



Khorramshahr University of Marine Science & Technology

Faculty of Marine Science and Oceanography

**Department of Marine Biology** 

M.Sc. Thesis

# Study of Genetic Diversity of Orange-spotted Grouper, Epinephelus coioides (Hamilton, 1822), in the Persian Gulf Using Microsatellite Markers

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# **Proffer to:**

All Pigeons that flight amorous

Specially to Islam's adolescent headed, Martyr M. Jahanara and Khorramshahrian people in 34 days resistance, unknown Martyrs and all sincerely and brave warriors of Beit-Al Moghaddas sally.

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)

**Key Words:** Genetic diversity, Microsatellite, Persian Gulf, Orange-spotted grouper, *Epinephelus coioides*.

#### Abstract:

Genetic diversity of *Epinephelus coioides* (Hamilton, 1822), Orange-spotted grouper, that inhabits coastal reefs from the western Indian Ocean to the western Pacific Ocean, was studied based on six polymorphic microsatellite loci in the Persian Gulf waters. 3-5 gr of each individual's soft tissue were prepared through 120 hunted individuals. Samples were fixed in pure ethanol (96%) and transferred to the laboratory. Khuzestan, Bushehr, Dayyer and Bandar-Abbas were 4 sampling stations. Sampling accomplished during June and July 2010. DNA extraction was performed using a standard ammonium/acetate method. Quantification and quality control of DNA were done using spectrophotometery method and 1% agarose gel electrophoresis. The DNA of each individual was amplified via polymerase chain reaction (PCR), primed by six microsatellite primers. For better separation of DNA fragments, PCR products were run on polyacrylamide gel. Staining was done through silver nitrate. Mean number of real alleles for all loci in each population was between 4.667 (Khuzestan) and 6.333 (Bandar-Abbas). Statistical analysis represented mean of genetic parameters include real and effective alleles, observed and expected heterozygosity for over loci in all populations, 5.458, 3.793, 0.500 and 0.649 respectively. Chi-Square test showed that all populations in all studied loci probably due to homozygote excess or small effective population sizes were out of equilibrium with the exception of Khuzestan population in Em-10 loci. AMOVA test showed maximum rate of F<sub>st</sub> (0.086) and minimum rate of  $N_m$  (2.652) between Khuzestan and Bushehr populations. Also minimum rate of  $F_{st}$ (0.034) and maximum rate of N<sub>m</sub> (7.070) were between Dayyer and Bandar-Abbas populations. Maximum Nei genetic distance (0.226) and minimum Nei genetic identity (0.798) between Khuzestan and Bushehr population also minimum genetic distance (0.105) and maximum genetic identity (0.900) between Dayyer and Bandar-Abbas populations were represented. Khuzestan was the ultimate population and results were showed an intermediate separation from other studied populations; thereby separated fisheries management is recommended. Final results exhibited that there was no considerable genetic diversity variance among regions and populations (8%), therefore maximum variance percentage related to within population differences (92%).

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# CHAPTER 1:

Introduction and Generalities

# 1-1. Introduction

## **1-1-1. Importance and value of this survey:**

Orange-spotted grouper (*Epinephelus coioides*, Hamilton, 1822) is one of the most valuable and marketable fishes of the Persian Gulf. There is no study for genetic diversity and population structure of this species in the Persian Gulf so far. Thereby this survey can answer some questions about characteristic of this valuable and economic fish population in the Persian Gulf. Also *E.coioides* is in IUCN red list of near threatened species (iucnredlist, 2010) so this study can help in designing better protection programs.

Aquaculture management needs to have enough information about all characteristic of wild or cultured species. Molecular studies of valuable species may show variation of attributes important for aquaculture and preservation of genetic diversity.

#### **1-1-2.** Theories for this study:

1. Epinephelus coioides has different populations in the Persian Gulf.

2. Persian Gulf *E.coioides* containing high inter-species diversity.

3. Genetic variation of *E.coioides* populations is depending the geographical distance.

4. Microsatellite as a molecular marker has ability to appointment genetic diversity of *E.coioides* populations.

#### **1-1-3.** The aims for this study:

1. To recognition of probable populations of E.coioides in Iranian water of Persian Gulf.

2. To assess genetic diversity of Persian Gulf E.coioides.

3. To identity assessment of *E.coioides* populations in the Persian Gulf.

4. To determine phylogeny between Persian Gulf E.coioides populations.

# **1-2.** Generalities

#### 1-2-1. Persian Gulf:

The Persian Gulf is unique water extend, habitat for many fishes, crustaceans, birds and etc. contains single in kind marine mammals (dugongs), mangroves and coral islands. Persian Gulf located in Southwest of Asia flanked by Iran and the Arabian Peninsula and is extension of the Indian Ocean. Ecology of this unique water extend became in danger, has come under pressure from industrialization, and in particular, oil and petroleum leakage during wars specially Iran-Iraq war and Persian Gulf War in recent decades (Wikipedia, 2011).

