Karyotype Analysis in *Schizothorax zarudnyi* from Hamoon Lake

Seyyed Vali Hosseini[1], Mohammadreza Kalbassi[2]
1- Fishery Department, Faculty of Natural Resources and Marine Sciences, Tarbiat Modares University, Iran. Phone: +98-122-6253102 Email:valihosseini814@hotmail.com; 2- Fishery Department, Faculty of Natural Resources and Marine Sciences, Tarbiat Modares University, Iran. Phone: +98-122-6253102 Email: Kalbas_m@modares.ac.ir

Abstract

Karyological characters of *Schizothorax zarudnyi*, in Hamoon lake of Iran were studied by examining metaphase chromosome spreads from the kidney tissues.

The examination of 85 metaphases spread prepared from 20 fingerling specimens indicated that the chromosome numbers of this species was found 2n=96 and the arm number was determined as NF=142. The prepared karyotype of this species was consisted of 9 pairs of metacentric (m), 14 pairs submetacentric (sm) and 25 pairs subtelocentric (st) chromosomes. The chromosome formula can be stated as 2n = 9m + 14sm + 25st.

Karyological parameters shown that centromeric index, arm ratio, relative length and so length variation range of chromosomes of this fish are between 25.00 – 49.24, 1.03 – 3.55, 0.88 – 4.13 and 14.57 – 67.70 respectively and total length of chromosome is 1639.16µm. The largest chromosome in this species is a pair of the submetacentric chromosome. The sex chromosomes were cytologically indistinguishable.

With respect to the number of *S. zarudnyi* chromosomes and resistant of this fish to the environmental conditions, it seems to be tetra ploid origin fish.

Key words: Karyotype, Chromosome, *Schizothorax zarudnyi,* Hamoon lake-Iran.

Introduction

The family Cyprinidae is the richest and most important family of fish, and its members are distributed throughout the world (Al-Sabti, 1991). The vast majority of boned fish belongs to this family in Iran, and these are distributed widely in freshwater resources (Abdoli, 1999). Although this family is represented by approximately 1500 species worldwide (Gül et al., 2004) there are only 38 Geni and over 80 species in Iran (Firouz, 2000).

Systematically, *S. zarudnyi* belongs to teleostei class, cypriniformes order, cyprinidae family and schizothorax genus (Mostajeer and Vossoughi, 1994). The world distribution of *S. zarudnyi* is in semi-temporal freshwater of western Asia (Bianco and Banarescu, 1982). This fish is endemic in Iran and found in Sistan region mainly (Abdoli, 1999).

Since the 1960s, karyological studies in teleost fishes have made noteworthy contributions to increasing knowledge in the fields of genetics, taxonomy and environmental toxicology (Cucchi and Baruffaldi, 1990). The progress in increasing such knowledge has been closely related to the evolution of application methodologies (Rivlin et al., 1985). Studies of the chromosomes of fishes have not been as successful or widespread as in other vertebrate groups. Standard karyotypes are reported for less than 10% of more than 20000 extant species of fishes (Gold et al., 1990).

Cytogenetic studies in fish have not been comprehensive when compared to other vertebrate grops in Iran. In this respect, the most important karyological studies of

Cyprinids fishes in Iran are consist of *Rutilus frisii kutum* (Nowruzfashkhami and Khosroshahi, 1995), *Abramis brama* (Nahavandi et al., 2001), *Ctenopharyngodon idella* (Nowruzfashkhami et al., 2002) and *Hypophthalmichthys molitrix* (Varasteh et al., 2002).

As this species is a good candidate for other genetic investigations such as hybridization and chromosomal manipulation and so due to lack of their chromosomal data, this first report could be provide the detailed information on the chromosome number and karyotype of this species in the world.

Materials and methods

Twenty *S. zarudnyi,* weight 10 ± 1gr, were caught in Hamoon lake (Lat 31° 2′to 30° 54′, Long 30° 54′to 31° 2′) in eastern of Iran. The fishes were transported live to the laboratory, and kept in a well-aerated aquarium at 20-24˚C before analysis.

The stock solution of colchicine was made by dissolving 10 mg colchicine and 100 mg NaCl in 20 ml distilled water. The colchicine was administrated at dose of 25 and 50 µg/gr body weight (BW) and slowly injected into the intraperitoneal muscle. Then, fishes left in aquaria at 20-24˚C for 5-10 hours before sacrificing. After it, the fish were killed and their anterior kidneys removed, suspensioned and placed in hypotonic treatment (0.075M KCl and 1 % Sodium citrate solution) at two different temperature 4˚Cand 25˚C. Lasting time for hypotonisation treatment was 45-60 min (whit distance of 5 min).The swollen cell suspensions were fixed in 3:1 cooled Carnoy's fluid (3 parts methanol and 1 part glacial acetic acid) for 30 min, then, the old fixative was replaced with the fresh Carnoy's. Lasting time for fixation treatment was 60 min.

The slides, previously washed in alcohol and ether and kept at -1˚C, were prepared by letting two drops of the fixing solution containing the cell suspension fall onto the slide in different high (60, 90 and 120cm). Immediately thereafter the fixative was burned off, using the technique developed by Mellman (1965). The slides were stained in series of concentrations of Gimsa Merck solution in distilled water (5, 10 and 15%) and buffered by phosphate (40 mol Na_2HPO_4 and 26.6 mol K H_2PO_4) at PH 6.8 and were assessed at 7, 8, 9 and 10 min exposure times to determine optimum staining conditions. Slides were dipped into distilled water to wash off extra Giemsa solution and then were allowed to airdry at 25 jC for 2–3 h.

Metaphases were examined under a microscope (Leca SER. NO. 990398, Equipted with a green filter and digital camera) with an oil immersion lens at 1000 magnification. The chromosomes at the metaphase were photographed with a digital camera (Sony SSC-DC 58 AP) onto Kodak colour films (ASA 25). In the course of the microscopic examinations the chromosome sets of 80 cells were counted and 10 of the best metaphases were used to measure karyotypes.

For each chromosome, the average lengths of the short and long arms, and the centromeric index (CI, expressed as the ratio of the short arm length to the total length of chromosomes) were calculated. The relative length of each pair was expressed by the percentage of the absolute length of each chromosome pair divided by the sum of the absolute length of total chromosomes. The Excel application paired up all the chromosomes using criteria of maximum resemblance based on the total length and the centromere position. The homologous chromosome pairs were classified according to increasing differences between the homologous chromosomes. The total length of chromosome was computed by summing up the average chromatid lengths of each diploid complement. The length recorded in pixels by the Colour Image Analysis System Video Pro 32 (Leading Edge) was converted into micrometers after the scale factor was calibrated with a stage micrometer.

The chromosome pairs were classified following the recommendations of Macgregor (1993) into metacentric (M), submetacentric (SM), subtelocentric (ST) and telocentric(T), with CI ranges of 46-49, 26-45, 15-26 and less of 15, respectively.

Results

Relatively small and high numbers of chromosomes were observed in *S. zarudnyi*. The scattering of the diploid chromosome number values is shown in Fig. 1. The counts of chromosome ranged from 95 to 101 per metaphases with a mode at 96, representing 92.94% of the metaphases. In 85 metaphases from the anterior kidney cells of 20 *S. zarudnyi* specimens, the diploid chromosome number was 2n = 96 (Fig. 2), which is valid over 90٪ of metaphases cells. Cells lacking a normal number of chromosomes values (2n=95–101) were probably caused by losses during preparation or additions from nearby cells. All chromosomes in the karyotype have a homologous pair. Homologous pairs of chromosomes were arranged in decreasing size and centromeric indexes. The investigation of metaphases showed notable difference in size and type of chromosomes in *S. zarudnyi*. In addition to, the sex chromosomes could not be distinguished in this species.

The representative karyotype for *S. zarudnyi* is shown in Fig. 3. The karyotype of *S. zarudnyi* has 9 pairs metacentric, 14 pairs submetacentric and 25 pairs acro-telocentric chromosomes. The number of chromosome arms was determined NF=142 and chromosome formula can be expressed as 2n=9m + 14sm + 25a-t. The morphological and numerical data are summarized in Table 1. Other data represented in Table 2. According to this table, centromeric index, arm ratio, relative length and length variation range of chromosomes are between 25.00 – 49.24, 1.03 – 3.55, 0.88 – 4.13 and 14.57 – 67.70 respectively. Total length chromosomes was 1639.16µm. The largest chromosome is a pair of submetacentric chromosome. The ideogram of the *S. zarudnyi* was made on the basis of the karyotype (Fig. 4).

