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Random Amplified Polymorphic DNA - Polymerase Chain Reaction (RAPD-PCR) Technique of Genetic Diversity of Silurus glanis.

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Abstract. General Background Genetic diversity is essential for ensuring species resilience and supporting long-term ecological stability. Specific Background Silurus glanis, an ecologically and economically important freshwater fish in Iraq, is increasingly exposed to environmental pressures that may shape its genetic composition. Knowledge Gap Yet, information on the genetic variation between populations inhabiting the Euphrates and Tigris Rivers remains limited, particularly when assessed using RAPD-PCR molecular markers. Aims This study aims to evaluate the genetic diversity of S. glanis from both rivers using five RAPD primers to identify patterns of genetic similarity and differentiation. Results Analysis of 120 samples revealed 13 genetic loci, with 83.842% similarity and 16.154% genetic variation, indicating low diversity but high interpopulation resemblance. Novelty The study provides an updated genetic profile demonstrating the influence of local environmental conditions on the species' genetic structure and suggesting potential gene flow between riverine populations. Implications These findings highlight the need for strengthened river ecosystem management and the application of molecular biomarkers to guide conservation strategies capable of preventing further genetic erosion and supporting the long-term sustainability of S. glanis populations.

Highlights:

1. The results show measurable genetic variation and high similarity between the two river populations.
2. RAPD-PCR markers detected differences across loci that reflect environmental influences on genetic structure.
3. The findings highlight the importance of monitoring river conditions to maintain the species' genetic stability.

Keywords: Genetic diversity, RAPD-PCR, Silurus glanis, Euphrates and Tigris populations, molecular markers

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Introduction

A majority of aquatic lives are exposed to severe threats due to the presence of nanoparticle, pharmaceutical products, personal care products, pesticides, and other pollutants in the environment that contribute to toxicity of their genes and subsequent eventual prevalence of cancer in human beings and other living organisms[1] . Climatic changes, including rise of temperature and lack of oxygen, influence genetic diversity of marine fishes . The adverse effects of these climatic changes and toxic substances which destroy the genes in the aquatic environment necessitate laboratory tests and scrutinies to identify their effects[2]. RAPD technique does not assume any knowledge of the DNA sequence of the target organism as is the case with the conventional PCR analysis[3]. This method involves the application of 10-mer primers that amplify a fragment of DNA or fail to amplify one depending on the complementary sites of the sequences of the primers. The majority of RAPD primers are completely dominant such that one cannot tell whether a particular DNA fragment is being amplified out of a heterozygous or a homozygous locus[4]. RAPD is an enzyme reaction, hence, it depends on the lab and must have developed laboratory procedures. It is a dependable method, which entails amplification of random sequences of genomic DNA with brief random primers[5]. The technique is useful in the determination of species and strains , genetic diversity studies , in breeding programmes involving the use of markers, genetic variation detection and genotoxicity determination because of environmental pollutants.

In improving the sustainability of species and their capacity to adapt to environmental variation, genetic diversity is required[6]. Silurus glanis or European catfish is a significant environmental and economic fish in Iraq. The paper is meant to examine the genetic variation of such fish with the help of RAPD-PCR (Random Amplified Polymorphic DNA - Polymerase Chain Reaction) method that enables the identification of genetic variations through the use of random primers.

Methodology

Sample Collection: Samples of Silurus glanis 120 samples were taken with equal representation in two sites (60 samples in the Euphrates River in Al-Rumaitha district in the Al-Muthanna Governorate and 60 samples in the Tigris River in Al-Nasr district in the Dhi Qar Governorate).

Genomic DNA Extraction

DNA Amplification: This study involved the use of 5 primers consisting of 10 bases and having 60-70 percent C-G content (Table 1). The final reaction solution volume was 50 µL, which included: 5-10 µL of extracted DNA, 1 µL of primer, 1 µL of Tag Polymerase enzyme, 1 µL of 10 mM dNTPs, 2 µL of PCR buffer solution, 2 µL of MgCl2, and distilled water to reach the final reaction solution volume.

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Table 1. primers

NO.	
P1	CACCCGGATG
P2	TGGTCGCAGA
P3	GGACACCACT
P4	AAGCGGCCTC
P5	AAGCGGCCTC

RAPD-PCR Protocol

Preliminary heating phase at 95°C for 5 minutes followed by 35 cycles (DNA strand separation at 94°C for 30 seconds, primer annealing at 36°C for 1 minute, DNA elongation at 72°C for 2 minutes), and a final phase at 72°C for 10 minutes.

Separation and Analysis of RAPD-PCR Products

The products were separated using agarose gel electrophoresis at a concentration of 1.5% for 1 hour at 150 volts. The bands were stained with ethidium bromide and observed under UV light.

Statistical Analysis

The data from the bands of the primers in this study were tabulated in Excel and coded as 1 for the presence of bands and 0 for their absence. The genetic analysis was performed using POPGENE software.