#### Geography:

Persian Gulf with the extent of almost 251,000 Km<sup>2</sup> is an inland sea. Eastern limit is the Strait of Hormuz that is narrowest part of the Gulf whit 56 Km in wide. Total length of Persian Gulf is nearly 989 km that Iran covered most of the Northern coastlines. Persian Gulf is limited at west by the great river delta of the Arvandrud contain Karun and Shatt al-Arab (Shatt al-Arab itself create with joining of two famous rivers Euphrates and Tigris). The waters are overall very shallow, with a maximum depth of 90 meters and the mean depth of 50 meters. Persian Gulf contains several Islands. Some of them with an ecological importance like Kish as a coral island although there is more coral reefs and some with political and strategically position like Greater Tunb, Lesser Tunb and Abu Musa. The biggest island in the Persian Gulf is Qeshm Island located in the Strait of Hormuz. In recent years, the artificial islands although very small, have had a negative impact on the mangrove habitats upon which they are built, often causing unpredicted environmental issues (Wikipedia, 2011).

#### Wildlife:

Persian Gulf contains unique and diverse wildlife due to its separation from the international waters only breached by the narrow Strait of Hormuz. Persian Gulf is inhabited some of the most splendid marine fauna and flora, some of which are near extinction or at serious ecological threat. One of good examples of this beings are the mangroves which belonging to the genus Avicennia or Rhizophora require tidal flow and a combination of fresh and salt water for growth, and act as nurseries for many crabs, small fish, and insects; these fish and insects, are the source of food for many of the marine birds that feed on them. They are as critical an indicator of biological fitness on the surface of the water, as the corals are to biological fitness of the gulf in deeper waters (Yamada et al., 2002). Unfortunately however, mangroves, fishes spawning on them, the crustaceans that rely there and the species that feed on them all are at serious environmental risk. One of the most unique marine mammals living in the Persian Gulf is Dugong dugon, also called sea cows. These are gentle mammals that feed on the sea grass, and genetically resemble the land mammals more than the dolphins and the whales. 7,500 remaining dugongs making the Persian Gulf the second most important habitat for the species after Australian waters with some 80,000 dugong. Dugong's current number is decreasing and it is not clear as of now how many are currently alive (Picow, 2010a).

Coral is another important inhabitant of the Persian Gulf waters. Corals are vital ecosystems that support huge number of marine species, and whose health directly reflects the health of the gulf. Recent years have seen a severe decline in the coral population in the gulf, partially owing to global warming but majorly due to irresponsible dumping by surrounding countries (Picow, 2010b).

Also many migratory and local birds are chosen Persian Gulf as temporary or perpetual habitat. Persian Gulf is also habitat to over 700 species of fish, most of which are native to the gulf. Of these 700 species, more than 80% are coral reef related, and directly or indirectly depend on the coral reef for their survival (fishbase, 2011).

As it was mentioned this unique Gulf in the light of ecology, policy, economy and etc, is in danger seriously. Global mention or regional responsibility is necessary for preservation of this delicate ecosystem that been in harmony for hundreds of years.

## 1-2-2. Characteristics and Importance of Orange-spotted grouper:

*Epinephelus coioides*, the **Orange-spotted grouper**, belongs to the family Serranidae. *E.coioides* and other members of the genus Epinephelus and the two sister genera Plectropomus and Cromileptes have an important role in capture fisheries production, with almost 250,000 metric tons landed. Moreover, *E. coioides* is among a few species of *Epinephelus* that are chosen for aquaculture and showed an increasing trend for the world's annual production (Antoro *et al.*, 2006). Orange-spotted grouper is known as **Estuary cod** too (Cornish and Harmelin, 2004).

#### **1-2-2-1. Taxonomy:**

Taxonomy of studied species in present research, Orange-spotted grouper is exhibited in table 1-1.

	Taxonomy of epinephelus coioides
Kingdom	Animalia
Phylum	Chordata
Subphylum	Vertebrata
Superclass	Osteichthyes
Class	Actinopterygii
Subclass	Neopterygii
Infraclass	Actinopteri
Superorder	Acanthopterygii
Order	Perciformes
Suborder	Percoidei
Family	Serranidae
Genus	Epinephelus
Species	Epinephelus coioides (Hamilton, 1822)

#### Table 1-1. Epinephelus coioides taxonomy

## 1-2-2-2. Morphology:

Fusiform body little pressed from the sides, maximum length of 120 cm, 39 cm in standard length, 58-65 scales in lateral line, two teeth rows in downer maxilla, cetenoid scales in body sides, white brown of body color with 5-7 dark vertical lines, almost equal nose holes, total dorsal spines: 11, total dorsal soft rays: 13-16, anal spines: 3 and anal soft rays: 8 are some characteristics of this fish (Myers, 1999). Schematic image of *E. coioides* is apparent in figure 1-1.