In this study, the optimum colchicine concentration for *S. zarudnyi* was determined to be 50 µg/gr BW of colchicine solution for five hours. This concentration have effectively arrested dividing cells in metaphase. In addition to, the best chromosomal spread quality (well-spread metaphase) were obtained from treatment of cells with 1% Sodium citrate solution at $4^{o}C$ for 45min and high of dropping in 120cm.

The other hypotonic solution tested, 0.075M KCl, did not result in many scorable metaphases.

Discussion

Several techniques have been developed to examine chromosomes in tissues of adult fish. These include squashed (Ojima et al., 1963; Roberts, 1967), blood leucocyte culture (Barker, 1972; Hartley and Horne, 1985) and cell suspensions from tissues sach as gill, kidney and intestine(Gold, 1974; Klingerman and Bloom, 1977), cornea (Drewery, 1964) and scales (Denton and Howell, 1969). Each of these procedures was optimized to obtain large numbers of well-spread metaphases and was used regularly for karyotypeic analysis that in present lecture we utilized from anterior kidney.

Karyological study of teleost fishes presents technical difficulties which are not encountered in the study of other vertebrates, and these difficulties are due to the small size and high number of chromosomes (Cucchi and Baruffaldi, 1990). Different techniques are presently being used to perform such studies: direct, *in vivo*; and indirect, *in vitro*. With those forms employing direct techniques, the preparation of slides for optical microscopy is quite easy.

Karyological study has been in different steps. Each of the steps involved in the preparation of tissues and slides for cytogenetic analysis was important in attaining large number of well-spread metaphases. The first step in the procedure was treatment of the cells with colchicine, which arrests cell division at metaphase (Baski and Means, 1988). High concentration and long period of colchicine treatment effect on chromosome, causes to aggregate and smallish of chromosome and their arms, so it is difficult to identify short arm of acrocentric chromosomes and other chromosomes. This study suggests that colchicines concentrations of 50 µg/gr BW can effectively arrest dividing cells in metaphase in kidney tissues. But the maintenance periods may vary according to species.

Type of hypotonic treatment and the length of exposure to the tissue affect on the degree of chromosome spreading. In this study, 0.075M KCl hypotonic treatment was ineffective in obtaining well-spread metaphases. Although condensed chromosomes could be observed, they were often seen inside an intact cell or only slightly spread. Fixative treatment was not found to be as important as hypotonic treatment in obtaining well-spread metaphases.

The main difficulty in working with fish chromosomes is in obtaining high quality metaphase spreads. A few studies have used fish standard karyotypes to examine taxonomic or systematic problems (Bolla, 1987). The major difficulty encountered is the morphological variation existing even between homologous chromosomes in the same nucleus (Al-Sabti, 1991 and Levan et al., 1964). Sometimes it could happen that some chromosomes are more contracted than others, so chromosome measurements are very small chromosomes compared to those of man and mammals. Another problem is that fish karyotypes are not identical, as in human being or other animal species, so we canned have a standard karyotype for fish because not only are there differences between species, but polymorphism often occurs within the same fish species (Al-Sabti, 1991).

Several incomplete metaphases were encountered in the preparation, and these probably resulted from hypotonic over treatment (Nanda et al., 1995). The majority of authors classify uni-armed and bi-armed chromosomes according to the guidelines of Macgregor (1993). Where differences in the number of chromosome arms was seen, which, this is usually the result of a difference in the scoring of subtelocentric chromosomes by different authors (Philips and Rab, 2001).

The majority of cyprinid species have 2n = 50 chromosome (Al-Sabti, 1985), while *Cyprinus carpio* has 2n = 98-100 (Demirok and Ünlü, 2001) and polyploidy Barbus species from southern Africa has 2n = 148 or 150 chromosome (Oellerman & Skelton, 1990), and fishes which have several chromosome series(2n > 50) are called polyploids. The role of polyploidy in evaluation and survive of fishes is very important because it provide from natural selection pressure (Oellerman and Skelton, 1990). Khuda et al., in 1982, noticed that *S. nigar* was caught in India is a polyploid fish. In other species of cyprinids such as *Tor putitora, Tor khudree* and *Tor tor* polyploidy was reported, too (Anonym, 1982). So, with respect to the number of *S. zarudnyi* chromosomes and their resistant to the environmental conditions, it seems to be polyploidian fish.

Until now, karyotype of some member of Schizothorax genus was determined like as *S. richardsonii* (2n=98, NF=154, 2n=16m+40t+42a) and *S. kumaonensis* (2n=98, NF=126, 2n=18m+10t+70a) (Lakara et al., 1997) but there isn't any report about *S. zarudnyi* and our study is the first report of the karyotype of *S. zarudnyi.*

The karyotype analysis is a key step toward the stock improvement by polyploidy manipulation, hybridisation and related genetic engineering (Tan et al., 2004).

Therefor like to other animals, comprehensive genetic researches will need for this fish.

This paper is the first to provide the detailed information on the chromosome number and karyotype of *S. zarudnyi*.

Reference

Abdoli A (1999) The inland water fishes of Iran. Natural and wild life museum of Iran. 378

Al-Sabti k (1985) The karyotype of *Cyprinus carpio* and *Leuciscus cephalus* *Cytobios* 47: 19-25

Al-Sabti k (1991) Handbook of Genotoxic effects and fish chromosome. *Ljubljana*, 97

Anonym (1982)

Baksi SM, Means JC (1988) Preparation of chromosomes from early stages of fish for cytogenetic analysis. *J. Fish Biol.* 32: 321-325

Barker CJ (1972) A new method for the display of chromosomes of plaice, *Pleuronectes platessa,* and other marine species. *Copeia* 1972, 365-368

Bianco PG, Banarescu P, (1982) A contirbution to the knowledge of the cyprinidae of Iran (Pisces, Cypriniformes). *Cybium* 6(2), 75-96

Bolla S (1987) Cytogenetic studies in Atlantic salmon and rainbow trout embryos. *Hereditas* 106: 11-17

Cucchi C, Baruffaldi A 1990 Anew method for karyological studies in teleost fishes. *J. Fish Biol.* 37: 71-75

Demirok NK, Ünlü E (2001) Karyotype of cyprinid fish *capoeta trutta* and *capoeta capoeta umbla*(Cyprinidae) from the Tigris River .Turk. J. Zool. 25, 389-393

Denton TE, Howell WM (1969) A technique for obtaining chromosomes from the seale epithelium of teleost fishes. *Copeia*, 392-394

Drewry G (1964) Chromosom number bull. Thx. Mem. Mus. 5-72

Firouz E (2000) A guide to the fauna of Iran. Iran University Press. Tehran. 491.

Gold JR (1974) A fast and easy method for chromosome karyotyping in adult teleosts. *Progve Fish Cult*. 36: 169-171

Gold JR, Li YC, Shipley NS, Power PK (1990) Improved methods for working with fish chromosomes with a review of metaphase chromosome banding. *J. Fish Biol.* 37: 563-575

Gül S, Colak A, Kaloğlu B (2004) Karyotype analysis in *Alburnus heckeli* (Battalgil, 1943) from lake Hazer. *Turk. J. Vet. Anim. Sci*. 28: 309-314

Hartley SE, Horne MT (1985) Cytogenetic techniques in fish genetics. *J. Fish Biol.* 26: 575-582

Khuda – Bukhsh AR, Nayak K (1982) Karyomorphological studied in two species of hillstream fishes from Kashmir, India. Chromosome. *Inf. Serv*. 33: 12-14.

Klingerman AD, Bloom SE (1977) Rapid chromosome preparation from solid tissues of fish. *J. Fish. Res. Bd Can*. 34: 266-269

Lakara WS, John G, Barat A (1997) Cytogenetic studies on endangered and threatened fishes 2 karyotypes of two species of Snow-trout, *schizothorax richardsonii* (Gray) and *S. kumaonensis* (Menon). Proc Natl. Acad. Sci. India. *B Biol. Sci*. 67(1): 79-81

Levan A., Fredge K, Sandberg AA (1964) Nomenclature for centrometric position on chromosome. *Hereditas* 52: 201-202

Macgregor UC (1993) Chromosome preparation and analysis. Chapter 6, 177-186.

Mellman W (1965) Human chromosome methodology. New York: Achademic Press.

Mostajeer B, Vossoughi G (1994) Freshwater fishes. Tehran University Publications, 317

Nahavandi R, Amini F, Rezvani S (2001) Karyology of Abramis brama in southern waters of Caspian sea. *Iranian Journal of Fisheries Sciences.* 10(3): 89-100

Nanda L, Schartl M, Feichtinger W, Schlupp I, Parzefall J, Schmid M (1995) Chromosomal evidence for laboratory synthesis of a triploid hybrid between the gynogenetic teleost *Poecilia formosa* and host species. *J. Fish. Biol.* 47: 221-227

Nowruzfashkhami MR, Khosroshahi M (1995) Karyology of the Caspian sea kutum roach by with blood cells culture. *Iranian Journal of Fisheries*

Sciences. 4(1): 64-71
Nowruzfashkhami MR, Pourkazemi M, Kalbassi MR (2002) *Iranian Journal of Fisheries Sciences.* 11(3): 137-144.
Oellerman LK, Skeleton PH (1990) Hexaploidy in yellowfish species (Barbus, Pisces, Cyprinidae) from Southern Africa. *J. Fish. Biol.* 37:105-115
Ojima Y, Makei K, Takayama S, Nogusa S (1963) A cytotaxonomic study of the Salmonidae. *Nucleus (Calcutta)* 6: 91-98
Rivlin K, Rachlin JW, Dale G, (1985) A simple method for the preparation of fish chromosomes applicable to field work teaching and banding. *J. Fish Biol*. 26: 267-272.
Roberts EL (1967) Chromosome cytology of the Osteichthyes. *Progve Fish Cult.* 29: 75-83.
Varasteh A, Hossienzadeh MM, Pourkazemi M, (2002) Karyotyping and number of chromosomes of silver carp(*Hypophtalmichthys molitrix*). *Iranian Journal of Fisheries Sciences*. 11(1): 107-114.

Total length of haploid chromosome (µm)	**Total length lomg arm (µm)**	**Total longth short arm (µm)**	**2n**	**NF***
1638/58	1226/27	412/31	96	142

***** Arm number

Table 1. The morphological and numerical data are summarized in *S. zarudnyi*.

Chromosome pair no.	Total length (µm)	Long arm (µm)	Short arm (µm)	Relative length (%)	Centromeric index	Arm ratio	Classification
1	61/26	31/83	29/43	3/73	48/02	1/08	Metacentric
2	52/76	28/86	23/90	3/21	45/29	1/28	Metacentric
3	46/69	24/76	21/93	2/84	46/94	1/13	Metacentric
4	43/34	23/32	20/02	2/64	46/19	1/16	Metacentric
5	43/01	22/92	20/09	2/62	46/95	1/13	Metacentric
6	41/16	20/93	20/23	2/51	49/24	1/03	Metacentric
7	41/02	22/00	19/02	2/50	46/48	1/15	Metacentric
8	40/95	22/00	18/95	5/49	46/40	1/16	Metacentric
9	36/95	19/85	17/10	2/25	46/27	1/16	Metacentric
10	67/70	38/20	29/50	4/13	46/48	1/34	Submetacentric
11	57/16	34/24	22/92	3/48	40/01	1/49	Submetacentric
12	55/75	37/50	18/25	3/4	32/73	2/05	Submetacentric
13	53/48	32/26	21/22	3/26	39/68	1/52	Submetacentric
14	52/77	29/43	23/34	3/21	44/26	1/26	Submetacentric
15	41/73	23/34	18/39	2/54	43/83	1/03	Submetacentric
16	37/91	25/18	12/73	2/31	33/48	1/99	Submetacentric
17	37/37	26/88	10/49	2/27	28/68	3/55	Submetacentric
18	37/35	22/07	15/28	2/27	41/70	1/47	Submetacentric
19	37/18	26/99	10/19	2/26	27/43	2/64	Submetacentric
20	36/22	20/31	15/91	2/20	43/26	1/50	Submetacentric
21	35/64	26/17	9/47	2/17	26/56	2/49	Submetacentric
22	28/20	21/15	7/05	1/72	25/00	3/00	Submetacentric
23	27/20	20/30	6/90	1/65	25/36	2/94	Submetacentric
24	33/95	33/95	0/00	2/07	0/00	∞	Subtelocentric
25	32/00	32/00	0/00	1/95	0/00	∞	Subtelocentric
26	30/24	30/24	0/00	1/85	0/00	∞	Subtelocentric
27	28/69	28/69	0/00	1/75	0/00	∞	Subtelocentric

28	28/58	28/58	0/00	1/74	0/00	∞	Subtelocentric
29	28/30	28/30	0/00	1/72	0/00	∞	Subtelocentric
30	28/02	28/02	0/00	1/70	0/00	∞	Subtelocentric
31	28/01	28/01	0/00	1/70	0/00	∞	Subtelocentric
32	27/16	27/16	0/00	1/66	0/00	∞	Subtelocentric
33	26/60	26/60	0/00	1/62	0/00	∞	Subtelocentric
34	25/53	25/53	0/00	1/55	0/00	∞	Subtelocentric
35	25/48	25/48	0/00	1/55	0/00	∞	Subtelocentric
36	25/25	25/25	0/00	1/54	0/00	∞	Subtelocentric
37	24/81	24/81	0/00	1/51	0/00	∞	Subtelocentric
38	24/76	24/76	0/00	1/51	0/00	∞	Subtelocentric
39	24/37	24/37	0/00	1/48	0/00	∞	Subtelocentric
40	24/05	24/05	0/00	1/46	0/00	∞	Subtelocentric
41	23/75	23/75	0/00	1/44	0/00	∞	Subtelocentric
42	23/91	23/91	0/00	1/45	0/00	∞	Subtelocentric
43	22/64	22/64	0/00	1/38	0/00	∞	Subtelocentric
44	21/93	21/93	0/00	1/33	0/00	∞	Subtelocentric
45	21/01	21/01	0/00	1/28	0/00	∞	Subtelocentric
46	16/29	16/29	0/00	0/99	0/00	∞	Subtelocentric
47	15/70	15/70	0/00	0/99	0/00	∞	Subtelocentric
48	14/57	14/57	0/00	0/88	0/00	∞	Subtelocentric

Table 2. Numeral characteristics of the karyotype of *S. zarudnyi* showing the mean values of measurements from ten best mitotic metaphases.

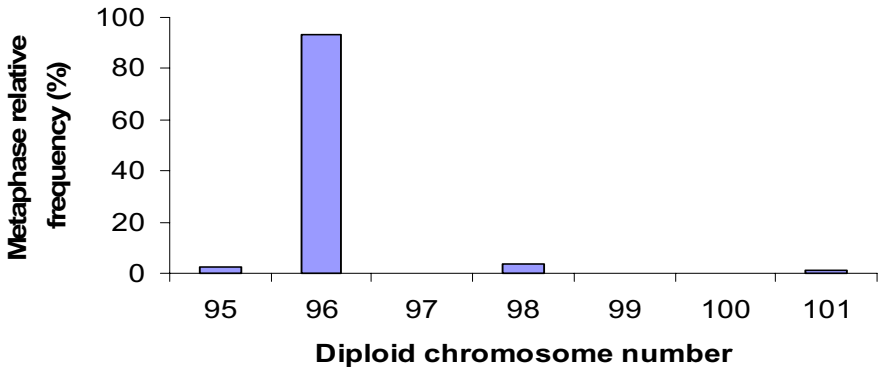


Fig. 1. Distribution of chromosome number recorded in 85 diploid metaphases of *S. zarudnyi.*

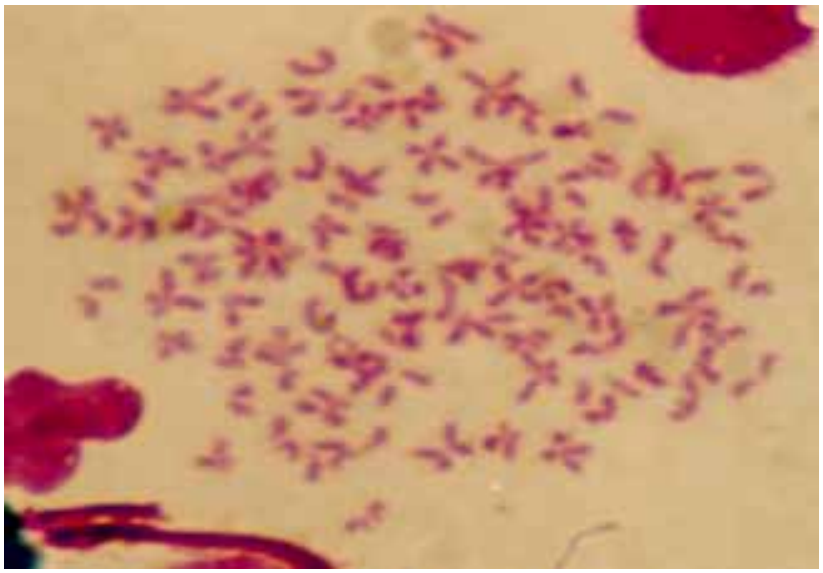


Fig. 2. Metaphase spread from kidney tissue of *S. zarudnyi* from Hamoon lake ×1000, 2n=96.

