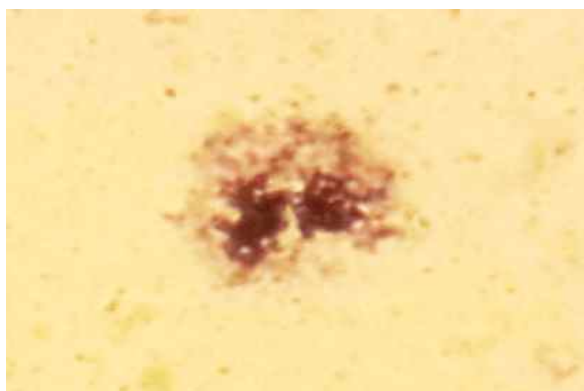
Table 1. Frequency of chromosomes aberration induced by electrical shock fishing in kidney tissues of *Poecilia latipinna* (Sailfin Molly) fishes.

Group	Mean of weight gram	Time exposure to electrical voltage in time seconds	Total no of metaphase analyzed	Mitotic index MI	Metaphase with chromosomes aberration	Chromosomes aberration Type of CA			
						Broken chromosomes mean min-minute fragment	Disturbed Metaphase and Anaphase mean	Stiky chromosomes mean	Aberration % N2
			N1	N1/N2					
control									
T1	3.16*	-	150	0.88	2	1	0	1	1.33
T2	4.82*	10	150	26.22	59	21	8	30	39.33
T3	4.49*	15	Non	ND	ND	ND	ND	ND	ND

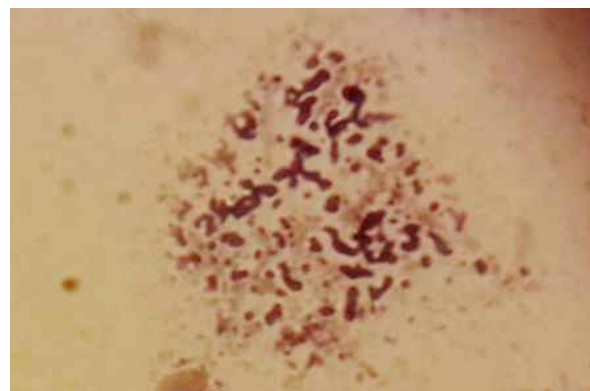
*Weight Mean in gram's of three value for each group ND All fish in the sample was died.



(a)



(b)



(c)

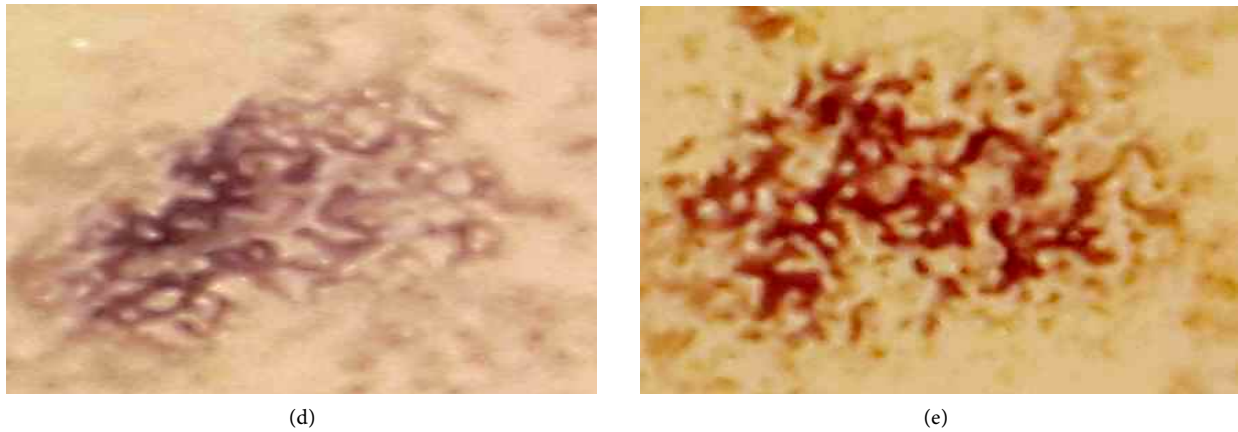


Figure 3. Chromosomes aberration observed in kidney tissues of *Poecilia latipinna*.