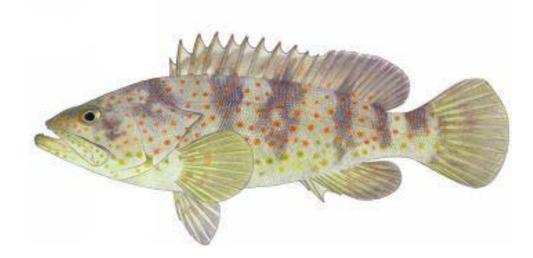


Figure 1-1. Schematic image of Epinephelus coioides (fishbase, 2010)

# 1-2-2-3. Habitat:

Its natural habitats are subtropical or tropical mangrove forests, turbid coastal reefs, shallow seas, subtidal aquatic beds, coral reefs, estuarine waters, intertidal flats, and coastal saline lagoons. Juveniles are common in shallow waters of estuaries over sand, mud and gravel and among mangroves. *E. coioides* threatened by habitat loss (Cornish and Harmelin, 2004).

## 1-2-2-4. Feeding:

Orange-spotted grouper is carnivorous species and usually feed on small fishes, shrimps, and crabs (Shapiro, 1987).

#### 1-2-2-5. Global dispersion:

*E.coioides* inhabits areas from the Red Sea, south to at least Durban, east to the western Pacific from the Ryukyu Islands to Australia, and eastward to Palau and Fiji (Heemstra and Randall, 1993). *E.coioides* reported from Eastern Mediterranean waters recently (fishbase, 2010).

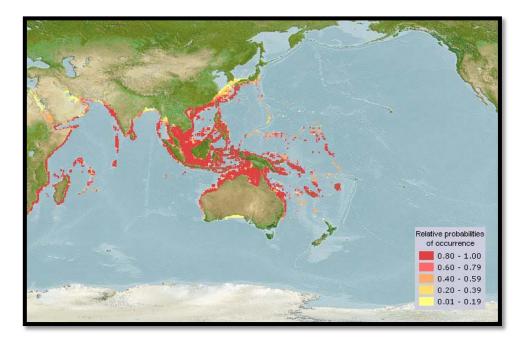


Figure 1-2. Global distribution map of E. coioides (fishbase, 2011)

## 1-2-2-6. Reproduction:

Sex reversal is one of the main characteristics of this species. Juveniles are female. Females are mature at 25–30 cm total length (2–3 years old), sexual transition occurs at a length of 55–75 cm, and the major spawning period in the Persian Gulf is from March to June. Probably spawn during restricted periods and form aggregations when doing so. Eggs and early larvae of *E. coioides* are probably pelagic (Heemstra and Randal, 1993).

# 1-2-3. Terminologies of Population Genetics

#### 1-2-3-1. Population:

A group of organisms of the same species living within a distinguished geographical area will be called as a population. Sometimes the area is large; like that time North American sparrows are object or may even include the entire earth, as in mention to the human population. More commonly, the area within which individuals in the population are likely to find mates is considered. Many geographically prevalent species are subdivided into several distinct breeding groups, called subpopulations; that live within limited geographical areas. Each subpopulation is a local population (Hartl and Jones, 1998).

#### 1-2-3-2. Population genetics:

Four main evolutionary processes inclusive natural selection, genetic drift, mutation and gene flow influence on allele frequency distribution and allele frequency vary under the pressure of them. Study of this variations, population subdivision and population structure located in population genetics domain. It effort is to explain such phenomena as adaptation and speciation (Postlethwalt *et al.*, 2009). Population genetics was a vital part in the emergence of the modern evolutionary synthesis. Its chief primary founders were Sewall Wright, J.B.S. Haldane and R.A. Fisher, who also laid the basics for the related discipline of quantitative genetics (Bowler, 2003).

## 1-2-3-2-1. Natural selection:

Natural selection is the progression by nature permit those individuals are compatible with natural conditions, those are more powerful and better fighter to survive and have successful reproduction. Factors which affect reproductive success are also important an issue which Charles Darwin developed in his ideas on sexual selection. Natural selection takes action on the phenotype, or the observable characteristics of an organism, but the genetic is root of any phenotype which gives a reproductive advantage will become more common in a population. Over time, this process can result in adaptations that specialized organisms for particular ecological niches and may finally result in generation of new species. Natural selection is one of the bases of modern biology. The term was introduced by Darwin in his revolutionary 1859 book "On the Origin of Species" (Huxley, 2003).

## 1-2-3-2-2. Genetic drift:

Genetic drift is accidental changes in allele frequency specially occurs in small population. That is, the alleles in the brood of the population are a random sample of those in the parents. And chance has a role in assessment whether a given individual survives and reproduces. A population's allele frequency is the fraction or percentage of its gene copies compared to the total number of gene alleles that share a particular form (Futuyma, 1998).

Genetic drift can decrease homozygosity and diversity. In contrast to natural selection, which makes gene variants more common or less common depending on their reproductive success, (Avers, 1989) the changes due to genetic drift are not driven by environmental or adaptive pressures, and may be beneficial, neutral, or detrimental to reproductive success. The effect of genetic drift is larger in small populations, and smaller in large populations. Discussion among scientists over the relative importance of genetic drift compared with natural selection. Ronald Fisher held the view that genetic drift plays at the most a minor role in evolution, and this remained the dominant view for several decades. In 1968 Motoo Kimura rekindled the debate with his neutral theory of molecular evolution which claims that most of the changes in the genetic material are caused by genetic drift (Futuyma, 1998).

#### 1-2-3-2-3. Mutation: