**In the name of God**

**FINAL DRAFT REPORT ON:**

# CONSERVATION AND RESTORATION OF CASPIAN TROUT

(*Salmo trutta caspius*)

## STOCKS IN THE SOUTHERN CASPIAN SEA


Conducted by:

**S.M.Jalil Zorriehzahra D.V.M & Ph.D**

*Manager of Caspian Sea Environment Program (CEP)*

*Project in Coldwater Fishes Research Center (CFRC)*


March 2012

## Table of contents

## 1) Background:

Biodiversity includes the variety of living organisms at genetic, species, and higher levels of taxonomy, as well as the variety of habitats and ecosystems and the processes that occur in them. The loss of biological diversity (as defined above) has disrupted the Caspian ecosystem in fundamental ways. Biodiversity is intimately linked to ecosystem function. Healthy, resilient ecosystems – those that contain natural assemblages of organisms, habitats, interactions and processes – can sustain exploitation. Disrupted ecosystems collapse. The Caspian salmon, *Salmo trutta caspius* (Kessler, 1877), originally widely distributed from the area surrounding the Mediterranean basin to north Scandinavia and Soviet Union, and from Spain to the Caspian Sea. This species must also have had different stocks or genetically distinct units. In the Pacific Northwest of North America, the US FWS has identified over 100 genetically distinct units or races of wild salmon, specifically adapted to individual river conditions and seasonal distinctions. The same level of intra-species diversity may still exist for *Salmo trutta caspius*, although this is currently unknown. The Caspian region is the global center for diversity and endemism of members of the genus *Salmo*, especially the brown trout (*Salmo trutta*). Caspian salmon (*Salmo trutta caspius*-Kessler 1877) are believed to be the largest of the European salmon, which includes the Atlantic salmon (*Salmo salar*) and brown trout (*S. trutta*). The Caspian salmon is listed in the Red Books of Russia, Kazakhstan, and Turkmenistan. In Azerbaijan, it is characterized as a sharply declining species. It is critically endangered in the southern part of the Caspian Sea according to IUCN criteria (Kiabi *et al.*, 1999; Coad, 2000) .The The Caspian salmon, is one of the most valuable fish in Iranian waters of the Caspian Sea. The Caspian salmon is anadromous and migrates up rivers in Northern Iran (mainly in Karganrud, Navrud, Tonekabon, and Sardabrud rivers) to spawn. The Caspian salmon attains the greatest length, weight, and growth rate within *Salmo trutta* complex (Sedgwick, 1995). Natural populations of Caspian salmon have declined drastically in recent decades as a result of over-fishing, poaching, river pollution, destruction of natural spawning areas, and drought (Abdoli, 2000; Barannik *et al.*, 2004; Niksirat & Abdoli, 2009). Due to sharp decline in population of this species, artificial breeding has been attempted in I.R.Iran, and huge investments by government have been made for enhancement of wild populations through releasing of fingerlings produced in hatchery (Jalali and Amiri, 2009).
Stocking can be regarded as a useful management technique for improving stocks of salmonids (Aprahamian *et al.*, 2003).

Captive breeding and conservation programs have been initiated to produce, restore, and protect populations of Caspian salmon (Sarvi *et al.,* 2006; Jalali & Amiri, 2009).

Stocking is defined as "the repeated injection of fish into an ecosystem in which a population of that species already exists from one external to it, i.e. a stocked species may be either already native to the recipient water body or exotic to it but previously introduced" (Cowx, 1998). The maintenance of genetic diversity is a primary goal for conservation breeding programs, so, its appropriate management is essential for species conservation programs (O'Connell & Wright, 1997).

The Shahid Bahonar hatchery center (Kelardasht ) on the River Sardabrud is the only active stocking center of Caspian salmon in I.R.Iran. The broodstocks are captured each year in the mouth of the Tonekabon river (Kiabi *et al.,* 1999). The broodstocks are captured in the autumn which runs up rivers in October–November. About 500,000 fingerlings are produced annually and released into the Caspian Sea . It takes 2 years before the fingerlings reach a weight of 15–20 g, corresponding to a length of 10–15 cm, which is considered suitable for releasing.

Unfortunately the broodstocks just supplied from Cheshmeh kileh (Tonekaboon) river and other rivers in the western region do not take into account for collection of broodstocks restocking purposes (Kiabi *et al.* 1999; Hatef *et al.,* 2007).

The stocking programme started in 1983 and between 1983 till 1995 the releasing of fingerlings had increasing trend. Totally 8.6 million fingerlings released into the rivers (mostly in Tonekaboon and Sardabrud rivers ) between 1983 till 2010 and in the same period 83.2 tons Caspian salmon has been caught totally ( Fig. 1 ).

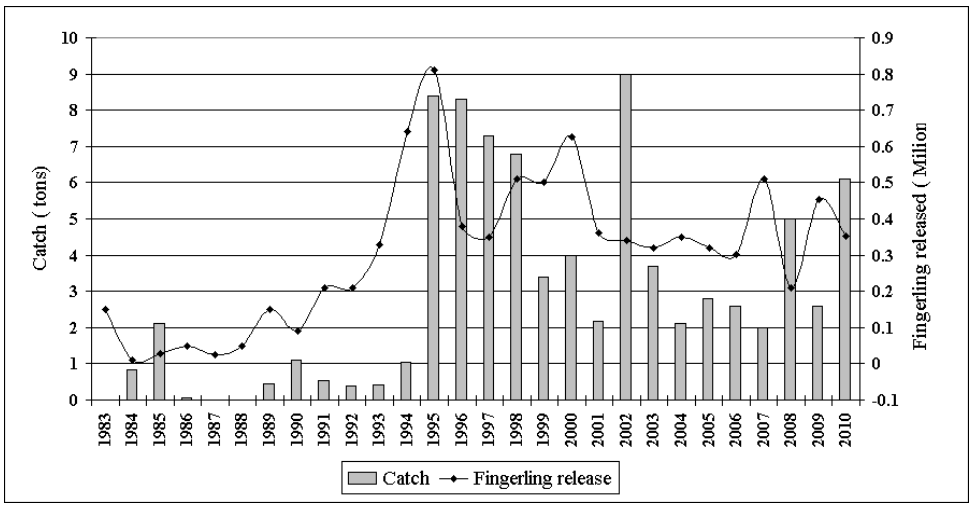



Figure 1: Historical trend of total catch and releasing of fingerlings of Caspian salmon in Iranian waters of the Caspian Sea

## 2) Introduction:

Caspian Sea (ancient Caspium Mare or Hyrcanium Mare), saltwater lake in southeastern Europe and southwestern Asia is the largest inland body of water in the world. The Caspian Sea is bordered on the west by Azerbaijan and Russia, on the northeast and east by Kazakhstan, on the east by Turkmenistan, and on the south by Iran. It extends about 1210 km (about 750 mi) in a northern and southern direction and about 210 to 436 km (about 130 to 271 mi) in an eastern and western direction. It has an area of 371,000 sq km (143,000 sq mi). The Caspian coastline is irregular, with large gulfs on the east, including Krasnovodsk Gulf and the very shallow Garabogazk Gulf, which acts as an evaporation basin and is the site of a major chemical plant that extracts salts from the deposits. The Caspian Sea has a mean depth of about 170 m (about 550 ft) and is deepest in the south. Its level varies from year to year but averages about 28 m (92 ft) below sea level. In the 1960s and 1970s the level fell substantially, partly because water was withdrawn from tributary rivers for irrigation and other purposes (Fig. 2.)

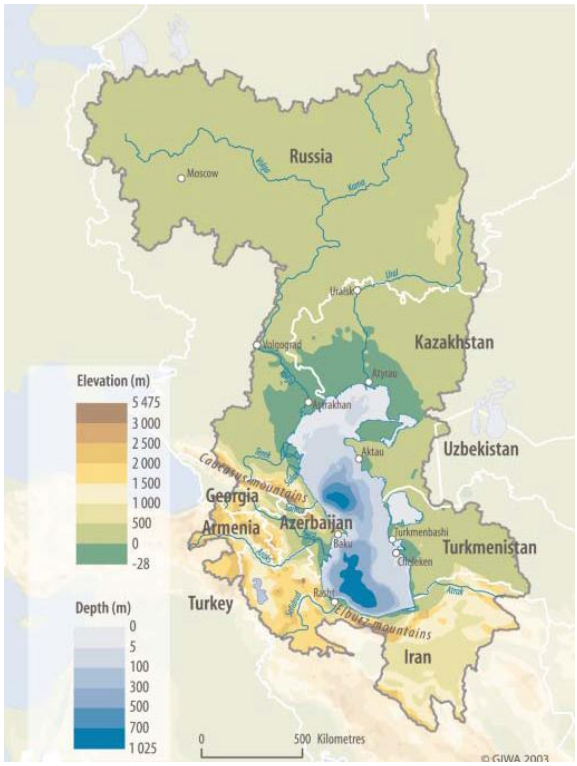



Figure2: Map of the Global International Waters Assessment (GIWA) Caspian subregion (Drainage basin of the Caspian Sea) Source: GIWA Core Team, 2003.

In 1980, a dike was built across the mouth of Garabogazk Gulf to reduce water loss, creating a lake that was expected to last for several years. Instead, the gulf dried up completely by 1983. In the meantime, the level of the Caspian Sea began rising again at a rate of about 14 to 20 cm (about 6 to 8 in) annually. To restore water flow into Garabogazk Gulf an aqueduct was built.

The southern and southwestern shorelines of the Caspian Sea are bordered by the Elburz Mountains and the Caucasus Mountains. The sea has numerous tributaries, notably the Volga, Ural, and Zhem rivers, all of which flow into it from the north. Other tributaries include the Gorgan (Gurgan) and Atrek rivers, flowing from the east, and the Kura River, flowing from the west. The sea has no outlet. The Caspian Sea is linked to the Baltic Sea, the White Sea, and the Black Sea by an extensive network of inland waterways, chief of which is the Volga River. These waterways provide an outlet to northern Europe for the oil fields of Baku, Azerbaijan on the Abşeron Peninsula. The Caspian Sea also contains highly productive fisheries, yielding valuable catches of sturgeon (the chief source of caviar), salmon, perch, herring, and carp. Other animal life in the Caspian Sea includes tortoises, porpoises, and seals.

Dam construction, irrigation, sand and gravel excavation from rivers have reduced river loads to 40 %. This culminates in river mouth erosion and retreat of seaward edge of deltas. Moreover, these changes have caused significant ecological impacts in the river mouths and the Caspian Sea. A sharp decrease in the fish stock of the Caspian Sea during the past few decades can be attributed to a reduction in the fish migration into the river mouth in spawning period (Saiko, 1996)

All 61 rivers that flow from Iranian coast to the Caspian Sea are subject to intensive human activities. A densely populated coastal area (7 million settlements in 15000 Km2) and distribution of high water consuming crops such as rice and cotton makes the Iranian coast of the Caspian Sea different from other parts of the sea (Lahijani, 2004).

## 2-1) The Caspian Sea and its Catchment Area:

The Caspian Sea is a semi-eclipse basin oriented in a N-S direction with a length of about 1,200 km and width of about 400 km. The sea surface (at a 28 m height) is around 360,000 km2 and the water volume exceeds 78,000 km3 (Nikolaeva, 1971). On the basis of the sea floor morphology, the Caspian Sea is divided into three sub-basins: northern, middle and southern with the average, maximum depth increasing from North to South (Voropaev,

1986). The Caspian Sea catchment basin covers an area of 3.5 km2, which is mainly located in the littoral states of Iran, Turkmenistan, Kazakhstan, Russia and Azerbaijan, and with small parts in Turkey, Armenia and Georgia (Mikhailov, 1997; Voropaev, 1986) (Fig.3).

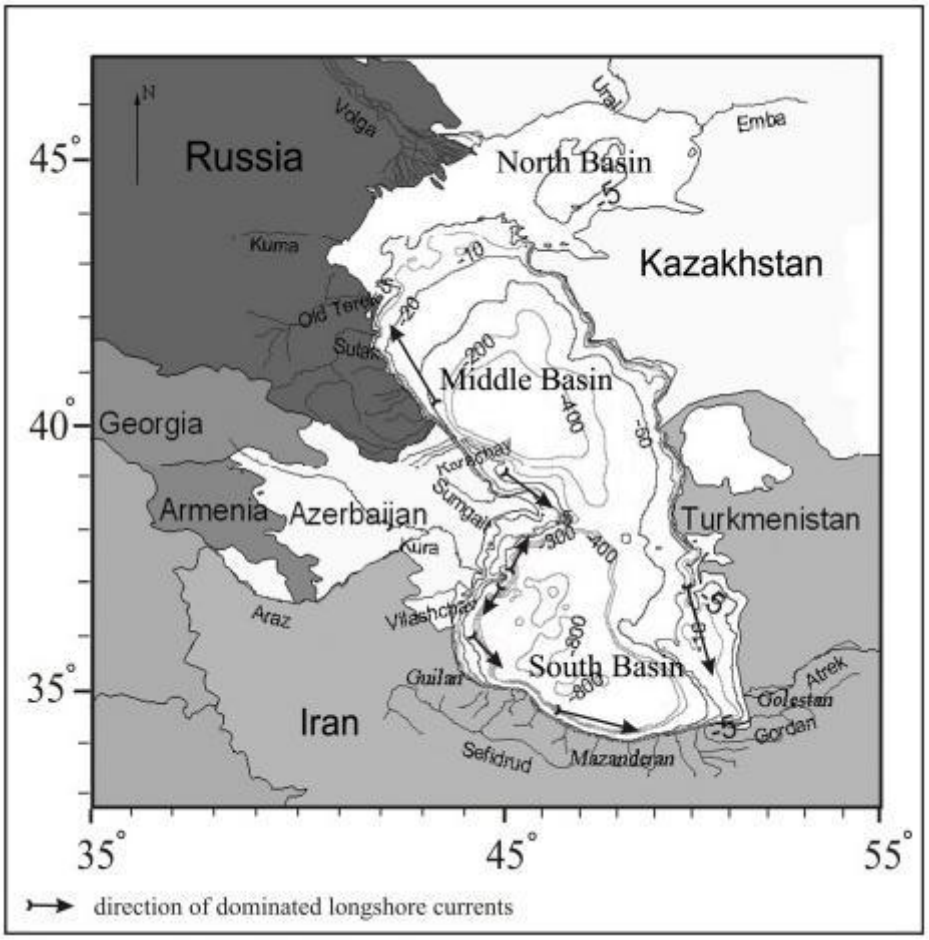



Figure 3: Caspian Basin including bathymetry and major rivers

The northern part of the basin is located in high latitude forests, middle Volga steppe and Pre-Caspian deserts. The western and southern parts of the basin are situated in the high mountain ranges of the Caucusus and Alborz, respectively. The Copet-Daq Mountains cover the southeastern part and whole of the eastern part comprises a wide area of deserts (Voropaev, 1986). The Caspian drainage basin in Iran covers an area of 185,000 km2, which encompasses the whole of the northern part of Iran. The northwestern and northeastern rivers of Iran flow into the sea through Azerbaijan and Turkmenistan, respectively (Afshin, 1993; Lahijani, 1997). The rivers that flow to the Caspian Sea through the Iranian coast have a drainage basin of 135,000 km2, most of which is located on the northern flank of the Alborz Mountain range. The Sefidrud as the greatest river of the Iranian coast drains various parts of

the Zagros and Alborz mountain ranges. Gorganrud on the East coast is another river that cut through Alborz and drains the Copet-Daq mountain range. Moderate rivers originate in the northern slopes of the Alborz range. Their headwater is located in a mountainous area with little vegetation covering that gradually changes to a densely forested
area, then they pass through the South Caspian coastal plain. The small rivers mainly begin from densely vegetated hills with average an elevation of 200-300 m (Fig.4).

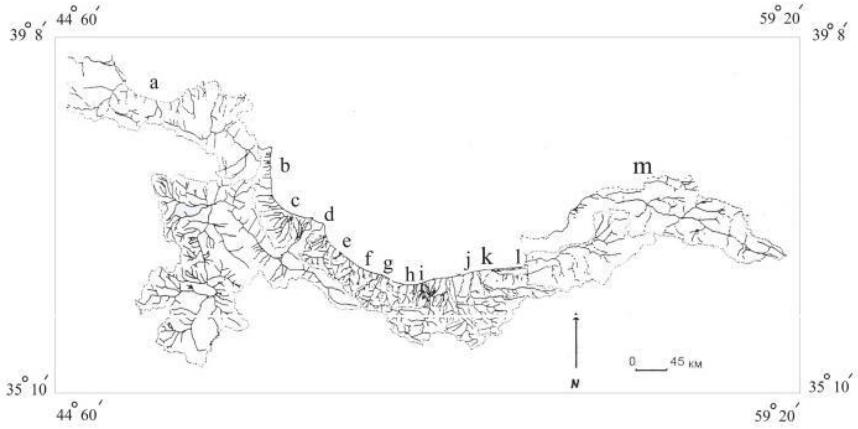



Figure 4: Caspian Catchment basin in Iran.

(a): Water of north-west basin of Iran flows through Azerbaijan territory to the Caspian Sea (Arkas- Kura). (b): West Gilan basin, main river: Karganrud. (c): Anzali Lagoon basin, main river: Pasikhan. (d): Sefidrud Basin,(e): East Gilan basin, main river: Polrud.(f): West Mazanderan basin, main river: Cheshmehkile.(g): Central Mazanderan basin, main river: Chalus. (h): Haraz basin. (i): Talar and Babolrud basin. (j): Tajan basin. (k): East Mazanderan basin, main river: Neka. (l): Golestan basin, main river: Gorganrud. (m):
Water of north- east Iran flows through Turkmenistan (Atrek River) to the Caspian Sea.

Annual precipitation in the south Caspian coasts varies from 1,500 mm in west to 150 mm in the east. Vegetation, humidity and temperature of the coasts change from subtropical in the west to arid/semiarid in the east (Khaleghizavareh, 2005). Precipitation over the upstream of the Sefidrud and Gorganrud Rivers is about 300 mm and 200 mm, respectively.
Precipitation in the coastal plain and forested areas, occurring mostly as rain, is distributed throughout the year. Upstream of the major and minor rivers receive their water as snow that falls during cold season (November to March).

## 2-2) Environmental Impacts of Climate Changes and Human Activities

The Caspian Sea as a closed basin has a unique natural and some socio-economic features, whose interaction is subject to environmental tension (Golubev, 1996). It is obvious that human activities are major factors in the environmental changes, but nature is also capable of rapid and dramatic changes (Leroy, 2005). The Caspian Sea and its catchment areas experienced different environmental changes during the Quaternary period (Varushchenko et al., 1987). Variations in humidity, precipitation, river influx and the sea level fluctuations have been the main consequences of the Caspian environmental changes (Klige, 1980; Meshcherskaya, 2001). The Caspian Sea as the world's largest land-locked water basin has drawn human attention since the late Pleistocene-early Holocene period coinciding with the early civilizations forming along the southern coast of Caspian Sea. Important sites in the eastern part of the South coast are Krasnovodsk, Ashghabad, Gorgan and Behshahr. In the western part Roudbar, Hashtpar and Baku are the identical points. They are mainly located above the Khavalyn Sea level from the Paleolithic to Neolithic ages (Ravasani, 1994). In the human occupation sites, plentiful amounts of animal bones have been found which belong to species currently living in the Alborz forests. After the primary civilization which was scattered along the Caspian coast, the Persian, Hun, Khazar, Ottoman and Russian empires were the dominant powers in circum-Caspian region during succeeding historic eras. Iran, Russia and the newly-independent Republics of Turkmenistan, Kazakhstan and Azerbaijan which were formed after the collapse of the Soviet Union in 1991 are located on the Caspian coast. The population settling in the Caspian catchment area is estimated to be around 80 million, most of which lives in Russia (73 %), Iran (13 %) and Azerbaijan (10 %) (Lahijani, 2001) (Table 1).

**Table 1- Description of circum-Caspian states**

| Country | Area of the country ($Km^2$) | Area located in Caspian watershed ($Km^2$) | Population of country (Million) | Population settled in Caspian watershed |
|---|---|---|---|---|
| Iran | 1685000 | 185000 | 65 | 11 |
| Turkmenistan | 488100 | 400000 | 4.1 | 0.4 |
| Kazakhstan | 2717300 | 695000 | 17.1 | 0.4 |
| Russia | 17075400 | 1800000 | 148 | 60 |
| Azerbaijan | 86600 | 86600 | 7.6 | 7.6 |

Widespread human activities in the Caspian region deeply have affected its natural environment. Developments in industry, agriculture, fishery, marine transportation, and urbanization from the middle $20^{th}$ century in the Caspian basin have increased the human impacts on the Caspian Sea (Komarov, 1996; Mikhailov, 1997). Both influencing factors, i.e. natural and human induced, control the river mouth configuration by changes in fluvial discharge and basinal parameters mainly of the sea level.

## 2-3) Species and habitats

The biological diversity of the Caspian and its coastal zone makes the region one of the most valuable ecosystems in the world and the loss of biological diversity, at the genetic, species and ecosystems levels has disrupted the Caspian Sea ecosystem and correspondingly the fisheries of the Caspian in fundamental ways. Biodiversity is intimately linked to ecosystem function.

There are over 120 fish species in the southern part of the Caspian Sea, which are commercially divided in two groups of sturgeons and bony fishes. The bony fishes are also divided into kilka and other species.

The Caspian salmon, *Salmo trutta caspius* Kessler, is one of the nine subspecies of brown trout *Salmo trutta* in the world and native to the Caspian Sea (Quillet, *et al.*, 1992).

Due to diversity of conditions existing in different parts of the Caspian Sea, certain of water areas and the coastal zone serve as habitats for certain groups of organisms.

## 2-4) Main fish habitats

Fishes have three main habitats through their life: spawning ground, feeding ground and wintering ground. For freshwater fishes (fishes making short migrations) these areas overlap. Semi-anadromous fish species feed in the sea and return to rivers only for wintering and spawning. Anadromous species live in the sea, return to rivers only for spawning. The pelagic species spend their whole life in the sea. Some of them migrate to considerable distances within the sea, others spend their life on a rather small area. Majority of Caspian fishes range in coastal zones at the depths of 50-75 m.

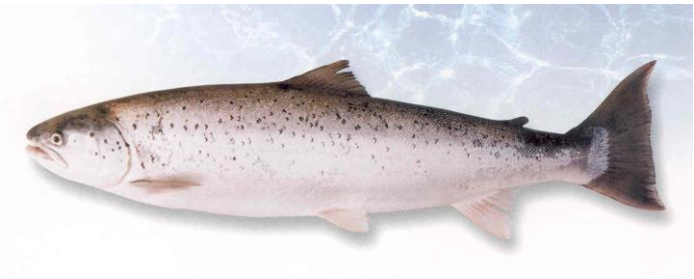
## 2-5) Semi-anadromous fishes

Semi-anadromous fishes spawn in lower stretches and deltas of the rivers. Therefore lower stretches and the deltas practically of all rivers are of high value as spawning grounds. Semi-anadromous fishes feed in areas of the sea with fresher waters. For majority of commercial species, the optimum salinity is up to 8 gr/l. During high water years, such an area with low salinity embraces the entire Northern Caspian and estuaries of the rivers of the Middle Caspian. Semi-anadromous fishes winter in lower stretches of the rivers, deltas or estuaries. In the Northern Caspian, semi-anadromous fishes give preference to the estuary of the Ural, Volga and Terek, though they feed in all parts of the area.

Fries of anadromous and semi-anadromous fishes are fattening in the cis-Kura region, Kyzyl-Agaj gulf and Lenkoran coast of the Azerbaijan sector of the Caspian. Besides, broodstocks of anadromous and semi-anadromous fishes gather in this area of the Southern Caspian, in front of the mouth of the Kura, ready for spawning. This region becomes especially important during vernal-estival period and, to less extent, in autumnal period.

At the territory of Turkmenistan, semi-anadromous fishes spawn in the Atrek. Damming of this river at the territory of Iran had led to loss spawning grounds of semi-anadromous fishes, such as zander *(Lucioperca lucioperca)*, carp *(Cyprinus carpio)*, Caspian roach *(Rutilus caspius)* and even sturgeons.

## 2-6) Anadromous fishes



The Caspian salmon, *Salmo trutta caspius* (Kessler, 1877), is one of the most valuable fishes, which are spawning in the rivers of the Caspian basin. They can run upstream for hundreds kilometers. Caspian salmon prefer pebbly and solid sandy ground select for spawning. Damming of the lower extents of the rivers (except for the Ural) has restricted access of fishes to spawning ground and sharply reduced the number of accessible spawning grounds, which had adversely affected on the extent of natural reproduction.

Nowadays, the Caspian salmon, *Salmo trutta caspius* (Kessler, 1877), is one of the nine subspecies of brown trout *Salmo trutta* (Quillet *et al.*, 1992). This subspecies attains the greatest size, weight and growth rate of all brown trout and lives in the Caspian Sea (Sedgwick, 1995). An endangered species mainly found in the southwestern Caspian Sea in Iranian waters, *S. trutta* used to be heavily fished especially during spawning migration to fresh waters (Kiabi *et al.*, 1999) because of their very high marketability and value (in 2011, about 60 US$ per kg in local markets of northern Iran). More recently, the Caspian salmon has attracted interest for aquaculture in cages and raceways in Iran, with emphasis on using triploid populations to omit problems associated with sexual maturation, which can reduce commercial benefits of salmonid culture, especially beyond the maturation phase (Thorgaard, 1983; Hulata, 2001).

The Caspian salmon, (*Salmo trutta caspius*) is considered as critically endangered according to IUCN criteria. This species is an endangered vulnerable anadromous species that has been considered for a biological conservation program in the southern part of the Caspian Sea (Coad, 2000; Vera *et al.*, 2011; Kiabi, Abdoli & Naderi 1999; Niksirat & Abdoli, 2009). The Caspian salmon, is anadromous and migrates up rivers in Northern Iran (mainly in Karganrud, Navrud, Tonekabon, and Sardabrud rivers) to spawn (Kazancheyev, 1981). The Caspian salmon is listed in the Red Books of Russia, Kazakhstan and Turkmenistan. In Azerbaijan and Iran, it is characterized as a sharply declining species.

The endemic Caspian salmon *(Salmo trutta caspius*) is one of the largest trout of the world (Abdoli , Niksirat and Patimar ,2011) that attains the greatest length, weight, and growth rate within *Salmo trutta complex* (Sedgwick, 1995). Natural

In I.R.Iran, Caspian salmon populations have experienced a strong decline during the past two decades as a result of overfishing pressure, habitat pollution and reduction in spawning areas. This fish migrates to some Southern and Northern Caspian Sea rives for spawning (www.caspianenvironment.org). Caspian salmon forms local stocks confined to certain rivers such as Kura, Samur and Yalama, which differ in morphological features, age and interval of maturity (Kazancheev, 1981).

The most important Iranian rivers for their spawning consist of, Karganrood, Navrood, Astarachiy in Gilan province and Tonekabon (Cheshmekileh), Chaloos, Sardab-rood in Mazandaran province. Distribution of subspecies occurs commonly at the Western and Southern coasts, from Terek to Sefid-Rud river and is seldom found in the Northern part (www.caspianenvironment.org).

Caspian salmon spawns in Terek, Kura, Sefid-Rud and other minor rivers of the Western and Southern coasts of the Caspian Sea. In respect to its reproductive life cycle, two immigrant forms namely fall-run and spring-run were observed. In local areas, spring-run and fall-run are named Salmon and Tian. Spawning migration to Kura takes place from November till February. The temperature range of salmon migration is rather wide, though the majority (appr.75%, immature fish with undeveloped gonads) enters the rivers at a comparatively low temperature, 8.2 – 12.8oC in November-December. Spawning takes place only a year later, starts in October and completes in early January. Eggs are released in the bottom and buried in substrates. Incubation period continues for 30-50 days. Afterwards, spawners either die (in Kura river), or return to the sea (Kazancheev, 1981). Spring-runs differ from fall-runs by a greater body depth and silver color body; they migrate with unripe gonads to rivers at the end of winter and beginning of spring and stay in rivers until autumn for spawning. Fall-runs migrate with mature gonads and spawn at the end of winter.

The Caspian region is the global center for diversity and endemism of members of the genus Salmo, especially the bull trout or large sea-trout *(Salmo trutta*). Caspian salmon (*Salmo trutta caspius*-Kessler 1877) are believed to be the largest of the European salmon, which includes the Atlantic salmon *(Salmo salar)* and brown trout *(S. trutta).*

Populations of Caspian salmon have declined drastically in recent decades as a result of over-fishing, poaching, river pollution, destruction of natural spawning areas, and drought (Abdoli,

2000; Barannik *et al.,* 2004 ; Niksirat & Abdoli, 2009 ; Jalali and Amiri, 2009 see also the Iranian Fisheries Organization ( IFO ) webpage http://www . shilat.com). This species is caught by local fishermen in relatively commercial quantities in the south and west of the Caspian Sea, but now barely survives in extremely small populations.

The catch of Caspian brown trout in Iranian waters of the Caspian Sea sharply declined from 20 tons in 1947 reached to 2 tons in 2007 ( Iranian Fisheries Organization Yearbook ,2008). So the Iranian Fisheries Organization has been conducting artificial reproduction of the brood stocks that enter rivers south of the lake for spawning. This organization also has been growing fry to the smolt level and releasing them (Mojazi *et al.,* 2005).

Shaheed Bahonar (Kelardasht) salmon culture center was designed in collaboration with experts from the former USSR. Following the site selection in Rodbarak village in the Kelardasht zone the construction started in 1979 and the center started operating in 1983 to breed; culture and release Caspian salmon for enforce recruitment and restocking of this species. The primary program was releasing of 100 thousands of Caspian salmon in suitable rivers annually and this amount increased to 300 thousands smolts in recent years.

During last decades, the artificial seed production has been a strategy for replenishing of Caspian salmon stocks in Iranian waters of the Caspian Sea and Iranian Fisheries Company has considered captive breeding and biological conservation programs to produce, protect and restock populations (Sarvi *et al.,* 2006).

Studies indicate that in recent decades over fishing along with the destruction of habitats for juveniles, reduction of genetic diversity and poaching have been contributed to the collapse of the natural population of the species . In the spawning season of 2007, only 64 adults (34 females and 30 males) were captured by the Iranian Fisheries Organization Authorities for artificial propagation purposes (Niksirat & Abdoli, 2009).

To increase reproductive success and diversifying of the genetic stocks of Caspian salmon, the CaspEco ( The Caspian Sea: Restoring Depleted Fisheries and Consolidation of a Permanent Regional Environmental Governance Framework ) project has been establishes to supports the efforts to halt the decline in bio-resources and to restore critically endangered Caspian Sea salmon *(Salmo trutta caspius*) fisheries in the Caspian Sea, through the implementation of agreed actions defined in the Caspian Strategic Action Plan .

**Table Catch rate (tons) and release (thousand pieces) of Caspian salmon in the Caspian Sea between the years 1990 to 2009**

| Release (thousand pieces) | Catch (tons) | Year |
|---|---|---|
| 155 | 1.1 | 1990 |
| 155 | 0.53 | 1991 |
| 359 | 0.4 | 1992 |
| 335 | 0.41 | 1993 |
| 640 | 1.04 | 1994 |
| 800 | 8.4 | 1995 |
| 424 | 8.3 | 1996 |
| 349 | 7.3 | 1997 |
| 510 | 6.8 | 1998 |
| 500 | 3.4 | 1999 |
| 626 | 4 | 2000 |
| 362 | 2.16 | 2001 |
| 340 | 9 | 2002 |
| 321 | 3.7 | 2003 |
| 300 | 2.1 | 2004 |
| 306 | 2.8 | 2005 |
| 555.7 | 2.4 | 2006 |
| 463.4 | 2 | 2007 |
| 210 | 1.7 | 2008 |
| 470 | 2.58 | 2009 |

## 3) Project importance:

Preservation and restoration fish stocks, especially endangered fishes are one of the most pivotal duties of responsible institutions. Caspian salmon *(Salmo trutta caspius)* according to IUCN criteria lie among the red list of endangered fish. Preservation and restoration of wild stocks in the internal waters is one of the main branches of research in fisheries. Major

carried out activities on restoration of Caspian salmon stocks was artificial reproduction, production and release of fry in rivers and estuaries. Despite of these efforts, there is no reports in relation to outcome of fry release especially re-capture coefficients. Caspian salmon catch rates have had so undulations in the past years as have significant reduction in recent years. So we can do considerable assist to preservation and restoration stock of this valuable species in country with implementation of this project and aiming the objectives and study on Caspian salmon brood-stock quality and produced fry and supplementary genetic researches same creation gene and sperm bank.

Despite of its significance, there are a few scientific studies on this species. These note the efforts made by the governments, in particular the high level of investment by Iran on restocking operations in recent years, but at the same time highlight the lower than expected results of these efforts and point to the need for the implementation of a Caspian salmon stocks rehabilitation program.

The Coldwater Fishes Research Center (CFRC) as a part of the Iranian Fisheries Research Organization (IFRO) was founded in 2005 and designated as the Network of Aquaculture Centers in Asia (NACA) and Pacific Regional Lead Center for Coldwater Aquaculture (RLCIr) on 17th Governing Council Meeting, Tehran, I.R. Iran, 25-28 February 2006. The center concentrated its activities towards sustainable coldwater aquaculture and fisheries development also concentrating on promotion of knowledge of aquarists and increasing aquaculture and fisheries productions for rural development. At the international level, CFRC provided a framework for cooperative activities between the NACA members by transferring research achievements and technologies to the members, introducing experts to countries at the basis of TCDC programs and implementation the joint research projects.

Iranian Fisheries Organization (IFO) have arranged for the capture of wild broodstocks in the estuaries of some specific rivers, e.g. Tonekabon (Cheshmeh kileh) River, as the main migratory route for the species, every year and then the smolts are artificially propagated in the Shahid Bahonar Kelardasht Hatchery Center. This hatchery is located in high altitude area (over 2000 m) with low annual mean temperature and cold climate. The environmental conditions are very different from the natural habitats of the species. The chilly water coupled with the other environmental characters of the area lead to the production of smolts with low weight even of fairly aged old individual fish. Duration of smoltification, lasts to more than 2

years while according to our investigations it carry out just in 5 months in CFRC. Moreover, the released juveniles reared in a safe condition in artificial environment and fed just by dry pellets. Juveniles have no experiences to forage behaviors. Thus, the survivals of the new juveniles in the wild habitats can be directly affected. Despite of previous long efforts during last three decades and mainly high amount of budget, there are no significant or desirable outputs and successes.

**Project Duration:** Sep. 2009 – Jan. 2012

## 4) Goals and Objectives:

The main objective of the project was production of high quality viable juveniles with forage behaviors, better growth performance and survival rates in an assessment environment to restore salmons in the Caspian Sea.

Specific objectives:

1- *Collection of trout brood-stocks, production, tagging, tracking and release near 20000 trout's smolts with high quality into the Caspian Sea.*
2- *Establishment live broodstocks gene bank from different rivers of southern Caspian Sea.*
3- *Establishment sperm bank from different races of Caspian salmon in the southern Caspian Sea.*
4- *Capacity built for community engagement in Caspian trout conservation.*

## 5) Main Activities:

Activities were done in seven major categories namely as follows:

5-1) Broodstocks collection
5-2) Juvenile Production
5-3) Tagging and Tracking
5-4) Release Operation
5-5) Gene bank
5-6) Sperm Bank
5-7) Capacity Building for Community Engagement and Regional Dissemination of Findings.

### *5-1) Collection related activities :*

#### 5-1-1 Broodstocks catch:

According to signed contract, Just 90-110 breeders of Caspian salmon should be caught from the estuary of Tonekabon as well as 9 significant migratory rivers in Gilan and Mazandaran provinces in each year. But fortunately with warm collaboration with Iranian Fisheries Organization (IFO) and Shahid Bahonar Hatchery Center (Kelardasht) we succeed to obtained more than 150 broodstocks from various rivers in above provinces in north of I.R.Iran. It is highly important to diversify the sources of importation in order to increase the genetic variability of the mentioned brood-stock.

This part of the project of brood-stock supply in south western of the Caspian Sea ( Gilan and Mazandaran provinces) started from 23rd of Sep. 2010 (opening of the fishing season ) Collection of specimens was limited by scarcity of Caspian salmon in Iranian waters of the Caspian Sea. So wild broodstocks of Caspian salmon were collected in the most representative beach seine cooperatives in Gilan and Mazandaran provinces based on the catch statistics of recent 10 years (Fig.5). The cooperatives (Fig.6) have been supplied by 0.4 $m^3$ tanks and oxygen capsules and manometers.

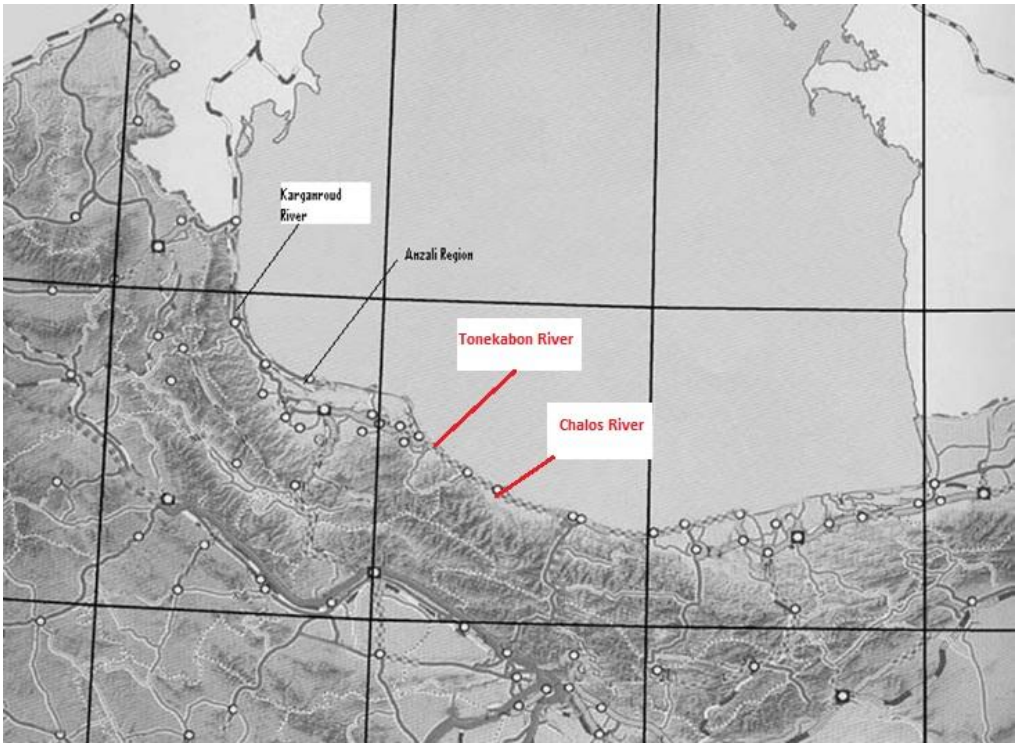



Figure 5:Places of collection of Caspian salmon sampling in Gilan and Mazandaran provinces

Figure 6 : Beach seine cooperatives used for catch of Caspian salmon broodstocks

When Caspian salmon broodstocks were caught, they were carried with soft gauze and put in the tanks (Fig.7, 8, 9).

Figure 7, 8, and 9: Caspian salmon broodstock in the tank supplied with oxygen

The captured fishes has been tagged in the water using marking of fish with tags which sewed to the first dorsal fin (Fig.10)

Figure 10: The captured Caspian salmon accompanied by tag

Regarding to unready situation of CFRC hatchery salon and absence of request equipment's that should be prepared according to signed contract by CEP, we obligate to transfer the captured brood-stock to ponds in standard situation at Mr.Jahankhah coldwater fish farm that located in Asalem to Khalkhal road for future artificial breeding plans (Fig.11).

This condition was similar to captured broodstock of Mazandaran province that we ought to transfer them to Shahid Bahonar (Kelardasht) Hatchery Center due to next step or artificial breeding program.

Quarantine operation was done and captured broodstock were stocked in quarantine place before transfer to final distention.

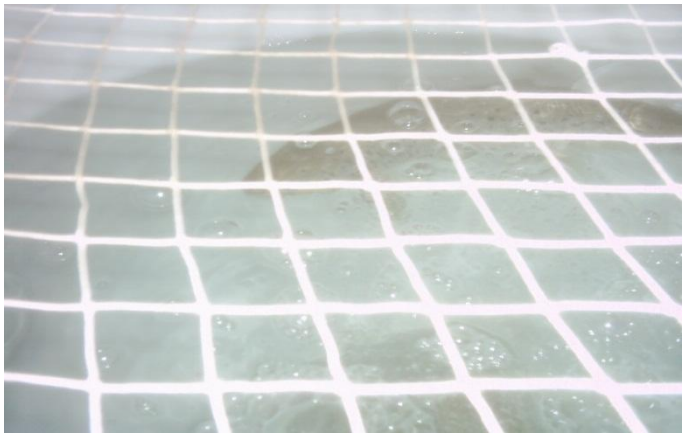


Figure 11: Perspective of Mr.Jahankhah coldwater fish farm in Asalem-Khalkhal road

Figure 12: Transport facility for transfer of captured Caspian salmon to coldwater fish farm

The fishes were kept to this farm and have been supervised regularly for inspection of maturity and evaluation of fungal or bacterial infection. (Fig.13)

Figure 13: inspection of captured Caspian salmon in the fish farm for maturity statute and evaluation of antifungal activities and tagging.

Figure 14: Protection of captured Caspian salmon in the fish farm and evaluation them for maturity statute and probable infection

**5-1-2) Main Achievements:**

From the start of the project (6th of Oct. 2010) till first of Nov. 2011, totally 44 specimen of Caspian salmon broodstock have been caught. The first brood fish have been caught in 6th of Oct. 2010 when the temperature was 17 °C.

9 of this brood fish died in fish farm and 34 is alive .The dead fish have been transferred to Inland water aquaculture research institute for further inspection (Fig.15).

Figure 15: Dead Caspian salmon in the lab and biometry of fishes in the lab.

Table 2 shows the biometrical result of captured Caspian salmon in Gilan province, Iran.

**Table 2: The mean length, weight and location of capture of Caspian salmon in Gilan province, Iran**

| Locality ( name of Cooperative ) | Region | Number | Min-Max length (cm) and weight (g) | Mean length ( cm ,Mean±SD) | Mean weight ( g, Mean±SD ) |
|---|---|---|---|---|---|
| Poshte-e-Jokandan | Karganrud river | 8 | 67-78 cm , 3000-5000 g | 70.8 ± 3.3 | 3550 ±656.8 |
| Shohad-e-Jokandan | Karganrud river | 19 | 58-75 cm , 2300 – 4000 g | 68.3 ± 4.6 | 3078.9 ±603.9 |
| Omid-e-Chobar | Karganrud river | 1 | | 63 | 2300 |
| Haft-e-Tir | Shafarud river | 8 | 60 – 70 cm 2500 – 3500 g | 66.7 ± 3.8 | 3000 ±267.3 |
| Karim Bakhsh | Anzali Region | 3 | 67 - 68 cm 2500 | 67.3 ± 0.6 | 2500 ± 0 |
| Isargaran | Anzali Region | 4 | 70 – 80 cm 3000 – 4200 g | 72.5 ± 5 | 3300 ± 600 |
| Omid | Anzali Region | 1 | | 70 | 3500 |
| Total | | 44 | 58 – 80 cm 2300 – 5000 g | 68.7 ± 4.3 | 3122 ±547.7 |

Results showed that in the Karganroud river region, Shafaroud river and Anzali region totally 28, 8 and 8 individuals have been caught respectively.

Meanwhile, from the start of the project (6th of Oct. 2010) till first of Nov. 2011, totally 105 specimen of Caspian salmon brood-stock have been caught in Mazandaran province. Same to Gilan province all of captured broodstock were transferred to Shahid Bahonar (Kelardasht) Hatchery Center in order to lack of request facilities in CFRC hatchery. Three broodstocks (1 male & 2 female) were caught from Sardabrood river (Chalous) and about 140 specimen of Caspian salmon broodstock have been captured from Tonekabon (Cheshmeh kileh) and transferred to Kelardasht hatchery. In fact, 150 broodstocks have been caught during CFRC project in Gilan & Mazandaran provinces.

### *5-2) Juvenile production related activities:*

This step was divided two stages as follows:

#### 5-2-1) Practice phase:

Presently Iranian Fisheries Organization (IFO) arranges for the capture of wild broodstocks in the estuary of some specific rivers, e.g. Tonekabon Cheshmehkileh River, as the main migratory route for the species every year and then the smolts are artificially propagated in the Kelardasht hatchery. This hatchery is located in an area of high altitude over 2000 m with a very low mean temperature and cold climate regime.

The environmental conditions are very different from the natural habitats of the species. The chilly water coupled with the other environmental characteristics of the area lead to the production of smolt juveniles with low weight even of fairly aged old individual fish.

Duration of smoltification, lasts to more than 2 years while according to a series of investigations carried out in the CFRC, the smoltification duration can be implemented just in 5 months. So in this phase two private farms were nominated and selected as propagation farm and artificial breeding of CFRC 700 domestic broodstocks that were adapted in Fresh water in ponds of CFRC.

In addition of participation of some local fishermen for request wild salmon broodstock in region, two private cold water hatchery and rearing fish farms selected (Mr.Kazemi in Asal mahaleh region in north of 2000 Road and Mr.Tajik in 3000 Road in Tonekabon)

They succeed to produce more than 150000 Caspian smolts with 20gr. weight as first time in last season in 2010. This practice was useful for CFRC experts in order to obtained necessary experiences and skills in Caspian salmon artificial breeding and propagation.



**5-2-2) Project official phase:**

In this period that started from 2011 catch season, propagation room was established private farm belongs to Mr. Jahankhah in Gilan province in order to propagate the brood stock fish in Gilan – this work was done for the first time in Iran as an innovatory event.

In order to artificial fertilization, mature spawners were selected with high quality performances and transmitted to a hygienic and separated room. Followed by aesthetic operation with Iranian herbal plant (clove extract), after extracting the gametes, "dry method", were conducted according to the standard manuals.

Then more than 100000 eyed eggs after incubation time were transferred to CFRC hatchery room in order to convert to next stage of salmon life cycle. Fecund eggs were held in 32 California trough tanks in Hatchery room of CFRC. This procedure for alevin production from green eggs, eyed eggs and alevins was considered and carried out. Feeding operations were started as long as alevins appear to surface grazing. They fed with Artemia nauplii and Gammarus larva as well as with high quality starter. The new hatched Caspian salmon reared to 0.4 g in troughs. All photoperiod, light intensity and light spectrum operations were considered according to the standard protocols for Salmonids. Therefore more than 90000 yolk sac larvae were produced in CFRC and in the final stage propagation and larvae produce were applied and near 80000 smolts were reared in CFRC and more than 90000 larvae were produced from 55 Broodstocks in Kelardasht in Mazandaran province parallel.

The main targets of rearing of juveniles were production of juveniles with high quality performance and improvement of survival rate from 25-30% presently obtained in current hatchery to 70% rate.

So far, rate of Fry Mortality Syndrome were increased in salmonids farms in the country. Some multi factors and complex causative agents were identified as main etiology factors. So improving the survival rate of Caspian salmon, from larval to juvenile stage, requires a multidiscipline approach, as no single factor is responsible for the mortality observed up to now in the hatcheries and is predominant for supporting high growth rate. Breeder origin and management, feeding conditions (live feed composition, quantity, frequency and feeding level), environmental conditions (tank size and materials, light conditions, water quality and flow, etc) and stocking procedures (larval density, grading, etc.) were all variables that can significantly affect the success of larval rearing, by influencing the growth rate or modifying the impact of cannibalism and the rate of morphological deformities.

So in this project, major variables were tested and sanitary evaluation were employed in order to improving survival rates, reducing mortality, cannibalism and deformities, and improving growth rate and feed efficiency.

### 5-2-3) Sanitary evaluation:

In this project the health condition of Caspian salmon, have been investigated in Mazandaran and Gilan provinces since 2010. According to the standard methods and manuals, provincial fish health technicians collected samples from moribund fish to detect bacteria, fungi, viruses and parasites and to determine farm-level disease events.

The samples were punctuated which showed changes in behavior, suffering from a disease, parasite, or other physical affliction that were abnormal distribution in pond, such as riding the surface, gathering at the pond sides or in slack water, and crowding the head or tail screens, flashing, scraping on bottom or projecting objects, darting, whirling, or twisting, and loss of equilibrium, and loss of vitality, weakness, and loss of ability to stand handling during grading, seining, loading, or transportation.

They were controlled for gross external symptoms such as discolored areas on the body, eroded areas or sores on the surface of the body, head, and fins, swelling on the body or gills, pop eye, hemorrhages and cysts containing parasites and also were examined gross internal symptoms like color changes of organs or tissue (Pale liver or kidney or congested organs), hemorrhages in organs or other tissues, swollen or boil-like lesions, change in texture of organs or tissues accumulated fluid in body cavities, and cysts containing parasites. The main infections that were considered for pathogen detection were external bacterial diseases: columnaris, bacterial gill disease, Peduncle disease, fin rot and internal bacterial diseases: Furunculosis, Ulcer disease, Redmouth disease, Fish tuberculosis, kidney disease and external protozoan diseases: *trichodina spp, epistylis spp, chilodon spp, costia spp.* The results showed some parasites exist in digestive tract of breeders. Study on larvae of Caspian salmon in throughs showed no parasites five days after exogenous feeding initiation. Survey on quality and quantity assessments of rearing water and fungal flora in eggs, yolk sac larvae and exogenous feeding larvae carried out. In the eggs and rearing water fungi such as Penicilium, Mucor and Acremonium showed the most abundance, respectively. Also, in yolk sac larvae and rearing water Mucor and Penicilium were the most abundant. Moreover, in exogenous feeding larvae and rearing water the most abundant larvae were Penicilium. *Saprolegnia sp*. separated from eggs, larvae and rearing water in all of the mentioned stages.

A few bacteria including Entrobacteriaceae, *Aeromonas spp, Pseudomonas spp, Bacillus spp,* determined in spawners.

In order to determine viruses in Caspian salmon spawners, PCR identification kit and various cell lines applied. No viral infections in Caspian salmon spawners have been found yet.

Different Caspian salmon abnormalities observed in hatcheries, which include lethargy, emaciation, scoliosis, head deformation.

### *5-3 Tagging and tracking related activities*

Passive Integrated Tag (PIT) was employed in order to identification and traceability of each broad-stock consists of brooders from various rivers. PIT tagging was inserted in special point of selected parents and all broad-stock were individually tagged by Passive Integrated Tag (PIT). This method was a very innovative approach to identify individuals and present the description of the broodstocks of Caspian salmon, with special characteristics e.g., date, size, weight, age, sex, code number and catch origin. This approach was usable for genetic variability and reproductive competency including fecundity and sperm quality.

In order to study tagging effects on the survival rate of Caspian Sea salmon *(salmo trutta caspius)* fries before releasing into the natural habitat, planning is made to tag 10% of produced smolts during the project.

Two kinds of tags including Elastomer tag and Coded Wire Tag were employed Tagged fish are kept in small plastic tanks containing about 200 liters of water and fries behavior, body surface (skin) reflection and mortality were monitored during 5-7 days after tagging.

In this project in order to trace the produced juveniles, about 10% individuals of produced smolts were tagged by Coded Wire Tag before releasing operations.

Tagged juveniles will be tracked by reports of local and regional legal catch communities and local fisherman in the future. In this regards some specific incentive will be considered to persuade fishermen to report CFRC. So additional financial resources were needed and special budget should be allocated to CFRC in next year.

.

### *5-4 Releasing Operations related activities*

After smoltification of produced juveniles in CFRC releasing operation were done. Smoltification is complex approach and some multi factors could be affected them. Maturation and on-time smoltification allows the Caspian salmon juveniles to become adapted to the current environmental conditions including hydrological and hydrochemical composition of the river, familiarize themselves with the local landmarks for orientation and navigation, recover from transportation, and develop cohesive social bonds wherever appropriate.

According to last research findings and comprehensive information release site were selected. It consists of choosing the appropriate right location and the time of year for the release of captive-reared fish especially with reference to flow rates, habitat quality including stream-bed structure, prey, predator and competitor abundance, etc. and restocking protocols. In this project requested protocol and guidelines were prepared through consult with professional experts in Iranian Fisheries Organization (IFO), Iranian Fisheries Research Organization (IFRO) and distinguished professors in merit university in order to establishing how best to release hatchery-reared Caspian salmon.

Some hatchery-reared Caspian salmon were released in river primary branches in the mountain of nominated rivers. This innovative action was imagined according to some new approach in project consultant meeting and some research findings.

The smoltification procedures of Caspian salmon has been determined by some several studies namely Bahramian & *et al.,* 2006 and Sayad borani & *et al,* 2008 in Inland Aquaculture Research Center. They acted this action as one of project key managers. According to the findings of these investigations, the Caspian salmon juveniles of 20 gr, average size of 122 mm, are qualified for smoltification. According to new findings last stage of PARR step when reached to 15-17 gr. is the best situation for releasing purposes by considerations of osmoregulatory adaptations. So smoltification period was done in sufficient time in releasing action during juveniles were reached to the Caspian Sea.

Thus, the juveniles of 15-17 gr were released at the considered sites.

*Main achievements and innovative approach were documented as a standard protocol for the Iranian Fisheries Organization (IFO) and other Caspian countries that will be minimize methodological errors, maximizes the validity and consistency of data, and allows them to make reliable comparisons and reasonable conclusions and result for Caspian salmon and related bony fish restocking activities.*

### *5-5 Gene bank (Briefly)*

**Developing and establishing Gene Bank of Live Broodstocks of Caspian salmon (*Salmo trutta caspius)* in CFRC**

In this activity, specific scheme was designed by Prof.Dr.Sohrab Rezvani Gill Kolaei as Head of Biotechnology Dept. of IFRO. He has been carried out this objective through Molecular Genetics assay in *Salmo trutta caspius* population. It will be designed in two fish migration forms (spring and autumn seasons) in Southern Caspian Sea using Microsatellite marker method basin and established an alive Caspian trout gene bank. This activity would be coordinated and carried out in national level and all affiliated branches of IFRO and Iranian Fisheries Organization (IVO) are participated.

Objectives of mentioned activity are as follows:

1) Molecular population genetic study on *Salmo trutta caspiu*s from five rivers of Southern Caspian Sea
2) Molecular genetics on spring and autumn races of *Salmo trutta caspius*
3) Molecular phylogeny study on different Salmon species from Caspian basin
4) Establish of Gene Bank of different alive population of *Salmo trutta caspius* from five main rivers that release to the Caspian Sea.

**Sampling procedure:**

1. Sixty fish fin tissue were collected from 5 rivers for molecular analysis and 5-10 pairs broodstocks for alive gene bank.

2. Prime coordination was carried out with Molecular Laboratory in Caspian Sea Ecology Research Center (Khazar Abad –Sari) and selected exist primers for Microsatellite and PCR-Sequencing purposes.
3. A technical meeting was carried out with Head and experts of Enhancement Center of Salmon in Kelardasht for coordinating of fishing and culturing of broodstocks from 5 main rivers as following: Sardab Roud, Cheshmeh Kileh, Kagran Roud, Shafa Roud and Nav Roud. These broodstocks were kept in separate ponds for each river or with tagging were kept in the same ponds.
4. More than 20 fish fins tissue from Cheshmeh Kileh was collected by Kelardasht Salmon enhancement Center experts who are cooperator of this project.
5. List of necessary materials were prepared and offered to CRFC-CEP manager in order to purchase or preparation.
6. Preparation of technical bulletins and executive protocols about establishment of Caspian trout Gene Bank has been started.

Caspian Salmon *(Salmo trutta caspius)* is one of more economic and expensive fish species in north of Iran. This species also includes as endangered species in CITES list. The objective of this project is including: population genetic study of the species from 5 rivers of Caspian costal in north of Iran which entrance in the Caspian Sea and determination of genetic variation between spring and autumn races. This genetic information is more important for managing of enhancement program in IFO. At least 30 samples were collected from each river but in first step DNA was extracted using phenol and chloroform method from fin tissues of 60 fish individual. Both nuclear DNA and mitochondrial DNA were used for polymerase chain reaction amplification using 12 pair microsatellite primers and the universal primers of D-loop or control region part of mitochondrial DNA molecular. The main result reveals that there are enough high polymorphism within and between captured fishes from Gilan and mazandran provinces. Also the result shows that the genetic structure of fish from Gilan is not more depend to enhancement program of IFO but might be depend to wild stocks of the Caspian Sea.

### *5-6 Sperm Bank (briefly)*

**Developing Sperm Bank from different races of Caspian salmon (*Salmo trutta caspius)* in CFRC:**

This constructive aspiration would be done through sign of subcontract with International Sturgeon Research Institute (ISRI) in Rasht. In order to establish of mentioned target, some coordination meeting and professional visit were performed with participation of executive managers and related experts of CFRC in ISRI laboratory of Sperm Bank. Using of modern facilities and merit experiences of scientific board and trained experts in ISRI could be main approach. In this manner, It could be ideal opportunity for increscent of experts skills in CFRC and executive potential of ISRI members as both affiliated centers of IFRO.

Regarding to successful experience in establishment of Sperm Bank of Sturgeon in ISRI, Mr.Shahroz Baradaran Noeiri were selected as responsible executor in mentioned activity and his working group consist of related experts in ISRI would be supported him. Some interested experts in CFRC accompanying them in development of Sperm Bank scheme in district. Special draft was designed by him and executive sampling were examined on exist broodstocks of *Salmo trutta caspius* as experimental layout in CFRC. This performance would be sustained on Caspian trout broads in capture seasons (Final report is available on pages of 54-68 in current report).

### *5-7 Capacity Building for Community Engagement and Regional Dissemination of Findings.*

These activities were carried out through some official meetings and advertising programs in CFRC and IFRO continuously.

#### 5-7-1 Internal consultants meeting in IFRO (Monday, 5th July 2010)

Coordination meeting was attended by nine internal consultants and head of Iranian Fisheries Research Organization (IFRO) in CEP project to create more coordination about implementation of the project process (on Monday 2010/July/5).





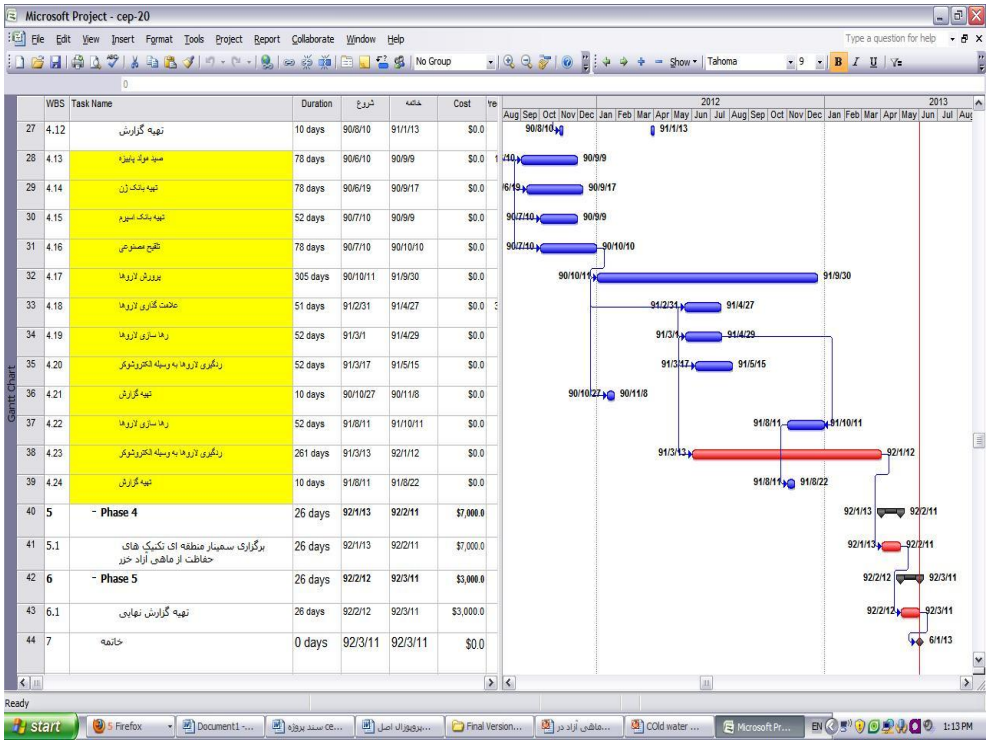

In this meeting, all nine internal consultant such as Dr.Sharif Rohani as Research Deputy of IFRO, Dr.Taghavi as Head of Iranian Fisheries Organization (IFO), Dr.Pourkazemi as Head of Sturgeon International Research Institute (Rasht), Dr.Fallahi as Head of Inland Aquaculture Research Center (Anzali), Dr.Soltani as Professor of Tehran University, Dr.Gholizadeh as Director Manager of private Company in Aquatic Engineering, Dr.Salehi as

Head of Socio-Economic Dept. of IFRO, Dr.Abdolhay as substitute of Aquaculture Dept. of IFRO, were invited but seven consultants were participated actively.

### 5-7-2 Holding 20 expertise meeting around issues of CEP project and forming some working groups in CFRC

### 5-7-3 Application of Special software (Microsoft Project) in order to Project Manager

It was nominated in order to CEP project management as first time in IFRO and it demonstrated as oral presentation in mentioned meeting for invited consultants. Almost of them approved application of mentioned software in CFRC-CEP project.




- Training courses entitled "Introduction preliminary software Microsoft Project" to increase efficiency in research projects with emphasis on CEP project in CFRC (on Wednesday 2010/ June/ 23).

- Design of special web site for CEP projects in internet as follows:

http://salmotruttacaspius.ifro.ir/portal.aspx

- Preparation of posters and brochures in the limited number and distribution in coastal cities of Mazandaran, Golestan and Gilan provinces.

The following topics for inclusion posters were approved:

- Biology and reproduction
- Maintaining and rebuilding reserves
- Ecological and environmental considerations

- Color brochures about *S. trutta caspius* were published in 1000 issues.

Five different posters were designed and published in 5000 issues (1000 copy for each subject)

- Distributing and installing the prepared posters and brochure in 25 cities of Gilan and Mazandran provinces (each city including 10-12 government departments, totally 250-300 departments).


- Preparation a new model for analysis of the effect of information CFRC-CEP project in awareness, attitude and performance of the local people and fishermen to protect and restore Caspian trout *(Salmo trutta caspius)* stocks in the Caspian Sea (Through questionnaires) .

Preparation of the mentioned questionnaire including 4 sections:

1) Demographic section (12 questions)
2) Awareness section (25 questions)
3) Attitude section (12 questions)
4) Performance section (8 questions)

- Distributing and completing the project questionnaires in 16 cities of Gilan and Mazandran provinces.
- Questionnaires analysis was applied after end of data collection.

Regarding to importance of CEP-CFRC join-venture project, several technical and coordination meeting were hold in CFRC.

Last meeting were performed with attendance of Dr.Motallebi as Head of IFRO ( May,2011) and in last week were participated with Dr.Sharif Rohani, Research Deputy of IFRO, Dr.Zorriehzahra as Manager of project, Dr.Rezvani as Head of Biotechnology Dept. of IFRO and Dr.Mehrabi as representative of Aquatic Animal Health & Diseases of IFRO with active attendance of Dr.Borani Head and substitute of CEP project manager in CFRC and his colleagues and staff in district.

Fortunately, executive stages of CEP-CFRC went well according to submitted TOR and work plan in IFRO and CFRC.

### 5-7-4 Last coordination & professional meeting that hold in 14 and 15th June, 2011 in CFRC

Report of 1st Local attachment of Caspian trout stocks conservation considerations which was held on 8th August 2010, 09:00 am - 16:00 pm in CFRC would be presented as follows:

### 5-7-5 **Orientation meeting with fishery communities and inhabitants in the vicinity of coastline south of the Caspian Sea who are relevant to spawning, migration routs of Caspian trout and inviting them to participate in the project,**

Targeted participants via letters, face to face and telephone calls for collaborators in project were invited. Coldwater Fishes Research Center (CFRC) hosted this meeting.

Dialogue, views exchange, questions & answers regarding to Caspian trout life history, origin of today management problems and Caspian trout conservation requirements performed between 12 participants from governmental section, 12 participants contain old fishermen those were professional hunter of Caspian trout in revering also maritime conditions, well-informed local citizens, NGOs, Governors of targeted cities of those rivers are involved in the project, project collaborators team from CFRC contains 12 individuals of scientific board, senior experts and technician.

All talks of 36 participants were recorded on hardcopy, and filmed by the camera.

In break and after lunch time, an exhibition of ancient and new catch equipments of Caspian trout were demonstrated to CFRC-CEP project guests.

All dialogues replayed by project collaborators in CFRC, and those talks were relevant to conservation requirements of Caspian trout were summarized.

Results and findings of 1st local attachment will support 1st National meeting of Caspian trout conservation considerations which is organizing for forthcoming autumn in Tonekabon. Via local, national & regional attachments-technical meetings, the subject of project would be supported by new approach to fisheries for Caspian trout stocks conservation plan.

### 5-7-6 Orientation meeting with local fishery communities and inhabitants in the vicinity of coastline south of the Caspian Sea (8th Aug. 2010)

Report of the first national meeting on considerations of Caspian salmon stocks rehabilitation which held by CFRC on 10th November, 2010 would be presented as follows:

### 5-7-7 First national meeting on considerations of Caspian trout stocks rehabilitation

National meeting on considerations of Caspian trout stocks rehabilitation was held by CFRC on 10th November, 2010. This meeting arranged by invitation to Iran Fisheries Organization (IFO), Iran Environment Protection Organization (IEPO), Water Resource Management (Ministry of Energy) and some university senior professors, with high knowledge and experience on Caspian trout migration behavior, and the area governor.

A report session was arranged to dedicate time (15-20 minutes) to any representative of organizations to present their activities report on the fish and environment rehabilitation and problems which they may encounter in fulfilling their duties.

At the end of report session, roundtable session was held to consult and make suggestions to the governmental managers about approaches to Caspian trout rehabilitation (discussion and conclusion).

All talks of participants and oral presentations were recorded on hardcopy, and filmed by the camera.

**First national meeting on considerations of Caspian trout stocks rehabilitation (10th Nov.2010)**



**Details of National Meeting on considerations of**

**Caspian salmon stocks rehabilitation**

Regarding to work plan of joint project between Iranian Fisheries Research Organization (IFRO), Coldwater Fishes Research Center (CFRC) and the Caspian Sea Environment Program (CEP) second cconference as a consultative meeting was held on Wednesday 19/8/1389 equal to 10th Nov.2010 in Tonekabon.

.

The conference agenda was designed base on responsible and relevant organizations such as representatives of the Iranian Fisheries Research Organization (Dr.Kaymaram as Head of Fish Stock Assessment Dept. and Dr.Ghasemi and Dr.Sharifian) and fisheries research centers in the neighboring provinces such as Dr.Pourkazemi as Head of Sturgeon International Research Institute (Rasht), Dr.Fallahi as Head of Inland Aquaculture Research Center (Anzali) , Iran Fisheries Organization, Mr. Mokarami as Director of Fish Preservation and Restoration Section and his colleagues (as well as fisheries administration branches in the neighboring districts from Mazandaran and Gilan provinces), the Environmental Protection Agency (State and city), Ministry of Energy, several professors from the University of Tehran, Shahid Beheshti, Tarbiat Modarres University and some local authorities (governors and mayors of the adjacent towns, chief of Fisheries Resource Protector in district and representatives of local fishing cooperatives were invited.

The conference was held in two panels as follows:

In the first part of the meeting, Dr.Zorriehzahra as Head of CFRC welcomed to invited guests and presented his initial description about CFRC-CEP project. Then representatives of Iran Fisheries Organization, Environmental Protection Agency and governor of Tonekabon presented their opinions and activities in relation to the preservation and restoration of Caspian trout (*Salmo trutta caspius*) stocks in the Caspian Sea. Then three university professors ( Prof.Amiri Mojazi from Tehran University, Prof.Abdoli from Shahid Beheshti University and Dr. Kalbasi from Tarbiat Modarres University) presented their results of related studies and opinions in relation to preservation of Caspian trout stocks separately.

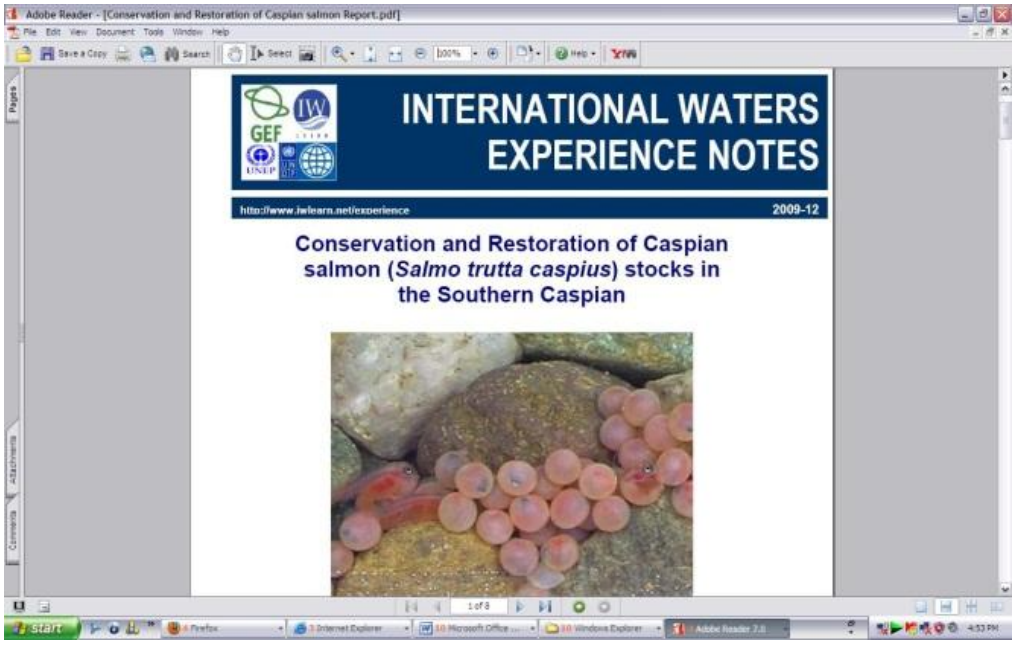
The second part of meeting allocated to discuss about problems and requirements to rebuild Caspian trout stocks in the Caspian Sea. In this part of the meeting, executive section, research parts and academic departments emphasizing to the necessity and benefits of such joint meetings to express their views about failure of achieve the desired results and finding the reasons and solutions to improve the activities in relation to rebuild and conservation of the Caspian trout stocks. All participants also praised the activities of Iran Fisheries Organization that being done by production and releasing of fry Caspian trout in recent decade.

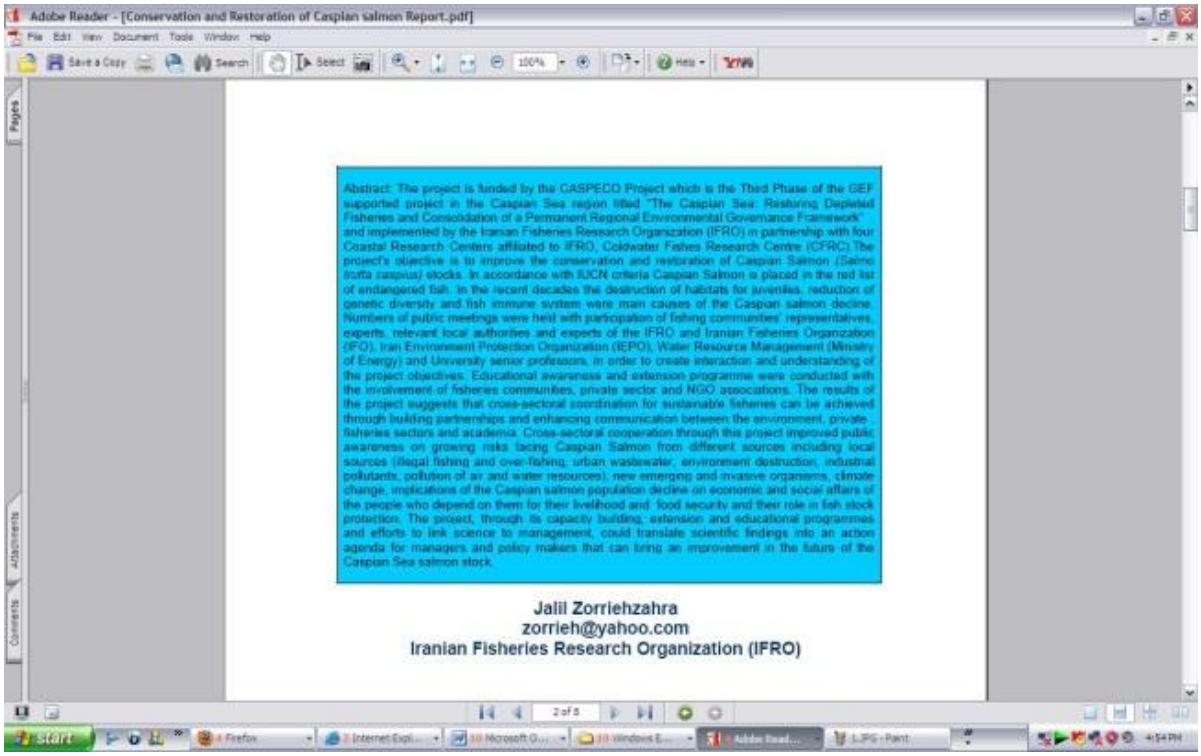
Meanwhile, the develop strategies to rebuild stocks of the Caspian trout should be focus on basic scientific principles with emphasis on the centrality of the importance to improve the environment, migration routes and spawning locations of the Caspian trout. Accompaniment and coordination between responsible organizations were emphasized again.

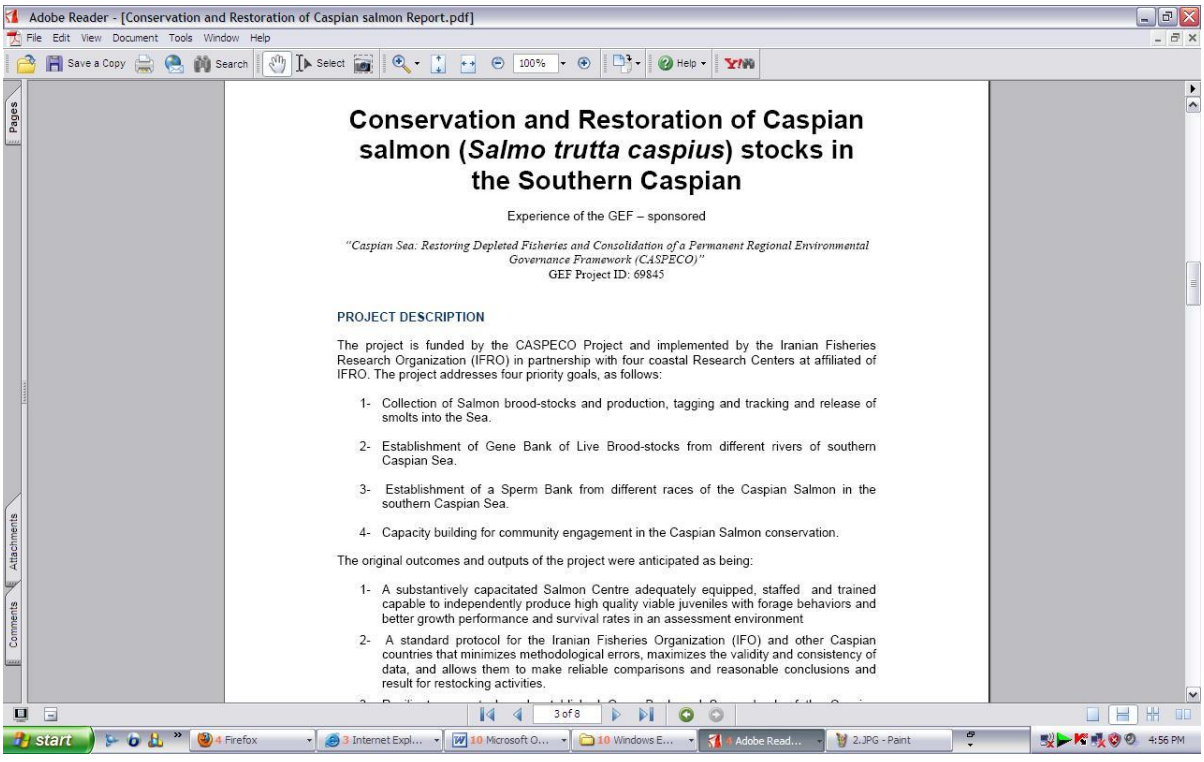
All talks of participants and oral presentations were recorded on hardcopy, and filmed by the camera. It will be documented as official findings and publish as executive protocols in order to extension and executive solutions in near future.

### 5-7-8 Preparation of Experience Note:

Regarding to advice of Dr.Farshchi as CASPECO manager, and consideration of technical and executive experiences of CFRC members in Conservation and Restoration of Caspian trout *(Salmo trutta caspius)* stocks in Caspian Sea and related rivers in district, especial pamphlet was wrote. This manner was prepared in order to share experiences and information distribution between countryside neighbors in district. It was submitted in UNDP web site and would be available through internet. As follows:




Adobe Reader - [Conservation and Restoration of Caspian salmon Report.pdf]
Abstract: The project is funded by the CASPECO Project which is the Third Phase of the GEF supported project in the Caspian Sea region titled "The Caspian Sea: Restoring Depleted Fisheries and Consolidation of a Permanent Regional Environmental Governance Framework" and implemented by the Iranian Fisheries Research Organization (IFRO) in partnership with four Coastal Research Centers affiliated to IFRO, Coldwater Fishes Research Centre (CFRC).The project's objective is to improve the conservation and restoration of Caspian Salmon (Salmo trutta caspius) stocks. In accordance with IUCN criteria Caspian Salmon is placed in the red list of endangered fish. In the recent decades the destruction of habitats for juveniles, reduction of genetic diversity and fish immune system were main causes of the Caspian salmon decline. Numbers of public meetings were held with participation of fishing communities' representatives, experts, relevant local authorities and experts of the IFRO and Iranian Fisheries Organization (IFO), Iran Environment Protection Organization (IEPO), Water Resource Management (Ministry of Energy) and University senior professors, in order to create interaction and understanding of the project objectives. Educational awareness and extension programme were conducted with the involvement of fisheries communities, private sector and NGO associations. The results of the project suggests that cross-sectoral coordination for sustainable fisheries can be achieved through building partnerships and enhancing communication between the environment, private, fisheries sectors and academia. Cross-sectoral cooperation through this project improved public awareness on growing risks facing Caspian Salmon from different sources including local sources (illegal fishing and over-fishing, urban wastewater, environment destruction, industrial pollutants, pollution of air and water resources), new emerging and invasive organisms, climate change, implications of the Caspian salmon population decline on economic and social affairs of the people who depend on them for their livelihood and food security and their role in fish stock protection. The project, through its capacity building, extension and educational programmes and efforts to link science to management, could translate scientific findings into an action agenda for managers and policy makers that can bring an improvement in the future of the Caspian Sea salmon stock.
Jalil Zorriehzahra
zorrieh@yahoo.com
Iranian Fisheries Research Organization (IFRO)


Adobe Reader - [Conservation and Restoration of Caspian salmon Report.pdf]
Conservation and Restoration of Caspian salmon (Salmo trutta caspius) stocks in the Southern Caspian
Experience of the GEF – sponsored
"Caspian Sea: Restoring Depleted Fisheries and Consolidation of a Permanent Regional Environmental Governance Framework (CASPECO)"
GEF Project ID: 69845
PROJECT DESCRIPTION
The project is funded by the CASPECO Project and implemented by the Iranian Fisheries Research Organization (IFRO) in partnership with four coastal Research Centers at affiliated of IFRO. The project addresses four priority goals, as follows:
1- Collection of Salmon brood-stocks and production, tagging and tracking and release of smolts into the Sea.
2- Establishment of Gene Bank of Live Brood-stocks from different rivers of southern Caspian Sea.
3- Establishment of a Sperm Bank from different races of the Caspian Salmon in the southern Caspian Sea.
4- Capacity building for community engagement in the Caspian Salmon conservation.
The original outcomes and outputs of the project were anticipated as being:
1- A substantively capacitated Salmon Centre adequately equipped, staffed and trained capable to independently produce high quality viable juveniles with forage behaviors and better growth performance and survival rates in an assessment environment
2- A standard protocol for the Iranian Fisheries Organization (IFO) and other Caspian countries that minimizes methodological errors, maximizes the validity and consistency of data, and allows them to make reliable comparisons and reasonable conclusions and result for restocking activities.

## 5-7-9 Final Regional Meeting of the Caspian salmon Conservation Project

**Ramsar, I.R.Iran, 20-23 December, 2011**

Regarding to approved work plan, The Project, inviting at least one expert from each of the Caspian littoral countries to exchange views, share lessons learnt and exchange technical views in company with 2nd national attachment on Consideration of Caspian trout stocks rehabilitation by CFRC invitation to Iranian Fisheries Organization (IFO), Department of Environment (DOE), Coldwater Fishes Aquaculture Union , representatives of Local Fishermen, NGOs, Municipality, City council , Mayors and Governors of Coastline nominated cities.

Opening meeting was carried out on 20th in Ramsar and then invited participants visited from CaspEco projects in Iran (Tonkabone, Chaboksar & Rasht) as IFRO affiliated Research Centers (CFRC and ISRI) as well as Shahid Bahonar hatchery center (Kelardasht) in three days.

### List of Participants to Ramsar

| No. | Name | Nationality |
|---|---|---|
| 1 | Mr. Serik Akhmetov | Kazakhstan |
| 2 | Mr. Amerzhan Shaudanov | |
| 3 | Mr. Kuanysh Isbekov | |
| 4 | Ms. Yuliya Kim | |
| 5 | Ms. Yelena Bokova | |
| 6 | Ms. Anara Ayaganova | |
| 7 | Mr. Boris Morozov | Russia |
| 8 | Mr. Muhy Muhiyev | Turkmenistan |
| 9 | Mr. Orazmuhammet Myradov | |
| 10 | Ms. Jahan Annacharyyeva | |
| 11 | Mr. Tariyel Mammadli | Azerbaijan |
| 12 | Mr. Aghasadig Gasimov | |
| 13 | Ms. Tamara Zarbaliyeva | |
| 14 | Mr. Elchin Mamedov | |
| 15 | Interpreter | |
| 16 | Dr. Parvin Farshchi | I.R.Iran |
| 17 | Dr. Hossein Emadi | |
| 18 | Dr. Asghar Abdoli | |
| 19 | Mr. Reza Shahifar | |
| 20 | Mr.Feridone Owfi | |


Dear Dr.Farshchi(Caspeco P
and Repcted Delegations .Welcometo col
( Pilot Project Site )
FRC Manager .Sc Board


Dear Dr.Farshchi(Caspeco Project Manager)
and Repcted Delegations .Welcometo coldwater Fishes Research
( Pilot Project Site )
CFRC Manager .Scientific Board Memb Staff

## 5-7-10 Speech of Dr. Motallebi Head of Iranian Fisheries Research Organization (IFRO)CaspEco Final Meeting

***Dear Ladies and gentlemen, honorable guests, executive managers of CaspEco projects and respectable colleague****s*

I would like to express welcome width to all participants with regards and special levee in this priceless and important meeting.

I hereby beg to inform you during recent years on the one side we are faced in world with as a result of the appearance of climate exchange ,atmospheric phenomenon and different environmental fluctuation such as the El Nino, La Nina, tropical storms and cyclones, devastating floods, earth quick. These destructive natural phenomena are caused for the major changes of aquatics ecosystems and habitats in world.

With the other side the increase in aquaculture production during the past l00 years, is one of the most informant case for profound changes in food production globally.

The rapid population growth, in creasing human demand for seafood and change the level of fishing and fishery products into the marine aquaculture has expanded greatly and is rapidly becoming a major global industry. Currently, the economic contribution of aquaculture in many countries is an integral.

This is the main mover of Job creation and social / economic development in poor rural and coastal communities, particularly in Asia, and has caused the pressure on the sustainable harvesting of natural rivers; lakes and oceans will be reduced.

Are you aware of the Caspian Sea is the largest lake in the word, and located between 5 neighboring countries: I. R. Iran, Azerbaijan, Russia, Kazakhstan and Turkmenistan.

The Caspian Sea as a unique closed aquatic body is the sensitive and vulnerable ecosystem with diverse of habitats for different species of invaluable fauna and flora communities. So far 21 families, 64 genera, 114 species, 63 subspecies and 13 races of fishes have been identified, that Sturgeons, White Kutum, and Caspian Salmon are the obvious examples.

The various factors such as: destroying the ecological conditions of rivers, in creased by fishermen arbitrary banning and unauthorized and illegal fishing, diversion dams, river bed degradation due to pollutants of industrial wastes, Urban sewage, fertilizers , pesticides and herbicides to agricultural surpluses are causing pollution of rivers and mortality of fishes and other aquatics communities.

Unfavorable environmental conditions, the occurrence of petroleum hydrocarbons contamination are other problems for the Caspian Sea region, which has been the threat of aquatic communities.

All of these factors have caused the natural reproduction and migration of the Caspian Sea economic fishes has undergone many threats and factors affecting fertility of these stocks should be mentioned. In addition to causing the generation of these valves is threatened.

In this respect the executive managers of mentioned projects will be informed you the details of projects progress, methodology, field and laboratories activities and results.

Notably, which tease projects executed by cooperation and collaboration of fisheries research organizations and Iranian universities, private sectors and public unions and particularly the participation of local people, fishermen and fishing communities.

***Distinguished delegates, dear guests:***

It is my pleasure to inform that Iranian Fisheries Research Organization (IFRO) given the capabilities of skilled manpower (members of scientific boards) and efficient , diverse areas and subjects for study and research , and 6 research centers / institutes in the Caspian Sea border and equipped with modern scientific equipment (such as Gilan research vessel) is the biggest fisheries research communication network in Middle East and Western Asia which amount of 9 million $ annually for research and monitoring projects such as hydrology , hydrobiology , pollution, stock assessment , ecology and biology aspects of fauna and flora communities , biodiversity and environment will cost which all of them plays an important part and role in the progress of fisheries management and stock / habitat restoration and rehabilitation.

For example, the formulation and adoption of two major projects by CEP (Caspian Environment Program) in 2009 as following titles are the actions of IFRO among mentioned subjects:

- *Gene pool conservation of Sturgeon species in the Sepidrud River.*
- *Conservation and restoration of the Caspian Salmon stocks in the Southern Caspian Sea.*

I hope that during this short stay in Iran and Gilan and Mazandaran green provinces, is associated with good memories, and so the transfer and exchange experiences and strengthen regional cooperation in order to resolve existing problems and to achieve higher aims and goals of fisheries in the region to be successful.

At the end, I sincerely thank the Mrs. Dr. Farshchi (CaspEco project manager), Mr. Dr Ghaffarzadeh (the ex-manager of CaspEco project), project managers and the coordinators group and organization colleagues in order to make the necessary arrangements to travel to Iran and celebration of this important meeting.

I wish to good stay in Iran during the next few days, and I hope to enjoy and profit useful result and discussion for information exchange, scientific joint aims and good goals and experiences transition.

***With the best regards***

***A. A. Motallebi, Ph.D (Head of IFRO)***

***20. Dec.2011***
***Ramsar, I.R.Iran***

## 5-7-11 Bilateral Collaboration with Caspian Sea

# Ministry of Jihad-e-Agriculture

## IRANIAN FISHERIES RESEARCH ORGANIZATION

DATE. 14th March, 2012 NO. 10363 ENCL.

**From: Iranian Fisheries Research Organization (IFRO)**
**To: CaspEco Project Management and Coordination Unit (PMCU)**
**Subject: Bilateral cooperation**

To whom it may concern

I would like to inform you that we are very interesting to share our skills, knowledge and experiences in related field of Caspian salmon (*Salmo trutta caspius*) and Sturgeon fish with our colleagues in neighbor's countries.
Our proposed topics could be including of some theoretical and practical affaires such as:

1. Propagation and Hatching of Caspian salmon (*Salmo trutta caspius*) and Sturgeon fish
2. Salmon and Sturgeon fish Nutrition
3. Health & Diseases
4. Stock assessment
5. Biology & Physiology
6. Ecology
7. Establishment of Gene bank and Sperm bank
8. Fish biotechnology & fish processing

We are sure that bilateral cooperation with the experts from the Caspian littoral countries could be very useful for fish farmers and region development.
In fact, on the possibilities of sharing experiences / replication of the two CaspEco pilot projects that were applied in I.R.Iran with the other Caspian countries we can gain valuable achievements through training workshops and some educational affaires.
Please let me know final decisions in order to apply mentioned programs.

**Best regards**
**Dr.Abbas Ali Motallebi D.V.M & Ph.D**
**Head of IFRO**

Iranian Fisheries Research Organization

No.297, West Fatemi Avenue,Tehran - IRAN P.O.Box:14155 - 6116 Tel:0098 21 6420731
Fax:0098 21 6420732 http://www.ifro.org E-mail: info@ifro.org

# 6) Final Report

# Sperm bank creation of Caspian Sea trout (*Salmo trutta caspius*) broodstock

**CFRC Project Manager:**

**Dr.Jalil Zorriehzahra**

**Sperm Bank Project Executer:**

**Shahrouz Baradaran Noveiri**

**Team members:**

**Dr. M.Pourkazemi, S. Najjar Lashkari, Dr. M.Sayad Bourani, Mahdavi, Mr. Lashtu Aghaii, B.Bahramian, M.E.Rastravan, M.Rezvani, S.Zaroudi, D. Bighlarian**

## CONTENTS

**Topic** **Page No.**

**Problem statement:**

Fish are the largest phylum of living vertebrates, with around 30000 species out of approximately 50000 vertebrate species (www.fish base.com). They inhabits in almost every aquatic environment on the earth with a vide variation in temperature, salinity. oxygen and other physiochemical water properties.

Finfish culture is the fastest growing food production industry in the world. In 2005, a total of 28.3 million tones of finfish were produce, which is around 20% of the world's fisheries production (FAO, 2007). The number of species being cultured is also increasing rapidly, resulted double production from 1980 to 2005 (FAO, 2005). One of the most important aspects of the basis of this continuing increase in the number of cultured species is our growing understanding of the complexities of many different reproductive strategies which must be recognized, studied and taken into account when a species is brought into captivity as a brood stock for aquaculture production.

Total production of World Salmonidae based on aquaculture were more than 1.95 million tones, of which 1.23 million tones were Atlantic salmon (*Salmo salar*) and 0.48 tones rainbow trout (*Oncorhynchus mykiss*) mainly from Norway, Chile, Scotland and Canada (FAO, 2005).

The genus *Salmon*, has a plasticity gonochoristic maturation in which the environmental and genetic conditions determine the date of maturation completion (Thorpe et al., 1990; Thorpe 1986). The Caspian Sea trout (*Salmo trutta caspius*) is distributed in southern basin of this sea and the natural stocks of this fish is critically endangered (Kiabi et al., 1999).

Sperm cryopreservation is considered as a general component of effective strategies for saving the endangered species through preserving the gametes of breeders at the frozen state especially for different species of fishes (Gausen, 1993). It serves several benefits which could be briefly mentioned as follow (Cabrita et al., 2010):

1- Synchronization of gamete availability of both sexes

2- Simplification of brood stock management

3- Transport of gametes from different fish farms

4- Gamete storage for genetic selection programs of conservation

During the past few years, several studies have done to identify the mechanisms of intercellular aspects of fish sperm motility, but sperm movement is still the fastest determination way to its overall quality assessment (Bobe & Labbe 2010).

Sperm motility has been extensively studied in most fish species of interest in aquaculture or endangered species. The trigger factors involved in fish sperm motility activation could be divided into parameters, environmental factors after its release and factors present in the egg, coelomic fluid or seminal plasma (Morisawa et al., 1983).

Only those semen samples of good quality are susceptible to successfully cryopreseerved, as has been pointed out by several authors (Robles et al., 2003; Billard & Zhang, 2001). Despite the great deal of researchers of parameters affecting the sperm quality, the motility and fertilization ability of spermatozoa seems to be global (Lahnsteiner et al., 1996; Rurangwa et al., 2001). Parameters related to motility, especially the rate of progressive movement of cells, are widely used for routine field milt evaluation. Nevertheless, an accurate assessment should always be used on the combination of several traits and must include other general parameters of the sperm (sperm density, pH, plasma ion composition…).

**Objectives**

1. Cryopreservation of semen and long-term storage of suitable semen samples from male spawners of Caspian Sea trout caught in the south Caspian Sea
2. Long-term storage of excess semen of male breeders in hatcheries during the breeding season
3. Serving the preserved semen from valuable males to hatcheries if needed in future
4. Develop and extend the technique for the long-term preservation of semen in the management of Caspian Sea trout stocks

**Material and Methods**

The present study was conducted through the breeding seasons of Caspian Sea trout in Janurary 2012. The fresh caught spawners transferred to the Shahid Bahonar (Kelardasht) hatchery for artificial breeding programs and the old caught breeders transferred to the Mr. Kazemi's freshwater hatchery (Dohezar region,Tonekabon) (Fig. 1,2). Estimation of sperm male potential was carried on the ponds (Fig. 4) and sperm samples collected from a total of 12 male spawners which included 8 samples from Dohezar and 4 samples from Kelardasht. Semen was collected from 7 spawners that exhibited reasonable quality of sperms with 20-70% motility (Cabrita et al., 2010).

Semen samples were collected after anesthetized with clove for 10 minutes at water temperature of 9-9.4 ºC (Fig.3, 5).After cleaning the genital area with towel to avoid contamination of samples with water, and fecal mass and urine. (Sarvi et al., 2006) (Fig. 6), Semen was collected in containers by applying a slight pressure to the abdomen (Fig.7) .After a quick qualitative evaluation fresh semen was stored whole and undiluted at 3-4 °C on ice-water container (Fig.8). It was then mixed with a diluting solution which consisted of 0.3M Glucose, 10% Methanol and 10% egg yolk (Sarvi et al., 2006). Semen was diluted with diluting solution at a ratio of 1:3 proportions (Sarvi et al., 2006) (Fig. 9).

Semen was filled in the 0.5 mL straws (Fig. 10) with sampler (Fig.11). After the semen and the diluting solution had reached the same temperature (Fig. 12) the straws with semen were frozen with vapor of liquid nitrogen (Fig. 13) at 2 cm above surface of liquid nitrogen (freezing rate about ~30 ºC $min^{-1}$) (Sarvi et al., 2006) . Finally all frozen samples plunged into liquid nitrogen container (-196 ºC) (Fig. 14).

**Results and Discussion**

About 28 ml of semen from seven male spawners of Caspian trout were collected and cryopreserved. The main spermatological characteristics of each spawner were evaluated and summarized in Table 1. At present the frozen semen from this species is stored in liquid nitrogen at -196 °C in the Cryopreservation Laboratory of the International Surgeon Research Institute.

Table 1: Spermatological characteristics of Caspian Sea trout (*Salmo trutta caspius*) samples

| Sample number | pH | Density ($\times 10^9$ $ml^{-1}$) | Motility (%) | Motility (Sec) | Collected Volume (ml) |
|---|---|---|---|---|---|
| 1 | 8.29 | 0.1 | 10 | 22 | < 0.5 |
| 2 | 7.16 | 0.656 | 10 | 36 | < 0.5 |
| 3 | 6.61 | 0.768 | 0 | 0 | < 0.5 |
| 4 | 7.36 | 0.288 | 20 | 42 | 1.5 |
| 5 | 7.28 | 3.024 | 10 | 35 | 0.5 |
| 6 | 7.08 | 4.048 | 10 | 22 | 1.5 |
| 7 | 7.57 | 3.552 | 20 | 24 | 1 |
| 8 | 7.38 | 3.472 | 20 | 33 | 3 |
| 9 | 7.16 | 3.84 | 10 | 28 | 2.5 |
| 10 | 7.64 | 4.032 | 10 | 28 | 9 |
| 11 | 7.49 | 5.376 | 40 | 42 | 8 |
| 12 | 7.53 | 4.096 | 70 | 44 | 2.5 |

The total length and weight of spawners also are shown in Table 2.

Table 2: Total length and weight of male Caspian Sea trout (*Salmo trutta caspius*) according to the sample number in table 1(gray cells).

| Sample number | Total length (cm) | Weight (Kg) |
|---|---|---|
| **4** | 47 | 1520 |
| **5** | 35 | 580 |
| **6** | 40 | 860 |
| **7** | 35 | 580 |
| **8** | 42 | 1030 |
| **Mean ± SD of Dohezar** | 39.8 ± 5 | 975 ± 389 |
| **9** | 45 | 3200 |
| **10** | 48 | 3500 |
| **11** | 43 | 3100 |
| **12** | 52 | 3600 |
| **Mean ± SD of Kelardasht** | 47 ± 4 | 3350 ± 238 |

The results obtained from this study indicate that male spawners from Kelardasht region were potentially better than those the other region. The average total length and weight of males from Kelardash region (fresh wild broodstock) were significantly greater than of Dohezar's (captured previous broodstock) ($P > 0.05$). This difference is more effective by looking at the differences between mean semen values (5.5 vs 1.5 ml), sperm density ($4.3\times10^{9}$ $ml^{-1}$ vs $1.98\times10^{9}$ $ml^{-1}$) , sperm motility time (35.5 sec vs 26.3 sec) and the percentage of sperm motility (32.5% vs 25.7%).Therefore for further fertilization efforts, we strongly suggest using of freshly caught male Caspian Sea trout to maintain the best fertilizing ,hatching and larval rates.

Cryopreservation is of interest not only for fish farming but also for conservation and genetic improvement for wild resources. Regarding to the facilities available, this work had shown the potential of long-term storage of sperms from different number of breeders as well as other localities of Caspian Sea trout. The equipment available can be used to develop research related to cryopreservation of spermatozoa. Also it is advisable to find the fertilizing potential of frozen sample through next breeding season.

**Acknowledgements:**

I am very grateful to my collaborators who were of a great help as project chief manager: Dr.Jalil Zorriehzahra, Head of the International Sturgeon Research Institute : Dr. Mohammad Pourkazemi, colleagues of fish reproduction: Mr. Mahdavi, Mr. Lashtu Aghaii, Mr. Bahramian , Ms. Najar Lashgari and Dr.Mohammad Sayad Bourani, Head of Coldwater fishes Research Center (Tonekabon, Iran).

Also I would like to thank the collaboration of Mr. Kazemi's hatchery and Breeding and cultivation center of Kelardasht for their assistance on supplying the experimental materials and finally thank the confidence of CASPECO manager Ms. Dr.Farshchi (CEP) and Dr.Abbasali Motalebi as Head of IFRO (Iran).

**References:**

1. **Billard, R. and Zhang, T. (2001).** Technique of genetic resource banking in fish, in Cryobanking the Genetic Resource, Watson, P.F. and Holt, W.V., Eds., Taylor and Francis, London, p.22-144.
2. **Bobe,J. and Labbe, C.(2010).** Egg and sperm quality in fish. Gen. Com. Endocrinol., 165:535-548.
3. **Cabrita,E.,Saraquete,C., Martinez-Paramo,S., Robles,V., Beirao,J., Perez-Ceralez,S. and Herraez,M.P. (2010).** Cryopreservation of fish sperm: applications and prospective. J. Appl. Ichthyol., 26: 623-635.
4. **FAO, (2007),** The state of world fisheries and aquaculture 2006, Food and Agriculture Organization of the United Nations, Fisheries and Aquaculture Department, Rome,(www.fao.org/docrep/fao/009/a0699e/a0699e.pdf).
5. **FAO, (2005),** Aquaculture production; Quantities 1970-2005, Fisheries Department, Fishery Information, Data and Statistics Unit, FISHSTAT Plus: Universal software for fishery statistical time series, Version 2.3.2000.
6. **Gausen, D. (1993).** The Norwegian gene bank programme for Atlantic salmon (*Salmo trutta*). In: Claud,J.C., Thorgaad,G.H. (Eds),Genetic Conservation of Salmonid Fishes. Plenum, New York, pp: 181-187.
7. **Kiabi, B.H, Abdoli,A. and Naderi, M. (1999).** Status of fish fauna in the south Caspian basin of Iran. Zool. Middle East.18: 57-65.

8. **Lahnsteiner, F., Berger, B., Weismann, T., and Patzner, R.A. (1996).** Motility of spermatozoa of *Alburnus alburnus* (Cyprinidae) and its relationship to seminal plasma composition and sperm metabolism, Fish Physiol. Biochem., 15:167-179.
9. **Morisawa, M., Suzuki, K., and Morisawa, S. (1983).** Effect of potassium and osmolarity on spermatozoa motility of salmonid fishes, J Exp Biol, 107: 105-113.
10. **Robles, V., Cabrita, E., Cuñado, S. and Herráez, M.P. (2003).** Sperm cryopreservation of sex reversed rainbow trout (*Oncorhynchus mykiss*): parameters that affect its ability for freezing, Aquaculture, 224: 203- 212.
11. **Rurangwa,E., Volckaert,F.A.M., Huyskens,G., Kime,D.E. and Ollevier,F.(2001).** Quality control of refrigerated and cryopreserved semen using computer assisted sperm analysis (CASA), viable staining and standardized fertilization in African catfish (*Clarias gariepinus*), Theriogenology, 55(3):751-769.
12. **Sarvi,K., Niksirat,H., Mojazi Amiri,B.,Mirtorabi,S.M., Rafiee,G.R. and Bakhtyari,M. (2006)**. Cryopreservation of semen from endangered Caspian brown trout (*Salmo trutta caspius*). Aquaculture, 256:564-569.
13. **Thorpe, H.E. (1986).** Age at first maturity in Atlantic salmon, *Salmo salar*: Freshwater period influences and conflicts with smelting. In: Meerburg, D.J. (Eds), Salmonid age at maturity. Fish. Aquat. Sci., 89.
14. **Thorpe,J.E., Talbot,C., Miles,M.S. and Keay,D.S. (1990).** Control of maturation in cultured Atlantic salmon, *Salmo salar*, in pumped seawater tanks, by restricting food intake. Aquacultur,86(2-3):315-326.
15. **Lahnsteiner, F., Weismann, T. and Patzner, R.A., (1995).** A uniform method for cryopreservation of semen of the salmonid fishes *Oncorhynchus mykiss, Salmo trutta fario, Salmo trutta lacustris*, Coregonus sp. Aquac. Res., 26: 801-807.

Fig.1: The Caspian Sea trout previously caught broodstock (Dohazar)

Fig.2: The Caspian Sea trout fresh caught broodstock (Kelardasht)

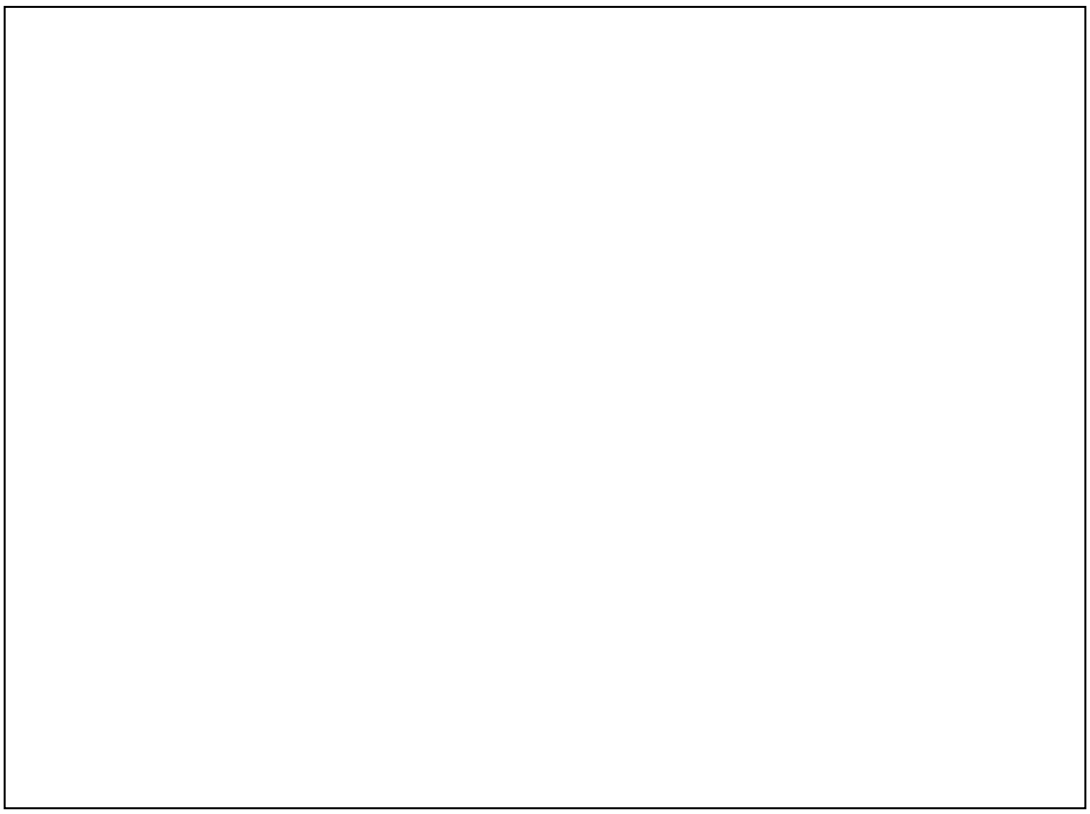


Fig.3: Collecting of broodstocks for anesthetizing

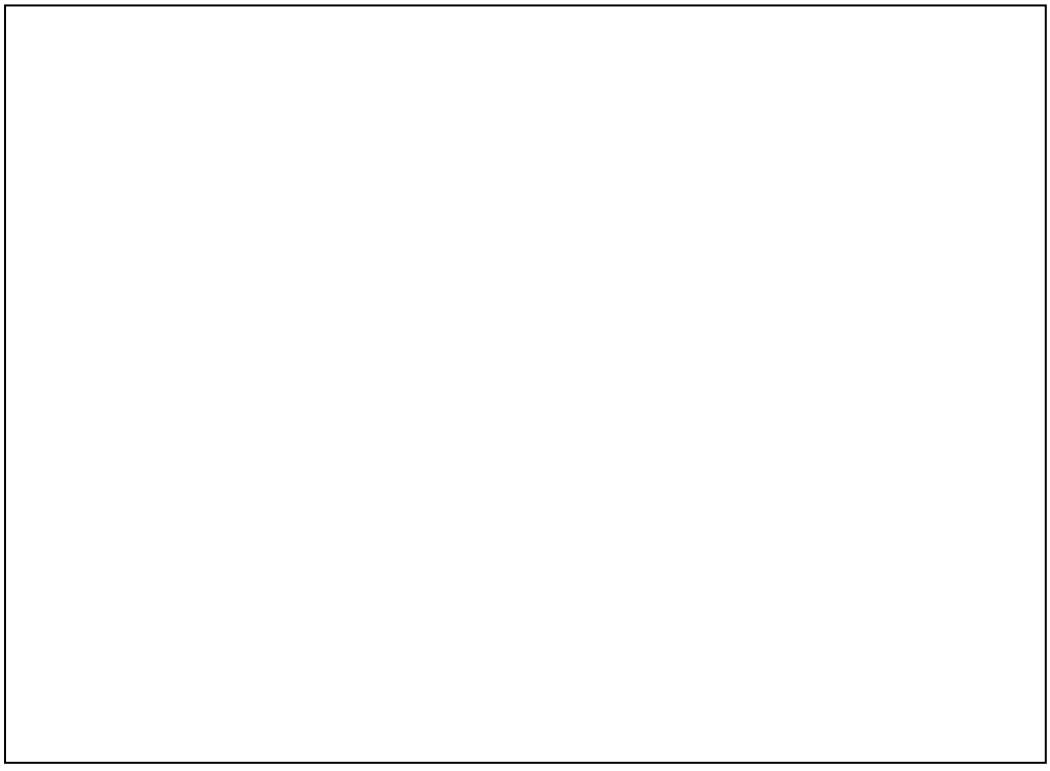


Fig.4: Checking the male potential for sperm releasing

Fig.5: Anesthetized male fish

Fig.6: Drying the genital area

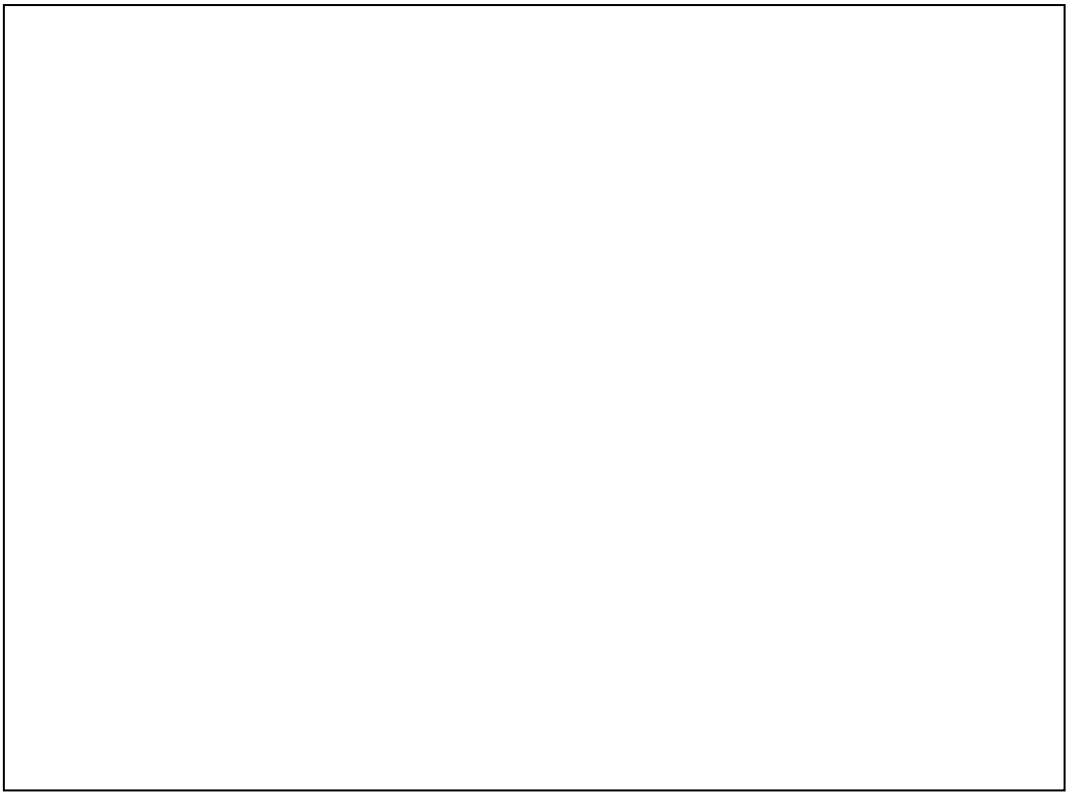


Fig.7: Hand stripping collection of sperm

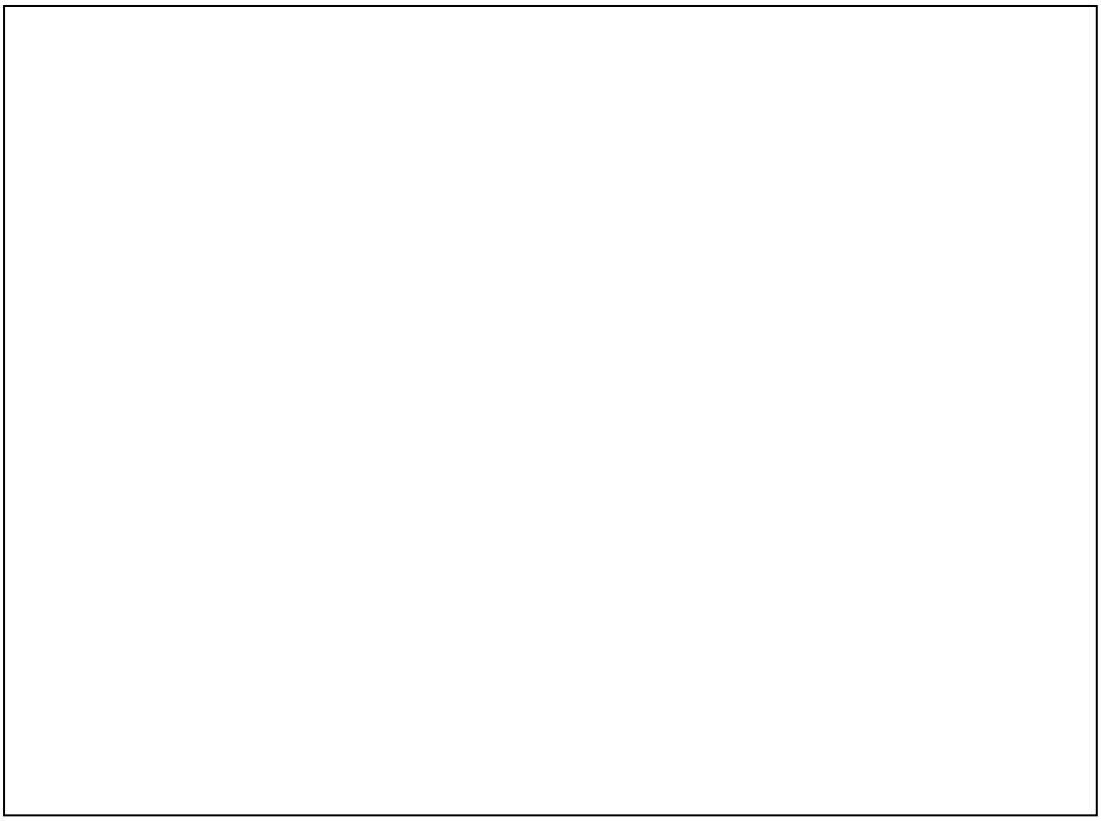


Fig.8: Holding of fresh samples at 4 ºC

Fig.9: Mixing of sperm and extender at the same temperature

Fig.10: Different colored 0.5 ml straw

Fig.11: Hand filling the straws

Fig.12: Equilibration of mixed sperm+extender before deep freezing

Fig.13: Freezing the samples on vapor of liquid nitrogen


Fig.14: Preserving the samples in liquid nitrogen

**List of figures:**

## 7) Final Report

# Gene Bank creation of Caspian Sea trout (*Salmo trutta caspius*) broodstock

**CFRC Project Manager:**

**Dr.Jalil Zorriehzahra**

**Gene Bank Project Executer:**

**Prof.Dr.Sohrab Rezvani Ghil kolaei**

**Team members:**

**S. Najjar Lashkari, Dr. H.A.Abdolhay, Dr. M.Sayad Bourani, B.Bahramian, M.E.Rastravan, M.Rezvani, F.Laloui, M.J. Taghavi, H. Pourgholam, A.Fahim, R.Safari, S.Zaroudi, D. Bighlarian**

## Introduction

Caspian Sea is the largest salt lake on the earth with old, long history and different habitats has created a unique aquatic ecosystem. Caspian Sea has been located in latitude 47º7' North 36º32'South and longitude 45º42' West and 54º20' East and has been located in cross the continent of Europe and Asia and is shared between five countries Russia, I.R.Iran, Turkmenistan, Armenian and Azerbaijan. Armenian and Azerbaijan are shared. 1030 km in length and width of 435-196 km is the range in narrowest. The South Caspian with maximum depth about 1025 meters and 60/6%the volume of water included 37% of the Caspian Sea surface (Messenger, 1384).

In terms of biodiversity about 584 animal species and over 500 plant species living in it. A number of animal and plant species in Caspian Sea were originated from the Black Sea and Azvef Sea. In the Caspian Sea, 871 species of invertebrates and 305 species of tiny macro benthic organisms have been reported. A total of 25 fish species with commercial and economic importance are exploiting in it (Ivanov, 2000) that one of the most important is Caspian salmon (*Salmo trutta caspius*).

Caspian salmon with scientific name *Salmo trutta caspius* Kessler, 1877 is a brown trout and including the migratory fish to the river (Anadromous) and is native to the Caspian Sea. Among the bony fishes of the Caspian Sea, It has been achieved high economic value and completely compatible with the culture and palate of northern people In Iran. So it is the most expensive marine fish in the north of the Iran. Caspian salmon migrate in autumn and spring in order to spawning and feeding. The fish are spawning in the mountain areas in the river bed with rubble stone, gravel and sand.

**Problem Statement:**

In the last decade, geneticists have helped aquaculture managers by implementing breeding programs and genetic variability to obtain higher productivity and sustainability in fish hatcheries. The use of molecular markers has significantly helped this goal. In particular, the use of microsatellites has allowed the determination of effective breeding numbers (Ne) through parentage assignments in several species, including Atlantic salmon (*Salmo salar*) (Norris et al., 2000)

Microsatellites are also useful for the characterization of genetic stocks (Chistiakov et al., 2006).This method provides significant information in genetic of fish. Investigation of genetic diversity by microsatellite have been described within genes of a variety of fish

species, including Caspian Sea *Cyprinus carpio* (Yousefian et al., 2011) , *Catla catla* (Alam & Islam, 2005), *Esox Lucius* (Lucentini et.al., 2006), *Oncorhynchus masou* (Kitanishi, et al., 2009) and Caspian Sea *Salmo trutta* (Yousefian et al., 2010). The brown trout, Salmo trutta L., is today widely distributed in many continents. It is a polytypic species with a wide variety of adaptations to different environments. On the southern Caspian (Iranian costal), Caspian salmon or Sea trout, *Salmo truta Caspius*, have access to approximately 5 streams for spawning, but mainly in Tonkabon (Cheshmeh kileh) river. The spawning migration normally extends from October to January. The timing of the spawning period is highly dependent on water flow but normally most of the sea trout spawning takes place in November and December. The eggs hatch during April-May and sea trout generally remain 2 years in fresh water before smoltification and migration to the sea. These streams run with clear water. Caspian salmon (*Salmo trutta caspius*) is an anadermus fish species and it is an important food source for the people of South Caspian Sea. Recently the populations of Caspian Sea trout are reduced in numbers due to human activities such as deforestation, drainage, and dam constructions. Many streams have become less suitable for spawning trout and their progeny. The depletion of wild fish stocks in the south Caspian Sea (Yousefian et al., 2010) led to artificial propagation of this fish. In hatchery, cultivated stocks have been reared for enhancement and protection of wild populations and maintenance of this species. To study the genetic structure of *Salmo trutta caspius* improve management plan that aim to conserve diversity of this species. In our previous research we studied the Stock Identification and Genetic variation at Microsatellite Loci of Caspian Sea Salmon *(Salmo trutta caspius)* to clarify the status of genetic structure of one of the mast valuable fish of Caspian Sea. In present study we analyzed the same microsatellite locus to identify genetic variation among the spring and autumn population that have been immigrated from Caspian Sea to mentioned rivers in order to spawning and evaluate the results of artificial propagation in gene conservation of *Salmo trutta Caspius*. So Molecular Genetics techniques were employed to study on population of *Salmo trutta caspius* spring and autumn forms in Southern Caspian Sea by using Microsatellite marker method basin and establish an alive gene bank

**Objectives:**

1) Molecular population genetic study on *Salmo trutta caspius* from five rivers of Southern Caspian Sea

2) Molecular genetics on spring and autumn races of *Salmo trutta caspius*

3) Molecular phylogeny study on different Salmon species from Caspian basin

4) Establish of Gene Bank of different alive population of *Salmo trutta caspius* from five main rivers that release to the Caspian Sea.

## Materials and Methods

### 1. Sampling

In this project, 3-5 gram of fin soft tissue samples of 352 *S.trutta caspius* broodstocks were collected. Broodstock (male and female)(Figure 1)were caught with several methods such as Shill dam (Koleham)( Figure 2), Beach seine (Parreh) (Figure 3), Cast net (Figure 4) and fishing net. Rivers of mentioned sampling were consisted of Sardabroud (Figure 5) and Cheshmeh Kileh rivers (Figure 6) in Mazandaran province that totally 189 samples were collected. In the other hand, Navroud and Shafaroud Rivers (Figure 7) and Karghan Roud River (Figure 8) were located in Gilan province with 53 samples. All samples were performed in autumn and winter in caught seasons.

All broadstocks were collected and then biometry affaires were done (Figures 9 & 10). Then Ovum and fertilized eggs were measured (Figure 11) by caliper tool. Also scale samples were taken in order to fish age estimation (Figure 12).

All mentioned tissue samples were collected by scissor (Figure 13) and were fixed in ethanol 96% (Figure 14) and finally delivered to Genetic Laboratory of Ecology Research Center in Sari city (Figure 15).

Figure 1 : Broodstoks of *Salmo trutta caspius* of Caspian sea (Male and Female)

Figure 2: Shill dam (Koleham) in Cheshmeh kileh River

Figure 3: Beach seine (Parreh)

Figure 4: Cast net

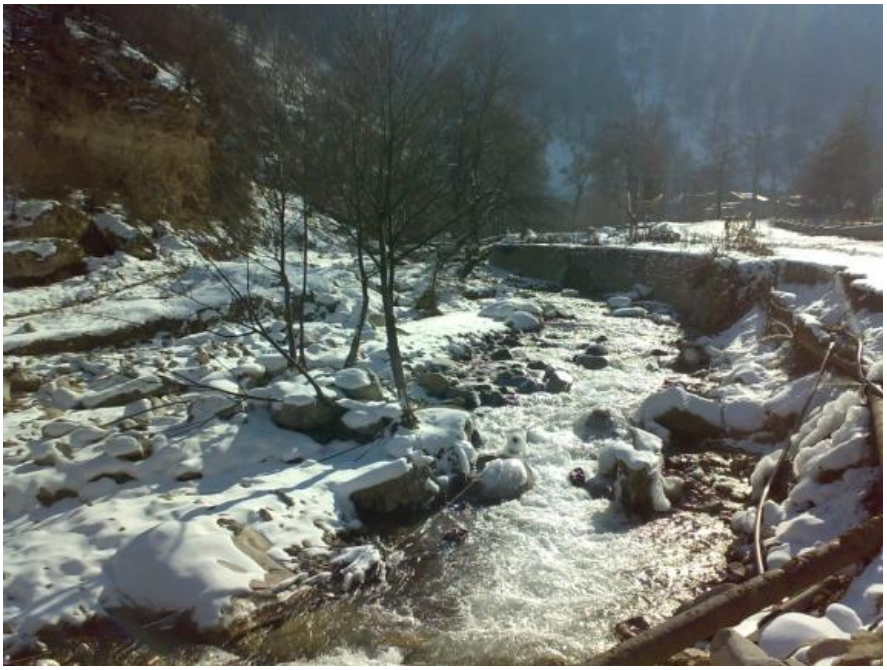


Figure 5: Sard Abroud River in Mazandaran province

Figure 6: Cheshmeh Kileh River in Mazandaran province

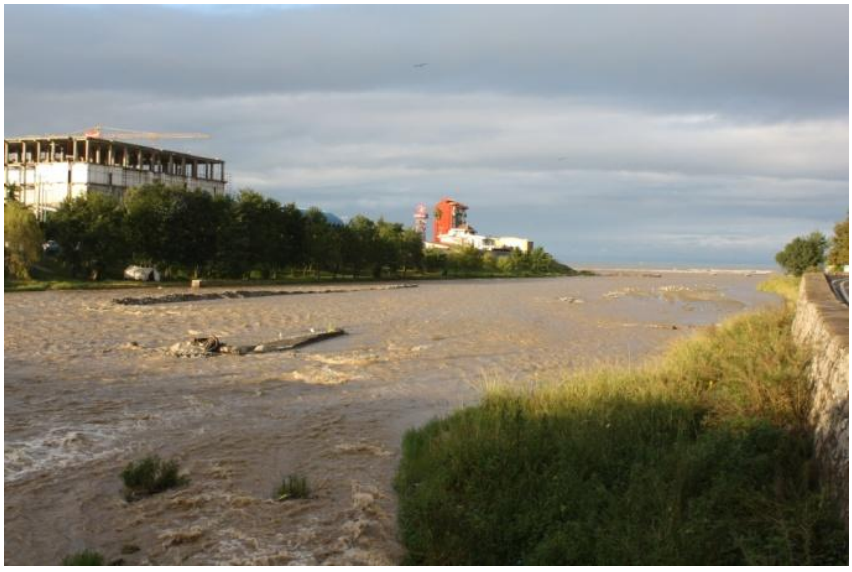


Figure 7: Shafa roud River in Gilan province

Figure 8: Karghan Roud River in Gilan province

Figure 8: Biometry and data recording

Figure 10: Broodstock biometry


Figure 11: Measuring of fertilized eggs by caliper tool

Figure 12: Scale removing from broodstock skin surface

Figure 13: Fin removing method from broodstock

Figure 14: Fin sample fixation method in ethanol 96%

Figure 15: Genetic Laboratory of Ecology Research Center in Sari

**2 – DNA extraction**

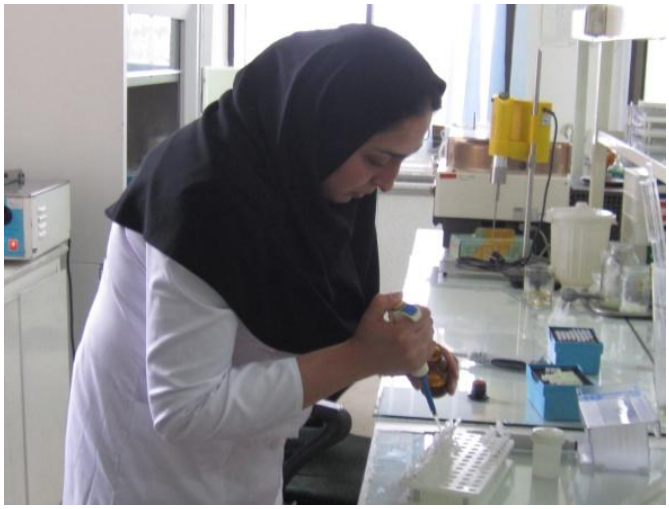
There are several methods for DNA extraction in this study, phenol – chloroform (Hilis & Moritz, 1990) to extract the DNA 352 productive salmon fishing in the Caspian (Mazandaran, Gilan and 274 samples, 78 samples) were used.

**A) Extraction process**

1. Approximately 50 mg of Caspian salmon fin tissue after completely dried by filter paper into vials or tubes 5/1 ml was transferred to the feedback (Figure 16).

Figeure16: Chopping and squashing of small fins

2. Then used to digest tissue proteins, especially on the 600 micro litres of buffer STE, 30 micro litres SDS (Sodium sulfate 2 Dsyl) 10% and 7-4 mg ml l K10 protease were added (Figure 17).



Figeure17: Adding the necessary substances into the digested tissue

3. In order to complete activate of proteinase enzyme, vials containing the samples were placed in thermomixer or water bath for 4-3 h at 50 to 55 ° C. (Figure 18) During this time the samples were completely digested and concentrate emulsion was formed.

Figeure18: Water bath/ bain-marie (thermomixer) during work

4. In order to protein denaturation and fat solving about 500 µl phenol (pH=8) was added, and then vortex-mixed by a few seconds (Figure 19) and for at least 1 hour it was shaked with  using a shaker (Figure 20) and immediately was centrifuged (Fig. 21) with 13000 rpm for 5 min.

Figure19: Vertex samples method


Figure 20: shaking of samples


Figure21: Centrifuges

5. Then upper phase was separated slowly by a sampler and poured into a new vial, to remove residual phenol 500 micro litre chloroform was added and process repeated again for 15 to 20 minutes and it centrifuged at 13000 rpm and then was shaked for 5 minutes. Then separation of upper phase was performed according to the previous step.
6. Then 800 micro litre pure ethanol and 40 micro litre 3M sodium acetate were added to upper phase, and then tubes were shaked gently by hand and were centrifuged for 15 min at 13000 rpm. White plate was formed in the tube that it was extracted DNA.

   Ethanol and acetate were thrown away after drying for 3 to 4 minutes, then rinsed with 70% ethanol and then centrifuged at 13000 rpm for 2 min. Then the upper phase was empty, for drying and evaporation of the ethanol from DNA plate, the samples were placed in room temperature for 30 minutes.
7. After drying, 100 microliters sterile distilled water and 3 microliters of RNase were added to the DNA plate (Figure 22), and were putted in water bath for 30 min at 37 ° C until DNA was completely dissolved in water and unwanted RNA fragments were digested, then it was transferred to the - 20 ° C freezer for long term storage.

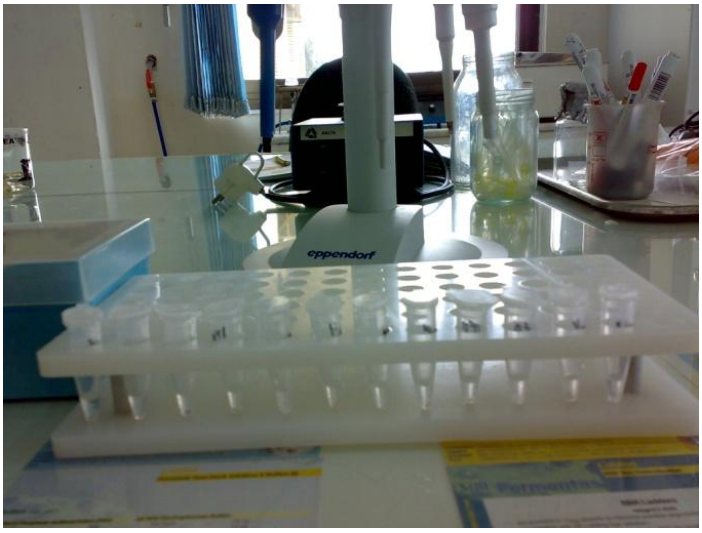



Figure 22: DNA dissolved in sterile distilled water

**3 – Determination of quantity and quality of extracted DNA**

Spectrophotometer and electrophoresis techniques were used in order to determination of the quantity, quality, concentration and purity of extracted DNA.

1. **Using of spectrophotometer for quantity of extracted DNA**

   In order to determination of the quantity of extracted DNA samples, first the calibration device of spectrophotometer CECIL (Model DE2040) was done with distilled water, 5 µl of genomic DNA by distilled water was adjusted volume to 300 µl, optical absorption of samples were measured and recorded by the device at a wavelength of 260 to 280 nm and A260/280 ratio and at the end DNA concentration was calculated by using formula 2-1.

   Formula 1-2

   $$50 \times D \times A260 = \text{DNA concentration in nano gr per ml (ng/ml)}$$

   A: Optical absorption at a wavelength of 260 nm

   D: Dilution ratio 60 = 300/5

   If the ratio of 8/1 = A1/A2 may be suitable for DNA and the 8/1 A1/A2> is the impurity of DNA and RNA absorb a proportion of 8/1 A1/A2 <is indicative of impurities and contamination with phenol and protein.

**2. Assessment of extracted DNA Quality by using agarose gel electrophoresis**

Used materials: 10X concentration TAE buffer (Tris acetate), agarose, heavy buffer, 1%ethidium bromide, injection distilled water

Needed equipment: Samplers and tip of them which can take a 20 micro liters volume of sample, gel tray and its comb, Disposable gloves, UV trans Sylva Native machine, Horizontal electrophoresis and a source of electric current 760l-EPS model Paya Pazhohesh Company

Assessment method: In this method, the extracted DNA using 1% agarose gel (depending on the size of DNA) was electrophoreses through the following steps.

1- Clean and dry gel electrophoresis tank was placed on a horizontal surface.
2- Special gel tray placed in the local flat and placed on the combs so that the gel to a foam tray with gel mm is the distance.
3- For preparing one present agarose gel, 3 ml of buffer TAE (10x) poured into the Erlenmeyer flask and 3/0 g agarose was added and its volume was brought to 30 ml with distilled water.
4- The obtained suspension was heated over a flame until the agarose is dissolved and clear, then flasks were placed at room temperature to cool.
5- Melt agarose gel poured into trays and allowed to be concluded

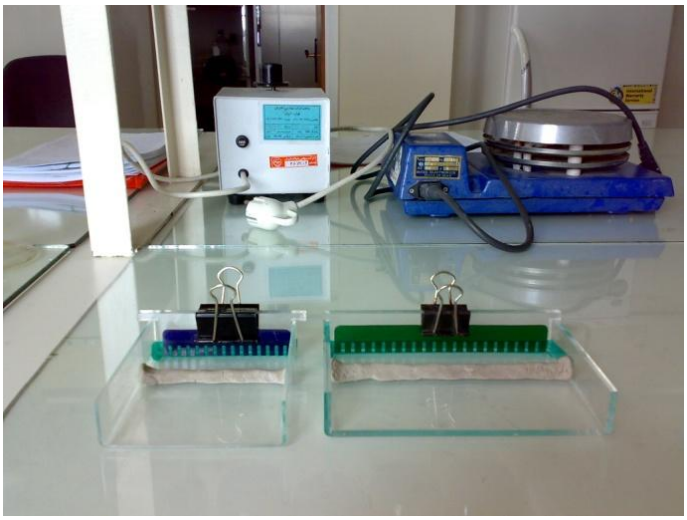


Figure 23: 1% agarose gel in the gel trays

6- The two sides of the tray open and the closed gel were gently placed into electrophoresis tank and then a while the comb was gently removed from the gel

7- 5 micro litre of genomic DNA with 3 micro litre of heavy buffer mixed completely and was poured into each of the gel wells (Figure 24).

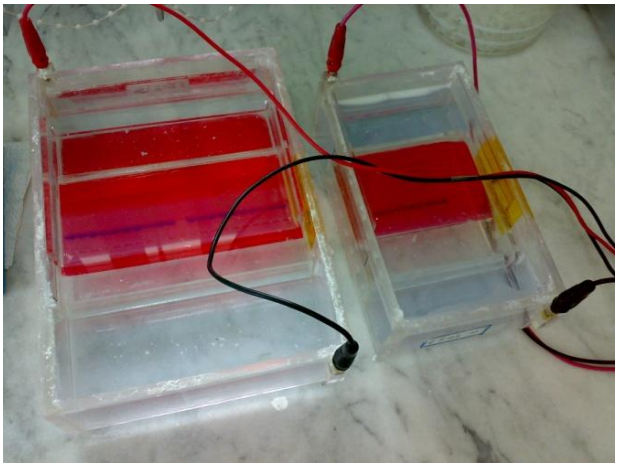


Figure 24: Horizontal electrophoresis tanks, were injected with the samples in the agarose gel well

Source of electricity connected to the electrophoresis tank the generator was set on 90 volts and 45 mA

8- After heavy buffer getting the end of the gel, the gel was stained with ethidium bromide solution and after washing with water and transferred to the gel doc and was exposed to UV (Figure 25). DNA bands on the gel had been seen orange color under UV light and DNA quality and concentration based on the observation of strong and weak bands, purity, phenolic pollution, protein and DNA breakage was evaluated.

Figure 25: Gel documentation (Gel Doc) with the gel to produce image

**4 – Primers**

Primers that were used in this study along with related sequences are listed in Table 1.

Table1: List of used primers with related sequences

| Primers Sequences | Replication | Size(bp) | Locus |
|---|---|---|---|
| 5'-AATCTCAAATCGATCAGAAG-3'<br>5'-AGCTATTTCAGACATCACC-3' | GT | ١٢٤-٢١٦ | Strutta 12 |
| 5'-AACAATGACTTTCTCTGAC-3'<br>5'-AAGGACTTGAAGGACGAC-3' | GT | ١٠٢-١٩٠ | Strutta 58 |
| 5'-AGATTTACCCAGCCAGGTAG-3'<br>5'-CATAGTCTGAACAGGGACAG-3' | GT | ١٨٧-٢٦٣ | OmyFgt1TUF |
| 5'-TTATTATCCAAAGGGGTCAAAA-3'<br>5'-GAGGTCGCTGGGGTTTACTAT-3' | GTGA | ٢٠١-٢٤٣ | Ssa 171 |

**5 – Amplification of gene fragments and polymerase chain reaction**

For PCR and amplification of target genes, 100 ng of extracted DNA, 1 µl of primers (30 Pico mole) plus 5/0 µl dNTP (10 mM), 2/0 µl Taq$(5u/\mu)$, 5/2 µl PCR bafr (10x), 8/0 µl MgC12 (50 mM ), were added to a 2/0 or 0/5 mL sterile vial that based on ice and adjust its volume with distilled water to 25 µl and was stirred well by sampler.

The vials were closed and centrifuged for 30 seconds until the contents were transferred to the bottom of the vials. The vials were in thermo cycler (Figure 26) and according to the plan (Table 2), PCR was performed. After the PCR products were transferred into the - 20 ° C freezer.

Figure 26: PCR products and thermo cycler

1. **Optimize the thermal profile and PCR**

   To optimize the PCR, the optimal concentration of each of the reaction components such as primers, enzymes, magnesium chloride, DNA template and thermo cycler program were obtained after several tests and repeated.

**6 – PCR product was electrophoresed, using 6%polyacrylamide gel**

After preparing 6%polyacrylamide gel electrophoresis and injected between two glass vertical electrophoresis devices, special comb was placed between two glass for 0/5 hours at the lab temperature until to be jell. Then remove the comb and wells were washed with TBE buffer 1x, then three micro litre of the PCR product and 2 micro litre of heavy buffer (Loading Dye) with a marker were transferred into the gel wells. Device connected to electricity with 160-150 volts and 80-60 mA for 2 hours until the gel was run. After Two hours, duplicated DNA sequences were separated based on their molecular weight along the gel.

1. **Methods for preparing the polyacrylamide gel (6%)**

   For preparing 6% polyacrylamide gel, at first 27/5 ml distilled water with 7/5 ml 30% polyacrylamide and 3/5 ml TBE buffer (10X) were poured into the balloon has a side arm and air was get by compressor device for 4 minutes. Then filled with 300 micro litre of 10% ammonium sulphate solution, and 32/5 micro litre of TEMED solution was added to the previous mixture. This combination was poured into the

space of between two glasses that its sides and bottom is blocked and a comb is placed on upper part. It is necessary at this stage to avoid creating air bubbles.

The time required for polymerization of the gel was about 30 minutes and after this period the comb remove gently from the upper part. After washing the created wells by a TBE solution (1x) (electrode buffer), the PCR samples were poured into wells and the gel was placed on vertical TBE buffer column (1X), so that the gel was been between two buffer tanks and the only connection between the buffers is possible through the gel. When the device is connected to the 150 volts generator, positive and negative pole is connected in order to the top and bottom of the tank. The flow from the top to the bottom side of the tank and led to move the loaded samples in this direction in wells, And amplified DNA fragments based on their molecular weight are separated along the gel( Figure 27).Samples electrophoresis was done in 3 hours. To appearance of DNA bands, the silver nitrate staining method was used and to ensure the reliability of test strips, experiment were repeated again at the same conditions.

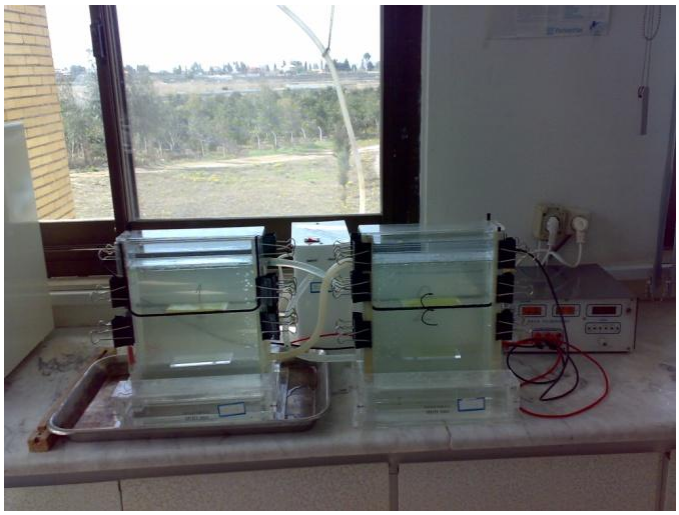


Figure 27: Vertical electrophoresis device and 8% polyacrylamide gel

**2. Stained polyacrylamide gel with silver nitrate**

For staining polyacrylamide gels, three types of solution were prepared as follows:

a) Solution A, 0/5% acetic acid buffer, and 10% ethanol

| | |
|---|---|
| Ethanol | 40 ml |
| Acetic acid | 20 ml |
| Distilled water | 360 ml |

b) Solution B, 1/0% silver nitrate buffer

| | |
|---|---|
| Silver nitrate | 0/2g |
| Distilled water | 200 mg |

c) Solution C,0/15% formaldehyde buffered, 0/14% $NaBH_4$ and 5/4% NaOH

| | |
|---|---|
| NaOH | 4/5 g |
| $NaBH_4$ | 0/03 g |
| Distilled water | 300 ml |
| Formaldehyde | 1/2 ml |

It is necessary to prepare the solution3, at first sodium hydroxide dissolved in distilled water and then other materials are poured on them.

After finishing the electrophoresis, gels from glass plates separated with a palette knife and for staining, the gel twice and each time for 3 minutes was placed inside the solution A on a shaker and then 10 minutes transferred to solution B, at the end of this term twice and each time was 1 minute, rinsed with distilled water. Finally, 10 minutes or until bands appear as dark colored bands was placed in the solution C. Stained gels for storage were transferred into pressing plastic covers.

According to the presence or absence of mutations in the special position of small satellite, DNA fragments are produced with different molecular weights from the PCR products So the difference between the bands and the difference in DNA molecular weight can lead to the production various genotypes, and by putting a detector (bp50) next to the sample can be estimated and used to identify genotypes .These detectors are specific bacteria genomes which have been cut into many pieces with specified molecular weight by various enzymes (Bassam *et al.*, 1991).

**7 - Recorded images**

Polyacrylamide gel images after staining, recorded and saved by trans-illuminators gel doc system DOC008.XD model that manufacturing UVI company with UVI DOC Version V.99.04 software in Biotechnology Laboratory, Ecological Institute of Caspian Sea in the Abad city of Sari ( Figure 28, 29).

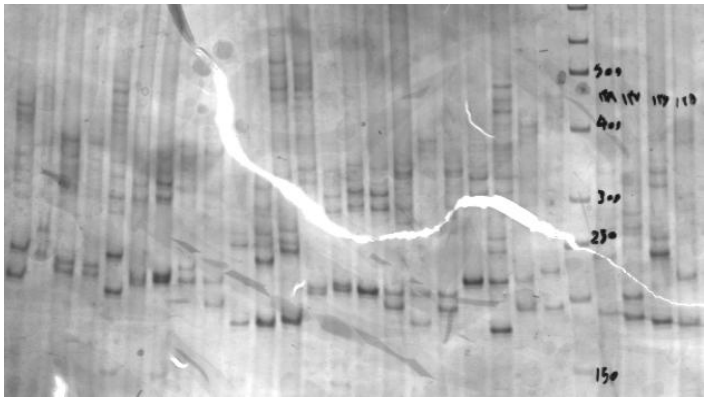


Figure 28: Polyacrylamide gel, DNA samples from Mazandaran

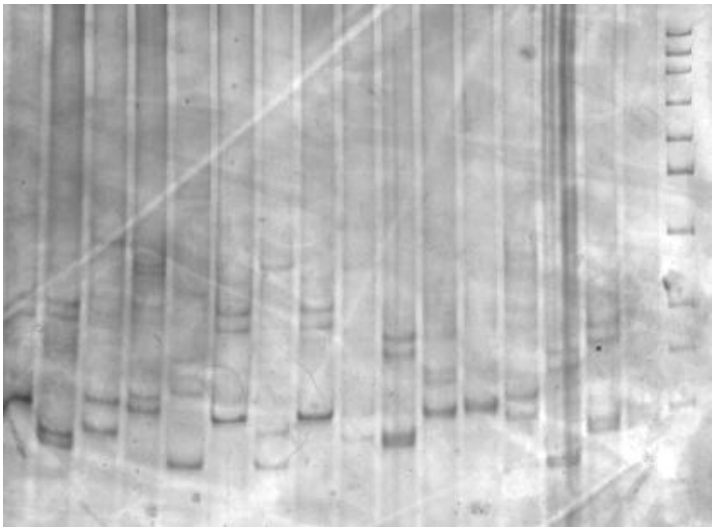


Figure 29: Polyacrylamide gel, DNA samples from Gilan

**8 - Measurement of PCR product molecular weight and bands rated**

Polyacrylamide gel images after being recorded by gel documentation system, to measure the molecular weight of PCR product bands, to obtain the types and sizes of alleles and determined genotypes was used Lab Image version 3.3.3 Computer software. In each genotype one band was to be homozygosis and see two bands, heterozygosis.

**9 - Statistical analysis**

Allele frequency, expected heterozygosis and observed heterozygosity, the number of the real and effective allele in micro-satellite sites, (Information index), the similarity matrix (Genetic similarity) and genetic distance (Genetic identity) according to Nei, 1978; Nei, 1972 and Hardy - Weinberg equilibrium based on the $X^2$ and the hierarchy of five regions population (Sardab rood river, Cheshmeh kileh, Navrood, Shafarood and Krganrood) and

two regions (Mazandaran and Gilan provinces) were calculated based on the test (Analysis of Molecular Variance) AMOVA levels 0/01 and using the Gene Alex software.

## Result

### 1. Evaluation Result of the quantity and quality of extracted DNA

DNA quality and quantity were evaluated in two ways that the results are as follows:

**A – Electrophoresis Method**

Evaluation of intensity and resolution of DNA bands on 1% agarose gel showed that extracted DNA from the fins by phenol chloroform method has acceptable quality and quantity for using in the PCR experiments (Figure 30).

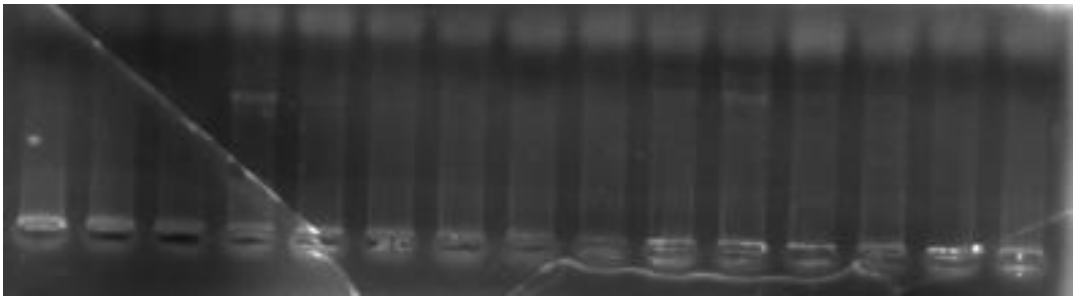


Figure 30: DNA samples on 1% agarose gel

**B– DNA Spectrophotometric**

Absorption was calculated by the spectrophotometer in the 280 and 260 nm wavelengths and the absorption ratio of 260 to 280 nm wavelength was considered as a quantity index. Samples have 1/8 to 2 ratio, were chosen and inappropriate extracted DNA samples were repeated again.

### 2. The results of the PCR products on 6% polyacrylamide gel (Vertical Electrophoresis)

After putting the PCR products of primers Struta 12, Struta 58 and OmyFgt1TUF on 6% polyacrylamide gel and stained by silver nitrate, a single or double bond observed,

the size were between bp 216 - 124, bp 190-102 and bp 263-18 and the total bands size of Sea 171 primer was 230 bp.

3. **The number of effective and observed alleles in *Salmo trutta caspius***

   The number of observed alleles in the 4 microsatellite position Strutta 12, Strutta 58, OmyFgt1TUF Ssa171 of *Salmo trutta caspius* are 15, 17, 16 and 2 in order and the number of effective alleles are also listed in fourth place, respectively, 8/328, 12/014, 8/623 and 2.

4. **Expected and observed heterozygosis at each position of a *Salmo trutta caspius* microsatellite**

   Amounts of expected heterozygosity (Ho) in four micro satellite *Salmo trutta caspius* position Strutta 12, Strutta 58, OmyFgtlTUF and Ssa171 are 0/911, 1/000, 0/764 and 000/0 in order and amounts ofobserved heterozygosity (He) is also listed in fourth place are 0/887,0/927, 0/882 and 1/000 in order.

5. **Shannon index values for each micro satellite position of a *Salmo trutta caspius***

   Shannon index values for 4 microsatellite position *S.trutta* 12, *S.trutta* 58, OmyFgtlTUF and Ssa171 a *Salmo trutta caspius* were obtained 2/57, 2/64, 2/38 and 0, in order.

6. **Hardy - Weinberg equilibrium**

   Values of Hardy - Weinberg equilibrium in the three polymorphs micro satellite position of a *Salmo trutta caspius (S.trutta* 12, *S.trutta* 58 and OmyFgtlTUF were obtained 187/5, 2/173 and 193/7, in order.

## Conclusion:

One of the main issues for geneticists and statistics to determine the minimum sample size is required for the assessment of microsatellite variability and provide understandable interpretations of the data. Since the number of observed alleles in microsatellite is usually high and allele frequency may be down 40 to 100number of samples are needed for statistical analysis, although this number depends on the number and frequency of alleles.

In this study, 189 and 53 samples of Mazandaran and Gilan in order were used to review the micro satellite methods.

In this study the average number of alleles in each locus of *Salmo trutta caspius* micro satellite was obtained 16/7 which compared to the number obtained from Norris and colleagues research (1999) on Atlantic salmon (*Salmo salar*) the (17/8) was lower and the obtained range by Andrews and colleagues (1995) on Atlantic salmon (*Salmo salar)* was (35-13).

In this study the average value of heterozygosis of each microsatellite position Caspian salmon, was obtained 0/8 and compared to the obtained number from R Norris and colleagues (1999) on Atlantic salmon (*Salmo salar*) the (0/75) was higher and the range obtained by Andrews and colleagues (1995) on Atlantic salmon (*Salmo salar*) was the (1-0/82).

In conducted research on the Caspian salmon using Markers has been observed deviation from Hardy - Weinberg equilibrium and since, according to studies done by other researchers proved that several factors such as other fertilization (Rico et al., 1997), low sample size (Reilly et a1., 1999), the null allele and relative crosses (Zhao et a1., 2005), the sampling error (Appleyard et a1., 2002 ), hybridization (Tonteri, 2005) etc., can cause deviations from Hardy - Weinberg equilibrium in the study using Microsatellite Markers in different species. In this research effective factors on observing deviation of should be evaluated and studied.

## General conclusion:

Results of this study revealed that the gene with more alleles had more heterozygosis. Since the average heterozygosis and inside populations diversity are equivalent and these values with the average effective alleles, identify the mode and how of genetic variation in a population, so it can be concluded that genetic diversity within populations of studied salmon, sea Caspian is high.

The number of observed alleles in the examined *Salmo trutta caspius* populations in this study was lower than the number of alleles have been reported in some studies by other researchers which shows *Salmo trutta caspius* less variation than the elsewhere fish.

Due to the artificial propagation and restoration of salmon Caspian stocks by the Propagation Center of Shahid Bahonar (Kelardasht), and illegal overfishing, lack of

necessary supervision to protect the Sea and rivers, was expected less diversity genetic which this study showed that there is variation genetic within populations of *Salmo trutta caspius* of South shore of the Caspian Sea especially in Gilan province.

Reduce the genetic diversity of salmon Caspian Sea in Mazandaran province is related to the proliferation of fish because the current program of the Iranian Fisheries Organization is rebuilding stocks of salmon that focused mainly on the Cheshmeh Kyleh river but the proliferation of these fish began in early 1360, parent fish were collected from different rivers of Mazandaran and Gilan and fish propagation was performed without regard to the location of their fishing.

This action was mixed salmon populations and consequently had a high genetic diversity and this high genetic diversity could be due to high recombination, mutation or mixing with other populations of *Salmo trutta caspius*. high polymorphisms among samples of Gilan province compared to Mazandaran province due to restructuring annual storage of this type in Mazandaran is showed that salmon stocks in rivers of Gilan in terms of natural propagation, is less influenced by artificial propagation and stores reconstruction programs so the genetic structure of salmon populations in the Caspian Sea in Gilan is partly kept.

All named location in this study show high heterozygosis. Due to the observed number of alleles in the studied population was concluded that the polymorphism and performance of used primers were suitable and can be used with confidence.

This study revealed that microsatellite primers *S.trutta* 58 and *S.trutta* 12 and OmyFgt1TUF can be applied to genetic diversity research within populations of salmon the Caspian Sea and further study is being done on the number of primers.

The Living Bank Caspian salmon was established by using plaque with salmon stores in the Propagation Center Shahid Bahonar and examine the population genetic structure is being done by using micro-satellites and sequencing.

## Acknowledgement:

We are very grateful to our collaborators who were of a great help as project chief manager: Dr.Jalil Zorriehzahra, Head of the Caspian Sea Ecology Research Institute (Sari): Dr. Reza Pourgholam and his colleagues, Mr. F.Laloui, Mr.M.J. Taghavi, Mr. H. Pourgholam, Mr.A.Fahim, Mr.R.Safari and colleagues of fish reproduction: Ms. Najar Lashgari, Mr.

Bahramian , Mr.Rastravan, and Dr.Mohammad Sayad Bourani as Head of Coldwater fishes Research Center (Tonekabon, Iran).
Also we would like to thank the collaboration of Mr. Mostafa Rezvani as head of Breeding and cultivation center of Kelardasht, Mr.D.Bighlarian and Mr.Zaroudi for their assistances on supplying the experimental materials and finally thank the confidence of CASPECO manager Ms. Dr.Farshchi (CEP) and Dr.Abbasali Motalebi as Head of IFRO (Iran).

## 3) References:

1. **Adams, B. K. and Hutchings, A., 2003.** Micro geographic population structure of *Brook charr*: a comparison of microsatellite and mark-recapture data. *Jornal of Fish Biology*, 62: 517-533.

2. **Angers, B., Bernatchez, L., Angers, A and Desgroseilles, L., 1995.** Specific microsatellite loci for *Brook charr* reveal strong population subdivision on a micro geographic scale. *Journal of Fish Biology*, 47: (Supplement A) 177-185.

3. **Appleyard, S. A., Ward, R. D. and Grewe, P. M., 2002.** Genetic stock structure of big eye tuna in the Indian Ocean using mitochondrial DNA and microsatellite. *Journal of Fish Biology*, 60. 767-770.

4. **Bahramian, B. (1995)**. Production report of Breeding and cultivation center of Shahid Bahonar (Kelardasht),Iranian Fisheries Organization Publication, Pages: 19

5. **Banks, M. A., Rashbrook, V. K., Calavetta, M. J., Dean,C. A., Hedgecock, D. ,2000.** Analysis of microsatellite DNA resolves genetic structure and diversity of Chinook salmon.

6. **Bassam , B. J., Caetano – Anolles, G. and Gresshoff, P. M., 1991.** Fast and sensitive silver staining of DNA in polyacrylamide gels. *Analytical biochemistry*, 196: 80-83.

7. **Beacham, T. D. and Dempson, J. B., 1997.** Population structure of Atlantic salamon from the conne River, Newfoundland as determined from microsatellite DNA. *Journal of Fish Biology*, 52: 665-676.

8. **Busack, C. A., and Currens, K. P., 1995.** Genetic risks and hazards in hatchery operations: fundamental concepts and issues. Pages 71-80 in Schramm and Piper (1995).

9. **Cheshmei, M.R. (2006).** Study on Genetic diversity of Pike with Microsatellite method in Gilan region, M.Sc Thesis, Gilan University, pp: 185-196.

10. **Chakmeh doz Ghasemi, F. (2009).** Study on Genetically Differentiation and comparison of Genetic structure of *(Rutilus frisii kutum)* in spring and autumn population with using of (DNA Sequencing) and Microsatellite. M.Sc Thesis, Azad University, (Lahijan Branch), pp: 155-176.

11. **Ivanov, V. P., 2000.** Biological Resources of the Caspian Sea. Astrakhan Kasp NIRKH. Pp. 96.

12. **Miller, W. H., Coley, T. C., Burge, H. L. and Kisanuki, T. T., 1990.** Analysis of salmon and steelhead supplementation: emphasis on unpublished reports and present programs. Part 1 in W. H. Miller, editor. Analysis of salmon and steelhead supplementation, parts 1-3. Bonneville Power Administration, Technical Report 88-100, U. S. Department of energy, Portland, Oregon.

13. **Mullis , K. B., Saiki , R. K., Scharf , S., Faloona, F., Horn , G. T., Erlich, H. A. and Arnheim, N., 1985 .** Enzymate a malification of beta – globin genomic sequences and restriction site analysis for diagnosis of sickle cell anemia. *Science*, 239: 487-491.

14. **Navidi Moghadam Foumani, R. (2005).** Study on Genetically diversity of Caspian salmon (*Salmo trutta caspius*) in Tonekabone population with Microsatellite technique. M.Sc Thesis, Gilan University, Basic Science Faculty, pp: 98-118.

15. **Nei, M., 1972.** Genetic distance between populations. American Naturalist, 106: 283-292.

16. **Nei, M., 1978.** Estimation of average heterozygosity and Genetic distance from a small number of individuals. Genetics, 89: 583-590.

17. **Nickelson, T. E., Solazzi, M. F. and Johnson, S. L., 1986.** Use of hatchery Coho salmon (*Oncorhynchus kisutch*) pre smolts to rebuild wild populations in Oregon coastal streams. *Canadian Journal of Fisheries and Aquatic Sciences*, 43: 2443-2449.

18. **Norris, A.T., Bradley, D.G. and Cunningham, E.P., 1999.** Microsatellite genetic variation between and within farmed and wild Atlantic salmon (*Salmo salar*) populations. Aquaculture 180, 247–264.

19. **Rafiee, A. (2006).** Study on Genetically diversity of Caspian salmon (*Salmo trutta caspius*) M.Sc Thesis, Gilan University, Basic Science Faculty, pp: 85-88.

20. **Rasouli,B. (2005).** Study on environmental factors and quality condition of Shiroud River in maturation of *(Rutilus frisii kutum)*. M.Sc Seminar, Azad University, (Lahijan Branch), pp: 8-21.

21. **Reilly, A. and Ward, D., 1999.** Microsatellite loci to determine population structure of the Patagonian tooth fish *Dissostichus eleginoides*. *Molcular Ecology*, 8: 1753-1755.

22. **Reisenbichler, R. R., and Rubin, S. P., 1999.** Genetic changes from artificial propagation of Pacific salmon affect the productivity and viability of supplemented populations. *ICES Journal of Marin Sciences*, 56: 459-466.

**23. Rezvani Gilkolaei S., 1997**. Molecular population genetic studies of sturgeon species in the south Caspian Sea. Ph. D Thesis, School of Biological Sciences, University of Wales, Swan Sea, 196 P.

**24. Rezvani Gilkolaei S., 1999.** Polymerase Chain Reaction (PCR) and direct sequence of mtDNA from ND5/6 gene region in Persian sturgeon (*Acipenser persicus*) from the southern Caspian Sea. *Iranian Journal of Fisheries Sciences*, Vol. 1, No. 1: 24-34.

25. **Rezvani Gilkolaei S., 2000.** Study of mtDNA variation of Russian sturgeon population from south Caspian Sea using RFLP analysis of PCR amplified ND5/6 gene region. *Iranian Journal of fisheries Science*s, Vol. 2, No. 1: 13-36.

26. **Rico, C., Ibrahim, K. M., Rico, I. and Hewitt, G. M., 1997.** Stock composition in North Atlantic population of whiting using micro satellite markers. *Journal of Fish Biology*, 51: 462-475.

27. **Ryman, N., 1991.** Conservation genetics considerations in fishery management. *Journal of Fish Biology*, 39 (Supplement A): 211-224.

28. **Sajedi, H. (1999)**. Study on Genetically diversity of Rainbow trout *(O.mykiss)* broodstock with using of PCR-RFLP technique on Mitochondria genome. M.Sc

Thesis in Biochemistry course, Tarbiat-e- Modarres University, Basic Science Faculty, pp: 65-78.

29. **Skaala, O., Hoyheim, B., Glover, K. and Dahle, G., 2004**. Microsatellite analysis in domesticated and wild Atlantic salmon (*Salmo salar* L.): allelic diversity and identification individuals. *Aquaculture*, 240: 131-143.

30. **Sunsik, S., Snoj, A. and Dovc, P. 2001.** Microsatellite in gayling (*Thymalus thymalus*): comparison of two geographically remote population from the Danubian and Adriatic river basin in Slovenia. *Molecular Ecol*ogy,8: 1756-1758.

31. **Yoon, M., Sato, S., Azoma, N., Seeb, J. E., Brykov, V., Seeb, L. W., Varnavskaya, N., Wilmot, R. L., Jin, D. H., Urava, S., Urano, A. and Abe, S., 2007.** Congruence of Population Genetic Profiles Obtained from Mitochondrial and Microsatellite DNA Analysis in the Pacific Rim Chum Salmon Populations. North Pacific Anadramous Fish Commission, Technical Report, 7: 121-123.

32. **Yousefian, M. (2011).** Evaluation of Genetic Variation Among Endangered Sea Brown Trout Young Fish for Restocking to Caspian Sea. Advances in Environmental Biology, 5(9): 2769-2775.

33. **Zhao, N. A., Shao, W., Zhu, Z., Brosse, B. and Chang J., 2005.** Microsatellite assessment of Chinese sturgeon (*Acipencer sinensis* Gray) genetic variability. Journal of Applied Ichthyology, Vol. 21, PP. 7-13.

## 8) CFRC Project Budget

### TITLE OF PROJECT

**CONSERVATION AND RESTORATION OF CASPIAN TROUT (*SALMO TRUTTA CASPIUS*) STOCKS IN THE SOUTHERN CASPIAN**


- Total Budget: 1.1 Million USD
- CEP allocated Budget : 250 Thousands USD
- IFRO/CFRC shared Budget: 910000 USD

### Project Budget

| Items | IFRO/CFRC Inputs ($) | Caspeco total inputs ($) | Caspeco inputs under this TOR*($) | Total ($) |
|---|---|---|---|---|
| International Consultants | 0 | 50,000 | 0 | 50,000 |
| National Consultants | 8,000 | 25,000 | 25,000 | 28,000 |
| Training/capacity building | 17,000 | 20,000 | 10,000 | 37,000 |
| Equipment | 47,000 | 110,000 | 40,000 | 157,000 |
| Consumables | 115,000 | 0 | 0 | 15,000 |
| Construction/buildings | 510,000 | 0 | 0 | 510,000 |
| Sub-contracts | 28,000 | 20,000 | 0 | 48,000 |
| Outreach/information Dissemination | 35,000 | 20,000 | 10,000 | 55,000 |
| Misc | 150,000 | 5,000 | 5,000 | 155,000 |
| Total | 910,000 | 250,000 | 90,000 | 1,160,000 |

| No. | **First payment and expenses** Activities | Total costs (USD) |
|---|---|---|
| 1 | Holding 10 separate orientations meeting with participation of government officials including: Director of Fishery Dept., Iranian Environment Protection Organization (IEPO) in Mazandaran and Gilan provinces, Stock enhancement Mangers, Fishery experts, Coldwater Fishes Research Center (CFRC) staff. | 350 |
| 2 | Orientation meeting with local fishery communities and inhabitants in the vicinity of coastline south of the Caspian Sea (8th Aug. 2010). | 2600 |
| 3 | Several visits to the estuaries of Caspian Sea and Shahid Bahonar Salmonids hatchery and rearing Center in kelardasht during in project. | 500 |
| 4 | Costs (travel, petrol, car, etc.) for team traveling in order to meeting coordination in region and other provinces. | 1000 |
| 5 | Training courses entitled "Introduction preliminary software Microsoft Project" to increase efficiency in research projects with emphasis on CEP project in CFRC. | 200 |
| 6 | Purchase two fiberglass tanks (each $9m^{3)}$ to hold wild salmon brood-stock | 2800 |
| 7 | First national meeting on considerations of Caspian trout stocks rehabilitation (10th Nov.2010) | 3350 |
| 8 | Expenses of color brochures publication (1000 Issues) consisted of preparation of scientific materials, designing, publish, laminate and distribution in three provinces. | 700 |
| 9 | Expenses of five different posters about *S. trutta caspius* that published in 5000 issues (1000 copy for each subject) consisted of preparation of scientific materials, designing, publish , laminate and distribution in three provinces. | 1500 |
| 10 | Distribution of 5000 issue of published Posters and 1000 color brochures between three northern provinces and related questionnaires for broadcasting and propagation of *S. trutta caspius* in district. | 5000 |
| Total | | 18000 |

| No. | **Second payment and expenses** Activities | Total costs (USD) |
|---|---|---|
| 1 | Subcontract with International Sturgeon Research Institute (ISRI) – Rasht in order to establish Sperm Bank and Gen Bank | 7000 |
| 2 | Costs to purchase Chemicals and Instruments in order to establish Sperm Bank of Live Broodstocks from different races of *S. trutta caspius* | 8000 |
| 3 | Costs to purchase Chemicals and instruments for molecular genetics examinations in order to establish *S. trutta caspius* Gen Bank | 10000 |
| 4 | Preparation educational leaflets and color brochures in order to distributing in fishery community and inhabitants in the Caspian Sea coastline. | 1500 |
| 5 | Contract to regional Media and TV to introduce the project and preparation project entitled "Documentary film about the cycle life's *S. trutta caspius*". | 2500 |
| 6 | Organizing a technical workshop and training courses in CFRC-Iran for information sharing and field regional program about Caspian trout artificial breeding and Juvenile rearing in Feb. 2011. | 2000 |
| 7 | Costs to purchase 20000 PIT tags and also costs to tagging, Parrs of produced *S. trutta caspius*. | 5000 |
| Total | | 36000 |

### 9) Replication:

It is anticipated that the experiences gained in this project will be suitable for application in anadromous and endangered fish in other marine ecosystems. Many past marine protected areas which are under the influence of over-fishing and the use of inappropriate fishing gear are significant impediments to more sustainable exploitation of fisheries resources. The same geographical and natural disturbances and proposed solution approaches in the mentioned project could be replicated in other fisheries regions. Water pollution, over-fishing, the destructive effects of humans on rivers and dam construction against fish pathway in migration seasons could be replicated in other marine ecosystems. The solution approaches are also under discussion by members of the scientific board of the IFRO, university senior professors, experts of IEPO and IFO as well as some experienced local fishermen and NGO associations. These approaches were designed for implementation in the rehabilitation methods and approving executive instruction needed for *S. trutta caspius* stocks in the Caspian Sea. Many past marine ecosystems established around the world have been promoted in terms of their potential to improve the state of fisheries and their habitats, but have rarely included mechanisms to ensure the effective integration of fisheries considerations into management. In contrast fisheries departments and ministries largely focus on achieving sustainable yields from marine fish stocks. Experiences in our project suggest that cross-sectoral coordination can be achieved through the sustainable fisheries concept that has provided a platform for building partnerships and enhancing communication between the environment, private sector, scientific members and fisheries sectors. A second anticipated challenge in replicating the experience is generating sufficient support at the fishing community level for proposed interventions. At present many small-scale fishing communities and fisheries government managers are involved together in marine fishing in coastal regions. Absence of Marine Protected Areas (MPA) and equated area-based (zoning) approaches to fisheries management destroyed fish marine stocks, therefore multidisciplinary approaches and ultra-sectoral cooperation lead to joint resolution in Caspian salmon stocks rehabilitation.

The latter are often viewed as unacceptable at the fishing community level since they are rarely designated in locations of importance to the life-cycle of important fish species and they neither improve fish stocks, nor the community's income. So increscent of private section and NGO position and involving them to make decisions and executive management could be effective in Conservation and Restoration of Caspian salmon *(Salmo*

*trutta caspius)* stocks. So the mentioned fisheries management measures could also be replicated in other marine ecosystems in the world.

### 10) Significance:

This project, working in a multidisciplinary approach with cross-sectoral coordination, consists of Governmental and private sectors and NGO communities as well as old traditional local fishermen included through its regional centers to increase awareness of the growing risks facing Caspian salmon from local sources, climate changes and the implications in economic and social affairs of the people who's livelihood, food security and fish stock protection depends on them. Through targeted research, this project is using robust science to help measure these risks and better understand Caspian Salmon behavior, migration, changes of the ecosystem, stocks rehabilitation and find ways to eradicate these destructive risks. Through its capacity building, extension and educational program and efforts to link science to management, it is translating these findings into an action agenda for managers and policy makers which can make an improvement in the future of the Caspian Sea salmon stocks.


### 11) Acknowledgements:

I would like to thank to my dear colleagues in IFRO and affiliated Research Centers in North of I.R.Iran (CFRC, ISRI and others) who were of a great assistance in our project Dr.Abbasali Motalebi as Head of IFRO, Dr.Mostafa Sharif Rohani as Deputy Research of IFRO (Iran). Also my dear colleagues in Coldwater Fishes Research Center (Tonekabon) CFRC and Head of the International Sturgeon Research Institute: Dr.Mohammad Pourkazemi, and his colleagues in ISRI. Also Mr. Mostafa Rezvani as Head of Shahid Bahonar Breeding and Cultivation center of Kelardasht, as well as Mr.Mokarami, Mr. Saeidi from Iranian Fisheries Organization (IFO) for their warm assistance.

Also I am very grateful the collaboration of the Mrs. Dr. Farshchi (CaspEco project manager), Mr. Dr Ghaffarzadeh (the ex-manager of CaspEco project), and their kind colleagues in CEP for their kindness and collaboration duration of CFRC project.


**With the sincere and best regards**


**Dr.Jalil Zorriehzahra Ph.D**

**Manager of (CEP) Project in Coldwater Fishes Research Center (CFRC)**

# ANNEXES

# ANNEX (I)

## International Senior Consultancy

| Name | Specialization | Nationality / Institution | Period of service |
|---|---|---|---|
| Prof.Dr. Ian G. Cowx | Applied Fisheries Science | Britain , Director of Hull International Fisheries Institute | 10 days visiting consultancy 2 years contract consultancy |
| Prof. Dr. Patrick Sorgeloos | Live feeds | Belgium , University of Gent | 10 days visiting consultancy 2 years contract consultancy |
| Prof. Dr. Igor Shchelkunov | Aquatic Animal health &Fish Diseases | Russia, Head of All Russian Research Institute of Veterinary, Virology and Microbiology, Pokrov | 10 days visiting consultancy 2 years contract consultancy |
| Prof. Gunnar Kullenberg | Physical Oceanographic & Fisheries | Sweden | 3-5 visiting 2 years contract consultancy |

## National Consultants

| Name | Specialization | Institution |
|---|---|---|
| Dr.Hosein Abdolhay | Aquaculture | Iranian Fisheries Research Organization (IFRO) |
| Dr.Mohamad Pourkazemi | Genetic and Molecular Biology | Sturgeon International Research Institute |
| Dr.Maryam Falahi | Live food and Nutrition | Guilan Fisheries Research Center |
| Dr.Hassan Salehi | Social economic | Iranian Fisheries Research Organization (IFRO) |
| Dr.Mostafa Sharif Rohani | Aquatic Animal Health & Diseases | Iranian Fisheries Research Organization (IFRO) |
| Dr.Aminallah Taghavi | Stock Assessment | Iranian Fisheries Research Organization (IFRO) |
| Dr.Mohammad Gholizadeh | Aquaculture Engineering | Private Section (Pay ab Novin Co.) |
| Dr.Mehdi Soltani | Aquatic Animal Health & Diseases | Tehran University |
| Dr.Bahram Kiabi | Biodiversity | Shahid Beheshti University |

## Key Managers

| Manager | Activity |
|---|---|
| Shahram Abdolmaleki | Collection of Brood-stocks |
| Behrouz Bahramian | Juvenile production |
| Hajat Safikhani | Tagging and tracking |
| Mohammad Reza Mehrabi | Health Monitoring |
| Sohrab Rezvani Ghil Kolaei | Gene Bank of Live Broodstocks |
| Shahrouz Baradaran Noveiri | Sperm Bank creation |

## Operational personnel in CEP project

| Research Center / Activities | Coldwater Fishes Research Center (CFRC) | Sturgeon International Research Institute | Caspian Sea Ecology Research Center (Mazandaran Fisheries research center) | Gilan Fisheries Research Center |
|---|---|---|---|---|
| Collection<br>Brood-stocks catch<br>From significant migratory rivers in region<br>Transport brood-stocks<br>Quarantine operation<br>Head of activity:<br>Shahram Abdolmaleki | M.Rastravan<br>S.Najar Lashghari<br>Hajat Safikhani<br>Maysam Tavoli<br>Nourallah Khodaparast<br>Rahmat Yousefi<br>Abolfath Rezvani | Hamidreza Pourali<br>Mahmoud Shakorian | Mahmoud Ghanei tehrani<br>Hamid Ramazani | Kayvan Abassi<br>Mehdi Moradi |
| Juvenile production:<br>Artificial fertilization<br>Incubation<br>Rearing of juveniles<br>Head of activity:<br>Behroz Bahramian | M.Rastravan<br>S.Najar Lashghari<br>Mansour Zabihi<br>Mostafa Mahdavai | Sohrab Dejandian | Abdolhamid Azari | Mohammad Sayyad Borani<br>Afshin Amiri |
| Tagging and tracking<br>PIT tagging of Brood-stocks<br>Tagging of Juveniles<br>Tracking<br>Head of activity:<br>Hajat Safikhani | Maysam Tavoli<br>Maysam Samadi<br>Nourallah Khodaparast | Behrouz Fadaei<br>Hashem Joushideh | Gholamreza Daryanavard | Reza Nahrvar<br>Kambiz Khedmati |
| Health Monitoring<br>Head of activity:<br>Mohammad Reza Mehrabi | Masoud Haghighi<br>Hosein Asaeian<br>Samaneh Mousavi | Soheil Bazari Moghadam<br>Mehdi Alizadeh | Ali Asghar Saeidi<br>Maryam Ghiasi | Mohaddas Ghasemi<br>Monireh Foeid |

| | | | | |
|---|---|---|---|---|
| | S.Najar Lashkari | | | Babak Ramazani |
| Gene Bank of Live Brood-stocks<br>Head of activity:<br>Sohrab Rezvani | Gh. Lashtou Aghaei<br>S.Najar Lashkari<br>H. Sasani<br>E. Hatamian | Mohammad Pourkazemi<br>Shahrouz Baradaran Noveirri<br>Mohammad Hassanzadeh saber | Mehdi Yousefian<br>Faramarz Laloui | |
| Sperm Bank Creation<br>Head of activity:<br>Shahrouz Baradaran Noveirri | S. Najjar Lashkari,<br>Dr. M.Sayad Bourani,<br>M.Mahdavi,<br>Gh. Lashtu Aghaii,<br>B.Bahramian, | Dr. M.Pourkazemi, | M.Rezvani,<br>S.Zaroudi,<br>D. Bighlarian | |
| Totally personnel | 25 | 11 | 11 | 9 |

## ANNEX (2)

# WORKPLAN

### CONSERVATION AND RESTORATION OF CASPIAN TROUT(*Salmo trutta caspius*) STOCKS IN THE SOUTHERN CASPIAN

| PROJECT WORK PLAN | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **PROJECT ACTIVITIES** | **May 2010** | **Jun 2010** | **Jul 2010** | **Aug 2010** | **Sep 2010** | **Oct 2010** | **Nov 2010** | **Dec 2010** | **Jan 2011** | **Feb 2011** | **Mar 2011** | **Apr 2011** | **May 2011** | **Expenses (CASPECO) (USD)** |
| **Phase 1: Project Start up** | | | | | | | | | | | | | | |
| Project execution team meeting and arrangements in Coldwater Fishes Research Center (CFRC) – Tonekabon | X | | | | | | | | | | | | | 1500 |
| Contract with International Consultants | | X | | | | | | | | | | | | 50000 |
| Contract with National Consultants | | X | | | | | | | | | | | | 25000 |
| Distribution color printed brochures and leaflets between fishery community and inhabitants in the Caspian Sea coastline by emphasize to the vicinity of the Cheshmeh kileh River- Tonekabon | | X | | | | | | | | | | | | 1500 |
| Orientation meeting with fishery communities and inhabitants in the vicinity of South coastline of the Caspian Sea those are relevant to spawning migration routs of Caspian trout and inviting them to participate in the project | | | X | | | X | | | X | | | | | |
| Dissemination of information to the fishery community by printed information to publicize the study at specific intervals | | | X | | X | | X | | X | | X | | X | 4000 |
| Prepare Progress Report for CASPECO | | | | X | | | | | | | | | | 0 |
| **Phase 2:** | | | | | | | | | | | | | | |
| Equipping CFRC Salmonids Hatchery, Laboratories & Fish Tank Vehicles. Procurement of necessary equipment and their transfer to CFRC and setting up Fishing groups to catch Caspian trout live broodstocks from Tonekabon Cheshmeh kileh river and nominated adjacent rivers | | X | X | X | X | | | | | | | | | 110000 |

| | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1st national attachment on Consideration of Caspian trout stocks rehabilitation by CFRC invitation to Iranian Fisheries Organization (IFO), Department of Environment (DOE), Coldwater Fishes Aquaculture Union, representatives of Local Fishermen, NGOs, Municipality, City council , Mayors and Governors of targeted cities of those rivers are involved in the project. | | | | **X** | | | | | | | | | | 3000 |
| Sub-contracts with ISRI, Local unions and third parties | | | | **X** | | | | | | | | | | 25000 |
| Overseas and Local training courses for nominated project staffs | | | | | **X** | **X** | **X** | **X** | **X** | **X** | | | | 20000 |
| **Phase 3:** | **May 2010** | **Jun 2010** | **Jul 2010** | **Aug 2010** | **Sep 2010** | **Oct 2010** | **Nov 2010** | **Dec 2010** | **Jan 2011** | **Feb 2011** | **Mar 2011** | **Apr 2011** | **May 2011** | **Expenses (CASPECO) (USD)** |
| Caught and Collecting Autumn form of Caspian trout live broodstocks from nominated Rivers by assigned team and from fishermen via local subcontracts | | | | | **X** | **X** | **X** | | | | | | | |
| Developing Gene Bank of Live Brood-stocks in CFRC specific constructed cement and earthen ponds | | | | | **X** | **X** | **X** | | | | | | | |
| Developing Sperm Bank from different races via Subcontract with International Sturgeon Research Institute (ISRI) - Rasht | | | | | | **X** | **X** | | | | | | | |
| Caspian trout artificial breeding and Juvenile rearing – CFRC Salmonids Hatchery by local subcontracts | | | | | | **X** | **X** | **X** | | | | | | |
| Caspian trout Juvenile rearing – CFRC Salmonids Hatchery by local subcontracts | | | | | | | | | **X** | **X** | **X** | **X** | **X** | |
| Tagging, Parrs of produced Caspian trout | | | | | | | | | X | X | | | | |
| Prrs & Pre Smolts Caspian trout Releasing Operations | | | | | | | | | | | X | X | | |
| Tracking Caspian trout juveniles in rivers by LR-24 Smith-Root Electroshocker | | | | | | | | | | | | X | X | |
| Prepare Progress Report for CASPECO | | | | | | | | | **X** | | | | **X** | 0 |
| PROJECT ACTIVITIES | **May 2010** | **Jun 2010** | **Jul 2010** | **Aug 2010** | **Sep 2010** | **Oct 2010** | **Nov 2010** | **Dec 2010** | **Jan 2011** | **Feb 2011** | **Mar 2011** | **Apr 2011** | **May 2011** | **Expenses (CASPECO) (USD)** |
| Caspian trout Juvenile rearing – CFRC Salmonids Hatchery by local subcontracts | X | X | X | X | X | X | X | | | | | | | |
| Prrs & Pre Smolts Caspian trout Releasing Operations | | | | | | X | X | | | | | | | |
| Tracking Caspian trout juveniles in rivers by LR-24 Smith-Root Electroshocker | X | X | X | X | X | X | X | X | X | X | | | | |
| Prepare Progress Report for CASPECO | | | | | X | | | | | X | | | | 0 |

| | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Phase 4: | | | | | | | | | | | | | | |
| 1st Regional technical meeting on Caspian trout Conservation. The Project, inviting at least one expert from each of the Caspian littoral countries to exchange views, share lessons learnt and exchange technical views in company with 2nd national attachment on Consideration of Caspian trout stocks rehabilitation by CFRC invitation to Iranian Fisheries Organization (IFO), Department of Environment (DOE), Coldwater Fishes Aquaculture Union , representatives of Local Fishermen, NGOs, Municipality, City council , Mayors and Governors of Coastline nominated cities. | | | | | | | | | | | | x | | 7000 |
| Phase 5: | | | | | | | | | | | | | | |
| Prepare Final Report | | | | | | | | | | | | | x | 2000 |

## 12) References:


1 -Abdoli, A., 2000. Inland Water Fishes of Iran. 1st ed. Iranian Museum of Nature and Wild Life, Tehran.

2 - Abdoli ,A., Niksirat, H. and Patimar, R., 2011. Variation of some biological characteristics of Caspian brown trout in southern Caspian Sea .The Fourth International Symposium on Stock Enhancement and Sea Ranching.April 21st - April 23rd, 2011. Shanghai Ocean University .Shanghai, China .

3 - Aprahamian , M.W., Martin Smith ,K., McGinnity, P., McKelvey, S. and Taylor , J.,2003. Restocking of salmonids—opportunities and limitations . Fisheries Research, Vol. 62 : PP 211–227.

4 - Barannik, V., O. Borysova & F. Stolberg, 2004. The Caspian Sea region: environmental change. Ambio 33: 45–51.

5 - Coad, B.W., 2000. Criteria for assessing the conservation status of taxa (as applied to Iranian freshwater fishes). Biologia, Bratislava, Vol. 55.No. 5: PP. 539–557

6 - Cowx, I.G. (Ed.), 1998. Stocking and Introduction of Fish. Fishing News Books, Oxford.

7 - Iranian Fisheries Organization Yearbook ,2008. Fish and fishery statistics of IRAN. Iranian Fisheries Organization, Tehran .56 P.

8 - Jalali, M. A. & B. M. Amiri, 2009. Threatened fishes of the world: *Salmo trutta caspius* (Kessler, 1877) (Salmoniforms:Salmonidae). Environmental Biology of Fishes 86:375–376.

9 - Kazancheyev, E. N. 1981. Fishes of the Caspian Sea. Food Industry Publication, Moscow. 166 pp (in Russian).

10 - Kiabi, B.H., Abdoli A, and Naderi, M., 1999. Status of fish fauna in the south Caspian basin of Iran. . Journal of Zoology in the Middle East 18: 57–65.

11 - Mojazi, A.B., Bahrekazemi, M., Pousti, I., Vilaki, A. S. (2005) A histological study on the development of the digestive tract of Caspian brown trout, *Salmo trutta caspius* (Kessleri), from hatching to Parr stage. I.F.R.O., 5: 63-84.

12 - Niksirat, H. & A. Abdoli, 2009. On the status of the critically endangered Caspian brown trout, *Salmo trutta caspius*, during recent decades in the southern Caspian Sea basin. Zoology in the Middle East 46: 55–60.

13 - O'connell, M. & J. M. Wright, 1997. Microsatellite DNA in fishes. Reviews in Fish Biology and Fisheries 7: 331–363.

14 - Quillet, E., Faure, A., Chevassus, B., Kreig, F., Harache, Y., Arzel, J., Metailler, R. ,et al., 1992. The potential of trout (*Salmo trutta* L.) for mariculture in temperate waters. Icelandic Agricultural Sciences, 6, 63-76.

15– Sarvi, K., Niksirat, H., Mojazi Amiri, B., Mirtorabi, S.M., Rafiee, G.R. and Bakhtiyari, M., 2006. Cryopreservation of semen from the endangered Caspian brown trout (*Salmo trutta caspius*).Aquaculture 256:564–569

16 - Sedgwick, S. D., 1995. Trout farming handbook. Alden Press,Oxford.

17- Vera, M., Sourinejad, I., Bouza , C., Vilas, R., Pino-Querido, A., Kalbassi , M. R. and Martı´nez, P., 2011. Phylogeography, genetic structure, and conservation of the endangered Caspian brown trout, *Salmo trutta caspius* (Kessler, 1877), from Iran. Hydrobiologia, Vol. 664: PP. 51–67.