KÜBRA SARI

M.S. Thesis In Biology

July - 2009

FISH AQUACULTURE SUPPORTING DNA-BASED MOLECULAR WORKS IN TURKEY

by

KÜBRA SARI

July 2009

FISH AQUACULTURE SUPPORTING DNA-BASED MOLECULAR WORKS IN TURKEY

by

KÜBRA SARI

A thesis submitted to

the Graduate Institute of Sciences and Engineering

of

Fatih University

in partial fulfillment of the requirements for the degree of

Master of Science

in

Biology

July 2009
Istanbul, Turkey

APPROVAL PAGE

I certify that this thesis satisfies all the requirements as a thesis for the degree of Master of Science.

Assist. Prof. M. Serdal SAKÇALI
Head of Department

This is to certify that I have read this thesis and that in my opinion it is fully adequate, in scope and quality, as a thesis for the degree of Master of Science.

Assist. Prof. I. Irem UZONUR
Supervisor

Examining Committee Members

Assist. Prof. İ. İrem UZONUR ……………………….

Prof. Dr. Fahrettin GÜCİN ……………………….

Assist. Prof. Mustafa PETEK ……………………….

It is approved that this thesis has been written in compliance with the formatting rules laid down by the Graduate Institute of Sciences and Engineering.

Assoc. Prof. Dr. Nurullah ARSLAN
Director

FISH AQUACULTURE SUPPORTING DNA-BASED MOLACULER WORKS IN TURKEY

KÜBRA SARI

M. S. Thesis - Biology
July 2009

Supervisor: Assist. Prof. İ. İrem UZONUR

ABSTRACT

Fish as an important nutritional source can be provided by traditional methods and modern technologies in fish farms, called aquaculture. New modern technologies are quite popular in world fisheries. Depending on the rapid reproduction characteristics of fish, aquaculture is more advantageous than the culture and farming of other land animals making it more efficient.

In aquaculture, depending on scientific developments, DNA based molecular techniques are used and these techniques are increasing over time. In this study, we evaluated the results of used molecular techniques and advantages and disadvantages were put in place.

In this study DNA of rainbow-trout samples which were taken from five different regions were examined by RAPD and RAMD-PCR analysis Diversity profiles of rainbow-trout in farms, genetic variation due to various exposures as either DNA damage or mutation, somatic or gonadal mutations have been assessed. Muscle, liver and gonad tissue DNAs have been analyzed from trout samples from different farms and same farms with varying sizes and the effect of feeding (natural/commercial) on the RAPD-PCR profiles as an indication of DNA damage and mutation have been assessed.

Keywords: *Oncorhynchus mykiss,* Rainbow-trout aquaculture, RAPD-PCR, DNA damage, somatic mosaicism, gonadal mosaicism

TÜRKİYE’DE KÜLTÜR BALIKÇILIĞINI DESTEKLEYEN DNA-TEMELLİ MOLEKÜLER ÇALIŞMALAR

Kübra SARI

Yüksek Lisans Tezi – Biyoloji
Temmuz 2009

Tez Yöneticisi: Yrd. Doç. Dr. İ. İREM UZONUR

ÖZ

Değerli bir besin kaynağı olan balık, geleneksel yöntemlerin yanında çiftliklerde modern teknolojilerle üretilmektedir ve bu yeni yöntemler dünya balıkçılığında oldukça rağbet görmektedir. Kültür balıkçılığı, balıkların biyolojik özelliklerine bağlı olarak diğer kara hayvanlarına göre daha verimli olması ve balıkların daha hızlı üremeleri nedeniyle önemli bir avantaja sahiptir.

Kültür balıkçılığında bilimsel gelişmelere bağlı olarak, DNA-temelli moleküler teknikler kullanılmakta ve bu teknikler gittikçe artmaktadır. Bu çalışmada moleküler tekniklerle kültür alabalık DNAsı üzerinde çeşitli değerlendirmeler yapılmıştır.

Bu çalışmada yaptığımız deneylerde 5 farklı çiftlikten aldığımız alabalık örneklerinin DNA’larının değişikliklerini etkin sonuç veren RAPD PCR yöntemi ile incelendi.. Bu örneklerden çıkarılan kas, karaciğer ve gonad dokularının DNA yapılarında farklılaşma araştırılmasında RAPD bant profilleri kullanılarak farklılıkları ortaya çıkarıldı ve somatik ve gonad mosaisizmi belirlendi. Aynı yetişme koşullarında sadece yemleri farklı olan alabalık örneklerinde yemin RAPD PCR profillerine etkisi belirlendi.

Anahtar Kelimeler: *Oncorhynchus mykiss,* Gökkuşağı Alabalığı, Kültür Balıkçılığı, RAPD-PZR, DNA hasarı, gonad mozaisizmi, somatik mozaisizm

DEDICATION

To İsak Şahin and my parents

ACKNOWLEDGEMENT

I express sincere appreciation to Assist. Prof. Dr. İ. İrem UZONUR and biology faculty member Prof. Dr. Fahrettin GÜCİN for their scientific guides, valuable suggestions, motivation, patience and insight throughout the research.

I express my thanks and appreciation to my family and husband for their understanding, motivation and patience. Lastly, but in no sense the least, I am thankful to all colleagues and friends who made my stay at the university a memorable and valuable experience.

TABLE OF CONTENTS

ABSTRACT …………………………………………………………………………. **iii**

ÖZ …………………………………………………………………………………. **iv**

DEDICATION …………………………………………………………………….. **v**

ACKNOWLEDGMENT………………………………………………………….. **vi**

TABLE OF CONTENTS ……………………………………………………… **vii**

LIST OF TABLES ……………………………………………………………….. **viii**

LIST OF FIGURES ……………………………………………………………… **ix**

LIST OF SYMBOLS AND ABBREVIATIONS ……………………………….. **x**

CHAPTER 1 INTRODUCTION ………………………………………………… **1**

1.1 Aquaculture ……………………………………………………………….. **1**

1.2 Types of Aquaculture…………………………………………………….. **3**

1.2.1 Algaculture……………………………………………………………….. **3**

1.2.2 Fish Farming………………………………………………………………. **3**

1.2.3 Freshwater Prawn Farming…………………………………………….... **3**

1.2.4 Integrated Multi-Trophic Aquaculture………………………………… **3**

1.2.5 Mariculture……………………………………………………………….. **4**

1.2.6 Shrimp Farming…………………………………………………………. **4**

1.3 The Situation of Aquaculture……………………………………………….. **4**

1.3.1 The situation of aquaculture in Turkey………………………………… **4**

1.3.2 The situation of Aquaculture in the World……………………………… **8**

1.4 Rainbow Trout……………………………………………………................... **12**

1.4.1 Habitat Requirements……………………………………………………. **12**

1.4.2 Food…………………………………………………………….................................. **13**

1.4.3 Oncorhynchus mykiss……………………………………………………. **16**

1.5 DNA Based Work On Aquaculture…………………………………………….. **17**

1.5.1 Applications of DNA markers in aquaculture genetics ………….................... **19**

1.6 Genetic Diversity………………………………………………………………… **20**

1.6.1 Genetic diversity in marine species ………………………………………….. 23

1.6.1.1 Measuring genetic diversity ………………………………………………. 23

1.6.2 Levels of genetic diversity ……………………………………. …………….. 24

1.6.3 Pollution induced genetic changes…………………………………………….. 24

1.7 GENETIC VARIATION……………………………………………………….. 25

1.7.1 Epigenetics and its molecular basis …………………………………………... 27

1.7.2 Mosaicism ……………………………………………………………………. 27

1.7.2.1 Somatic Mosaicism …………………………………………………….... 28

1.7.2.2 Gonadal Mosaicism …………………………………………………….. 28

1.7.3 Threats to Global Biodiversity - Inbreeding and Genetic Deterioration…… 29

1.7.4 Methods in Molecular Evaluation of Genetic Diversity …………………….. 29

1.7.4.1 RAPD-PCR ……………………………………………………………... 29

1.7.5 Applications of Molecular Methods in Environmental Problems…………... 30

CHAPTER 2 MATERIALS & METHODS………………………………………. 32

2.1 Materials………………………………………………………………………….. 32

2.1.1 General Reagents……………………………………………........................... 32

2.1.2 Equipment……………………………………………………………………. 32

2.1.3 Fish Sampling………………………………………………………………... 32

2.1.4 DNA Isolation Reagent……………………………………………………… 35

2.1.5 RAPD-PCR Reagent………………………………………………………… 35

2.1.6 Polymerase Chain Reaction Reagent ……………………………………….. 36

2.1.7 Agarose Gel Electrophoresis ………………………………………………… 36

2.1.8 DNA Size Marker …………………………………………………………….. 37

2.2 Method………………………………………………………………………….. 38

2.2.1 DNA Isolation Assay………………………………………………………. 38

2.2.2 DNA Concentration Assay …………………………………………………... 38

2.2.3 RAPD-PCR Protocol & Modified RAPD Random Amplified Mosaic DNA-PCR (RAMD-PCR ………………………………………………………………….. 39

2.2.4 Agarose Gel Electrophoresis ………………………………………………… 41

CHAPTER 3 RESULTS…………………………………………………………. 43

3.1 EVALUATION of SAMPLING SITES ………………………………………. 43

3.2 DETERMINATION of GENETIC DIVERSITY USING RAPD-PCR ………. 45

3.3 GENETIC VARIATION DETERMINATION …………………………………. 47

3.3.1 Genetic Variation due to Size (Age, exposure level) ………………………… 47

3.3.2 Genetic Variation due to Different FoodTypes …………………………… ... 52

3.4 DETERMINATION of SOMATIC and GONADAL MOSAICISM using RAMD-PCR…………………………………………………………………............................. 53

CHAPTER DISCUSSION ……………………………………………………….. 60

CHAPTER CONCLUSION ……………………………………………………... 72

REFERENCES ………………………………………………………………… 74

APPENDIX A ………………………………………………………………….. 79

LIST OF TABLE

Table 1.1 Aquaculture areas in Turkey…………………………………………… 6
Table 1.2 Number of fish farms, their capacity and amount of production ……… 6
Table 1.3 Production of fishery products in Turkey by years……………………... 7
Table 1.4 Aquaculture of different species in Turkey…………………………….. 8
Table 1.5 World fisheries and aquaculture production........................………… 10
Table 1.6 World aquaculture production....……………………………………. 11
Table 1.7 Commercial trout diet ingredients with percentages…………………… 14
Table 1.8 Energy values of commercial trout diets……………………………….. 14
Table 1.9 Vitamins in commercial trout diets…………………………………….. 14
Table 1.10 Life cycle of Rainbow trout……………………………………….. 15
Table 1.11 Types of DNA markers, their characteristic and potential applications 18
Table 1.12 Applications of DNA markers in aquaculture genetics……………….. 20
Table 2.1 Fish sampling sites and sample information …………………………... 33
Table 2.2 Samples according to their food ……………………………………... 34
Table 2.3 Samples obtained from liver tissues of indicated fish …………………. 34
Table 2.4 Samples obtained from gonad tissue of indicated fish …………………. 35
Table 2.5 Specific base sequences of the Operon Technologies 10-mer primers…. 36
Table 2.6 DNA concentration assay……………………………………………. 39
Table 2.7 RAPD PCR ingredients ……………………………………………... 40
Table 3.1 Rainbow trout culture facility water quality criteria that should be met.. 45
Table A.1 Ministry of agriculture and rural affairs heavy metal analysis results of fish 79
Table A.2 Ministry of agriculture and rural affairs antibiotics analysis of fish samples 79
Table A.3 Sülfonamit Analysis Results ………………………………………………. 80
Table A.4 Physical Properties of Water of Gökkuşağı Aquaculture Facility…………. 80
Table A.5 Chemical Properties of Water of Gökkuşağı Aquaculture Facility ……… 80
Table A.6 Physical Properties of Water of Çağlayan Aquaculture Facility ....… 81
Table A.7 Chemical Properties of Water of Çağlayan Aquaculture Facility ………… 81

LIST OF FIGURES

FIGURE

Figure 2.1 Information of Fast Ruler DNA size marker………………………...... 37

Figure 2.2 Information of Bioron DNA size marker…………………………... 37

Figure 2.3 Representation of RAMD-PCR approach ……………………………. 41

Figure 3.1 a Kahramanmaraş-Çağlayan Rainbow-trout Culture Facility ……….. 44

Figure 3.1 b Kahramanmaraş-Gökkuşağı Rainbow-Trout Culture Facility ……... 44

Figure 3.2 Genetic diversity results using OPA-8 Primer ……………………….. 46

Figure 3.3 Genetic diversity results using OPB-18 Primer ………………………. 46

Figure 3.4 x3 RAMD-PCR of three different biggest fish of equal size from two different facilities using OPB-18 primer …………………………………………………… 48

Figure 3.5 x4 RAMD-PCR profiles of same sized fish from the same facility using OPA-08 and OPB-18 primers respectively …………………………………………. 49

Figure 3.6a x4 RAMD profiles of ten samples with sizes given below using OPB-18 primer………………………………………………………………………………… 49

Figure 3.6 b x4 RAMD profiles of ten samples with sizes given below using OPB-18 primer……………………………………………………………………………….. 50

Figure 3.7a x4 RAMD-PCR profiles of different sized fish samples from neighboring facilities on the same river using OPB-18 primer ………………………………… 50

Figure 3.7.b x4 RAMD-PCR profiles of different sized fish samples from neighboring facilities on the same river using OPB-18………………………………………… 51

Figure 3.8a x5 RAMD-PCR profiles of different fish samples from different facilities using OPB-18 primer …………………………………………………………………… 51

Figure 3.8b x5 RAMD-PCR profiles of different fish samples from different facilities using OPB-18 primer …………………………………………………………………….. 52

Figure 3.9 x3 RAMD-PCR profiles using OPB-18 primer. Comparison of RAPD profiles according to food of fish sample ………………………………………………… 53
Figure 3.10 x3 RAMD-PCR Profiles of liver tissue DNA extracts using OPB-18 primer………………………………………………………………………… 54
Figure 3.11 OPA-8 primer comparison of the same conditions for liver sample………. 55
Figure 3.12 x3 RAMD-PCR Profiles of the gonad tissue DNAs of below sized fish …. 55
Figure 3.13 x8 RAMD Profiles of gonad tissue samples of two different fish with the same primer ………………………………………………………………………. 56
Figure 3.14 x5 RAPD Profiles of liver tissue and gonad tissue samples for the below samples respectively …………………………………………………................. 57
Figure 3.15 Comparison of tissues with two different primers ………………………. 58
Figure 3.16 a x3 RAMD-PCR Profiles of muscle tissue samples ……………………. 58
Figure 3.16 b x3 RAMD-PCR Profiles of liver tissue samples ………………………. 59
Figure 3.16 c x3 RAMD-PCR Profiles of gonad tissue samples ……………………… 59
Figure 4.1: Genetic diversity results using OPA- 08 primer and OPB-18 primer……… 63
Figure 4.2: Intra-individual genetic variation, mosaicism detection with x4 RAMD-PCR…………………………………………………………………………….. 64
Figure 4.3: Genetic variation mosaicism detection with x4 RAMD PCR …….......... 65
Figure 4.4: x4 RAMD-PCR profiles of different sized fish samples from neighboring facilities on the same dam in Kahramanmaraş using OPB-18 primer………………….. 65
Figure 4.5: x4 RAMD-PCR profiles of different sized fish samples from neighboring facilities on the same river using OPB-18 …………………………………………….. 66
Figure 4.6: x5 RAMD-PCR profiles of different fish samples from different facilities using OPB-18 primer ……………………………………………………………………. 66
Figure 4.7: x3 RAMD-PCR profiles using OPB-18 primer. Comparison of RAPD profiles according to food of fish sample …………………………………………………….. 67
Figure 4.8: OPA-8 primer comparison of the same conditions for liver sample ………. 68
Figure 4.9: x3 RAMD-PCR Profiles of the gonad tissue DNAs of below sized fish………………………………………………………………………………. 69
Figure 4.10: x3 RAMD-PCR Profiles of muscle tissue samples ……………………… 69
Figure 4.11: Liver tissue x3 RAMD-PCR profiles ……………………………......... 70
Figure 4.12: x3 RAMD-PCR Profiles of gonad tissue samples …………………... 70

Figure 4.13: Comparison of all tissues for the same fish (10 cm) with two different primers………………………………………………………………………………… 71

LIST OF SYSMBOLS AND ABBREVIATIONS

SYMBOL/ABBREVIATION

AFLP	: Amplified Fragment Length Polymorphism
BC	: Before Christ
DNA	: Deoxyribonucleic acid
EST	: Expressed Sequence Taq
FAO	: Food and Agriculture Organization
IMTA	: Integrated Multi Trophic Aquaculture
Mt DNA	: Mitochondrial DNA
OM	: *Oncorhynchus mykiss*
PCR	: Polymerase Chain Reaction
RAPD	: Random Amplified Polymorphism DNA
RFLP	: Restriction Fragment Length Polymorphism
SNP	: Single Nucleotide Polymorphism
SSR	: Microsatellites

CHAPTER 1

INTRODUCTION

1.1 AQUACULTURE

Aquaculture is a type of agriculture of aquatic organisms in fresh, brackish or salt water and it is the practice of cultivating aquatic animals and plants in managed aquatic environments. A wide variety of aquatic organisms are produced through aquaculture, including fishes, crustaceans, mollusks, algae, and aquatic plants. Farming implies individual or corporate ownership of cultivated stock and some forms of intervention in the rearing process to enhance production, such as regular stocking, feeding, and protection from predators [1].

Unlike fishing, aquaculture, also known as aquafarming, implies the cultivation of aquatic populations under controlled conditions. Mariculture refers to aquaculture practiced in marine environments. Particular kinds of aquaculture include algaculture (the production of kelp/seaweed and other algae), fish farming, shrimp farming, oyster farming, and the growing of cultured pearls. Particular methods include aquaponics, which integrates fish farming and plant farming. Fish culture, or pisciculture, refers to the husbandry of finfish. The most popular aquaculture species are finfish grown in fresh waters, accounting for over 40 % of total aquaculture production [2].

Aquaculture in salt-water or marine environments is called mariculture. Fish culture, or pisciculture, refers to the husbandry of finfish. The most popular aquaculture species are finfish grown in fresh waters, accounting for over 40 percent of total aquaculture production [3].

Aquaculture is considered an agricultural activity, despite the many differences between aquaculture and terrestrial agriculture. Aquaculture mainly produces protein crops, while starchy staple crops are the primary products of terrestrial agriculture. In

addition, terrestrial animal waste can be disposed of off-site, whereas in aquaculture such waste accumulates in the culture environment. Consequently, aquaculturists must carefully manage their production units to ensure that water quality does not deteriorate and become stressful to the culture organisms [4].

Aquaculture has a number of economic and other benefits. The most economically important form of aquaculture is fish farming, an industry that accounts for an ever increasing share of world fisheries production. Formerly a business for small farms, it is now also pursued by large agribusinesses, and by the early 2000s it had become almost as significant a source of fish as the as wild fisheries [5].

Aquaculture has a long history. First of all, aquaculture has been used in China since circa 2500 BC. Aquaculture developed more than 2000 years ago in countries such as China, Rome and Egypt. In the past when the waters lowered after river floods, people held some fishes, mainly carp in artificial lakes. Not long after, aquacultural practices in Europe, China, and Japan commonly involved stocking wild-caught seed, for example, carp *fingerlings* (juvenile fish) captured from rivers, in ponds or other bodies of water for further growth.

Hawaiians practiced aquaculture by constructing fish ponds (see Hawaiian aquaculture). A remarkable example is a fish pond dating from at least 1,000 years ago, at Alekoko. Legend says that it was constructed by the mythical Menehune, the Japanese cultivated seaweed by providing bamboo poles and, later, nets and oyster shells to serve as anchoring surfaces for spores. The Romans bred fish in ponds.

In central Europe, early Christian monasteries adopted Roman aquacultural practices [6]. Aquaculture spread in Europe during the Middle Ages, since away from the seacoasts and the big rivers, fish were scarce/expensive. Improvements in transportation during the 19th century made fish easily available and inexpensive, even in inland areas, making aquaculture less popular.

1.2. TYPES OF AQUACULTURE

1.2.1 Algaculture

Algaculture is a form of aquaculture involving the farming of species of algae. The majority of algae that are intentionally cultivated fall into the category of microalgae, also referred to as phytoplankton, microphytes, or planktonic algae. Macroalgae, commonly known as seaweeds, also have many commercial and industrial uses, but due to their size and the specific requirements of the environment in which they need to grow, they do not lend themselves as readily to cultivation on a large scale as microalgae and are most often harvested wild from the ocean.

1.2.2 Fish Farming

Fish farming is the principal form of aquaculture, while other methods may fall under mariculture. It involves raising fish commercially in tanks or enclosures, usually for food. It is a facility that releases juvenile fish into the wild for recreational fishing or to supplement a species' natural numbers, generally referred to as a fish hatchery. Fish species raised by fish farms include salmon, catfish, tilapia, cod, carp, trout and others.

Increasing demands on wild fisheries by commercial fishing operations have caused widespread overfishing. Fish farming offers an alternative solution to the increasing market demand for fish and fish protein.

1.2.3 Freshwater Prawn Farming

A freshwater prawn farm is an aquaculture business designed to raise and produce freshwater prawn or shrimp for human consumption. Freshwater prawn farming shares many characteristics with and many of the same problems as, marine shrimp farming. Unique problems are introduced by the developmental life cycle of the main species [7].