Results

All five primers used in this study produced a number of bands ranging in molecular weight from 400 to 1600 base pairs distributed over 13 genetic loci (Figure 1). The samples showed similarity at approximately 10 loci and differed at only two genetic loci[7].

The RAPD-PCR technique results showed a genetic variation of 16.154% and a similarity of 83.842% between the samples taken from the Euphrates and Tigris Rivers[8]. There was a decrease in genetic diversity among and within Silurus glanis samples, with a value of H = 0.06. The obtained results are indicative of the high genetic variations between Silurus glanis in the two areas, which points to how varied environments influence the genetic make-up of these fish (Table 2 and 3).

Table 2. Values of genetic diversity

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PRIMER	POPULATION1		POPULATION2		BOTH POPULATION	
	H	I	H	I	H	I
P1	0.0592	0.0864	0.0592	0.0864	0.0801	0.1193
P2	0.0296	0.0432	0.0296	0.0432	0.0505	0.0762
P3	0.0296	0.0432	0.0296	0.0432	0.0505	0.0762
P4	0.0592	0.0864	0.0592	0.0864	0.0592	0.0864
P5	0.0592	0.0864	0.0592	0.0864	0.0592	0.0864
MEAN	0.04736	0.06912	0.04736	0.06912	0.0599	0.0889

Table 3. Percentage difference and similarity.

primer	Total loci		The number of polymorphic loci		The percentage of polymorphic loci		The number of monomorphic loci		The percentage of monomorphic loci	
	Pop1	Pop2	Pop1	Pop2	Pop1	Pop2	Pop1	Pop2	Pop1	Pop2
P1	12	12	2	2	16.67	16.67	10	10	83.33	83.33
Both	12		2		16.67		10		83.33	
P2	13	13	1	1	7.69	7.69	12	12	92.30	92.30

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both	13		2		15.38		11		84.61	
P3	13	12	1	1	7.69	8.33	12	11	92.30	91.66
both	13		2		15.38		11		84.61	
P4	12	12	2	2	16.67	16.67	10	10	83.33	83.33
both	12		2		16.67		10		83.33	
P5	12	12	2	2	16.67	16.67	10	10	83.33	83.33
both	12		2		16.67		10		83.33	
mean	12.4		2		16.154		10.4		83.842	

The large similarity percentage implies that the Silurus glanis in the Euphrates and Tigris Rivers have a high genetic similarity since there is a commonality of environmental factors or common environmental activities in the two rivers[9]. This can also be a sign that there is genetic exchange between the populations in the adjacent rivers, which increases the continuation of the genetic patterns[10].

The difference of 16.154 percent shows that we have realized genetic variation that can be credited to the capacity to respond to the local environment differences[11]. This difference may be as a result of difference in the environmental conditions like quality of water, water temperature or food quality.

In another research that was conducted on the premise of RAPD-PCR, the outcome of the research indicated that the process of investigating the genetic variation of fish in the various aquatic environments were largely contingent on the environmental factors like pollution and climatic changes . Their finding also included the fact that genetic diversity was lower in those areas that were more polluted like with our Silurus glanis case[12].

The conclusions made in the research work of Gholami et al., confirmed that the genetic variation of the riverine fish in the contaminated water (with industrial waste and toxic substances) was smaller, thus, making them more prone to diseases, and reducing the adaptive capacity to the environment, which can also be explained by the results of our study on the Silurus glanis[13].

In an experiment of how the climate change like increase in temperature and low oxygen

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levels affected the genetic diversity of the marine fish, Shah et al[14], discovered that the changes resulted in reduced genetic diversity of the fish, which affected their health and adaptation to the changes in the environmental condition[15]. This conforms to what we were able to experience in our study of Silurus glanis.

Conclusion

The findings provided in the given paper prove that the Euphrates and Tigris Rivers have a high level of genetic correspondence and a low level of genetic diversity of Silurus glanis under the influence of the local environmental factors. This correlates with the results of the current research where RAPD-PCR method was used to examine genetic variations of fish. The paper acknowledges that the environmental consideration needs to be put in place in dealing with genetic diversity and offers workable methods of preserving species.Furthermore, the study underscores the significance of environmental and anthropogenic factors—such as pollution, water salinity, agricultural runoff, and habitat degradation—in shaping the genetic structure of aquatic species. These factors can reduce effective population size and hinder natural genetic exchange, thereby increasing vulnerability to diseases and environmental stressors.

Therefore, it becomes evident that maintaining and improving the ecological health of river systems is crucial for conserving the genetic diversity of aquatic organisms. Environmental management strategies, including habitat restoration, pollution control, and sustainable water resource use, are essential to prevent further genetic erosion. The integration of molecular genetic tools such as RAPD-PCR with ecological monitoring can provide more precise insights into the adaptive potential of fish populations and guide conservation policies more effectively.

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