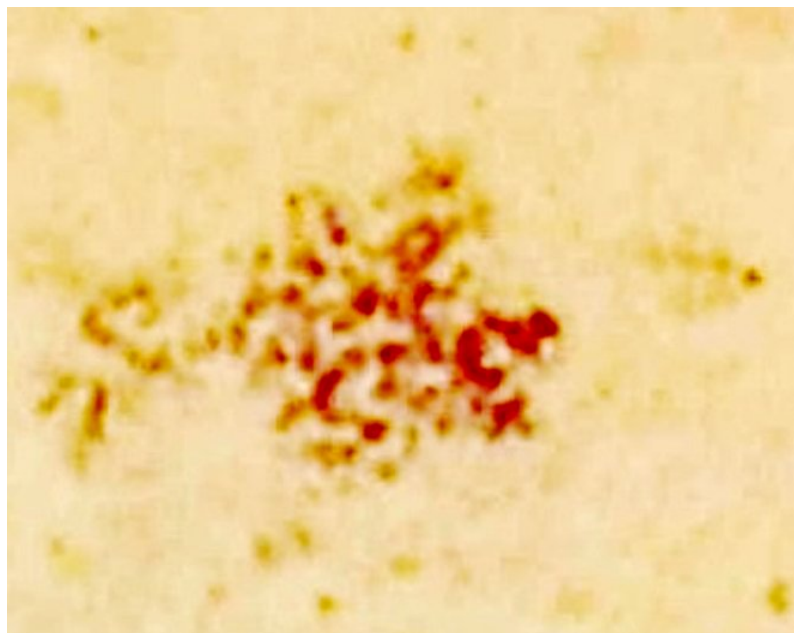


Figure 4. Chromosomes aberration observed in (T1) kidney tissues of *Poecilia latipinna* (Sailfin Molly) fishes.

However, increase chromosome aberration with time of exposed to electrical shock correlation analysis of frequencies of chromosome aberration, for the same time showed the higher positive correlations between total aberration frequencies in T2 group, while, all fishes died in T3 group (**Figure 5**).

4. Discussion

Despite all of the available knowledge on the effects of electrofishing on *Poecilia latipinna* (Sailfin Molly) fishes, we are aware of only a few studies in Iraq that have examined chromosomal aberration by exposed to apparatus generates voltage ranged between 40 - 280 volts (0 HZ). Chromosomal aberration is small fraction of a huge amount of changes in chromosomal DNA and reflects an enormous plasticity of the genome which has far-reaching consequences for



(a)



(b)

Figure 5. Showing the sever hemorrhage with one or more large wounds in hade of *Poecilia latipinna*, the sailfin molly fish exposed to electrofishing fields 15 seconds (a) (b).

evolution [20]. This study has focused on the Chromosomal aberration caused by electro fishing which may be induced the formation of chromosomal break, disturbed metaphase and anaphase, sticky chromosomes, ring chromosomes, fragments. It is also clear from present study that Chromosomal aberration increased initially exposed for electro fishing voltage group 1 and group 2 (**Table 1, Figure 3, Figure 4**) and then decreased caused by all fishes in group 3 all adied in increased time of exposure to 15 seconds in field to apparatus generates voltage ranged between 40 - 280 volts (0 HZ) (**Table 1, Figure 5**), these results agreement with [14] concluded that electrofishing caused on overall long-term effects on growth, in addition to physical injuries caused by hemorrhagic trauma and spinal compressions misalignments and fractures, fishes also may undergo a variety of stress related effects resulting from electrofishing that could have short-term or long-term implication for their health. The authors [21] found plasma cortisol and blood glucose levels significantly elevated by 3-hrs post shocking and which typically returned to control levels after 24-hrs. Eukaryotic

chromosomes are enormous. They contain one continuous DNA molecule in the pre-synthetic phase of cell cycle, which is replicated during the S-phase. During S-phase these DNA molecules are extremely long and fibrillar structure. While in metaphase chromosome is about 10 μm long. These packaging of chromosomes are associated with various types of protein. Due to their enormous dimension, DNA molecules in chromosome are permanent of physical damage of diverse origin such as chromosomal aberration [22]. We also observed different types of chromosomal aberration such as chromatic gap and deletion, dicentrics and corresponding fused acentric fragments in present studies by the exposure of apparatus generates voltage ranged between 40 - 280 volts (0 HZ). We also found that the electrofishing can also harmful on reproduction and early life stage. In same as, we result of electrofishing field can cause significant damage to premature expulsion of, reduce viability of subsequently fertilized eggs, exposure of recently hatched larvae might not cause significant mortality but can reduce growth rates for at last a few weeks [23].

5. Conclusion

Despite all of the available knowledge on the effects of electrofishing on fishes' chromosomal aberration, it is evident that the MI in Kidney tissue of *Poecilia latipinna* fish evaluates the kinetics of cytogenetic alterations under electrofishing influence. So that, in the present study we can say that there is a positive association between electrofishing and chromosome aberrations. Therefore it is important to prevent electrofishing in fresh rivers water.

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