1.2.4 Integrated Multi-trophic Aquaculture

Integrated Multi-Trophic Aquaculture (IMTA) is a practice in which the by-products (wastes) from one species are recycled to become inputs (fertilizers, food) for

another. Fed aquaculture (e.g. fish, shrimp) is combined with inorganic extractive (e.g. seaweed) and organic extractive (e.g. shellfish) aquaculture to create balanced systems for environmental sustainability (biomitigation), economic stability (product diversification and risk reduction) and social acceptability (better management practices) [8].

1.2.5 Mariculture

Mariculture is a specialized branch of aquaculture involving the cultivation of marine organisms for food and other products in the open ocean, an enclosed section of the ocean, or in tanks, ponds or raceways which are filled with seawater. An example of the latter is the farming of marine fish, prawns, or oysters in saltwater ponds. Non-food products produced by mariculture include: fish meal, nutrient agar, jewelries (e.g. cultured pearls), and cosmetics [9].

1.2.6 Shrimp farming

A shrimp farm is an aquaculture business for the cultivation of marine shrimp for human consumption. Commercial shrimp farming began in the 1970s, and production grew steeply, particularly to match the market demands of the U.S., Japan and Western Europe. Global production reached more than 1.6 million tones in 2003, representing a value of nearly 9,000 million U.S. dollars. About 75% of farmed shrimp is produced in Asia, in particular in China and Thailand. The other 25% is produced mainly in Latin America, where Brazil is the largest producer. Thailand is the largest exporter [10].

1.3 The Situation of Aquaculture

1.3.1 Aquaculture in Turkey

Aquaculture in Turkey started with carp and trout farming in 1970s and gained momentum with commencement of gilthead seabream / seabass farming in the Aegean Sea and Mediterranean Sea beginning from the midst of 1980s; cage culture of trout in the Black Sea during 1990s; and tuna rearing in the Aegean Sea and the Mediterranean

Sea in early 2000s. In 1990s, the attempts for salmon culture in the Black Sea and shrimp culture in the Mediterranean Sea (Manavgat) have been made but have not been succeeded. As indicated in Table 1.1. inland culture of trout and carp and off-shore culture of gilthead seabream/ seabass are still being made [11]. These natural riches carry a major advantage in having a large variety of aquatic species. Although the surrounding seas are linked to one another, each one's biological content differs due to the dissimilarities in temperature, salt content etc [12].

On the other hand, the inland resources are also very rich in the country: (175 km rivers, 1 million ha natural lakes, 170 000 ha reservoirs, 70 000 ha lagoons, 700 small reservoirs [12].

Turkey import raw fish and export mollusks and crustacean. The need for raw fish may be met through aquaculture. 70%-80% of export is achieved to European Union countries (France, Germany and Belgium) and followed by Japan. In recent years exportation to China is increasing. In order to evaluate the present potentials of seas and inland waters of Turkey, necessary precautions to prevent pollution and decrease in fish stocks should be taken. It is necessary that new aquaculture methods in inland waters and dams be introduced and encouraged. In addition, fish varieties with high economic value should be adapted without harming the domestic fauna [13].

The species of trout becoming widespread in cultivation in Turkey is rainbow trout (*Oncorhynchus mykiss*, W. 1792). The trout cultivation began in 1968 in Akyazı in the country. As indicated in Table 1.2. it was cultivated in inland waters till 1990 when it also began to be cultivated in net cages in sea [14].

Table 1.1 Aquaculture areas in Turkey [15].

Place of production	**Area (ha)**
Mediterranean Sea, Blacksea, Aegean Sea, Marmara Sea	24.600.000
Naturel lake	1.000.000
Barrage	340.000
Pond	10.000
River	200.000
TOTAL	**26.150.000**

Table 1.2 Number of fish farms, their capacity and amount of production [11].

Species	**Number of fish farms**	**Capacity (tons/year)**	**Product (tons/year)**
Rainbow trout (fresh water)	1215	29998	39674
Rainbow trout (sea)	11	1139	1194
G.seabream and seabass	345	51211	37773
Mussel	2	320	815
Carp	86	2613	543
Total	1659	85281	79943

The production from farming has increased by more than 20% in the last decade and has reached to 61.165 tons in 2002 with 10% share in the total fisheries production (Table 1.3 and Table 1.4). This increase is expected to continue [11].

Table 1.3 Production of fishery products in Turkey by years [11]

Years	Fisheries		Aquaculture		Total	Consumption
	Sea (Tons)	Freshwater (Tons)	Tons	%	Tons	kg per capita
1986	539.565	40.280	3.075	0.5	582.920	8.5
1988	627.369	44.535	4.100	0.6	676.004	8.7
1990	342.017	37.315	5.782	1.5	285.114	6.2
1992	404.766	40.370	9.210	2.0	454.346	7.5
1994	542.268	42.838	15.998	2.7	601.104	8.2
1996	474.243	42.202	33.201	6.0	549.646	8.5
1998	432.700	54.500	56.700	10.4	543.900	8.3
2000	460.521	42.824	79.031	13.6	582.376	8.0
2001	484.410	43.323	67.244	11.3	594.977	7.6
2002	522.744	43.938	61.165	9.7	627.847	6.7

One of the typical characteristics of aquaculture in Turkey is that aquaculture is mostly based on the intensive production of carnivorous fish species. 98.5% of the production is from the carnivorous fish species (window trout, seabass, gilthead seabream and tuna). Trout ranks the first (56.4%) amongst the species cultured, followed by seabass (23.4%), gilthead seabream (19.1%), mussel and carp (543 tons) [11].

Table 1.4 Aquaculture of different species (ton/year) in Turkey [11].

Species	1986	1990	1995	2000	2001	2002
Rainbow trout (fresh water)	990	3.512	12.689	42.572	36.827	3.707
Rainbow trout (sea)	_	_	_	1.961	1.240	846
Gilthead seabream	_	102	4.847	17.877	15.546	14.339
Seabass	34	1.031	2.773	15.460	12.939	11.681
Carp	2.050	1.136	424	813	687	590
Mussel	_	_	180	321	5	2
Salmon	_	_	654	_	_	_
Prawn	_	_	40	27	_	_
Total	3.075	5.782	21.607	79.031	67.244	61.165

1.3.2 Aquaculture in the World

In 2006, the total world production of fisheries was 143.6 million tones of which aquaculture contributed 51.7 million tones or about 36% of the total world production [16]. The growth rate of worldwide aquaculture has been sustained and rapid, averaging about 8% per annum for over thirty years, while the contribution to the total from wild fisheries has been essentially flat for the last decade.

China is by far the largest producer of aquaculture products, accounting in 2006, 66.7% of the worldwide aquaculture output, with the rest of the Asia and Pacific region accounting for another 22.8% of the production, and the remaining 10.5% being distributed between Europe, Africa, the Middle East, and North and South America. In

China, the cyprinids (including carp) are the dominant farmed fish, while in Western Europe the salmonid (including Atlantic salmon) hold that position, and in North America the dominant aquaculture product is the catfish.

The contribution of aquaculture to global supplies of fish, crustaceans, molluscs and other aquatic animals 3 has continued to grow, increasing from 3.9% of total production by weight in 1970 to 36.0 % in 2006. In the same period, production from aquaculture easily outpaced population growth, with per capita supply from aquaculture increasing from 0.7 kg in 1970 to 7.8 kg in 2006, an average annual growth rate of 7.0%. Aquaculture accounted for 47 % of the world's fish food supply in 2006 (Table 1.5). In China, 90 % of fish food production comes from aquaculture (2006). This indicates that aquaculture production in the rest of the world accounts for 24 % of food fish supply [16].

In 2006, China contributed 67 % of the world's supply of cultured aquatic animals and 72 % of its supply of aquatic plants [16].

World aquaculture has grown dramatically in the last 50 years. From a production of less than 1 million tones in the early 1950s, production in 2006 was reported to have risen to 51.7 million tones, with a value of US$78.8 billion. This means that aquaculture continues to grow more rapidly than other animal food-producing sectors.

Table 1.5 World Fisheries and Aquaculture Production[16].

Production (Million tons)	**2002**	**2003**	**2004**	**2005**	**2006**
Inland					
Capture	8.7	9.0	8.9	9.7	10.1
Aquaculture	24.0	25.5	27.8	29.6	31.6
Total inland	32.7	34.4	36.7	39.3	41.7
Marine					
Capture	84.5	81.5	85.7	84.5	81.9
Aquaculture	16.4	17.2	18.1	18.9	20.1
Total marine	100.9	98.7	103.8	103.4	102.0
Total capture	93.2	90.5	94.6	94.2	92.0
Total aquaculture	40.4	42.7	45.9	48.5	51.7
Total World Fisheries	133.6	133.2	140.5	142.7	143.6

Table 1.6 refers to world production of fish products (excluding seaweed and marine mammals) is estimated to have reached 141.6 million tons in 2008, a slight increase over 2007, driven by a 2.5% expansion in aquaculture to 51.6 million tons, while capture fisheries remained stable around 90 million tons. Based on current estimates, aquaculture products contributed 45% of total food fish supply [16].

Table 1.6 World Aquaculture Production [16].

	2007	2008	2009	%
		Million tons		
WORLD BALANCE				
Production	**140.4**	**141.6**	**142.0**	**0.3**
Capture fisheries	90.1	90.0	90.0	-0.0
Aquaculture	50.3	51.6	52.0	0.8
Trade value (exports billion USD)	92.8	99.5	98.0	-1.5
Trade volume(live weight)	52.9	52.6	52.0	-0.1
Total utilization				
Food	112.8	113.9	114.4	0.4
Feed	20.8	20.6	20.4	-1.0
Other uses	6.8	7.1	7.2	
Supply and Demand Indicators				
Per caput food/consumption				
Food fish (kg/year)	16.9	16.9	16.8	-0.3
From capture fisheries (kg/year)	9.4	9.3	9.2	-1.3
From aquaculture (kg/year)	7.5	7.6	7.6	1.0

1.4 RAINBOW TROUT (*Oncorhynchus mykiss*)

The rainbow trout (*Oncorhynchus mykiss*) is a species of salmonid native to tributaries of the Pacific Ocean in Asia and North America as well as much of the central, western, eastern, and especially the northern portions of the United States. The species was originally named by Johann Julius Walbaum in 1792 based on type specimens from Kamchatka. Rainbow trout prefer clear, cool, high quality water [17].

Rainbow trout, also called redband trout, are gorgeous fish, with coloring and patterns that vary widely depending on habitat, age, and spawning condition. They are torpedo-shaped and generally blue-green or yellow-green in color with a pink streak along their sides, white underbelly, and small black spots on their back and fins [18].

Rainbows may live up to 11 years, but the usual life span is four to six years. Life cycle related information is summarized in Table 1.11. Growth is highly variable, depending on the habitat. A typical stream-dwelling rainbow grows to weigh about 0.5 kg in four years. Efforts are being made by fishery managers to improve habitat so more wild trout can be produced, and fewer fish will be raised in hatcheries [19].

According to the water temperature, trout is raised to portion size starting from 7 months to 12 months in Turkey. The portion size differs from 170 g to 250 g. When they are 80-100 g, the trouts raised in the Black Sea region are put in net cages in the sea (in late September and early October). They are fed in these cages till they are 600-1000 g in the following period of 7-8 months. As the water temperature increases in June, the fish are taken from the cages and they are ready for consumption. The matured and unsold stock is transferred back to the inland ponds [14].

1.4.1. Habitat Requirements

Cold headwaters, creeks, small to large rivers, cool lakes, estuaries, and oceans comprise the habitats collectively used by the different populations of rainbow trout. Depending on the genetic makeup of a trout population and the habitat conditions, rainbow trout will use some or all of these aquatic habitats during their lives as indicated in Table 1.10 [20].

Prime trout waters are clear, clean and cold. Good trout stream habitat is complex, consisting of an array of riffles and pools, submerged wood, boulders, undercut banks, and aquatic vegetation. The ability to swim to and from different habitats from ocean to headwaters, or from tributary confluence to headwaters, increases the value of individual habitat components. Assuring fish passage through artificial barriers in a system of connected habitats greatly enhances the capability of an aquatic system to sustain rainbow trout populations.

1.4.2. Food

Rainbow trout's are opportunistic feeders that rely on a wide variety of food items ranging from small insects to crayfish. Trout inhabiting streams with a significant amount of riparian vegetation often feed heavily on terrestrial insects, such as grasshoppers and ants that fall into the stream. Rocky stream riffles produce bottom-

dwelling aquatic invertebrates, such as insects and crustaceans that are also fed upon. In lakes and streams, invertebrates such as plankton, crustaceans, snails, and leeches, as well as small fish and fish eggs also serve as food for trout [20].

Rainbow trout are predators with a varied diet, and will eat nearly anything they can grab, in contrast to the legendary, selective image people often have of the fish's dietary habits. Rainbows are not quite as piscivorous or aggressive as the brown trout or lake trout. When young, insects make up a large portion of the diet, smaller fish (up to 1/3 of their length), along with crayfish and other crustaceans make up the remainder. As they grow, though, the proportion of fish increases in most all populations. Some lake dwelling lines may become planktonic feeders. While in flowing waters populated with salmon, trout will eat salmon eggs, salmon fry, and even salmon carcasses.

Commercial trout food related information is given in Tables 1.7-1.9.

Table 1.7 Commercial trout diet ingredients with percentages [21].

Ingredients	**Percentage**
Protein	45.0 % (min)
Oil	20.0 % (min)
Humidity	8.5 % (max)
Ash	11.0 % (max)
Cellulose	3.0 % (max)
NFE	12.5 % (max)
Calcium	1.0 – 3.0 % (min – max)
Phosphorus	1.5 % (min)

Table 1.8 Energy values of commercial trout diets [21].

Energy	**Amount**
GE (Gross energy)	5124 kcal/kg (min)
DE (Digested energy)	4125 kcal/kg (min)
ME (Metabolism energy)	3742 kcal/kg (min)

Table 1.9 Vitamins in commercial trout diets [21].

Vitamins	**Amount**
Vitamin A	5.000 IU/kg
Vitamin D	1.500 IU/kg
Vitamin E	100 mg/kg
Vitamin K	20 mg/kg

fish	**age**	**length**	**wt**	**name**	**habitat**	**life style**
Embryonic stage	0	4mm dia	.01g	Egg micropyle yolk	hatchery	Eggs and ovarian fluid from 3 females are stripped into a bowl (containing no water, so that the micropyles remain open for longer). Milt from a male fish is stripped into the bowl and thoroughly mixed with the eggs.
	1-2 sec	4mm dia	.01g	green egg	hatchery	The head of the sperm makes its entry through the micropyle, the tail which has propelled it, being left outside. The nuclei of egg and sperm fuse and this single cell immediately divides into two.
	14 days	4mm dia	.01g	eyed egg	hatchery	The embryo has developed sufficiently for the eyes to be seen as two black dots.
hatching stage	30-100 days	5mm dia	.01g	hatching egg	hatchery	°C Temperature Days to Hatch The time of hatching depends on the water temperature. An enzyme is secreted which softens the eggshell and allows the alevin to break through.
larval stage	30-100 days	16mm	-	alevin	hatchery	When hatched the alevin retains its yolk sac and this may be referred to as the SWIM-UP stage.
	further 1 month	26mm	-	alevin	hatchery	Yolk has been absorbed and the alevin is fed on a high protein diet.
juvenile	further 1 month	-	-	fry	hatchery	Gradually acquire characteristic body markings of bluish or purple colour on the back and 7-11 oval spots of the same colour (parr marks) along the middle of each side.
rainbow trout	3-4 months	>10cm	-	fingerlings	hatchery	May - September are active feeding months. The fish are fed on high protein pellets.
	20 months	**30cm**	**250g**	**'portion size'**	**hatchery**	**Supermarket fish**
	24 months	25-45cm	1Kg	mature male	freshwater	Put-and-take fishery trout are usually infertile.
	36 months	25-45cm	1Kg	mature female		
	5-6 years	45cm	3Kg	normal lifespan	freshwater	Mature trout released into the reservoirs at the 36 month stage will grow on to about 8lb.
				record	freshwater	The record-size trout are specially bred for the purpose

Table 1.10 Life Cycle of Rainbow Trout [22].

1.4.3 *Oncorhynchus mykiss* Taxonomy

Taxonavigation of the Oncorhynchus mykiss is given bellow [23].

Cladus: Eukaryota
Supergroup: Opisthokonta
Regnum: Animalia
Subregnum: Eumetazoa
Cladus: Bilateria
Cladus: Nephrozoa
Cladus: Deuterostomia
Phylum: Chordata
Subphylum: Vertebrata
Infraphylum: Gnathostomata
Superclassis: Osteichthyes
Classis: Actinopterygii
Subclassis: Neopterygii
Infraclassis: Teleostei
Superordo: Protacanthopterygii
Ordo: Salmoniformes
Familia: Salmonidae
Subfamilia: Salmoninae
Genus: *Oncorhynchus*
Species: *Oncorhynchus mykiss*
Subspecies*: O. m. aguabonita* - *O. m.gairdnerii* - *O. m. gilberti* - *O. m. irideus* - *O. m. mykiss* - *O. m. nelsoni* - *O. m. stonei* - *O. m. whitei*

1.5 DNA BASED WORK ON AQUACULTURE

The development of DNA-based genetic markers has had a revolutionary impact on animal genetics. With DNA markers, it is theoretically possible to observe and exploit genetic variation in the entire genome. Popular genetic markers in the aquaculture community include allozymes, mitochondrial DNA, RFLP, RAPD, AFLP, microsatellite, SNP, and EST markers. The application of DNA markers has allowed rapid progress in aquaculture investigations of genetic variability and inbreeding, parentage assignments, species and strain identification, and the construction of high-resolution genetic linkage maps for aquaculture species [24].

Several marker types are highly popular in aquaculture genetics. In the past, allozyme and mtDNA markers have been popular in aquaculture genetics research. More recent marker types that are finding service in this field include restriction fragment length polymorphism (RFLP), randomly amplified polymorphic DNA (RAPD), amplified fragment length polymorphism (AFLP), microsatellite, single nucleotide polymorphism (SNP), and expressed sequence tag (EST) markers. Table 1.12 summarizes the basic properties of these marker types, and each is discussed in detail below.

Table 1.11 Types of DNA markers, their characteristic and potential applications [24].

Marker type	Acronym or alias	Requires prior molecular information	Mode of inheritance	Locus under investigation	Likely allele numbers	Major applications
Allozyme		Yes	Mendelian codominant	Single	2-6	Linkage mapping
Mitochondrial DNA	mtDNA	No	Metarnal İnheritance		Multiple haplotypes	Maternal lineage
Restriction fragment length polymorphism	RLFP	Yes	Mendelian codominant	Single	2	Linkage mapping
Random amplified DNA	RAPD AP-PCR	No	Mendelian dominant	Multiple	2	Fingerprinting for population studies Hybrid identification
Amplified fragment length polymorphism	AFLP	No	Mendelian dominant	Multiple	2	Linkage mapping Population studies
Microsatellites	SSR	Yes	Mendelian codominant	Single	Multiple	Linkage mapping Population studies

Molecular markers are classified into two categories: type I are markers associated with genes of known function, while type II markers are associated with anonymous genomic segments (Table1.12). Under this classification, most RFLP markers are type I markers because they were identified during analysis of known genes. Likewise, allozymes markers are type I markers because the protein they encode has known function. RAPD markers are type II markers because RAPD bands are amplified from anonymous genomic regions via the polymerase chain reaction (PCR). AFLP markers are type II because they are also amplified from anonymous genomic

regions. Microsatellite markers are type II markers unless they are associated with genes of known function. EST markers are type I markers because they represent transcripts of genes. SNP markers are mostly type II markers unless they are developed from expressed sequences (eSNP or cSNP). Indels are becoming more widely used as markers since they often are discovered during genomic or transcriptomic sequencing projects; they can be either type I or type II markers depending on whether they are located in genes [24].

1.5.1 Applications of DNA Markers in Aquaculture Genetics

One of the questions at the beginning of any genome research is what type of marker is most suitable for the species of interest. There is no simple answer to this question, and much depends on the specific objectives of the study (Table 1.13). However, with a good understanding of the DNA marker technologies, appropriate decisions can be reached.

Table 1.12 Applications of DNA Markers in Aquaculture Genetics [24].

Task	Recommended Marker System	Other Useful Marker Types
Species identification	RAPD	AFLP, microsatellites, isozymes
Strain identification	AFLP, microsatellites	RAPD
Hybrid identification	RAPD	AFLP, microsatellites, mitochondria
Paternity determination	Microsatellites	
Genetic resource	AFLP, microsatellites	RAPD
Genetic mapping	Type I markers,	
	Microsatellites SNP	AFLP, RFLP
Comparative mapping	Type I markers	ESTs, conserved microsatellites

1.6 GENETIC DIVERSITY

Genetic diversity is a level of biodiversity that refers to the total number of genetic characteristics in the genetic makeup of a species. It is distinguished from genetic variability, which describes the tendency of genetic characteristics to vary.

The academic field of population genetics includes several hypotheses and theories regarding genetic diversity. The neutral theory of evolution proposes that diversity is the result of the accumulation of neutral substitutions. Diversifying selection is the hypothesis that two subpopulations of a species live in different environments that select for different alleles at a particular locus. This may occur, for instance, if a species has a large range relative to the mobility of individuals within it. Frequency-dependent selection is the hypothesis become more common, they become less fit. This is often invoked in host-pathogen interactions, where a high frequency of a defensive allele among the host means that it is more likely that a pathogen will spread if it is able to overcome that allele.

There are many different ways to measure genetic diversity. Many works for the loss of animal genetic diversity have been done [25, 26]. A 2007 study conducted by the National Science Foundation found that genetic diversity and biodiversity are dependent upon each other, that diversity within a species is necessary to maintain diversity among species, and vice versa. According to the lead researcher in the study, Dr. Richard Lankau, "If any one type is removed from the system, the cycle can break down, and the community becomes dominated by a single species"[27].

Genetic diversity plays a very important role in survival and adaptability of a species because when the environment of a species changes, slight gene variations are necessary for it to adapt and survive. A species that has a large degree of genetic diversity among its population will have more variations from which to choose the fittest alleles. Species that have very little genetic variation are at a great risk. With very little gene variation within the species, healthy reproduction becomes increasingly difficult, and offspring often deal with similar problems to those of inbreeding [28].

Genetic diversity refers to the variation at the level of individual genes (polymorphism), and provides a mechanism for populations to adapt to their ever-

changing environment. The more variation, the better the chance that at least some of the individual genetic diversity refers to any variation in the nucleotides, genes, chromosomes, or whole genomes of organisms (the genome is the entire complement of DNA within the cells or organelles of the organism). Genetic diversity at its most elementary level is represented by differences in the sequences of nucleotides (adenine, cytosine, guanine, and thymine) that form the DNA (deoxyribonucleic acid) within the cells of the organism. The DNA is contained in the chromosomes present within the cell; some chromosomes are contained within specific organelles in the cell (for example, the chromosomes of mitochondria and chloroplast). Nucleotide variation is measured for discrete sections of the chromosomes, called genes. Thus, each gene compromises a hereditary section of DNA that occupies a specific place of the chromosome, and controls a particular characteristic of an organism. Individuals will have an allelic variant that is suited for the new environment, and will produce offspring with the variant that will in turn reproduce and continue the population into subsequent generations [29].

Most organisms are diploid, having two sets of chromosomes, and therefore two copies (called alleles) of each gene. However, some organisms can be haploid, triploid, or tetraploid (having one, three, or four sets of chromosomes respectively). Within any single organism, there may be variation between the two (or more) alleles for each gene. This variation is introduced either through mutation of one of the alleles, or as a result of sexual reproduction. During sexual reproduction, offspring inherit alleles from both parents and these alleles might be slightly different, especially if there has been migration or hybridization of organisms, so that the parents may come from different populations and gene pools. Also, when the offspring's chromosomes are copied after fertilization, genes can be exchanged in a process called sexual recombination. Harmless mutations and sexual recombination may allow the evolution of new characteristics.

Each allele codes for the production of amino acids that string together to form proteins. Thus differences in the nucleotide sequences of alleles result in the production of slightly different strings of amino acids or variant forms of the proteins. These proteins code for the development of the anatomical and physiological characteristics of

the organism, which are also responsible for determining aspects of the behavior of the organism.

Different species can have different numbers of genes within the entire DNA or genome of the organism. However, a greater total number of genes might not correspond with a greater observable complexity in the anatomy and physiology of the organism (i.e. greater phenotypic complexity). For example, the predicted size of the human genome is not much larger than the genomes of some invertebrates and plants, and may even be smaller than the Indian rice genome in humans; more proteins are encoded per gene than in other species [30].

1.6.1 Genetic Diversity in Marine Species

1.6.1.1 Measuring Genetic Diversity

The rapid advances in molecular biology have provided a range of techniques for direct examination of variation in DNA. To date most populations studies have used restriction fragment length polymorphisms of the mitochondrial genome. The mitochondrial (mt) DNA is small and relatively easy to purify, and the fragments generated with restriction enzyme digests are easy to interpret. Variations in fragment numbers are generated by additions and deletions of restriction sites, and in fragment lengths by insertions or deletions of blocks of bases. Similar techniques can be applied to nuclear (n) DNA, but the considerably larger size of nDNA means that small pieces of the genome have to be analyzed with specific probes. Several regions of the nuclear genome contain multiple repeats of short minisatellite sequences which are resolved as DNA fingerprints. The hypervariable nature of these variable number tandem repeats has lead to widespread use of DNA fingerprinting in forensic studies, but the technique has had limited application in marine population studies [31].

The development of the polymerase chain reaction, PCR, method has provided the means to amplify small fragments of the genome. With appropriate size primers the method can be used to screen for genetic variation in individuals and populations, alternatively amplified fragments can be sequenced. Application of these new genetic methods may produce new insights into the genetic structure of natural populations, as did protein electrophoresis in the 1970s and 1980s, although to date the methods have not been used widely with marine species.

Karyological methods can be used to measure genetic variation, either as chromosome number or banding polymorphisms. The techniques are laborious in comparison with electrophoretic techniques and require the use of live fish for chromosome preparations, thereby reducing their potential application with many marine species.

Morphological characters, the tools of traditional taxonomy, have been used to describe variation among individuals and populations. The characters used are meristic (countable) such as number of fin rays or vertebrae and morphometric (measurable) expressed as ratios of standard length or fork length. Morphological characters have limitations for describing intraspecific genetic diversity as they are polygenic and expression can be modified by the environment. Their use in population-stock identification studies has been superseded for the most part by the development of direct genetic methods.

1.6.2 Levels of Genetic Diversity

Invertebrates generally have higher levels of genetic diversity than vertebrates as measured by protein electrophoresis [32]. Within the vertebrates amphibia have the highest and teleosts have the lowest levels of genetic diversity [33]. Marine invertebrates show wide variation in levels of genetic diversity. In 26 species of molluscs, heterozygosities range from 2 to 32 %. Crustacea have lower levels of genetic diversity ranging from 0.4 to 10.9 % in 44 species of decapod, from 0.8 to 6.4%.

1.6.3 Pollution Induced Genetic Changes

The effects of pollution on coastal resources are often dramatic with mass mortalities in local stocks, reduction in species diversity, and changes in species composition. Local areas may be closed to harvesting. Sources of pollution include heavy metals, pesticides, oils and detergents, and thermal and radioactive discharges. There are limited reports of genetic changes due to marine pollution, in part because of the difficulty of measuring genetic changes in fish populations in which recruitment can be from outside the polluted area. Most examples of pollution induced genetic changes are for species with limited dispersal abilities; molluscs may be recruited from outside the area of pollution but the juvenile and adult stages are sessile.

The extensive studies of Nevo and coworkers on genetics of pollution in the Mediterranean have shown that genetic changes occur in natural populations of marine organisms exposed to local pollution events [34]. As a result genetic markers have been proposed as a monitoring tool for marine pollution. Laboratory studies on mollusks and crustacea have demonstrated differential survival of allozyme genotypes exposed to heavy metals. Similar changes in gene frequencies have been detected in marine organisms exposed to crude-oil.

In laboratory tests on pairs of species exposed to marine pollutants those species with the higher level of genetic diversity showed greater survival.

1.7 GENETIC VARIATION

Genetic variability is a measure of the tendency of genotypes within a population to differentiate. Individuals of the same species are not identical. Although they are recognizable as belonging to the same species, there are many differences in form, function and behavior. For every characteristic of an organism, variations will exist within the species. For example, the jaguars of the Pantanal in Brazil are more than twice the size (100 kg) of Mexican jaguars (30 to 50 kg) yet they are the same species (*Panthera onca)* [35].

Variability is different from genetic diversity, which is the amount of variation seen in a particular population [36]. The variability of a trait describes how much that trait tends to vary in response to environmental and genetic influences. Genetic variability in a population is important for biodiversity, because without variability, it becomes difficult for a population to adapt to environmental changes and therefore makes it more prone to extinction. Variability is an important factor in evolution as it affects an individual's response to environmental stress and thus can lead to differential survival of organisms within a population due to natural selection of the most fit variants.

The most obvious cases of genetic variability are to be found in the domesticated species, where humans use the variability to create breeds and varieties of maize, beans, apples, pumpkins, horses, cattle, sheep, dogs and cats, among others [35].

Much of the variation in individuals comes from the genes, i.e., from genetic variability. This variability is caused by mutations, recombination's, and alterations in the karyotype (the number, shape, size and internal organization of the chromosomes). The processes that produce or eliminate genetic variability are called natural selection and genetic drift [35].

Genetic variability permits the evolution of species, since in each generation only a fraction of the population survives and reproduces transmitting particular characteristics to their offspring.

There are many sources of genetic variability in a population:

Homologous recombination is a significant source of variability. During meiosis in sexual organisms, two homologous chromosomes from the male and female parents cross over one another and exchange genetic material. The chromosomes then split apart and are ready to form an offspring. Chromosomal crossover is random and is governed by its own set of genes that code for where crossovers can occur (in cis) and for the mechanism behind the exchange of DNA chunks (in trans). Being controlled by genes means that recombination is also variable in frequency, location, thus it can be selected to increase fitness by nature, because the more recombination the more variability and the more variability the easier it is for the population to handle changes [37].

Immigration, emigration, and translocation – each of these is the movement of an individual into or out of a population. When an individual comes from a previously genetically isolated population into a new one it will increase the genetic variability of the next generation if it reproduces [38].

Polyploidy – having more than two homologous chromosomes allows for even more recombination during meiosis allowing for even more genetic variability in one's offspring.

Diffuse centromeres – in asexual organisms where the offspring is an exact genetic copy of the parent, there are limited sources of genetic variability. One thing that increased variability, however, is having diffused instead of localized centromeres.

Being diffused allows the chromatids to split apart in many different ways allowing for chromosome fragmentation and polyploidy creating more variability [39].

Genetic mutations – contribute to the genetic variability within a population and can have positive, negative, or neutral effects on fitness. This variability can be easily propagated throughout a population by natural selection if the mutation increases the affected individual's fitness and its effects will be minimized if the mutation is deleterious. However, the smaller a population and its genetic variability are, the more likely the recessive/hidden deleterious mutations will show up causing genetic drift [40].

1.7.1 Epigenetics and its molecular basis

In biology, the term epigenetics refers to changes in phenotype (appearance) or gene expression caused by mechanisms other than changes in the underlying DNA sequence. These changes may remain through cell divisions for the remainder of the cell's life and may also last for multiple generations. However, there is no change in the underlying DNA sequence of the organism; [41] instead, non-genetic factors cause the organism's genes to behave (or "express themselves") differently [42]. The best example of epigenetic changes in eukaryotic biology is the process of cellular differentiation. During morphogenesis, totipotent, stem cells become the various pluripotent cell lines of the embryo which in turn become fully differentiated cells. In other words, a single fertilized egg cell - the zygote - changes into the many cell types including neurons, muscle cells, epithelium, blood vessels et cetera as it continues to divide. It does so by activating some genes while inhibiting others [43].

The molecular basis of epigenetics is complex. It involves modifications of the activation of certain genes, but not the basic structure of DNA. Additionally, the chromatin proteins associated with DNA may be activated or silenced. This accounts for why the differentiated cells in a multi-cellular organism express only the genes that are necessary for their own activity. Epigenetic changes are preserved when cells divide. Most epigenetic changes only occur within the course of one individual organism's lifetime, but some epigenetic changes are inherited from one generation to the next [44]. Specific epigenetic processes include paramutation, bookmarking, imprinting, gene

silencing, X chromosome inactivation, position effect, reprogramming, transvection, maternal effects, the progress of carcinogenesis, many effects of teratogens, regulation of histone modifications and heterochromatin, and technical limitations affecting parthenogenesis and cloning.

1.7.2 Mosaicism

In genetic medicine, a mosaic or mosaicism denotes the presence of two populations of cells with different genotypes in one individual, who has developed from a single fertilized egg [45]. Mosaicism may result from a mutation during development which is propagated to only a subset of the adult cells.

Different types of mosaicism exist, such as gonadal mosaicism (restricted to the gametes) or tissue mosaicism; one of them is chimerism, where two or more genotypes arise from the fusion of more than one fertilized zygote in the early stages of embryonal development. In the more common mosaics, different genotypes arise from only a single fertilized egg cell, due to mitotic errors.

Post-zygotic mutations produce mosaics with two (or more) genetically distinct cell lines. The older literature on human mosaicism refers only to chromosomal mosaicism, because that was the only type of mosaicism that could be detected before DNA analysis was developed, but mosaicism for single gene mutations is at least as frequent and important.

1.7.2.1 Somatic Mosaicism

The phenomenon of somatic implies the presence of genetically different cell lines in a single organism. The differences between the lines may be caused by mutations, chromosomal rearrangements, modification in mini-and microsatellite loci at DNA replications, the loss of heterozygosity through mitotic recombination, and different epigenetic factors [46].

Somatic mosaicism, the presence of genetically distinct populations of somatic cells in a given organism is frequently masked, but it can also result in major

phenotypic changes and reveal the expression of otherwise lethal genetic mutations. Mosaicism can be caused by DNA mutations, epigenetic alterations of DNA, chromosomal abnormalities and the spontaneous reversion of inherited mutations. In this review, we discuss the human disorders that result from somatic mosaicism, as well as the molecular genetic mechanisms by which they arise. Specifically, we emphasize the role of selection in the phenotypic manifestations of mosaicism [47].

1.7.2.2 Gonadal Mosaicism

Gonadal mosaicism or Germline Mosaicism is a special form of mosaicism, where some gametes, i.e. either sperm or oocytes, carry a mutation, but the rest are normal. The cause is usually a mutation that occurred in an early stem cell that gave rise to all or part of the gonadal tissue. This can cause only some children to be affected, even for a dominant disease.

1.7.3 Threats to Global Biodiversity - Inbreeding and Genetic Deterioration

Genetic deterioration is likely to become an important issue in the future, given the effects of fragmentation and over-exploitation of wild populations. Whilst many believe that inbreeding depression and reduced heterozygosity are important features of a populations slide to extinction, they have rarely been shown conclusively in wild populations. However, a firm link between inbreeding and increased risk of extinction has recently been shown in natural populations of the Glanville fritillary butterfly (*Melitaea cinxia*).

"We found that extinction risk increased significantly with decreasing heterozygosity, an indication of inbreeding, even after accounting for the effects of relevant ecological factors."

This gives further evidence of the potential dangers of human-caused fragmentation of habitats, and suggests that the status of currently stable small populations of animals may deteriorate as the effects of inbreeding depression are realized. Current declines to genetic diversity are restricting the future prospects of

genetic engineering for advanced crops and medical science, which may be crucial for the maintenance and welfare of mankind's ever increasing population.

1.7.4 Methods in Molecular Evaluation of Genetic Diversity

1.7.4.1 RAPD-PCR

RAPD, developed by Williams et al. (1990) and Welsh and McClelland (1990), is a PCR-based technique that amplifies DNA fragments of genomic DNA with single short primers of arbitrary nucleotide sequence under low annealing conditions. This technique is used extensively for species classification, genetic mapping and phylogeny etc. In addition, their use in surveying genomic DNA for evidence of various types of DNA damage and mutation shows that RAPD may potentially form the basis of novel biomarker assays for the detection of DNA damage and mutational events (e.g. rearrangements, point mutation, small insert or deletions of DNA and ploidy changes) in cells of bacteria, plants, invertebrate and vertebrate animals [48].

Recent advances in molecular biology enabled the development of an assessment technique that has the potential of identifying both acute and chronic effects of pollution earlier than other presently used methods. This method uses RAPD-PCR to generate genetic profiles of individuals within populations. The combined analysis of the RAPD profiles can provide useful information regarding species endemic to sites impacted by pollution. RAPD profiles have proven effective in determining the overall genetic diversity levels harbored within populations of both aquatic and terrestrial species. By analyzing the genetic health of endemic populations, this protocol is capable of identifying populations at risk prior to their local disappearance. RAPD profiling is continuing to gain acceptance by the scientific community. However, continued refinement of the method is required to establish RAPD profiling as a standard bioindicator of environmental contamination.

1.7.5 Applications of Molecular Methods in Environmental Problems

Ecological Risk Assessment by RAPDs, RFLPs, allozymes as bioindicators of ecosystem health. Toxicogenetics, toxicogenomics, ecogenetics, proteomics are newly

emerging fields of molecular biology that are intensely related with especially environmental pollution problems.

There is a great concern about the effect of environmental contaminants on the genetic make-up of natural populations. One class of genetic effects includes alterations to the structure and function of DNA including DNA adducts DNA breakage, and mutations as a result of chemical exposure (genotoxic effects). However, indirect genetic effects can also arise as a consequence of the interactions of genotoxic agents with DNA. The RAPD and similar Technologies have been used to detect not only DNA damage and mutations but also changes in genetic diversity and gene frequencies. However, most of the studies indicate that the observed changes in RAPD profiles which occurred among the exposed population were the result of either DNA damage and mutations or population genetic effects [49].

The RAPD method was used to detect genetic diversity among populations which had been exposed to environmental contaminants, including well-known genotoxins. It was reported that fish populations in the contaminated sites were consistently less genetically distant from each other than they were from each of the reference sites [50].

CHAPTER 2

MATERIALS & METHODS

2.1 MATERIALS

2.1.1 General Reagents

All laboratory chemicals were analytical grade from Sigma Biosciences Chemical Company (USA), Merck (Germany), Applichem (Germany) and Fluka (Germany).

2.1.2 Equipment

Autoclave	: CERTO CLAW A-4050 Traun, Austria
Balance	: Sartorius, Wender Landstrasse 94-108 D-37075
Goettingen,	Germany
Centrifuge	: Hettich, Mikro 22
Electrophoresis Equipment	: Bio-Rad Sub Cell, GT
Power Supplies	: Bio-Rad Power PAC-300
Thermocyclers	: TECHNE TC-512
Transilluminator	: Bio-Rad GelDoc 2000
Vortex	: IKA LABOTECHNIK
Water Purification System	: Millipore

Capillary electrophoresis systems : *QIAGEN*

2.1.3 Fish Samples

Fish culture samples were collected from different locations in Turkey. In the thesis 5 different sampling sites have been used (Table 2.1). These aquaculture places are in Kahramanmaraş and Sakarya.

Table 2.1 Fish sampling sites and sample information.

Location of Facility	Facility	Size	DNA Sample Name	Food
Sakarya (Hendek)	Sakarya	17 cm	OM - 3	Pınar
Sakarya (Hendek)	Sakarya	18 cm	OM - 4	Pınar
Sakarya (Hendek)	Sakarya	22 cm	OM - 5	Pınar
Sakarya (Hendek)	Sakarya	26 cm	OM - 6	Pınar
Sakarya (Akyazı)	Altındere	3.5 cm	OM - 7	Ecobio
Sakarya (Akyazı)	Altindere	15.5 cm	OM - 8	Ecobio
Sakarya (Akyazı)	Altindere	26 cm	OM - 9	Ecobio
Sakarya (Adapazarı)	Burnaz	10 cm	OM – 10	Pınar
Sakarya (Adapazarı)	Burnaz	19 cm	OM - 11	Pınar
Sakarya (Adapazarı)	Burnaz	26 cm	OM -12	Pınar
Kahramanmaraş(Centre)	Gökkuşağı	18 cm	OM – 13	Ecobio
Kahramanmaraş(Centre)	Gökkuşağı	21 cm	OM - 14	Ecobio
Kahramanmaraş(Centre)	Gökkuşağı	24 cm	OM – 15	Ecobio
Kahramanmaraş(Centre)	Gökkuşağı	28 cm	OM - 16	Ecobio
Kahramanmaraş(Centre)	Çağlayan	19 cm	OM - 18	Pınar
Kahramanmaraş(Centre)	Çağlayan	24 cm	OM - 19	Pınar
Kahramanmaraş(Centre)	Çağlayan	27 cm	OM - 20	Pınar

Table 2.2 Samples according to their food (live or commercial pellet).

Location of Facility	Fish Name	DNA Sample Name	Size	FOOD
Kahramanmaraş	Çağlayan	OM - 21	24 cm	Live Feed
Kahramanmaraş	Çağlayan	OM - 22	25 cm	Live Feed
Kahramanmaraş	Çağlayan	OM - 23	26 cm	Live Feed
Kahramanmaraş	Çağlayan	OM - 24	23 cm	Ecobio
Kahramanmaraş	Çağlayan	OM - 25	24 cm	Ecobio
Kahramanmaraş	Çağlayan	OM - 26	25 cm	Ecobio

Table 2.3 Samples obtained from liver tissues of indicated fish.

Location of facility	**Fish Name**	**Size (cm)**	**Tissue Type**	**DNA sample name**
Sakarya (Adapazarı)	Burnaz OM - 10	10 cm	Liver	OM - 27
Kahramanmaraş	Gökkuşağı OM- 13	18 cm	Liver	OM - 28
Sakarya (Hendek)	Sakarya OM - 5	22 cm	Liver	OM - 29
Sakarya (Akyazı)	Altındere OM - 9	26 cm	Liver	OM - 30
Kahramanmaraş	Gökkuşağı OM -16	28 cm	Liver	OM - 31

Table 2.4 Samples obtained from gonad tissues of indicated fish.

Location of facility	Fish Name	Size (cm)	Tissue Type	DNA sample name
Sakarya (Adapazarı)	Burnaz OM - 10	10 cm	GONAD	OM - 32
Kahramanmaraş	Gökkuşağı OM - 13	18 cm	GONAD	OM - 33
Sakarya (Hendek)	Sakarya OM - 5	22 cm	GONAD	OM - 34
Sakarya (Akyazı)	Altındere OM - 9	26 cm	GONAD	OM - 35
Kahramanmaraş	Gökkuşağı OM - 16	28 cm	GONAD	OM - 36

2.1.4 DNA Isolation Reagents

Macherey Nagel Nucleospin Tissue Kit components are used in DNA isolation as stated below:
Lysis Buffer, Buffer B1, Buffer B2, Wash Buffer B5, Wash Buffer BW, Elution Buffer BE, Proteinase K (lyophilized), Proteinase Buffer PB.

2.1.5 RAPD-PCR Reagents

PCR kit component (Fermentas):

10X Taq Buffer with $(NH_4)_2SO_4$	750mM Tris-HCl (pH 8.8 at 25°C), 200mM $(NH_4)_2SO_4$, 0.1% Tween20.
$MgCl_2$	25mM $MgCl_2$
dNTP mix	1.5ml of 2mM aqueous solution of

	each dGTP, dATP, dCTP, dTTP
$MgCl_2$	25 mM
Primers	10 pmol/reaction
Taq DNA Polymerase	5U/µl

2.1.6 Polymerase Chain Reaction Reagents

Polymerase chain reaction kit was from Fermentas, Germany. Primers used were synthesized by IONTEK (Istanbul, TURKEY). Sequences of the primers used are given in Table 2.5.

Table 2.5 Specific base sequences of the Operon Technologies' 10-mer primers.

Primer	**Sequncece 5´to 3'**	**Primer**	**Sequncece 5´to 3'**
OPA 01	CAGGCCCTTC	OPB 01	GTTTCGCTCC
OPA 02	TGCCGAGCTG	OPB 02	TGATCCCTGG
OPA 03	AGTCAGCCAC	OPB 03	CATCCCCCTG
OPA 04	AATCGGGCTG	OPB 04	GGACTGGAGT
OPA 05	AGGGGTCTTG	OPB 05	TGCGCCCTTC
OPA 06	GGTCCCTGAC	OPB 06	TGCTCTGCCC
OPA 07	GAAACGGGTG	OPB 07	GGTGACGCAG
OPA 08	GTGACGTAGG	OPB 08	GTCCACACGG
OPA 09	GGGTAACGCC	OPB 09	TGGGGGACTC
OPA 10	GTGATCGCAG	OPB 10	CTGCTGGGAC
		OPB 18	CCACAGCAGT

2.1.7 Agarose Gel Electrophoresis

10X TBE	For 1 Liters 108g Tris base, 55g boric acid, 40 ml 0.5M EDTA (pH 8.0), autoclave for 20 min
6X Loading Dye	0.5 mM Tris-HCl (pH 7.6), 0.03%

	bromophenol blue, 0.03% xylene cyanol FF, 60 % glycerol, 1 mM EDTA.
GeneRuler™ 100 bp DNA Ladder	100 µl (0.5 µg/µl) 100 bp sized DNA fragments in 10mM Tris-HCl (pH 7.6), 1mM EDTA.

2.1.8 DNA Size Marker

Fast Ruler™ DNA Ladder, Low Range:

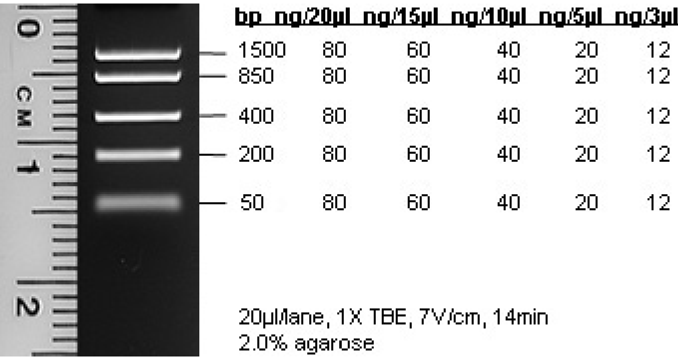


Figure 2.1 Information of Fast Ruler DNA Size Marker

Bioron 100 bp DNA Ladder:

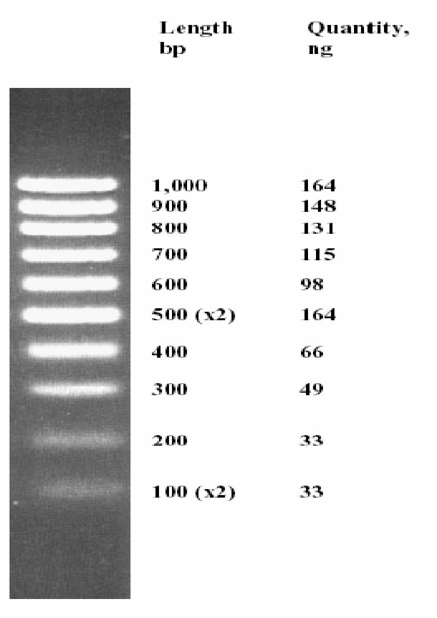


Figure 2.2 Information of Bioron DNA Size Marker

2.2 METHODS

2.2.1 DNA Isolation

DNA extraction was performed according to MN- Nucleospin tissue kit protocol step by step as described below:

· Cut 25 mg tissue in to small pieces in a final volume of 180 µl Buffer T1. 25 µl Proteinase K solution and 200 µl Buffer B3 were added. Samples were incubated at 70°C for 10 min.

· In order to adjust binding condition 210 µl ethanol (96-100%) was added to the sample and vortexed vigorously.

· For each sample, one NucleoSpin® Tissue Column was placed into a collection tube. The sample was loaded onto the column, was centrifuged for 1 min at 11.000 *g*. Flow-through was discarded and the column was placed back into the collection tube.

· 500 µl Buffer BW was added, centrifuged for 1 min at 11.000 *g*. Flow-through was discarded and the column was placed back into the collection tube.

· 600 µl Buffer B5 was added to the column and centrifuged for 1 min at 11.000*g*. Flow-through was discarded and placed the column back into the collection tube.

· In order to dry and remove the residual ethanol from membrane empty column was centrifuged for 1 min at 11.000 *g*.

· NucleoSpin® Tissue Column was placed into a 1.5 ml microcentrifuge tube and 100 µl prewarmed Elution Buffer BE (70°C) was added, incubated at room temperature for 1 min, centrifuged 1 min at 11.000 *g*.

· Highly pure DNA is extracted at the end of procedure and eluted DNA concentration and purity is calculated using Qubit Fluorometer.

2.2.2 DNA Concentration Assay

- Two tubes were used for the standards and one tube for each of our samples.

- Quant-iT Working Solution was prepared by diluting the Quant-iT reagent 1:200 in Quant-iT buffer. 200 µl of Working Solution were required for each sample and standard.

- Assay Tubes were prepared according to the table below.

Table 2.6 DNA Concentration Assay

	Standard Assay Tubes	**Sample Assay Tubes**
Volume of Working Solution to add	190 µL	180-199 µL
Volume of Standard to add	10 µL	—
Volume of Sample to add	—	1–20 µL
Total Volume in each Assay Tube	200 µL	200 µL

- Vortex all tubes for 2–3 seconds.
- Incubate the tubes for 2 minutes at room temperature
- Read tubes in Qubit fluorometer.
- Multiply by the dilution factor to determine concentration of original sample.

2.2.3 RAPD-PCR Protocol & Modified RAPD Random Amplified Mosaic DNA-PCR (RAMD-PCR)

The conditions of DNA amplification were optimized with some modifications. PCRs were performed in reaction mixture of 25 µl containing the ingredients given in Table 2.7:

Table 2.7 RAPD PCR Ingredients.

Reagent	Initial Concentration	Final Concentration	Final Volume
Taq Buffer	10X	1X	2.5 µl
dNTP	2 mM	0.2uM	1.5 µl
$MgCl_2$	25 mM	2 mM	3 µl
Primers (x6)	25 pmol/µl	25 pmol	4 µl
ddH2O	-	-	11.9 µl
Taq DNA Polymerase	5 U/µl	1 U	0.2 µl
Template DNA			5 µl
Total Reaction Volume			25 µl

The RAPD protocol consisted of an initial denaturing step of 5 min at 94 ºC, followed by 45 cycles at 94 ºC for 30 s (deneturation), 37ºC (in some cases 50°C to increase the stringency and confirm the repeatability of the bands) for 60 s (annealing) and 72 ºC for 60 s (extention), with an additional extension period of 10 min at 72 ºC.

- **94 ºC 5 minutes (initial denaturation)**
- **94 ºC 30 seconds (denaturation)**
- **37 ºC 60 seconds (annealing)**
- **72 ºC 60 seconds (extension)**
- **72 ºC 10 minutes (final extension)**

The RAMD-PCR was a modification of the RAPD-PCR; Figure 2.3 is a schematic representation of the applied method [51]. RAMD is random amplified mosaic DNA, here we assume that the tissues of the organism are highly exposed to many endogenous and exogenous exposures shown with lightenings here on the Figure 2.3. The DNA because of these many attacks are becoming a mosaic of DNA changes either DNA damage or mutations. After DNA extraction we use this mosaic DNA in the RAMD-PCR by preparing a mastermix containing the same mosaic DNA and aliquot,

distribute it to n tubes which are amplified in totally similar conditions having the same DNA. The amplification products are run on the same gel and the profiles theoretically should be the same because the conditions and the DNA are completely the same, but usually we obtain some different patterns to be discussed with other related information that may cause this variation in the profiles [52].

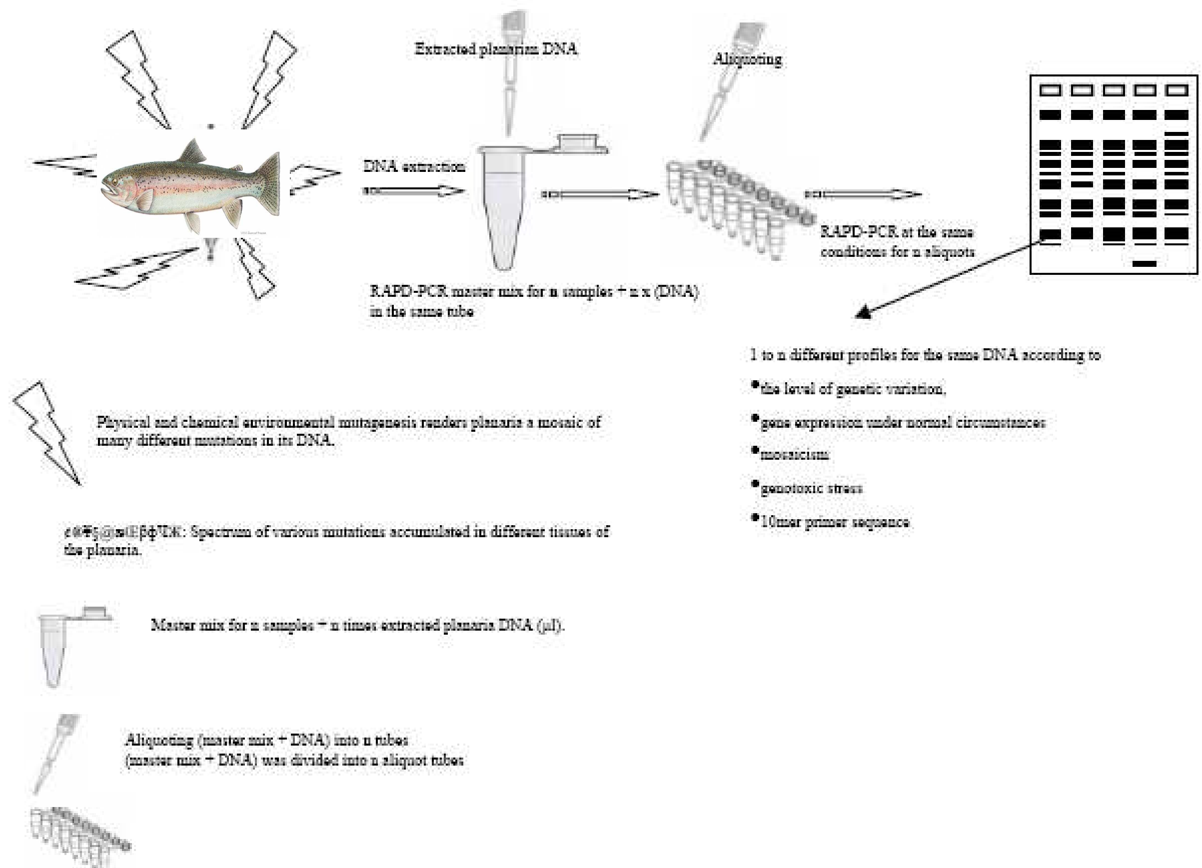


Figure 2.3 Representation of RAMD-PCR approach [53].

2.2.4 Agarose Gel Electrophoresis and Documentation

A 0.8 to 2 % agarose gel was used to detect PCR products and genomic DNAs, prepared as explained below:

Preparation of the gel:

1. 0.5 g of agarose (Sigma, St. Louis, USA) was mixed with 50 ml of 0.5X Tris-borate EDTA (TBE) buffer.
2. Then it was heated until boiling.

3. The gel was cooled to 50°C and 5 µl/100 µl Ethidium bromide was added. The gel was then poured and a comb was placed in the gel for the slots to be loaded after polymerization.

Loading:

1. 10 µl of PCR product was mixed with 2 µl of bromophenol blue as a tracking and loading dye.
2. 10 µl of PCR products were loaded in each slot mixed with loading buffer.
3. 1 µl of a 100 bp DNA Ladder (MBI Fermentas, Hanover, MD, USA) was mixed with 1 µl deionized water and 1 µl bromophenol blue. Then 2 µl of this mix was put into the side slot as a molecular marker.
4. The gel was run at 95 V in 0.5 X TBE buffer for 50 min.
5. The gel was placed in Gel Doc 2000 (Biorad, Milan, Italy) apparatus and the bands were detected under UV using a transilluminator.

CHAPTER 3

RESULTS

RAPD-PCR based approaches were used in this thesis to obtain the below results related to genetic diversity detection, genetic variation determination in relation to size and food of fish and mosaicism detection in somatic DNA and gonad DNA of rainbow trout.

We grouped our results under four basic headings; the first part is an evaluation of the rainbow trout sampling sites and water quality related criteria. The other three parts are related with sample DNA quality; the second one is determination of the level of genetic diversity which is a prerequisite for the downstream genetic variation determination works. The second one is determination of genetic variation due to different parameters: size of fish, feed of fish. The third one is the mosaicism detection using a modified RAPD-PCR protocol, RAMD-PCR: somatic mosaicism and gonadal mosaicism.

3.1 EVALUATION of SAMPLING SITES

Five different sampling sites have been worked out in this thesis from two cities of Turkey, Sakarya and Kahramanmaraş (Figure 3.1 a, b, c were photos taken during sampling from these facilities). Sakarya samples are from three different facilities at Hendek, Akyazı, Adapazarı; Kahramanmaraş samples are from two facilities at the center of Kahramanmaraş and Çağlayan. The water quality parameters for all the facilities were in compliance with the rules. Some of the water quality results of these facilities are given in appendix A. These parameters are in agreement with Table 3.1 values which are for the maximum of limits.

Figure 3.1 a Kahramanmaraş-Çağlayan Rainbow-trout Culture Facility

Figure 3.1 b Kahramanmaraş-Gökkuşağı Rainbow-Trout Culture Facility

Table 3.1 Culture facility water quality criteria that should be met [54].

Chemical	Upper level of continuous exposure (ppm)
Ammonia NH_3	0.0125 ppm
Cadmium	0.0004 ppm in soft water; 0.003 in raw water,
Chlorine	0.03 ppm
Copper	0.006 ppm in soft water.
H_2S	0.002 ppm.
Lead	0.03 ppm
Mercury	maximum 0.002 ppm; average 0.00005 ppm
Nitrogen	110% saturated, 0.1 ppm in soft water; 0.2 in raw water
Nitrite (NO_2)	0.03 and 0.06 ppm in soft and raw water, respectively
Ozone	0.005 ppm
PCB	0.002 ppm
Suspended particles	80 ppm or less
Zinc	0.03 ppm

3.2 DETERMINATION of GENETIC DIVERSITY USING RAPD-PCR

Genetic diversity is a level of biodiversity that refers to the total number of genetic characteristics in the genetic makeup of a species.

To determine genetic diversity of fish samples from all sampling sites have been used in the RAPD-PCR analysis using two different random 10-mer primers OPA-8 and OPB-18. The figures This PCR picture has low genetic diversity. Because there are 17 trout samples collected from different aquaculture and only one trout has two bands. It shows that means different size and different location chosen trout samples as the result it shows us low genetic diversity.

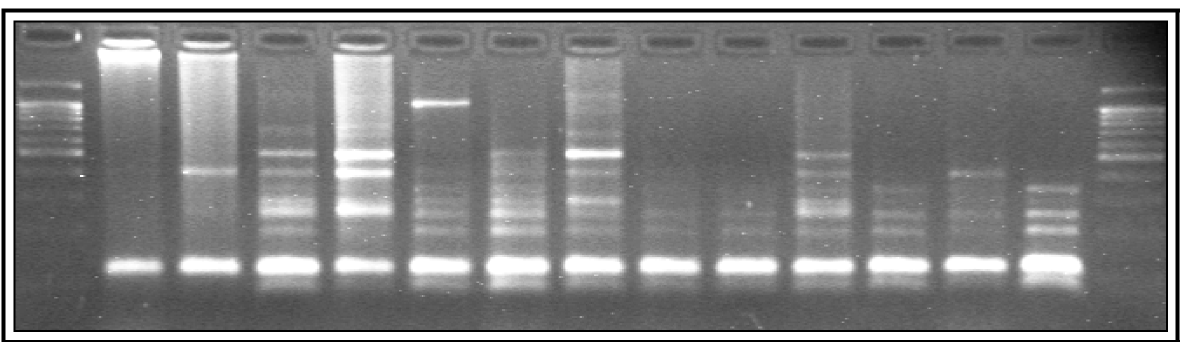


Figure 3.2 Genetic diversity results using OPA-8 Primer, RAPD-PCR amplifications of 13 samples obtained from different sampling sites. The sizes of fish loaded in each lane are indicated below. M: DNA size marker. **1)** 3.5 cm **2)** 10 cm **3)** 15.5 cm **4)** 17 cm **5)** 18 cm **6)** 19 cm **7)** 19 cm **8)** 19 cm **9)** 21 cm **10)** 26 cm **11)** 26 cm **12)** 27 cm **13)** 28 cm

RAPD profiles of 13 trout samples collected from 5 different fish aquaculture areas OPA-8 primer.

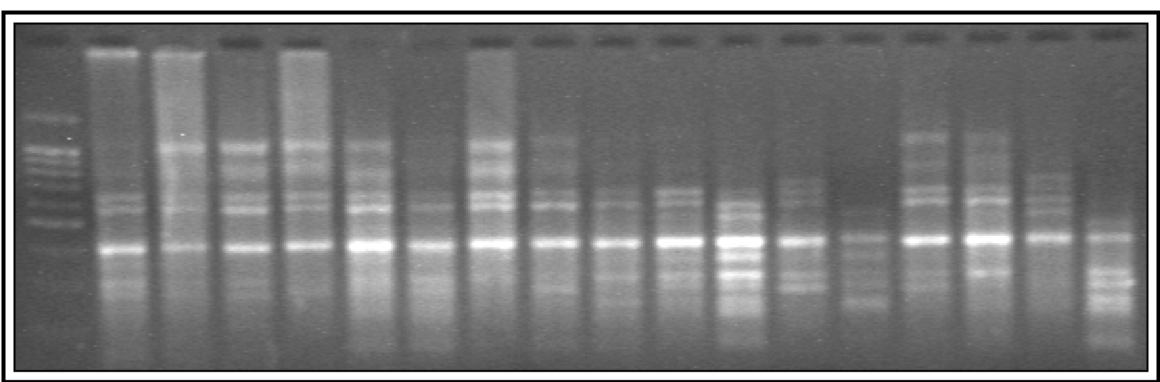


Figure 3.3 Genetic diversity results using OPB-18 Primer, RAPD-PCR amplifications of 17 samples obtained from different sampling sites. The sizes of fish loaded in each lane are indicated below. M: DNA size marker. **1)** 3.5 cm **2)** 10 cm **3)** 15.5 cm **4)** 17 cm **5)** 18 cm **6)** 18 cm **7)** 19 cm **8)** 19 cm **9)** 19 cm **10)** 21 cm **11)** 22 cm **12)** 24 cm **13)** 26 cm **14)** 26 cm **15)** 26 cm **16)** 27 cm **17)** 28 cm

3.3 GENETIC VARIATION DETERMINATION

Genetic variability is a measure of the tendency of genotypes within a population to differentiate. Individuals of the same species are not identical. Although they are recognizable as belonging to the same species, there are many differences in form, function and behavior.

3.3.1 Genetic Variation due to Size (Age, exposure level)

Genetic variation can be acquired due to various exposures of genotoxic agents that may cause DNA damage and mutations in the fish that may be accumulating them throughout their lives. In order to assess this type of an accumulated genetic variation a genome wide assessment method RAPD-PCR and its new modification done by us has been applied to our samples obtained from different facilities. In the first part of genetic variation determination sizes of the trout samples were taken into consideration. The assumption about the size is that the small sized ones are less exposed to the genotoxic agents throughout their lifetime when compared with bigger sized ones; roughly the size is considered to be directly related with age. Smaller ones are younger than the longer fish. The figures below are for the RAPD-PCR profiles of DNA extracted from muscle tissue of various sized trout samples from different facilities, same sized trout samples from different facilities, facilities that are neighbor and on the same river to control pollution parameters and provide a similar environment with the only controlled parameter being size.

21 different 10-mer primers (the information about them are given in Table 2.5) have been tried for achieving repeatability and high number of bands that enables the comparisons between various groups easier. OPA-8 and OPB-18 are chosen among them and in downstream applications they have been used as stated.

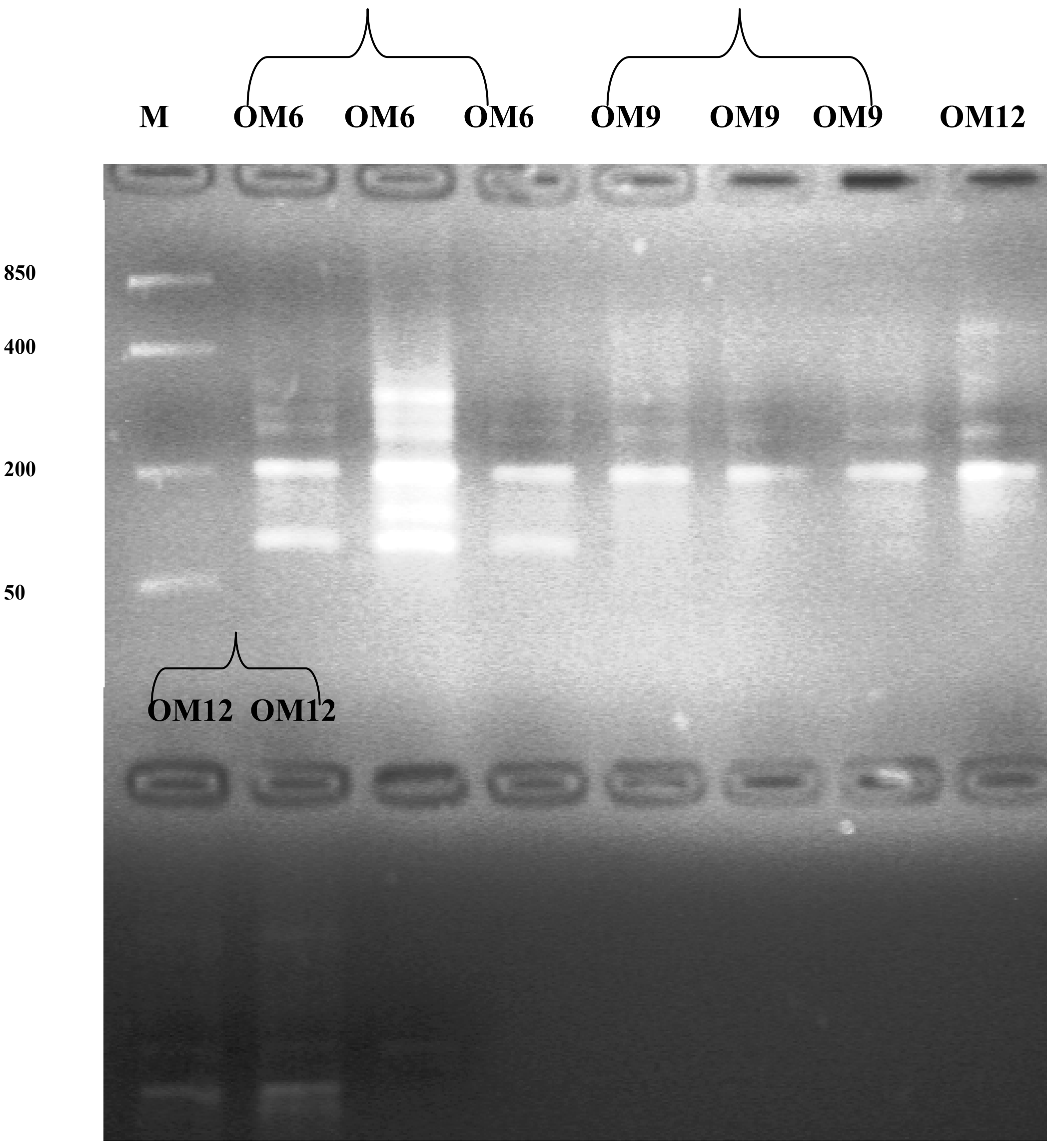


Figure 3.4 x3 RAMD-PCR of three different biggest fish of equal size from two different facilities using OPB-18 primer. M: DNA size marker

OM6: 26 cm **OM9:** 26 cm **OM12:** 26 cm

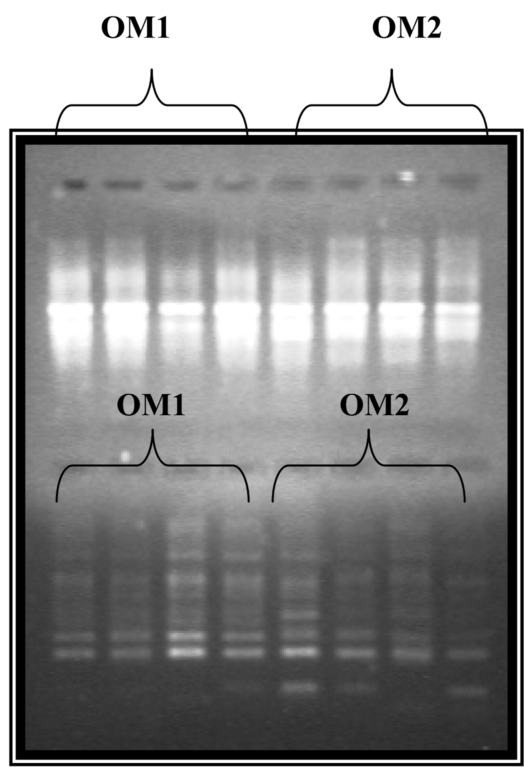


Figure 3.5 x4 RAMD-PCR profiles of same sized fish from the same facility using OPA-08 and OPB-18 primers respectively. **OM1:** 20 cm **OM2:** 21 cm

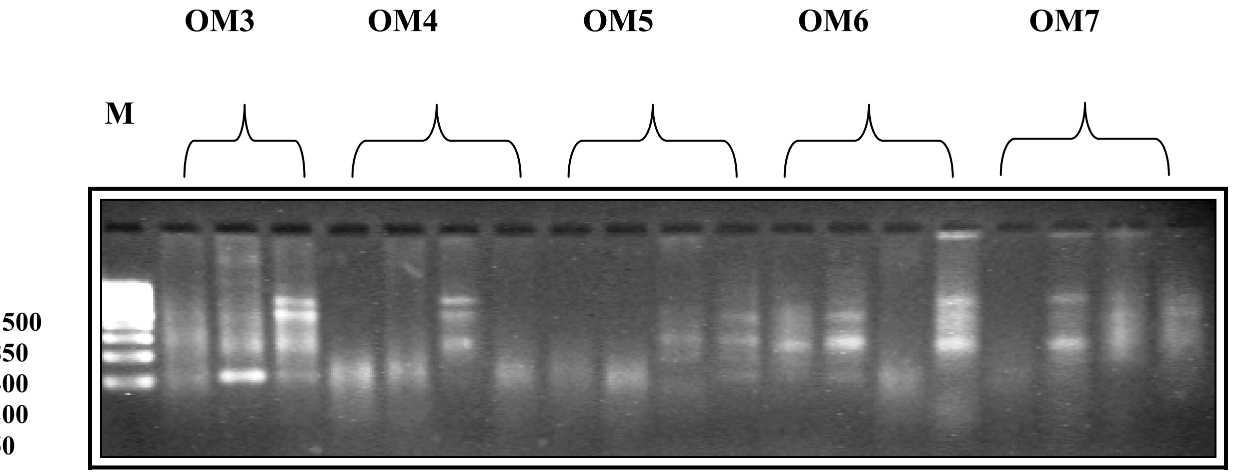


Figure 3.6a x4 RAMD profiles of ten samples with sizes given below using OPB-18 primer. Each quadruple is for the same sample DNA showing the intra individual genetic variation, mosaicism due to accumulation of various life-time exposures. M: DNA size marker.

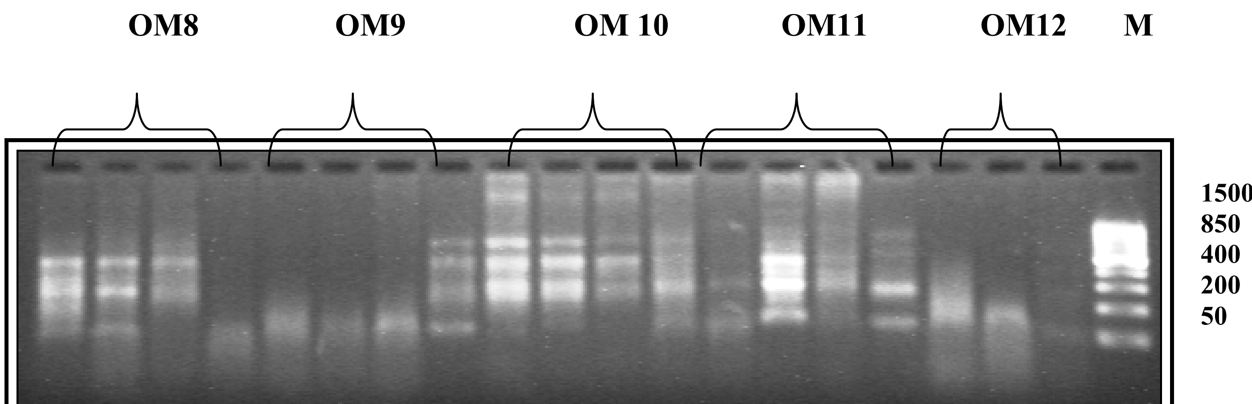


Figure 3.6 b x4 RAMD profiles of ten samples with sizes given below using OPB-18 primer.

OM3: 17 cm **OM4:** 18 cm **OM5:** 22 cm **OM6:** 26 cm **OM7:** 3.5 cm **OM8:** 15.5 cm **OM9:** 26 cm **OM10:** 10 cm **OM11:** 19 cm **OM12:** 26 cm

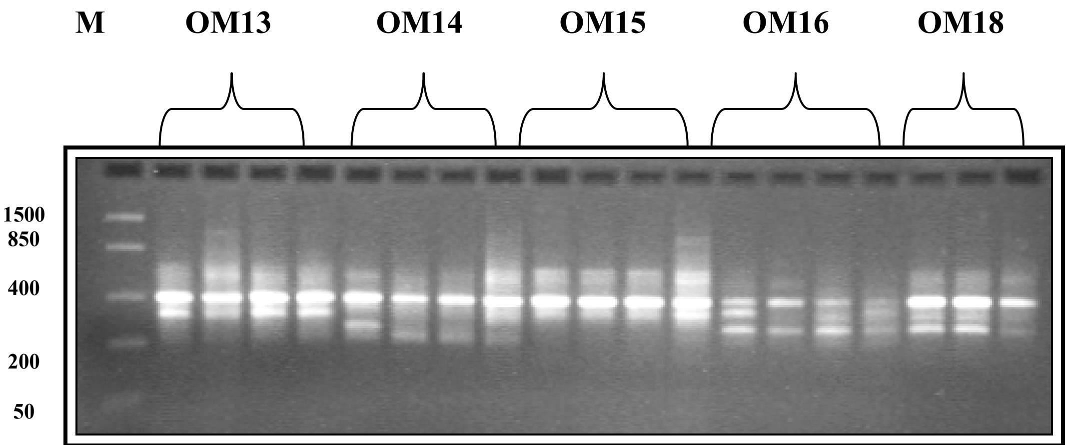


Figure 3.7a x4 RAMD-PCR profiles of different sized fish samples from neighboring facilities on the same river using OPB-18 primer. M: DNA size marker, B: blank no DNA sample; there is contamination but not fish DNA.

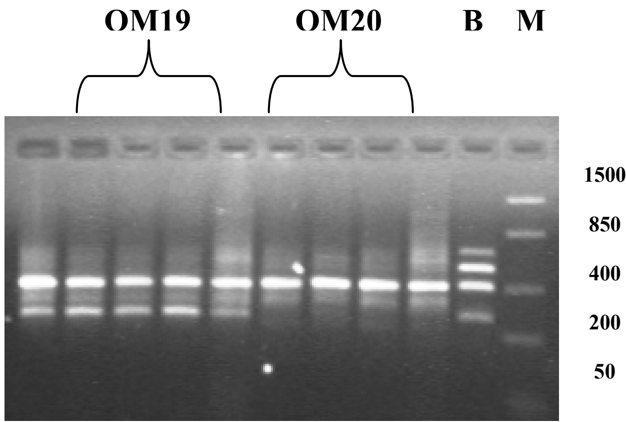


Figure 3.7.b x4 RAMD-PCR profiles of different sized fish samples from neighboring facilities on the same river using OPB-18. M: DNA size marker

OM13: 18 cm **OM14:** 21 cm **OM15:** 24 cm **OM16:** 28 cm **OM18:** 19 cm
OM19: 24 cm **OM20:** 27 cm

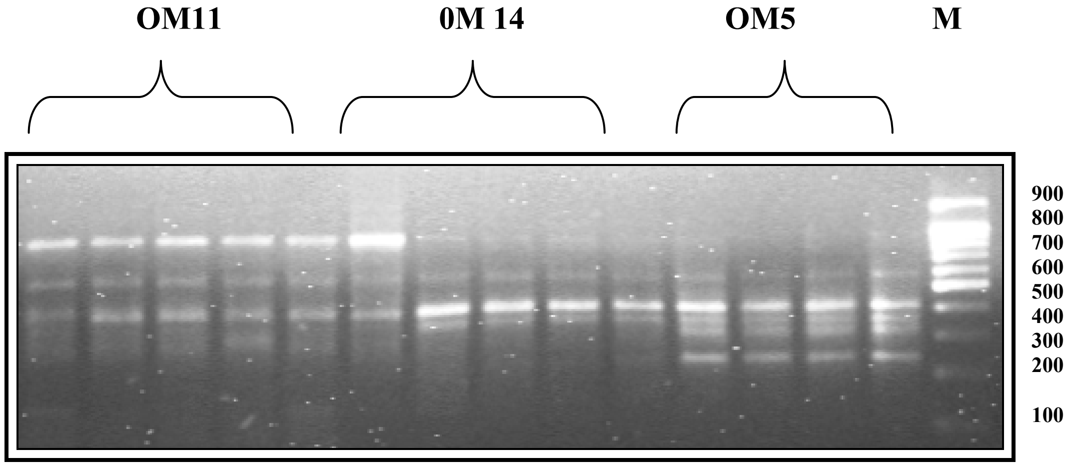


Figure 3.8a x5 RAMD-PCR profiles of different fish samples from different facilities using OPB-18 primer. X4 RAMD-PCR for OM-5 sample. M: DNA size marker. **OM11:** 19 cm **OM14:** 21 cm **OM5:** 22 cm

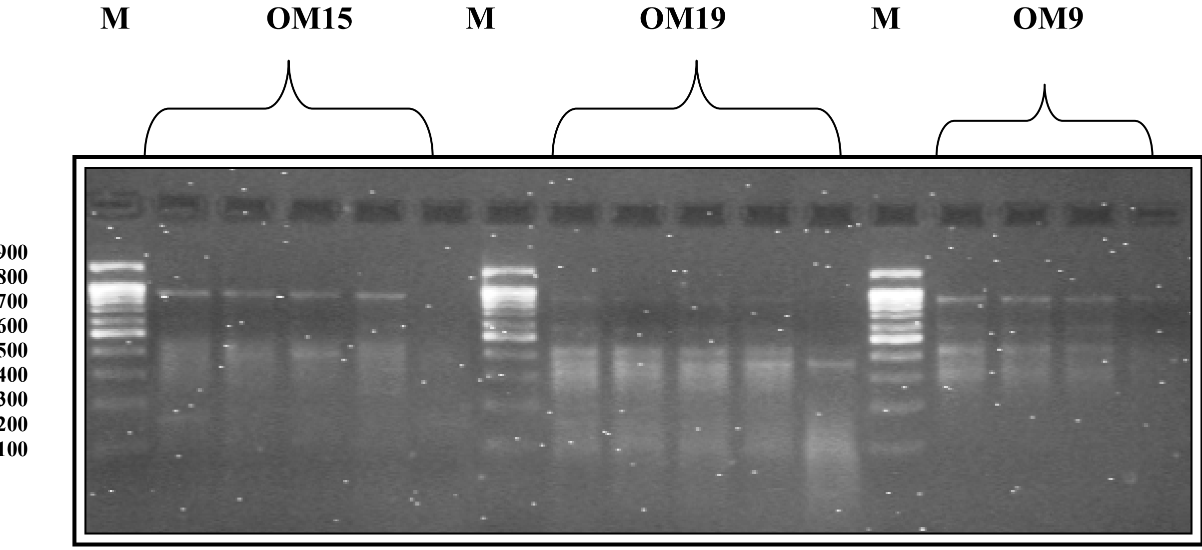


Figure 3.8b x5 RAMD-PCR profiles of different fish samples from different facilities using OPB-18 primer. x4 RAMD-PCR for OM-9.
OM15: 24 cm **OM19:** 24 cm **OM9:** 26 cm

3.3.2 Genetic Variation due to Different Food Types (Commercial food vs. live food)

In their natural habitats rainbow trouts are opportunistic feeders that rely on a wide variety of food items ranging from small insects to crayfish. Trout inhabiting streams with a significant amount of riparian vegetation often feed heavily on terrestrial insects, such as grasshoppers and ants, that fall into the stream. Rocky stream riffles produce bottom-dwelling aquatic invertebrates, such as insects and crustaceans, that are also fed upon. In lakes and streams, invertebrates such as plankton, crustaceans, snails, and leeches, as well as small fish and fish eggs also serve as food for trout.

Cultured fish are fed with commercial food containing the below mentioned ingredients. For our experimental group in order to assess the DNA effects of feeding behaviors of cultured trout we have produced two groups with the only difference their feeding, the first group of fish were numbered as OM-21, OM-22 and OM-23, they were fed with live food, their sizes were, 24 cm, 25 cm and 26 cm respectively whereas the other group of fish were fed with commercial food from ECOBIO and they were numbered as OM-24, OM-25 and OM-26 and their sizes were 23 cm, 24 cm and 25 cm

respectively. All two groups of fish were raised in the same conditions of the same facility from Kahramanmaraş.

OM-21, OM-22 and OM-23 which were fed with live food showed higher genetic variation and mosaicism when compared with commercial pellet fed ones; OM-24, OM-25 and OM-26 shown in Figure 3.12.

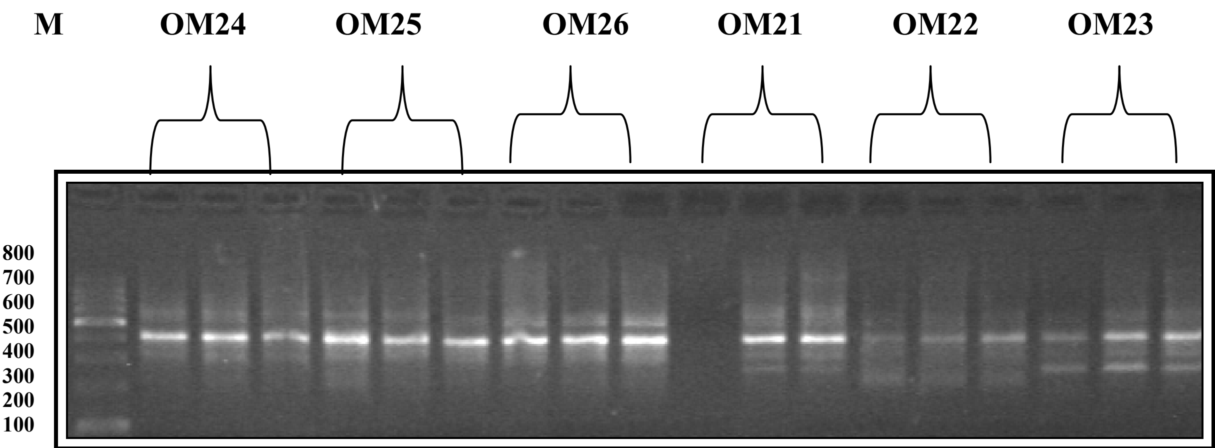


Figure 3.9 x3 RAMD-PCR profiles using OPB-18 primer. Comparison of RAPD profiles according to food of fish sample. **OM24:** 23 cm **OM25:** 24 cm **OM26:** 25 cm **OM21:** 24 cm **OM22:** 25 cm **OM23:** 26 cm

3.4 DETERMINATION of SOMATIC and GONADAL MOSAICISM using RAMD-PCR

In order to show mosaicism, muscle tissue, liver tissue and gonads were removed from five trout samples and DNAs of them were isolated separately.

The phenomenon of somatic mosaicism implies the presence of genetically different cell lines in a single organism. The differences between the lines may be caused by mutations, chromosomal rearrangements, modifications and different epigenetic factors.

For determination of somatic and gonadal mosaicism, three different tissues of OM-10, OM-13, OM-5, OM-9 and OM-16 have been dissected and the DNAs were isolated separately. Table 2.3 gives information from the liver tissues of indicated fish and Table 2.4 from the gonads of indicated fish. Liver tissue DNA are denoted as OM-27, OM-28, OM-29, OM-30 and OM-31 respectively and gonad tissues were denoted as OM-32, OM-33, OM-34, OM-35 and OM-36 respectively. The first group is for the muscle tissues.

For Figure 3.13 x3RAMD-PCR profiles showed a monomorphic pattern with OPB-18 primer. But the resolution of the gel system seemed to be low and that might be a reason for the monomorphic character of bands observed.

Some experimental optimizations are necessary to obtain the comparable patterns. To give another interesting example for the experimental necessities for optimizations Figure 3.14 is given. The experiment was designed to show the possible differences between different thermal cyclers. Every parameter except the thermal cycler were the same, but the results obtained looks quite different from each other indicating the importance of every detail.

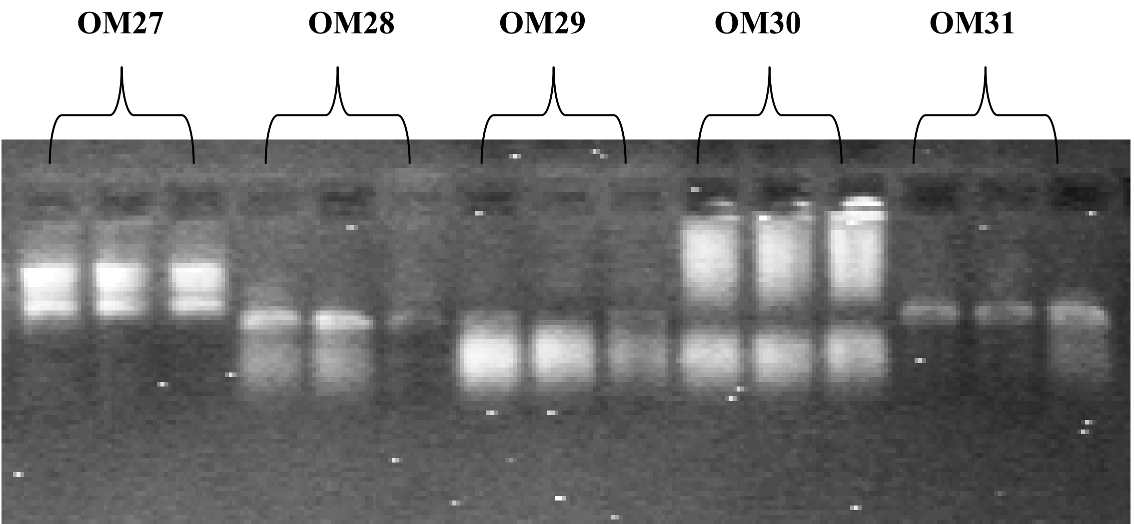


Figure 3.10 x3 RAMD-PCR Profiles of liver tissue DNA extracts using OPB-18 primer. **OM27:** 10 cm **OM28:** 18 cm **OM29:** 22 cm **OM30:** 26 cm **OM31:** 28 cm

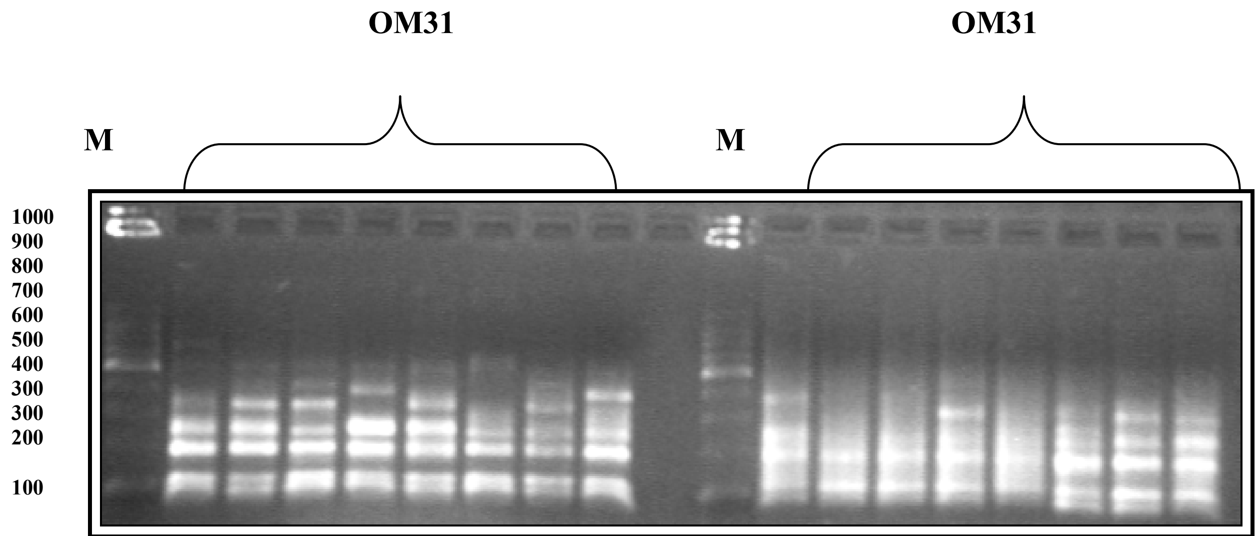


Figure 3.11 OPA-8 primer comparison of the same conditions for liver sample (OM-31) using two different PCR machines (Techne-TC512 gradient PCR and Techne Genious PCR machine) **OM31:** 28 cm Liver tissue.

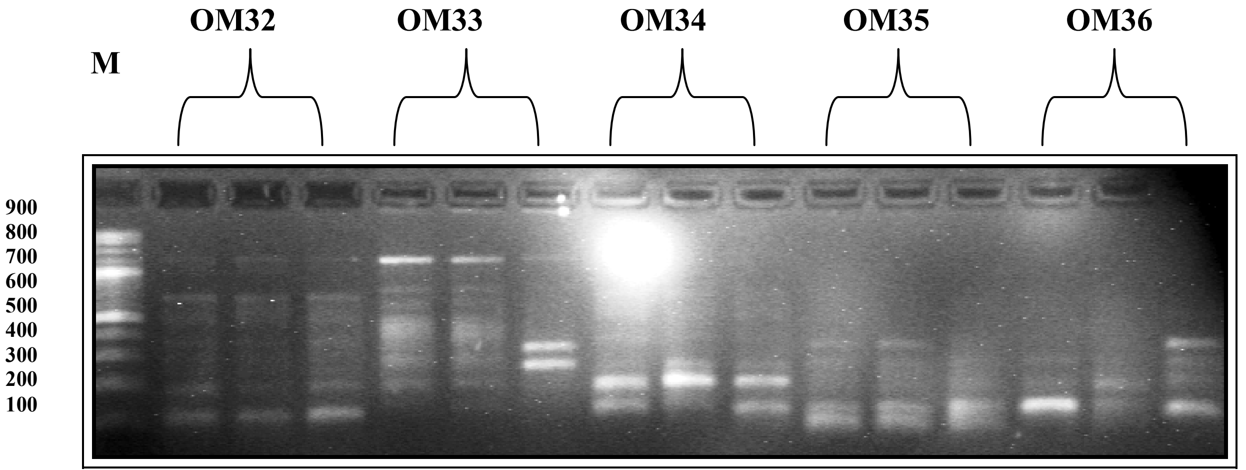


Figure 3.12 x3 RAMD-PCR Profiles of the gonad tissue DNAs of below sized fish. **OM32:** 10 cm **OM33:** 18 cm **OM34:** 22 cm **OM35:** 26 cm **OM36:** 28 cm

Figure 3.13 showed high genetic variation, mosaic behavior for all samples gonad tissue DNA with primer OPB-18.

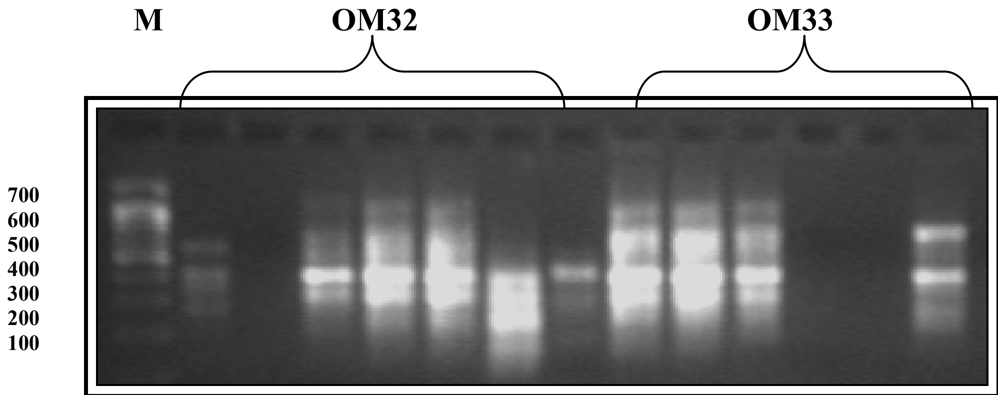


Figure 3.13 x8 RAMD Profiles of gonad tissue samples of two different fish with the same primer. **OM32:** 10cm Gonad tissue **OM33:** 18 cm Gonad tissue

High degree of variation, gonadal mosaicism exists for both samples OM32 and OM33. Although OM32 is the smallest fish that means the less exposed still has a highly detectable mosaic DNA for the gonad tissue.

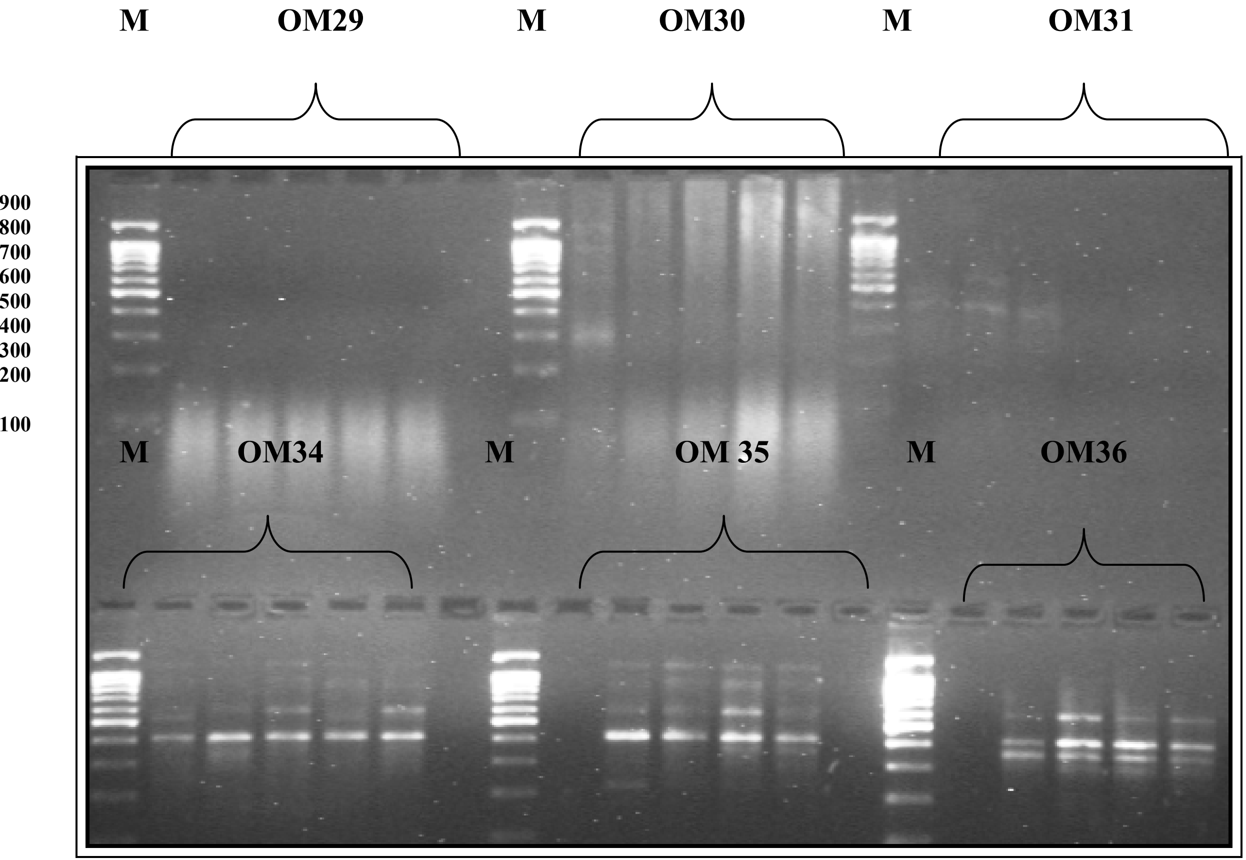


Figure 3.14 x5 RAPD Profiles of liver tissue and gonad tissue samples for the below samples respectively.

OM29: 22 cm **OM30:** 26 cm **OM31:** 28 cm**OM34:** 22 cm **OM35:** 26 cm **OM36:**28 cm

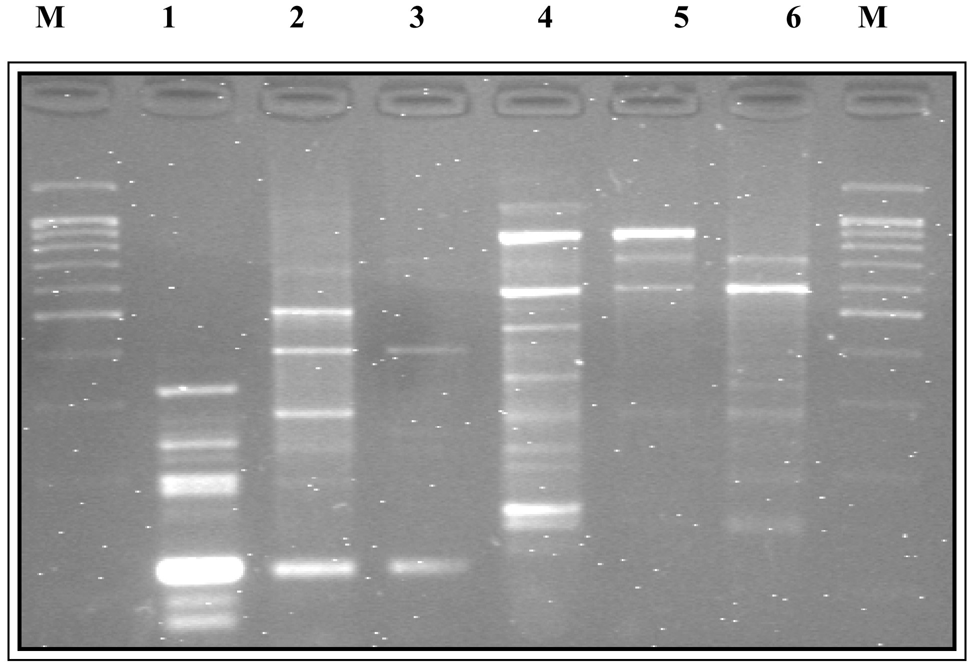


Figure 3.15 Comparison of tissues with two different primers.

1) 10 cm gonad tissue **2)** 10 cm liver tissue **3)** 10 cm muscle OPA-8 Primer

4) 10 cm gonad tissue **5)** 10 cm liver tissue **6)** 10 cm muscle OPB-18 Primer

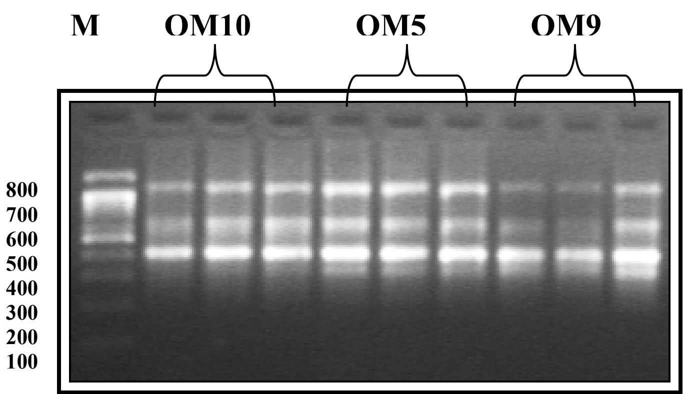


Figure 3.16 a x3 RAMD-PCR Profiles of muscle tissue samples

OM10: 10 cm **OM5:** 22 cm **OM9:** 26 cm

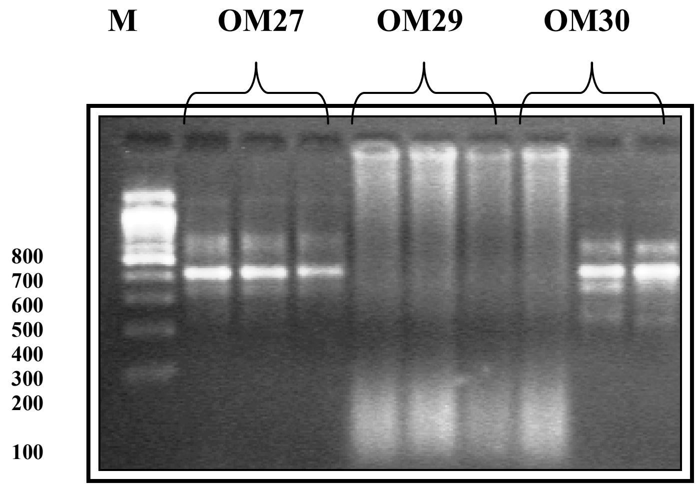


Figure 3.16 b x3 RAMD-PCR Profiles of liver tissue samples

OM27: 10 cm **OM29:** 22 cm **OM30:** 26 cm

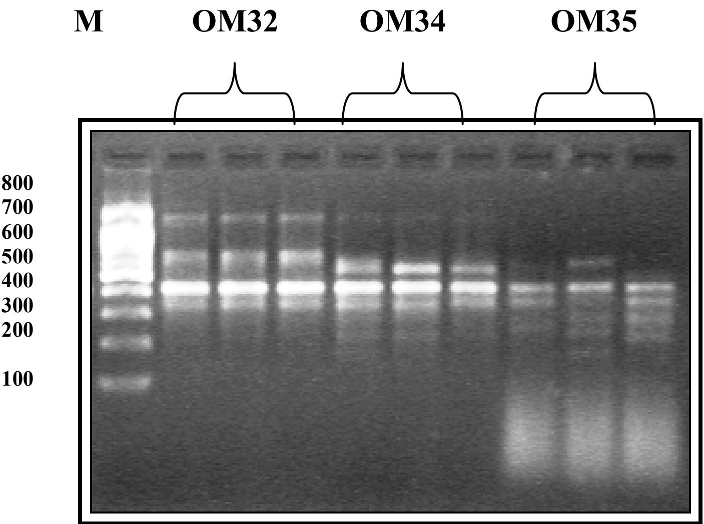


Figure 3.16 c x3 RAMD-PCR Profiles of gonad tissue samples

OM32 : 10 cm **OM34** : 22 cm **OM35 :** 26 cm

CHAPTER 4

DISCUSSION

This thesis contains many new applications that can be further be evaluated as RAPD-PCR based molecular approaches in fish-aquaculture with special emphasis on rainbow-trout culture.

Various optimizations of RAPD-PCR assay were done in the context of the thesis; on the choice of primer, annealing temperature, understanding the innate genetic diversity of cultured rainbow trout samples with RAPD-PCR using various primers, DNA quantification and qualification, showing the effect of different thermal cyclers, a new approach for confirmation of the repeatibility of the assay: RAMD-PCR has been optimized and all these optimisations were done to assess the genetic diversity of our samples obtained from five different facilities, comparative genetic variation which is dependent on differences in farm conditions like water quality, temperature, pollution and dependent on size and dependent on feed (live vs. Pellet: ready made feed) and to assess somatic mosaicism and gonadal mosaicism.

If the RAPD assay is used in genotoxicity studies, the choice of the species and level of genomic diversity among individuals of the same species are of fundamental importance. Indeed, it is necessary to know the level of genomic diversity among individuals of the same species before launching any genotoxicity studies. Because of this we have done a prior genetic diversity assessment. We also aimed to show genetic variation or variablity so below is some background information to support the results about genetic variation:

Genetic variability is a measure of the tendency of genotypes within a population to differentiate. Individuals of the same species are not identical. Although they are recognizable as belonging to the same species, there are many differences in form, function and behavior.

Variability is different from genetic diversity, which is the amount of variation seen in a particular population. The variability of a trait describes how much that trait tends to vary in response to environmental and genetic influences. Genetic variability in a population is important for biodiversity, because without variability, it becomes difficult for a population to adapt to environmental changes and therefore makes it more prone to extinction.

Next will be some very important conceptual background knowledge that will be very important in discussing our results and conclusion. The differentiation of DNA damage and mutation, because the variation observed in RAPD profiles are due to either DNA damage or mutations. Distinguishing between DNA damage and mutation is very important. They are the two major types of error in DNA. DNA damages and mutation are fundamentally different. Damages are physical abnormalities in the DNA, can be recognized by enzymes, and thus they can be correctly repaired. If a cell retains DNA damage, transcription of a gene can be prevented and thus translation into a protein will also be blocked. Replication may also be blocked and/or the cell may die. In contrast to DNA damage, a mutation is a change in the base sequence of the DNA that cannot be recognized by enzymes once the base change is present in both DNA strands, and thus a mutation cannot be repaired. At the cellular level, mutations can cause alterations in protein function and regulation. Mutations are replicated when the cell replicates. In a population of cells, mutant cells will increase or decrease in frequency according to the effects of the mutation on the ability of the cell to survive and reproduce.

Although distinctly different from each other, DNA damages and mutations are related because DNA damages often cause errors of DNA synthesis during replication or repair and these errors are a major source of mutation. Given these properties of DNA damage and mutation, it can be seen that DNA damages are a special problem in non-dividing or slowly dividing cells, where unrepaired damages will tend to accumulate over time. On the other hand, in rapidly dividing cells, unrepaired DNA

damages that do not kill the cell by blocking replication will tend to cause replication errors and thus mutation. The great majority of mutations that are not neutral in their effect are deleterious to a cell's survival. Thus, in a population of cells comprising a tissue with replicating cells, mutant cells will tend to be lost. However infrequent mutations that provide a survival advantage will tend to clonally expand at the expense of neighboring cells in the tissue. This advantage to the cell is disadvantageous to the whole organism, because such mutant cells can give rise to cancer. Thus DNA damages in frequently dividing cells, because they give rise to mutations, are a prominent cause of cancer. In contrast, DNA damages in infrequently dividing cells are likely a prominent cause of aging.

In tissues composed of non- or infrequently replicating cells, DNA damages can accumulate with age and lead either to loss of cells, or, in surviving cells, loss of gene expression.

Our results show that different tissues and organs of the same trout behaves differently that is because they have different renewal properties. Like the muscle cells which are less proliferative less dividing when compared with liver and gonad cells, we will be observing genetic changes that might be due to an accumulation of DNA damages in the muscle tissue. For the liver, hepatocytes although terminally differentiated non-dividing cells they retain the ability to proliferate when injured so both DNA damages and mutations can be observed for this organ. For the gonad it will be more like mutations that are not repaired because these cells are higly proliferative, dividing cells due to reproductive age of our samples.

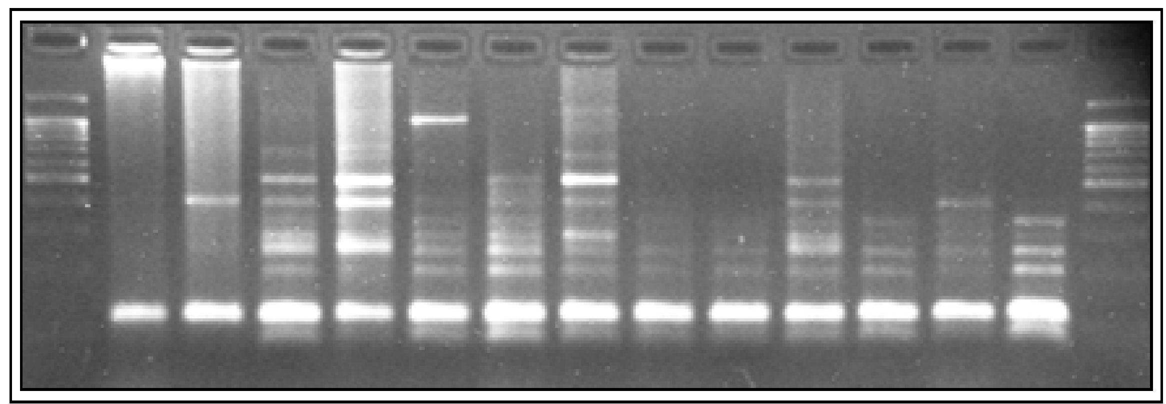
Either DNA damage or mutation the effects on RAPD profiles can be seen as loss of bands, appearance of bands, decrease in band intensity and increase in band intensity.

Now in view of these information the results are discussed.

The diversity evaluations were done using two different primers and the diversity was found to be very high for the samples we have as we expect it because they are from very different facilities from two different regions of Turkey. The Figure

4.1 is for OPA-8 primer for 13 samples from different places, nearly all the profiles are different from each other indicating high polymorphic character and high genetic diversity. Still with the other primer OPB-18 the genetic diversity is very high this time for 17 samples many different profiles are observable still some similar profiles are observed for the same city Sakarya Lanes 3, 4, 5, 7, 14 for the Figure 4.1 second part.

M 1 2 3 4 5 6 7 8 9 10 11 12 13 M



M 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17

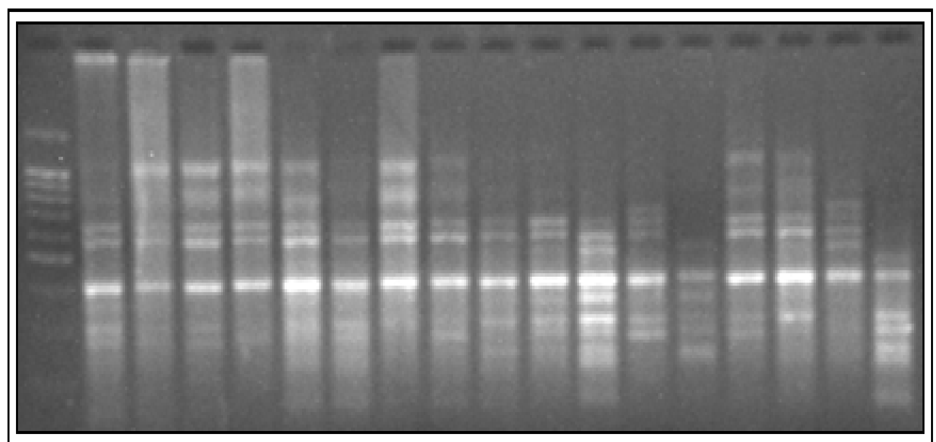


Figure 4.1: Genetic diversity results using OPA- 08 primer and OPB-18 primer, RAPD-PCR amplifications of 13 and 17 samples obtained from different sampling sites.

Muscle tissues of fish were used for genetic variation determination. The size changes of the fish samples were assumed to be roughly an indication for age and more exposure to environmental chemicals and physical agents. All have to some extend an

observable mosaic pattern accumulation of DNA damages in muscle DNA. All the samples have different sizes as indicated in the figure legends.

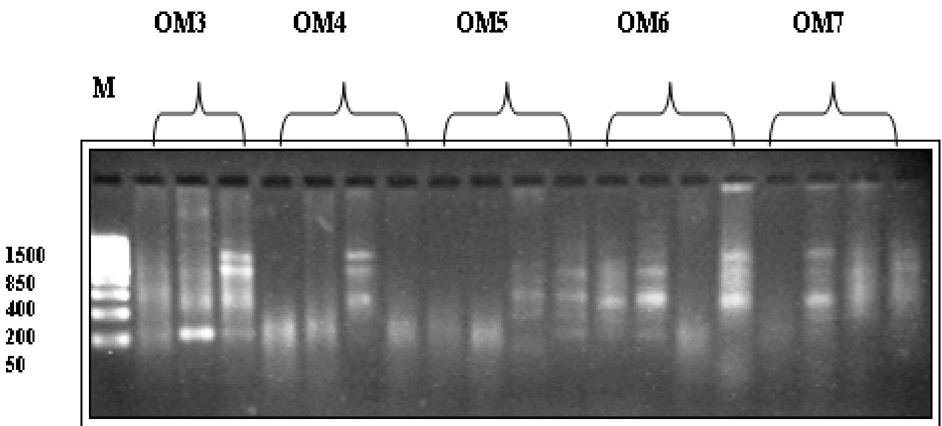


Figure 4.2: Intra-individual genetic variation, mosaicism detection with x4 RAMD-PCR. Each quadruple is for the same sample DNA showing the intra individual genetic variation, mosaicism due to accumulation of various life-time exposures. M: DNA size marker.

OM3: 17 cm **OM4:** 18 cm **OM5:** 22 cm **OM6:** 26 cm **OM7:** 3.5 cm

The same experiment results continue with the mentioned sized samples. To some extend all of them show genetic variation either due to environmental processes or ageing related accumulation of DNA damages.

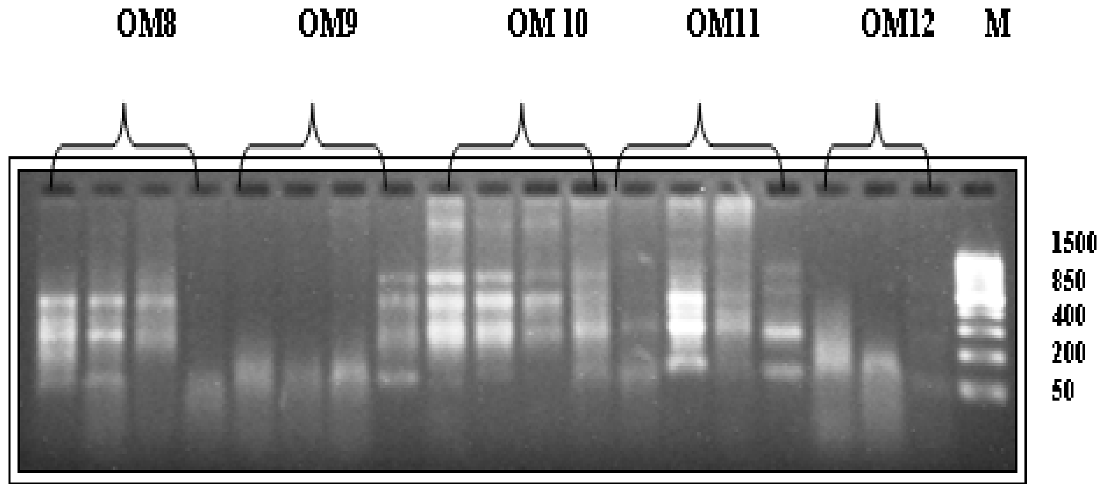


Figure 4.3: Genetic variation mosaicism detection with x4 RAMD PCR.
OM8: 15.5 cm **OM9:** 26 cm **OM10:** 10 cm **OM11:** 19 cm **OM12:** 26 cm

In order to control some parameters we chose our group now from the two neighbouring facilities. Until OM18 from one facility. The next figure is from the other facility. The patterns for this facility more variable whereas the other facility profiles are more homogenous. We have learnt that the first facility mixes antibiotics to the food of their fish to prevent an epidemic. It is a personal communication.

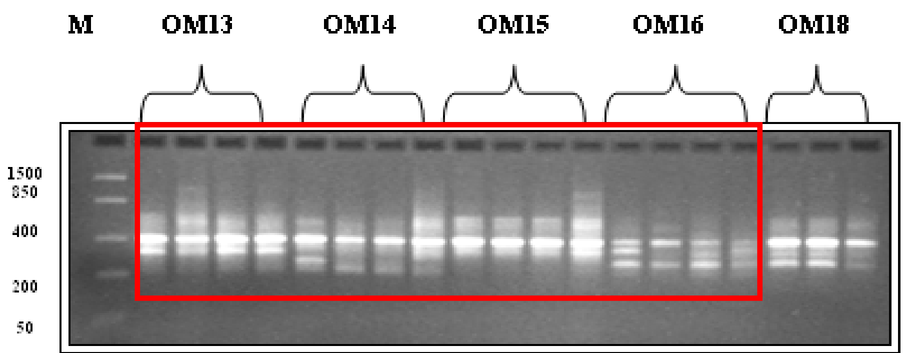


Figure 4.4: x4 RAMD-PCR profiles of different sized fish samples from neighboring facilities on the same dam in Kahramanmaraş using OPB-18 primer. M: DNA size marker, **OM13:** 18 cm **OM14:** 21 cm **OM15:** 24 cm **OM16:** 28 cm **OM18:** 19 cm

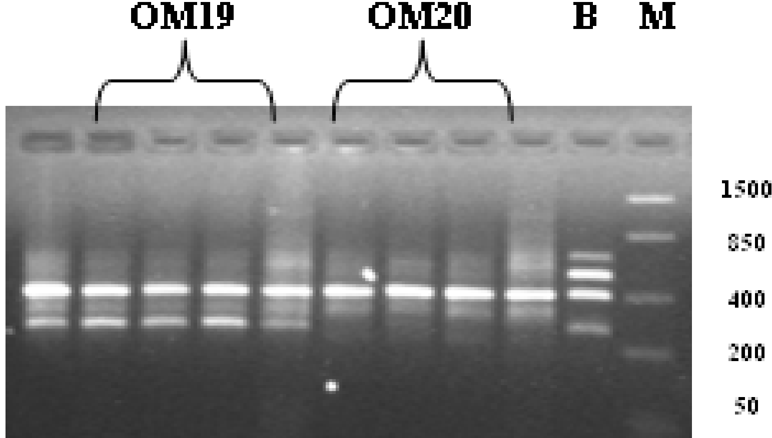


Figure 4.5: x4 RAMD-PCR profiles of different sized fish samples from neighboring facilities on the same river using OPB-18. M: DNA size marker, B: DNA sample of another species. **OM18:** 19 cm **OM19:** 24 cm **OM20:** 27 cm

Let me draw your attention to the below three samples in Figure 4.6, the first one OM 11 is from Sakarya, OM 14 is from Kahramanmaraş and OM 5 from Sakarya another facility. The circled band which is lacking in profiles of OM14 should have some interesting discussions it might be a mutation that is indicated as loss of bands in the rest and the other Sakarya sample. Or it might be a contamination which is not confirmed yet.

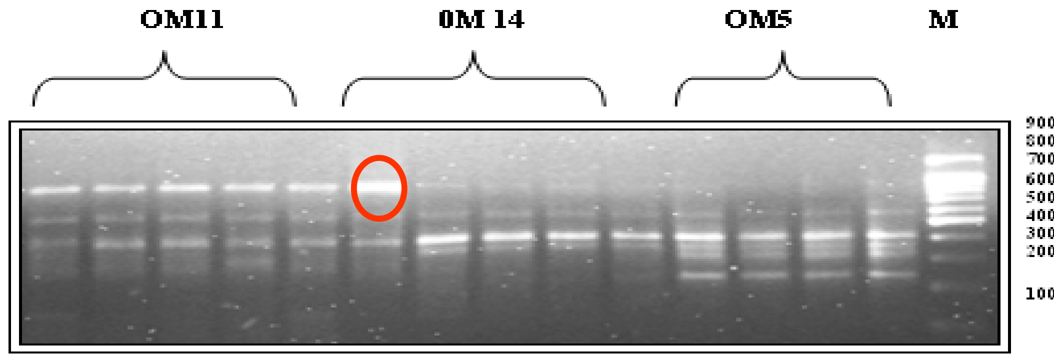


Figure 4.6: x5 RAMD-PCR profiles of different fish samples from different facilities using OPB-18 primer. X4 RAMD-PCR for OM-5 sample. M: DNA size marker.
OM11: 19 cm **OM14:** 21 cm **OM5:** 22 cm

Coming to genetic variation due to different food types commercial versus live food groups for the same facility. The first three triplets are for commercial food fed ones and the last three triplets are for the live food fed samples in Figure 4.7. We can see more variation in the live food fed ones whereas Commercial food seems to be more healthy in terms of somatic mosaicism. Those changes indicated with red arrows are for the most prominent observable changes that might be both mutations and DNA damages.

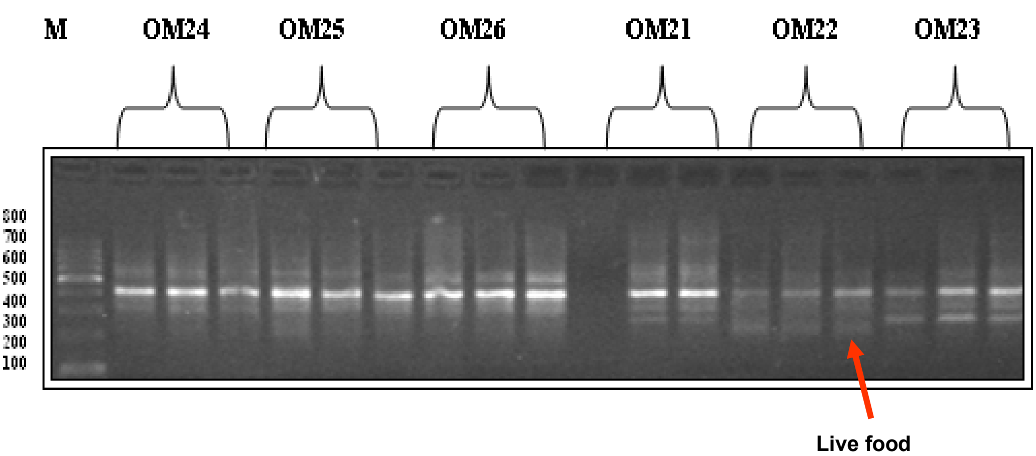


Figure 4.7: x3 RAMD-PCR profiles using OPB-18 primer. Comparison of RAPD profiles according to food of fish sample.

OM24: 23 cm **OM25:** 24 cm **OM26:** 25 cm **OM21:** 24 cm **OM22:** 25 cm **OM23:** 26 cm

The Figure 4.8 shows some optimization results and also somatic mosaicism detection results. Everything is the same for the left and right side of the patterns about the preparation of mixes and DNA; the only difference is the PCR machines used. On the left Techne-TC512 gradient PCR and Techne Genious on the right. The 8 times mastermix preparations show a high genetic variation for 28 cm liver tissue DNA sample which is an indication for somatic mosaicism in the liver tissue. You can easily see the 8 different patterns produced as a result of RAMD-PCR. Each lane having all sets of observable changes in RAPD profiles. They are loss of bands, gain of bands, increase and decrease in band intensities.

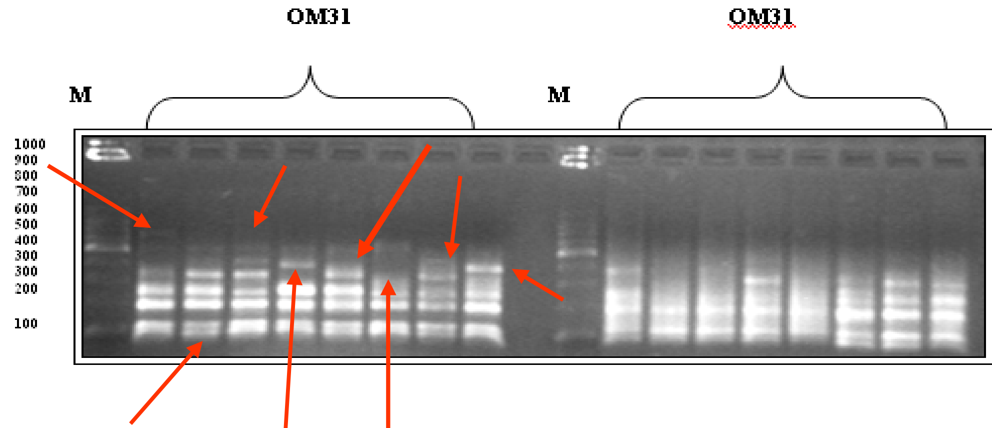


Figure 4.8: OPA-8 primer comparison of the same conditions for liver sample (OM-31) using two different PCR machines (Techne-TC512 gradient PCR and Techne Genious PCR machine) **OM31:** 28 cm Liver tissue

The Figure 4.9 very clearly shows the gonadal mosaicism in increasing size mostly due to accumulation of genotoxic effects. Look at the first triplicate for the smallest fish gonad tissue DNA that has the most homogenous profile whereas increasing size of the fish as 18cm, 22 cm, 26 cm and 28 cm shows us highly mosaic, polymorphic patterns due to accumulation of both mutations and DNA damages in the gonad tissue. These genomic changes if mutations can be carried on to the next generation deteriorating the genetic make-up of the offspring.

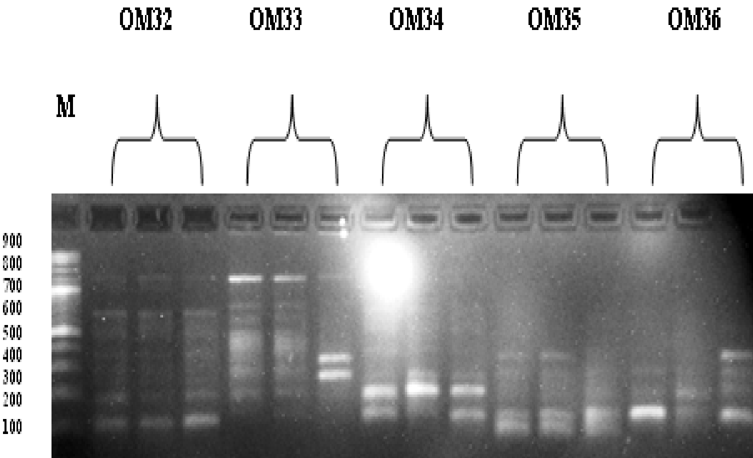


Figure 4.9: x3 RAMD-PCR Profiles of the gonad tissue DNAs of below sized fish. **OM32:** 10 cm **OM33:** 18 cm **OM34:** 22 cm **OM35:** 26 cm **OM36:** 28 cm

Three consecutive figures that are for the three trout samples' muscle, liver and the gonad tissues triplicate results are shown in Figures 4.10-4.12. When compared with the previous picture the profiles for muscle tissue is very homogenous only for the last one the somatic mosaicism is prominent. The band intensity changes can be observed in the last biggest fish sample.

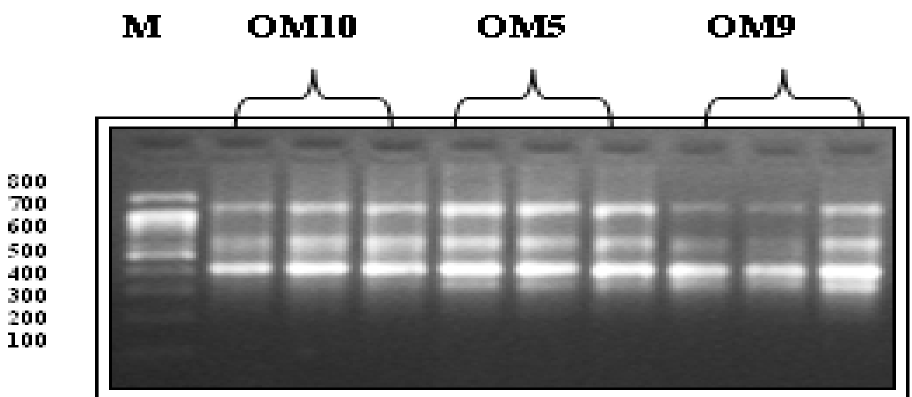


Figure 4.10: x3 RAMD-PCR Profiles of muscle tissue samples **OM10:** 10 cm **OM5:** 22 cm **OM9:** 26 cm

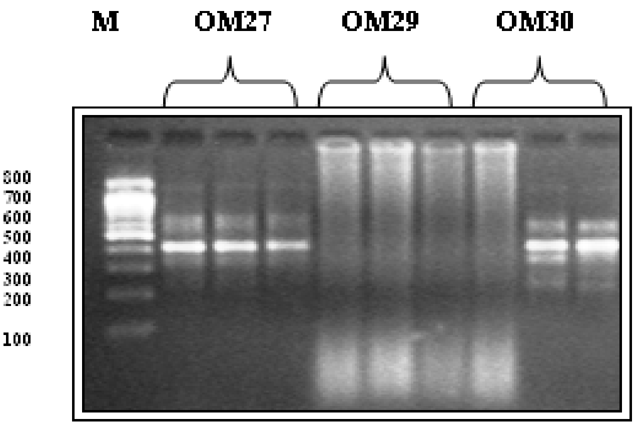


Figure 4.11: Liver tissue x3 RAMD-PCR profiles.

The Figure 4.11 is for the profiles of liver tissue DNA. For the first 10 cm fish there seems no change, but in the 22 cm and 26 cm samples' liver DNA seem to have DNA degradation type of a smear pattern.

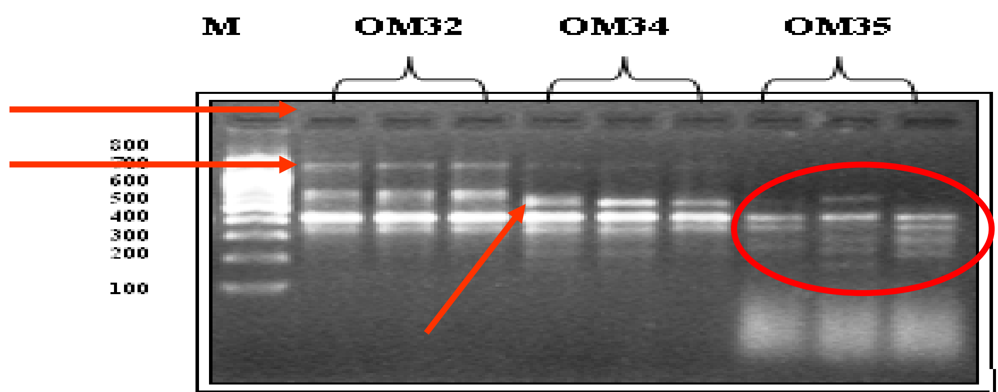


Figure 4.12: x3 RAMD-PCR Profiles of gonad tissue samples
OM32: 10 cm **OM34:** 22 cm **OM35:** 26 cm

The figure 4.12 is for the gonad tissue mosaicism for the small fish there seems to be no change in the 22 cm there is a small change and in the biggest fish which is 26 cm the pattern is highly mosaic, heterogeneous, polymorphic. Please concentrate on the 700 bp sized amplicon for all the samples which is lost in growing fish.

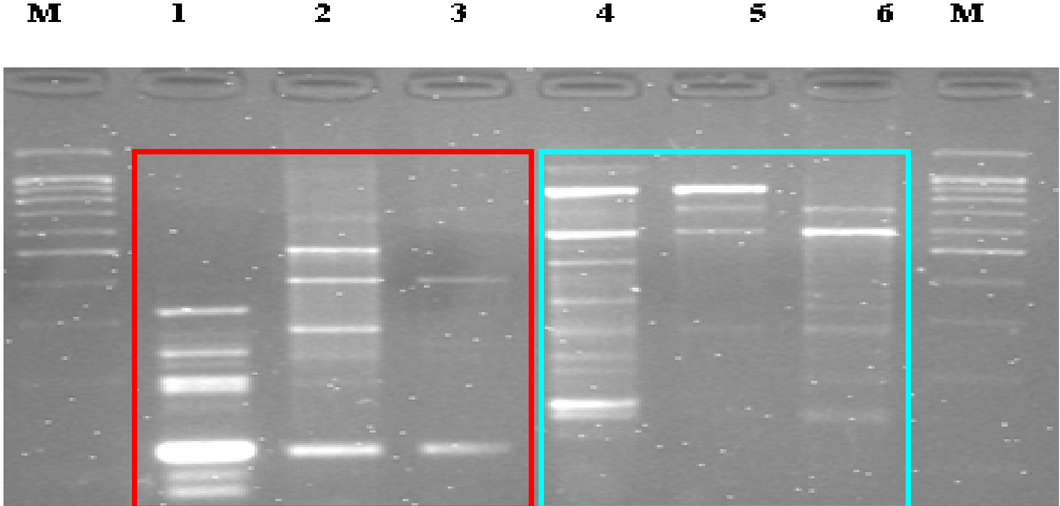


Figure 4.13: Comparison of all tissues for the same fish (10 cm) with two different primers.

1)10 cm gonad tissue **2)** 10 cm liver tissue **3)** 10 cm muscle OPA-8 Primer

4) 10 cm gonad tissue **5)** 10 cm liver tissue **6)** 10 cm muscle OPB-18 Primer

CHAPTER 5

CONCLUSION

Fish aquaculture is very important for Turkish food industry and economy, and should be enhanced for this sake with different scientific innovations. Genotyping is an important tool in selection and maintenance of domesticated fish strains. Molecular techniques based on the polymerase chain reaction are widely used for assessment of genetic variations between and within species, strains, populations, etc. In our thesis we applied DNA-based molecular biology and genetics tools to unravel various DNA-related problems or facts about fish-aquaculture in Turkey.

Genetic diversity of fish (especially *Oncorhyncus mykiss,* rainbow trout) used in aquaculture in Turkey, especially rainbow trout was assessed using RAPD-PCR based analysis. Genetic changes, modifications in cultured fish were evaluated for different cultivation parameters to increase the yield of fish aquaculture in Turkey. Genotoxic effects of environmental pollutants can threaten the survival of wild populations by modifying their genetic ability for adaptation to variable environmental conditions. For example, the genetic diversity in fish populations living in polluted sites was found to be altered, when compared with unpolluted areas. Studies in natural populations are associated with inherent methodological difficulties, particularly when evaluating chronic exposure situations.

In addition, these technologies are relatively cheap and do not require the use of specialized and expensive equipment. Another advantage is that after optimisation, the RAPD assay is very reliable. The assay also presents a number of advantages for the detection of genotoxic effects. And last but not the least, the RAPD method has the potential to detect a wide range of DNA damage (e.g. DNA adducts, DNA breakage) as well as mutations (point mutations and large rearrangements).

The RAPD method was used to detect genetic diversity among populations which had been exposed to environmental contaminants, including well-known genotoxins Nadig et al. reported that fish populations in the contaminated sites were consistently less genetically distant from each other than they were from each of the reference sites. The results of another study suggested that RAPD based measures of genetic diversity may be suitable for development as a sensitive means of directly assessing the impact of environmental contaminants upon ecosystems [48].

In conclusion rainbow trout is not a native fish species of Turkey, rainbow trout is native only to the Pacific slope of North America, but have been widely introduced on every continent, except Antarctica. The practice of releasing cultured fish into the wild is widespread, but without a careful genetic analysis, it may damage the goals of preservation, leading to the homogenization of populations and decreasing species diversity [15].

REFERENCES

1. U.S. Department of Agriculture. 1998 Census of Aquaculture. http://www.nass.usda.gov/census/census97/aquaculture/general.htm.

2. Schwartz, S. H., Aquaculture Research Trends, Nova Publishers, New York, 2008

3. Egna, S. H., and Boyd, C. E., Dynamics of Pond Aquaculture, CRC Press, USA, 1997.

4. Bartholomew, W. G. , B.A., M.S., Ph.D., Chief of Party and Principal Investigator, Aquaculture, International Center for Aquaculture and Aquatic Environments, Department of Fisheries and Allied Aquacultures, Auburn University, 2009.

5. Landau, M., Introduction to Aquaculture, John Wiley and Sons, USA, 1992.

6. Jhingran, V.G., Introduction to Aquaculture, United Nations Development Programme, Food and Agriculture Organization of the United Nations, Nigerian Institute for Oceanography and Marine Research, USA, 1987.

7. New, M. B., Farming Freshwater Prawns; FAO Fisheries Technical Paper, 428, ISSN 0429-9345, 2002.

8. Chopin, T., Buschmann, A. H., Halling, C., Troell, M., Kautsky N., Neori A., Kraemer, G. P., Gonzalez, J. A., Yarish, C and Neefus, C.," Integrating Seaweeds Into Marine Aquaculture Systems", A Key Toward Sustainability, Journal of Phycology 37: 975-986, 2001 .

9. Answers Corporation, 2009
http://www.answers.com/topic/mariculture#cite_note-0

10. Wikipedia, Encyclopedia, 2009.
http://en.wikipedia.org/wiki/Aquaculture#cite_note-figis_fresh-18

11. Akbulut, B., Aquaculture in Turkey, Su Ürünleri Merkez Araştırma Enstitüsü, pp. 4-6, 2006

12. Civaner, E. Ç., Fishery Products, Export Promotion Center Of Turkey, pp.1,Turkey 2007.

13. Civaner, E. Ç., Su Ürünleri Dış Pazar Araştırması, T.C. Başbakanlık Dış Ticaret Müsteşarlığı İhracatı Geliştirme Etüt Merkezi, pp. 75, Turkey, 2004.

14. Soylu M., Marketing Some Aquaculture Species in Turkey, Marmara University, Vocational School Of Technical Studies, Aquatic Products Department, pp.169, 1997.

15. Karakaş H. H., Türkoğlu H., Su Ürünlerinin Dünyada ve Türkiye'deki Durumu, Harran Üniversitesi Ziraat Fakültesi Dergisi, 9(3), 21-28, pp.24, Şanlıurfa, Turkey, 2005.

16. FAO Fisheries and Aquaculture Department, The State of World Fisheries and Aquaculture, Food and Agriculture Organization of the United Nations, pp. 3, Rome, Italy, 2006.

17. Behnke, R. J., Relationships of the Far Eastern Trout, Salmo Mykiss Walbaum Copeia, No. 2, pp. 346–348, 1966.

18. National Geographic, 2009
http://animals.nationalgeographic.com/animals/printable/rainbow-trout.html

19. Root, L., Department Game, Fish & Parks, Rapid City, SD 57702., USA, 1994.

20. Staley, K. and Mueller, J., Rainbow Trout, United States Department of Agriculture, Naturel Resources Conversation Service, Wildlife Habitat Management Institute, USA, 2000.

21. Ecobio Aqua Mar Products, 2009.
http://www.ecobio.com.tr/urun7.asp

22. Ken Baron, Life Cycle of Rainbow Trout, 30.11.2008.
http://eis.bris.ac.uk/~cckhrb/physiology/lc_rbow.htm

23. Integrated Taxonomic Information System (ITIS), 2009.
http://www.itis.gov/servlet/SingleRpt/SingleRpt?search_topic=all&search_value=Oncorhynchus+mykiss&search.htm

24. Liu, Z.J. and Cordes, J. F., DNA Marker Tecnologies and Their Applications in Aquaculture Genetics, Aquaculture, 238, pp 1-37, 2004

25. Groom, M.J., Meffe, G.K. and Carroll, C.R., Principles of Conservation Biology (3rd ed.). Sunderland, MA: Sinauer Associates, United Kingdom, 2006

26. Tisdell, C., Socioeconomic causes of loss of animal genetic diversity: analysis and assessment, Ecological Economics, 45(3), 365-376, 2003.

27. Lankau, R., Loss Of Genetic Diversity Threatens Species Diversity, USA, 2007

28.Genetic Diversity." National Biological Information Infrastructure. NBII. 2008
www.nbii.gov

29. Connexions, Genetic Diversity, 28.07.2004
http://cnx.org/content/m12158/latest/

30. Williams P.H., Humphries, C. J. and Gaston, K. J. (Ed.), Comparing character diversity among biotas, Biodiversity: a biology of numbers and difference. (pp. 54-76), U.K.: Blackwell Science Ltd, Oxford, 1996.

31. Baker, C.S., Maccarthy, M., Smith, P.J., Perry, A.P.and Chambers, G.K., DNA fingerprints of orange roughy, *Hoplostethus atlanticus*: a population comparison.Marine Biology 113: 561–567, 1992.

32. Nevo, E., Genetic variation in natural populations: patterns and theory. Theoretical Population Biology, 13: 121–177, 1978.

33. Ward, R. D., Skibinski, D. O. F., Woodwark, M., Protein heterozygosity, protein structure and taxonomic differentiation, In Hecht M.K., Wallace B., MacIntyre R.J. (eds), Evolutionary Biology. Plenum Press, London, pp 73–159, 1991

34. Nevo, E., Lavie, B., Noy, R., Mercury selection of allozymes in marine gastropods: prediction and verification in nature revisited. Environmental Monitoring and Assessment, 9: 233–238, 1987.

35. Mexican Biodiversity, 2009.
http://www.biodiversidad.gob.mx/v_ingles/genes/geneticvar.html.

36. Yale University, 2009.
http://www.yale.edu/old/cce/papers/ALife/node2.html

37. Burt, A., "Perspective: Sex, Recombination, and the Efficacy of Selection—Was Weismann Right?". Evolution: International Journal of Organic Evolution (54.2): 337–351, 2000.

38. Dorothy, E. and Jorde, P. E., "High Genetic Variability Despite High-Amplitude Population Cycles in Lemmings", *Journal of Mammalogy,* 86 (86.2): 380–385, 2005.

39. Linhart, Y. and Gehring, J., "Genetic Variability and its Ecological Implications in the Clonal Plant Carex scopulurum Holm, In Colorado Tundra", *Arctic, Antarctic and Alpine Research*, 35 (35.4): 429–433 2003.

40. Wills, C., *Genetic Variability*, New York: Oxford University Press, 1980.

41. Bird, A., "Perceptions of epigenetics", Nature 447: 396–398, 2007.

42. Philip Hunter, 24.05.2008 Issue 146
http://www.prospectmagazine.co.uk/2008/05/whatgenesremember/

43. Wolf, R., "Stability and Flexibility of Epigenetic Gene Regulation in Mammalian Development", Nature 447, 425–432, Retrieved on 2008.

44. V.L. Chandler, "Paramutation: From Maize to Mice". Cell 128: 641–645, 2007.

45. Strachan, T. and Read, A. P., Human molecular genetics 3, Garland Science, New York and London, 1999.

46. Butovskaia, P. R. and Pavlova, G. V.," Somatic mosaicism in mice detected using the RAPD-PCR method ", Mol Gen Mikrobiol Virusol (1): 3-7, 2009.

47. Youssoufian H. and Pyeritz R.E., "Mechanism and Consequences of Somatic Mosaicism in Humans", Nat Rev Genet **3**(10): 748-58, 2002.

48. Atienzar ,F. A. and Awadhesh, N. JHA.," The Random Amplified Polymorphic DNA (RAPD) Assay and Related Techniques Applied to Genotoxicity and Carcinogenesis Studies: A Critical Review", Mutat Res **613**(2-3): 76-102, 2006

49. Theodorakis, C. W., Elbl, T. and Shurgert, C. R., "Genetic Ecotoxicology IV: Survival and DNA Strand Breakage is Dependent on Genotype in Radionuclide Exposed Mosquitofish, Aquat", Toxicol, 451999.

50. Nadig, S. G., Lee, K. L. and Adams, S. M., Evaluating Alterations of Genetic Diversity in Sunfish Populations Exposed to Contaminants Using RAPD Assay, Aquat Toxicol, 43, 1998.

51. Uzonur I., Abasiyanik M.F., Bostanci B., Eyidemir M., Ocba N., Yanik C. and Petek M., Re-exploring planaria as a model organism for genotoxicity monitoring by an 'Improved Random Amplified Polymorphic DNA' approach, Fres. Environ. Bull., 13, 12a, 1420-1426, 2004a

52. Uzonur I., Abasiyanik M.F., Cam S., Cobanli K., Elmas A., Erdogan H., Hizal S., Karabulut D.S., Ozdemir M., Yesil F.A. and Petek M., A Preliminary report on target organ genotoxicity biomonitoring by an 'Improved Random Amplified Polymorphic DNA Assay'. Fres. Environ. Bull., 13, 12a, 1453-1456, 2004b.

53. Uzonur I., Genotoxicity monitoring with Improved RAPD Assay on Mytilus galloprovincialis as a model. PhD Dissertation, Istanbul University, Institute of Marine Sciences and Management, Physical Oceanography and Marine Biology Department, Istanbul, 2005.

54. Yılmaz, E., “Alabalık Yetiştiriciliği”, T.C Başbakanlık GAP Projesi Bölge Kalkınma İdaresi Başkanlığı, pp. 2-17, 2004.

APPENDIX A

DATA FOR MINISTRY OF AGRICULTURE AND RURAL AFFAIRS

The results of analysis of two fish farms from Kahramanmaraş Agriculture Management

Table A.1 Ministry of agriculture and rural affairs heavy metal analysis results of fish.

ANALYSIS	RESULT	METHOD	INSTRUMENT	LIMIT OF DETECTION
(Mercury)(mg/kg)	<0,001	Inhouse	ICP	0,001
Pb(Lead)(mg/kg)	<0,01	Inhouse	ICP	0,01
Zn(Zinc)(mg/kg)	4,02	Inhouse	ICP	0,05
As(Arsenic)(mg/kg)	<0,003	Inhouse	ICP	0,003
Cd(Cadmium)(mg/kg)	<0,01	Inhouse	ICP	0,01
Cu(Copper)(mg/kg)	0,25	Inhouse	ICP	0,01

Table A.2 Ministry of agriculture and rural affairs antibiotics analysis of fish samples.

ANALYSIS	METHOD	LIMIT OF DETECTION (μg/kg)	MRL (μg/kg)	RESULT OF ANALYSIS (μg/kg)
Oxytetracycline	CHARM-II	100	100	NEGATIVE
Tetracycline	CHARM-II	20	100	NEGATIVE
Chlorinetetracycline	CHARM-II	100	100	NEGATIVE
Doxycycline	CHARM-II	100	100	NEGATIVE

MRL (Maksimum Residue Limit)

Meat, milk and animal-source foods such as eggs in the determination of residual sulfonamite is important in terms of health.

Table A.3 Sülfonamit Analysis Results

ANALYSIS	METHOD	LIMIT OF DETECTION (μg/kg)	MRL (μg/kg)	RESULT OF ANALYSIS (μg/kg)
Sulfadiazine	CHARM-II	20	100	NEGATIVE
Sulfathiazole	CHARM-II	40	100	NEGATIVE
Sulfamerazin	CHARM-II	20	100	NEGATIVE
Sulfamethazin	CHARM-II	50	100	NEGATIVE
Sulfamethoxazol	CHARM-II	50	100	NEGATIVE
Sulfadimethoxine	CHARM-II	20	100	NEGATIVE

Water Analysis Report of the GÖKKUŞAĞI AQUACULTURE Facility in Kahramanmaraş

Table A.4 Physical Properties of Water of the Gökkuşağı Aquaculture

PH	8.41
COLOR	-
TURBİDİTY	-
ECX10^6 micromhos/cm	330

Table A.5 Chemical Properties of Water of the Gökkuşağı Aquaculture

Alkalinity carbonate, mg/l	0.0	Sodium DO, mg/1	8.8
Alkalinity bicarbonate, mg/l	195.2	Potassium, mg/1	_
Chloride, mg/l	7.0	Calcium, mg/1	62.12
Sulfate, mg/1	_	Magnesium, mg/1	13.37
Ammonia, mg/1	0.024	Total Iron, mg/1	_
Nitrite, mg/1	0.003	Total Manganese, mg/1	_
Nitrate, mg/1	1.20	Total NACI , mg/1	211.2
Chromium (Cr**) mg/1	_	*Suspension substance, mg/1	_
Phosphate, mg/1	0.0	*Organic matter, mg/1	0.56

Water Analysis Report of the ÇAĞLAYAN AQUACULTURE Facility in Kahramanmaraş

Table A.6 Physical Properties of Water of the Çağlayan Aquaculture

PH	8.29
COLOR	-
TURBİDİTY	-
ECX 10^6 micromoles/cm	341

Table A.7 Chemical Properties of Water of the Çağlayan Aquaculture

Alkalinity carbonate, mg/l	0.0	Sodium DO, mg/1	8.8
Alkalinity bicarbonate, mg/1	189.2	Potassium , mg/1	-
Chloride, mg/l	7.0	Calcium, mg/1	64.12
Sulfate, mg/1	-	Magnesium, mg/1	13.37
Ammonia, mg/1	0.034	Total Iron, mg/1	-
Nitrite, mg/1	0.004	Total Manganese, mg/1	-
Nitrate, mg/1	1.25	Total NACI, mg/1	218.2
Chromium (Cr**) mg/1	-	*Suspension substance, mg/1	-
Phosphate, mg/1	0.0	*Organic matter, mg/1	0.32