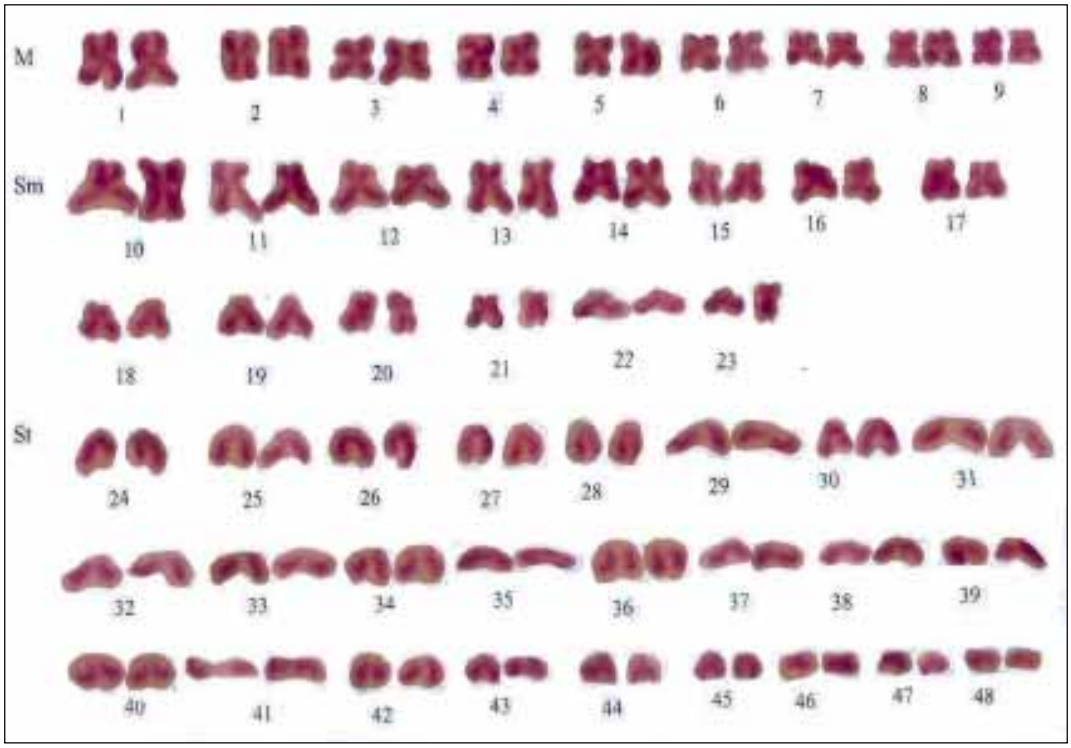


Fig. 3. Karyotype of S. zarudnyi from Hamoon lake, 2n = 96

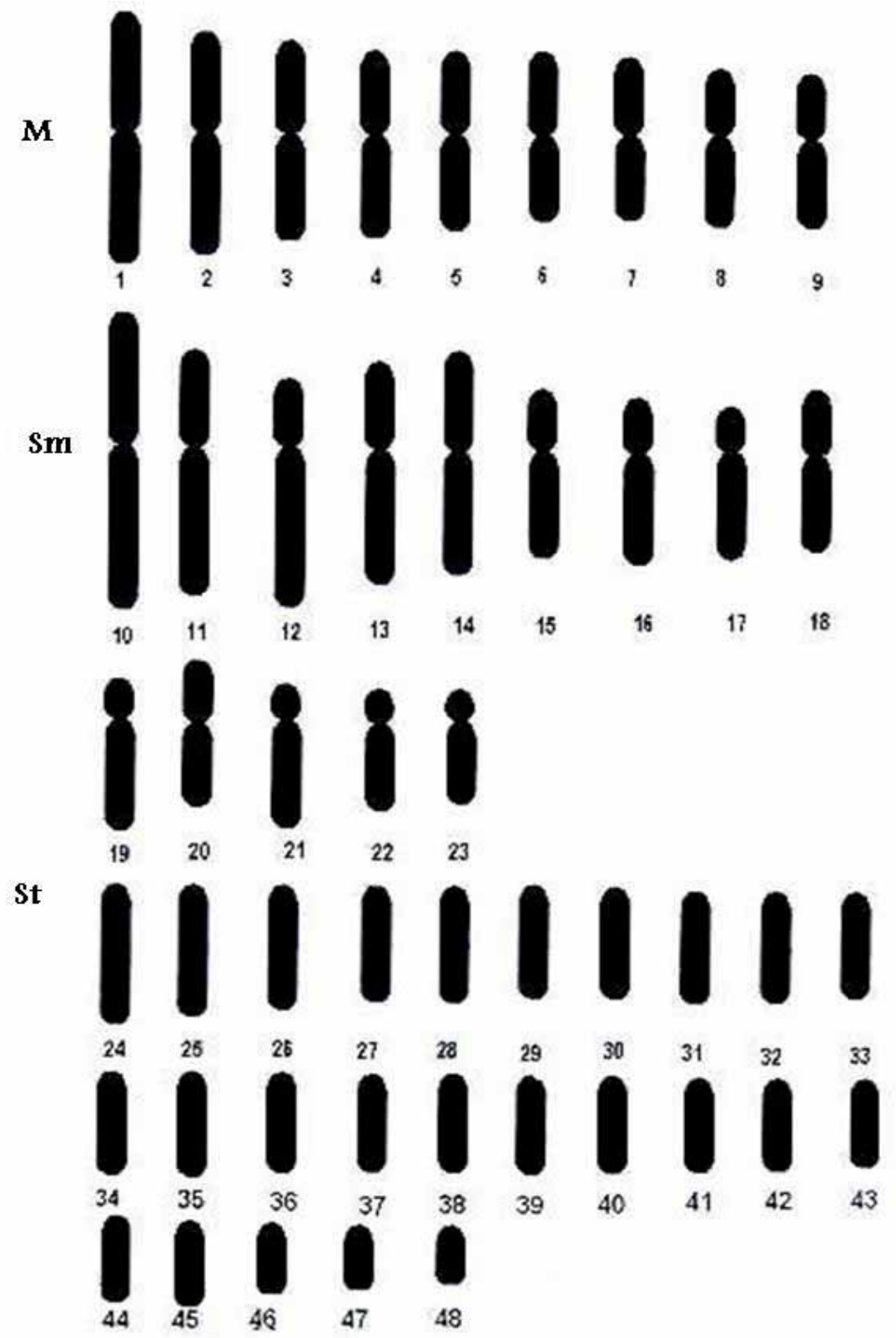


Fig. 4. Idiogram of *S.zarudnyi* from Hamon lake, n = 48

Study on Biology of *Pontogammarus maeoticus* in Caspian Sea southern shores

Lobat Jaber
[1] Dep. of Environmental Sciences, University of Allameh Mohaddes Noori, Noor, Iran ;Tel.: +98-122-6224499, lobatjaber@yahoo.com

Abstract:
Monthly sampling was carried out in southern littoral region of Caspian Sea to study biology of *Pontogammarus maeoticus*. Percentage of coupled population, length of the coupled females and males and number of eggs and babies which were carried by females were determined. Observations showed no resting period in reproductivity of this species. Number of coupled animal populations was determined with a maximum on August and a minimum on January. The shortest length of the animals was measured on December and the highest on February. Comparison between coupled males and females showed that females are longer than their paired males. Counted number of eggs indicated a decrease on June and an increase on April. However, number of babies was counted with a less number on October and a high number on November. A positive correlation was found between length of females and their eggs numbers. However no correlation was distinguished between their lengths and numbers of babies. SEM images showed that females have hollows between segments 1-2 and 5-6 on their dorsal sides. However males have no hollows on their dorsal sides. An aquarium study showed also a good adaptability of these animals to a fresh water condition. Feeding of themselves and dead algae and animals were observed as their nutritional behavior.

Keywords: Pontogammarus maeoticus, Caspian Sea, biology, sex discrimination, length, number of eggs and babies

Introduction

Identification of marine sources has a major importance; especially, fishes and other sources which are used for our nutrition even indirectly. For example, amphipoda are very important due to their major roles in providing bioresources for fish feeding. Unfortunately, few studies have been reported about amphipoda of Caspian Sea. However, amphipoda from other parts of the world have been studies intensively.
Alouf (1980) studied ecology, biology and reproduction of the amphipoda of Assai river in Lebanon. A study on biology of amphipoda showed that organic pollution affects their life cycles and delays their maturity and reproduction.
Among the studies, no investigation has been carried out about the reproduction and morphology of male and female animals.
Investigations on morphology of the males and females, reproduction, number of carried eggs and babies by females during a year were aimed in this study. Identification of amphipoda in littoral region was another approach in this study.

Material and Methods

Monthly samplings of *Pontogammarus maeoticus* were carried out in littoral region of Caspian Sea (52º 5' E, 36º 35' N) during a year. Number of coupled and single animals and total number of animals were determined during every sampling term. Some coupled and single animals were fixed in Ethanol 70% to measure their lengths. At least 60 couples were used for this purpose.

Morphology of coupled animals was studied under Scanning Electron Microscope (SEM) to identify their sex. Afterwards, a stereomicroscope was used for this purpose.
Coupled animal population was determined based on the total sampled animals. According to the identified patterns of the females, single female animals were identified separately and their eggs and babies were determined under a stereomicroscope.
Length of coupled animals (males and females) was measured by a ruler in millimeter. Study on the variation of animal length was carried out by measuring the whole sampled animals during every sampling term.
Difference between the lengths of the males and females was analyzed statistically by using a Paired Sample t-test with a confidence level of above 95%.
Correlation between the lengths of the females and numbers of their eggs and babies were analyzed based on the obtained data.
Some animals were caught and transferred into an aquarium filled with fresh tap water to study their behavior. Bottom part of aquarium was filled with few centimeters by sea sands. Their nutritional behavior and adaptability were studied by feeding them with rice, potato and algae as organic sources.

Results and discussion

Sex determination- Study on the morphology of males and females by a Scanning Electron Microscope and a stereomicroscope showed that they are morphologically very similar to each other. However, there are prominent differences on their dorsal sides. Microscopic studies showed that there are two *C-shaped hollows* on female's dorsal. The first hollow is located between segments number 1 and 2, and the second one is located on between segments number 5 and 6. These hollows are specified locations for connecting of male's gonadopodes during coupling period. It indicates that fertilization in *Pontogammarus maeoticus* should be an internal type.

Microscopic observations on abdominal side of females showed that they keep their eggs and babies between their coxal plates.

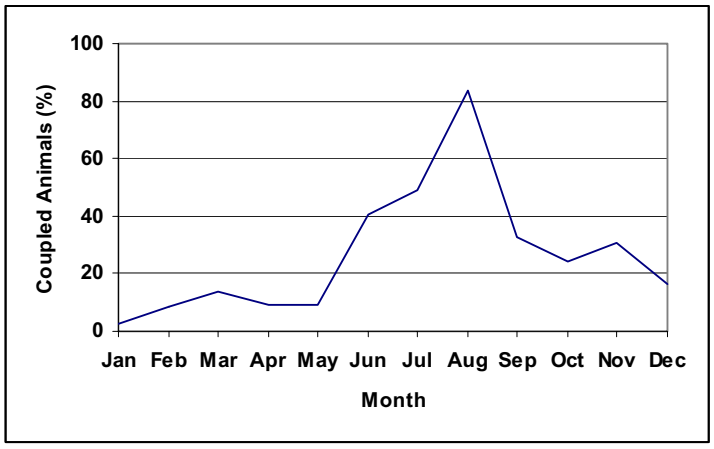


Fig. 1- Variation of coupled animal population during a year

Coupled animals population- Variation of coupled animal's population is shown in Fig. 1. It was revealed that the highest percentage of paired animals (83.64%) occurred in August and the lowest percentage of pairing (2.76%) was in January. Analyses showed that average coupling of animals was determined 26.9%.

It seems that climate has the major influence on their couplings. Stable water and warm weather during summer provide them a good opportunity to be paired. However, variation in climate and unstable water during winter time changes rapidly their habitat and does not permit them to be coupled easily.

Animal length- Comparison between paired male and female animal lengths showed that males (12.97mm) are slightly longer than females (12.32mm) (Fig. 2).

Study on variation of animal length during a year showed that the animals had the highest lengths (13.75mm) in February and their shortest lengths (7.23mm) were measured in December (Fig. 3). Measurements revealed that the average length of *Pontogammarus maeoticus* is 11.87mm.

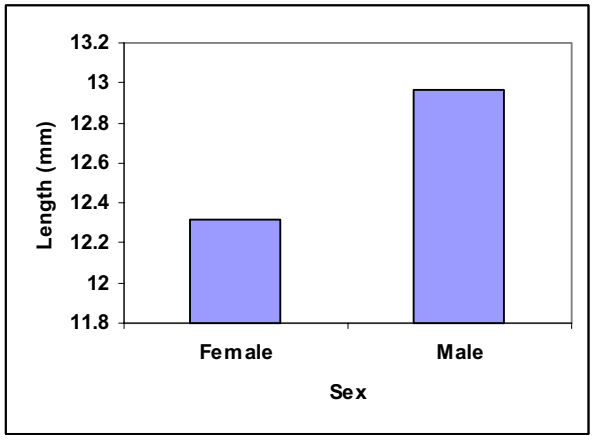


Fig. 2- Length of coupled animals

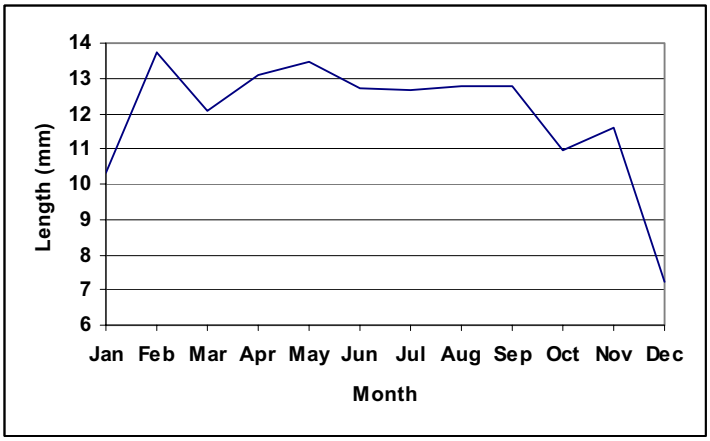


Fig. 3- Animal length variation during a year

Egg numbers per females- Study on the egg numbers per females showed that each female carried approximately 12.86 eggs. Number of the eggs which were carried during a year was varied. The highest number of the eggs per females (19.167) was determined in April and the lowest number (0) was determined in June (Fig. 4).

Study on the correlation between length of females and their egg numbers showed a positive relationship (Eq. 1). It was revealed that bigger females carried higher numbers of eggs.

Eq. 1 $Y= -4.13 + 1.28 X$

Where, *Y* indicates egg number and *X* indicate female length.

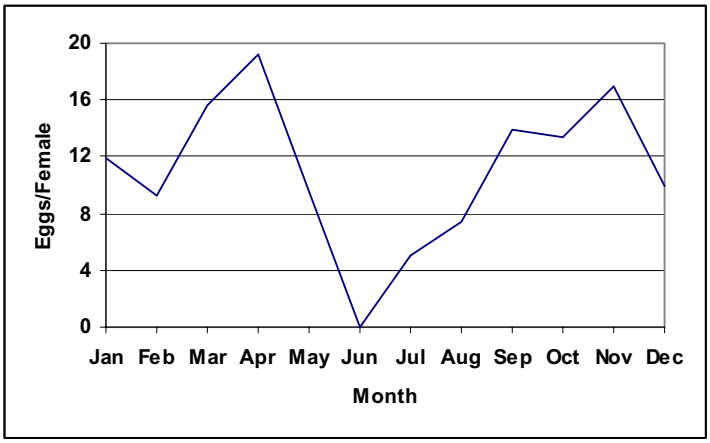


Fig. 4- Number of eggs per females

Number of babies per females- Study on the variation of carried babies by females showed that the highest number of babies was carried in November (10 babies per female) and the lowest number (4.7 babies per female) was carried in October (Fig. 5). It was determined that each female carried approximately 5.88 babies during a year.

Study on the correlation between length of females and their babies showed no relationships.

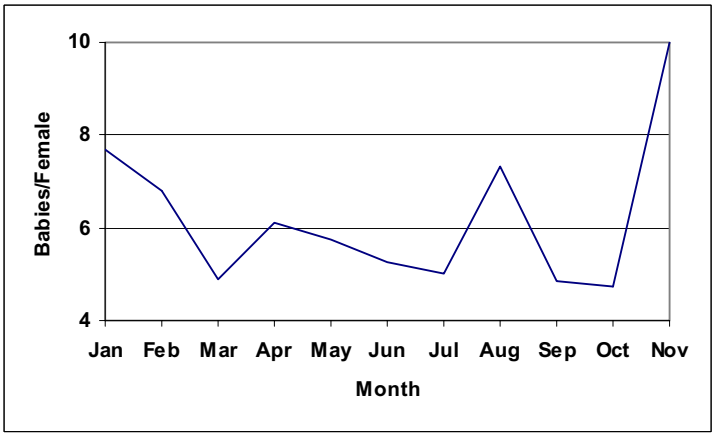


Fig. 5- Number of babies per females

Aquarium observation- Study of animal behavior in an aquarium showed that *Pontogammarus maeoticus* has a good adaptability to brackish water. Feeding with rice, potato and algae showed that this animal prefers algae. During the study, it was observed that the animals eat dead animals that were felled down. Using light source showed a photophobia in this animal. When they were exposed to a light source, they hide themselves under sea sands. After removing the light, they come out and continue their nutrition and swimming.

Conclusion

Study on the variation of the coupled animal population indicates a close relationship between their population and climate changes. Concerning the climate variation, coupled population increases since spring toward mid summer due to increase in temperature and then it decreases during autumn and winter due to decreased temperature and instability of sea water in winter period.

Study on the length variation showed that since season till autumn, the length of animals decrease and then toward winter it increases. It might be related to the increase in baby's population during the year.

A positive correlation between length of females and their egg numbers indicates that bigger animals carry more eggs than smaller animals.

Comparison between egg and baby numbers shows that females carry more eggs during spring and the eggs become hatched during summer. For this reason they have higher number of babies during summer. Also during the beginning of autumn number of the eggs raises and then toward the end of autumn and beginning of winter number of their babies become increased.

Concerning the results, it can be concluded that couplings, carrying eggs and babies are their reproductive behavior and there is no resting time in their reproduction during a year; although they vary during the year

Stock *et. al.* (personal communicatons) reported the length of *Pontogammarus sp.* is 12mm. This study indicates similar results. Alouf (1986) reported that males of *G. laticoxalis laticoxalis* are longer than females with lengths of 13mm and 11mm respectively. Kostalos (1979) also showed that males are longer than females.

Alouf (1986) reported no resting time in the reproduction of *Gammarus laticoxalis*. Also Alouf (1980) showed that there is no resting time the reproduction of river gammaruses (*G. syriacus, G. laticoxalis, G. oronticus*). Studies of Alouf (1979) and Steel & Steel (1972, 1973) showed that temperature had a major influence on population and reproduction of gammarus.

Kostalos (1979) showed female *G. minus* followed its reproduction throughout a year with no resting period. Also Halligan and Eaton (1978) reported that *G. pulex* has a higher reproduction during summer. Moor *et. al.* (1979) reported a higher reproduction in *G. salinus* during May.

References

Alouf N.J.; 1986: Biologie de *Gammarus laticoxalis laticoxalis* dans riviere du Liban; Hydrologia 133: 45-57.

Alouf N.J.; 1980: Ecologie, Biologie et cycle de reporductive des *Gammarus* du' Assai (=Oronte, Liban) (Crustaces, Amphipoda); Annl. Limno 16(2): 119-134.

Halligan B.J. & J.G. Eaton; 1978: Survival and reproduction of *Gammarus lacustris* and *G. pseudolimnaeus* under two experimental conditions; The Progressive Fish Culturist; Vol. 40(2):59-62.

Kostalos M.S.; 1979: Life history and ecology of *Gammarus minus* Say (Amphipoda, *Gammarus*); Crustaceana; 37(2): 113-122.

Moore J.W., I.A. Moore & P.N. Claridge; 1979: Seasonal changes in density, composition and reproductive biology of crustacean populations in the Severn Estuary; Crustaceana; 36(2): 113-122.

Stock J.H., A.R. Mirzajani, B. Kiabi & S. Naderi; personal communication: Limnic and brackish water Amphipoda (Crustacea) from Iran; Amsterdam Zoological Museum.

Taste preferences in fish and prospects for application of taste stimulants in aquaculture

Alexander Kasumyan

Department of Ichthyology, Moscow State University, 119899 Moscow, Russia. Phone: +007-095-9392725
Email:alex_kasumyan@mail.ru

Abstract:
Systematic investigations of fish taste preferences have only been performed during the last 10-15 years. Searching fish taste stimulants it was shown that many of free amino acids, betaine, nucleotides, nucleosides, amines, organic acids, salts and sugars had the same effect on feeding intake as a natural food extract. It was found that fish taste preferences are highly species specific and the differences among fish species are apparent when comparing the width and composition of spectra for both stimulants and deterrents. There is strong similarity in taste preferences between geographically isolated fish populations of the same species and there is similarity in taste preferences in males and females although taste preferences at the individual level may vary dramatically among conspecifics. Taste responses are more stable and invariable for highly palatable substances than for substances with a low level of palatability. There is a correspondence between oral and extra oral taste preferences in the same fish species; however, there is no correlation between smell and taste preferences. Taste preferences in fish show low plasticity (in relation to the diet), appear to be determined genetically and seem to be patroclinous. Fish feeding motivation and various environmental factors like water temperature and pollutants such as heavy metals and low pH water may shift fish taste preferences.
The growing world fish aquaculture demands sources for less expensive food for the fish. The use of additives that improve the palatability of artificial food for fish is a probable application. The eventual incorporation of feeding stimulants into diets consisting of cheap and normally non-palatable protein sources may be of practical significance to the fish farming industry. It will minimize wastage and the potentially deleterious effects of waste food on water quality. Introducing taste stimulants into modern fish feeds improved the feed intake and significantly increased the *ad libitum* metabolizable energy of the diets, which resulted in an increase in fish growth. If fish are fed to satiation with a diet of poor palatability and then received a more palatable diet, they will begin eating again until they reach a new satiation level. It has been shown that artificial feed with good chemosensory characteristics are digested by fish more effectively. Another possible application of feeding stimulants may be to mask deterrent feed ingredients like unpalatable plant proteins used as substitutes for fishmeal protein and certain antibiotics. Supplement of feeding stimulants might mask such ingredients and improve of feed intake. The palatability of artificial feed might also be raised by removing the components with bad taste or by replacing those components with equivalents properties, which do not provoke negative taste responses.

Keywords: aquaculture, chemoreception, feeding behavior, fish, taste stimulants.

Introduction

Feeding is an important life function and is a result of processes that are associated with searching, aiming, accepting, seizing, oral processing and evaluation of the quality of food objects. Swallowing, digestion, absorption and assimilation follow these processes. The

successes of these tasks, directly related to the satisfaction of energy requirements, is responsible for the rate of growth, maturation and fecundity of fishes, etc. Many sensory systems contribute to fish feeding behaviour, but their role and significance may profoundly differ at different phases of feeding behaviour. The gustatory sensory system provides the final evaluation in the feeding process. It is well known that the taste properties of feeds or baits have a dramatic influence on fish food consumption, growth rate and fishing success (Mackie, Mitchell, 1985; Takeda, Takii, 1992; Kasumyan, 1997).

Studies of the gustatory system of fish began in the 19th century, and for many years, morphological techniques dominated this area of research. It was shown that taste buds in fish have the same structure as in all gnathostomes - fish, amphibia, reptiles, birds and mammals. In fish, taste buds are situated not only within the oral cavity, pharynx, oesophagus and gills, but may also occur on the lips, barbels, and fins and over the entire body surface in many species (Kapoor et al., 1975; Jakubowski, Whitear, 1990; Reutter, 1992). The abundance of taste buds is another peculiarity of the fish gustatory system: taste buds are more numerous in fish than in any other animal. For instance, channel catfish 35-39.5 cm in body length have 680,000±36,000 taste buds on the entire body and fins surface (Finger et al., 1991) which is nearly 100 times more than is found in the oral cavity of an adult man. The taste buds density varies according to location and fish species. In the gular region the density of external taste buds reach up to 300 mm^{-2}, but is much lower in other areas of the body surface and fins (Gomahr et al., 1992). Based on the anatomical peculiarities, the gustatory system may by divided into two distinct, though interrelated subsystems: oral gustatory subsystem and extraoral gustatory subsystem (Finger, Morita, 1985).

In the last decades, electro-physiological methods have come into use to evaluate the properties of the gustatory system. It was found that fish gustatory system is highly responsive to many organic and inorganic chemicals as well as to natural food extracts. The responses from the gustatory nerves are fast adapting or phasic; the peak response is reached within the first few seconds of stimulation and returns to the baseline activity even with continued stimulation. The estimated thresholds for the most potent substances were less than 10^{-9} M. The dose-response relationships are variously expressed and are different for different taste substances; responses increase sharply with a logarithmic increase in concentration and eventually reach saturation (Caprio, 1984; Hara, 1994).

For many years a detailed knowledge of the structure and function of the gustatory system was not supported by the appropriate data on taste preferences of fishes. A decade ago data on the taste preferences were available for a few fish species only. Systematic investigations of fish taste preferences have been performed during the last 10-15 (Kasumyan, Døving, 2003). In the present article the appropriate information on taste preferences in fish, include unpublished data, are summarized and prospects for application of taste stimulants in fish aquaculture are discussed.

Materials and Methods

Several methods for presenting and quantifying fish taste preferences have been developed as bioassays. Many of these methods, though interesting and valuable, have limitations because they do not make it possible to distinguish between the taste and olfactory systems. Appropriate bioassay was developed only several years ago only. Following the procedure of the bioassay, fish are transported to the laboratory and are kept in a common aquarium during the first 1-2 weeks. Then they are placed one by one into separated aquaria. Each aquarium is equipped with

an aerator. The cover had a small feeding hole in the center. The water is replaced daily in part by fresh water. The fish are fed *ad libitum* once a day after the end of experiments.

Test procedure. After placing the fish in separate aquaria, they are trained to take the food dropped into the aquarium; first agar pellets containing a water extract of natural or artificial food. Pellets are dropped into the aquarium one by one, with an interval of 10-15 minutes. After about 3 days of training, a fish positioned itself under the hole of the aquarium's cover and took the pellet 2-5 seconds after its dropping into the water. Then a pellet containing one of the test substances is offered to the fish. In each trial, several parameters were registered: the number of grasps at the pellet; retention time of the pellet after the first grasp and the total retention time of all grasps; whether the pellet was swallowed or finally rejected by the fish. If the fish did not grasp the pellet within one minute, the trial was stopped and not registered. The pellets containing different taste substances should be offered to the fish at random sequence. Pellets rejected or not taken were removed from the aquarium immediately after the end of each trial (about 1 minute). Experiments showed that the pellet acceptance ratio as well as other characteristics of response to the pellet are independent of the sense of smell. Both, anosmic and intact fish have the same pattern of behavioral response to the same type of pellet; they also do not differ in the level of sensitivity to the substance contained in the pellet (Kasumyan, Morsy, 1996).

Preparation of agar pellets. The pellets are prepared from agar-agar gel (2%, Reanal) and had bright red colour. A dye solution (Ponceau 4R, 5 μM) is added to the gel together with one of the test substances or the food extract at the appropriate concentration. The control gel contained only the dye. Just before starting the experiment, each pellet is cut from the cool agar gel disk with stainless steel tube. The size of pellet varied and is depended from size of fish (for more details see Kasumyan, Sidorov, 1995; Kasumyan, Morsy, 1996). The Chi-square test (χ^2) is used for estimation of acceptance ratio and the Student's *t*-test for all other characteristics of taste response. For the assessment of the relationships between taste responses and taste preferences of different fish species, the Spearman rank correlation coefficient (r_s) is used.

Usinig this procedure the taste preferences are assessed now for more than 35 fish species belonging to Acipenseriformes, Salmoniformes, Cyprinifirmes, Poeciliformes, Gadiformes and several other orders of marine and freshwater fish. Some of these fish species are commercially valuable and are cultivated in many countries – common carp *Cyprinus carpio*, grass carp *Ctenopharyngodon idella*, tench *Tinca tinca*, rainbow trout *Oncorhyncus mykiss*, Atlantic salmon *Salmo salar*, Atlantic cod *Gadus morhua*, Siberian sturgeon *Acipenser baerii*, Russian sturgeon *Acipenser gueldenstaedtii*.

Results and Discussion

The basic knowledge in taste preferences in fish. During last decade the taste preferences to different types of substances including classical taste substances, free amino acids, betaine, nucleotides, nucleosides, amines, sugars and other hydrocarbons, organic acids, alcohols and aldehydes, and their mixtures, were assessed. It has been shown that chemical substances evoke different types of behavioral taste responses, thus they are perceived as different by fish. Many of amino acids have been found to be highly efficient taste stimulants for various fish species. In some species, one of free amino acid alone, had the same level of palatability as the whole extract of preferable food organisms. Not all amino acids, however, are palatable for fish. In a series of experiments, the oral taste responses evoked by common free amino acids were investigated for 21 fish species. The data reveal that the number of amino acids that acted as

stimulant ranged from zero to 13. There are only a few fish species for which the number of stimulatory amino acids reaches more than 10. For 13 fish species, the number of stimulatory amino acids was six or less. On average for all fish species tested 28% of the free amino acids were stimulatory. The range of free amino acids acting as incitants for fish is wide and include 14, 15 and 19 amino acids, as was found for three species of the genus *Acipenser* (Kasumyan, 1999). The spectra of stimulatory free amino acids are highly species specific and the list of palatable amino acids is different for different fish species. The palatable amino acids were not always among these essential amino acids. The amino acids are indifferent taste substances for fish more often than they are stimulants. Amino acids might have an aversive taste for fish and thus function as deterrents. Some of these substances significantly decrease the palatability of diets. The taste properties of amino acids are highly stereospecific. D-isomers of amino acids are usually deterrent or indifferent type of taste substances while the corresponding L-forms might be highly palatable for the same fish species. Betaine has important synergistic properties with amino acids or other substances.

When stimulants are given together in an experimental pellet or diet, there is often a synergistic effect. This synergism has been shown for pigfish, for which a synthetic mixture of betaine plus 19 amino acids was prepared so that the concentration of each substance was identical to that determined in an shrimp filtrate, and was as stimulatory as the filtrate itself. Both betaine and certain amino acids contributed to the activity of the synthetic mixture. A mixture containing the 19 amino acids alone was only about 28% as effective as the shrimp filtrate whereas a solution of betaine alone was only 39% as effective as the filtrate (Carr, 1976).

The minimal concentrations needed to give a positive or negative response of a fish toward a given taste stimulus is an important parameter when it comes to flavoring food or bait. Systematic behavioural studies of threshold parameters have been performed for a number of species. For the most common gustatory substances, the threshold concentrations are quite high and is usually around 10^{-2}-10^{-4} M. Based on threshold concentrations determined by behavioural assays it is possible to estimate the amount of substance in one pellet which is sufficient to release a significant taste response. These amounts were 4.27 and 3.39 µg for L-cysteine and citric acid (common carp, *Cyprinus carpio*), 0.236 µg for L-alanine (European minnow, *Phoxinus phoxinus*), 0.353 µg for L-aspartic acid (frolich char, *Salvelinus alpinis erythrinus*). These values correspond to 10^{-9}-10^{-10} mole or 10^{15}-10^{16} of molecules per pellet. However, all substances in a pellet do not stimulate the gustatory receptors, only those that are situated in the surface layer. The substance part inside the pellet is unavailable to taste receptors. Therefore, the actual quantities of the substance sufficient for eliciting a significant taste response would be lower than calculated by one to two orders of magnitude.

Comparative analyses of results obtained for more than 20 fish species reveal that oral taste preferences are highly species specific. The differences among fish species are apparent at a comparison of the width of spectra for stimulants and deterrents. The spectra of stimulant or deterrent amino acids differ not only in the width but also in composition. In many cases, the same amino acid evokes dramatically different oral taste response in fish, including closely related species from the same family or genus. The palatability of substances is different in sympatric fish that inhabit the same waters, and have the same biotopes and have similar feeding ecology and feeding behaviours. These data clearly demonstrate an important and undoubtedly leading role of gustatory reception in the feeding selectivity in fishes, and in their capacity to consume appropriate food items that are specific to them.

However, taste preferences do not have impressive interpopulation specificity in fish. It was found that taste preferences were similar in brown trout, *Salmo trutta* juveniles belonging to three populations from Caspian Sea, Baltic Sea and White Sea basins. It seems obvious that taste preferences in fish as in other vertebrates are genetically determined and seem to be patroclinous. Given the similarity between different fish population of the same species it is noteworthy that there is variability in taste preferences between individuals of a population. Taste responses are more stable and invariable for highly palatable substances than for substances with low level of palatability. Sex dimorphism with respect to taste preferences is not a characteristic attribute in fish.

Experiments performed on larvae and juveniles of Siberian sturgeon *Acipenser baerii* and stellate sturgeon *A. stellatus* have shown that, up to the time when it becomes necessary to estimate the gustatory qualities of food, fish have a well expressed ability to discriminate taste stimuli. fish larvae at the "start feed" stage have the ability to discriminate among taste substances and release an adequate behavioral taste response. Nevertheless, taste perception in fish larvae is less well developed than in adult fish.

Taste preferences show low plasticity in fishes. In other words, taste preference does not depend upon the fish diet. Fish conditioning with a long-term rearing on a selected food leads to only small shift in taste preferences and only a slight increase in palatability for the substances in the food on which they were reared. However, the fish diet might be reflected in taste preferences of fish to food, which have a more complicated composition. The fish feeding motivation strongly influences taste preferences: starvation during 17-18 hours caused a widening of both extraoral and oral taste spectrum in 4-6 cm long Siberian sturgeon juveniles in comparison with fish which had food *ad libidum* 1.0-1.5 hours before trials. The ambient water temperature is one of the most potent abiotic variables effecting on taste preferences in fish. Of particular interest is that the rank order of stimulatory efficiency differed between the amino acids under different temperature conditions.

Fish taste receptors are exposed to the environment and predisposed to the detrimental effects of water pollutants. It has been shown that many pollutants, especially heavy metals, affect fish taste reception by destroying both taste buds and reducing sensitivity to taste stimuli. These events occur after a short exposure of the fish to polluted water. In common carp, for example, the negative effect of heavy metals was evident after 15 min exposure. The exposure of fish during 1-3 hrs in heavy metal salt solution (1 μM) provide a strong and deep suppression on the taste responses of fish. Under these conditions, the fish more often rejected the pellets containing amino acids with very high palatability than did individuals in a control situation. The loss of taste responses in fish after the exposure to heavy metals is reversible. Some time after the cessation of exposure to toxic substances the taste responses of fish is improved to its former function. In common carp, the taste responses after an exposure to 0.1μM $HgCl_2$ for 3 hrs is recovered after 6 to 12 days at a temperature of 20°C and there appears to be a direct relationship between the duration of the exposure to a toxic substance and the duration of the recovery period.

Prospects for application of taste stimulants in fish aquaculture. Information about the taste preferences in fish might be of significant value when applied to fish cultivation. The growing use of fish in aquaculture demands sources for less expensive food for the fish. The use of additives that improve the palatability of artificial food for fish is a probable application. Knowledge of feeding behaviour of fish in aquaculture is important so that feeds and feeding techniques can be designed to encourage consumption and hence survival and growth whilst

minimizing metabolic energy expenditure in feeding. The eventual incorporation of taste stimulants into diets consisting of cheap and normally non-palatable protein sources may be of practical significance to the fish farming industry. Furthermore, wastage must be minimized because of high feed costs and the potentially deleterious effects of waste food on water quality. Non-acceptance of artificial food, which can cause large fish mortality, is a serious problem in aquaculture, especially in the rearing of marine fish larvae (De la Higuera, 2001). The variability in taste preferences between conspecifics can be used in aquaculture to promote fish strains that have particular taste preferences.

There are a several publications that show the importance of introducing taste stimulants into modern fish cultivation technologies. Addition of amino acids mixture significantly increased the *ad libitum* metabolizable energy of the diets (Heinsbroek, Kreuger, 1992). improved feed acceptability and growth performance of cultured fish, fed low cost, plant-based diets (Papatryphon, Soares, 2000).. Improvements in feed intake, weight gain and feed efficiency as a result of feeding stimulant supplementation have been previously observed in several cultivated fish like gilthead sea bream and European sea bass (Tandler et al., 1982; Dias et al., 1997). Fish fed a diet of poor palatability were satiated sooner than fish fed a more acceptable diet. If fish are fed to satiation with a diet of poor palatability and then given a more palatable diet, they will begin eating again until they reach a new satiation level (Ishiwata, 1968). It has been shown that artificial food with good chemosensory characteristics is digested by fish more effectively (Takeda, Takii, 1992).

Another possible application of feeding stimulants may be to mask deterrent ingredients that may lower the palatability of diets. Unpalatable plant proteins are used as substitutes for fishmeal protein in commercial diets. Certain antibiotics have been shown to reduce the palatability of feed. Supplement of feeding stimulants might mask deterrent ingredients and improve of feed intake (Toften et al., 1995; Dias et al., 1997; Papatryphon, Soares, 2000). Using special additives might increase palatability of artificial food; but it might also be raised by removing the components with bad taste or by replacing those components with equivalents, which do not provoke negative taste responses. To this end, one must estimate the palatability for each of the components that make up the feed. As was shown for sturgeon artificial food, many components have strongly different taste qualities for fish (Kasumyan et al., 1995). It was found that water extract of 13 of the components, mainly the fish protein concentrate, PVF enzymated yeast and premixes included in agar-agar pellets provoked the increase of grasp activity in stellate sturgeon *Acipenser stellatus* juveniles. Extracts of PF-2V premix, krill meal, fishmeal concentrate, Eprin yeast and coarsely cut Soya beans significantly increased the pellets consumption. The Japanese pilchard fishmeal stabilized by various antioxidants such as santochin, anfelan-1, anfelan-5, ionol, released different kind of behavioural taste responses in stellate sturgeon. The FinnStim (Finnish Sugar Co. Ltd.) was ineffective as both oral and extraoral taste stimuli. Based on the obtained results it was possible to correct the feed formulas so as to improve their palatability for fish (Kasumyan et al, 1995).

Acknowledgements
This study was supported by the Russian Foundation for the Basic Research, the European Science Foundation and the Norwegian Research Council.

References
Caprio J (1984) Olfaction and taste in fish. In: *Comparative physiology of sensory systems* (eds. Bolis L, Keynes RD, Maddrell SHP). Cambridge Univ. Press: 257-283.

Carr WES (1976) Chemoreception and feeding behaviour in the pigfish, Orthopristis chrysopterus: characterization and identification of stimulatory substances in a shrimp extract. *Comp. Biochem. Physiol.* 55A: 153-157.
De la Higuera M (2001) Effects of nutritional factors and feed characteristics on feed intake. In: *Food intake in fishEffects of nutritional factors and feed characteristics on feed intake* (eds Houlihan D, Boujard T and Jobling M). Blackwell Science, Oxford: 250-268.
Dias J, Gomes EF, Kaushik SJ (1997) Improvement of feed intake through supplementation with an attractant mix in European seabass fed plant-protein rich diets. *Aquatic Living Resources* 10: 385-389.
Finger TE, Drake SK, Kotrschal K, Womble M, Dockstader KC (1991) Postlarval growth of the peripheral gustatory system in the channel catfish, Ictalurus punctatus. *J. Comp. Neurology* 314: 55-66.
Finger TE, Morita Y (1985) Two gustatory systems: facial and vagal gustatory nuclei have different brainstem connections. *Science* 227: 776-778.
Gomahr A, Palzenberger M, Kotrschal K (1992) Density and distribution of external taste buds in cyprinids. *Environmen. Biol. Fish* 33: 125-134.
Hara TJ (1994) The diversity of chemical stimulation of fish olfaction and gustation. *Reviews in Fish Biol. Fish.* 4: 1-35.
Heinsbroek LTN, Kreuger JG (1992) Feeding and growth of glass eels, Anguilla anguilla L.: the effect of feeding stimulants on feed intake, energy metabolism and growth. *Aquaculture and Fisheries Management* 23: 327-336.
Ishiwata N (1968) Ecological studies on the feeding of fishes. VI. External factors affecting satiation amount. *Bull. Jap. Soc. Sci. Fish.* 34: 785-791.
Jakubowski M, Whitear M (1990) Comparative morphology and cytology of taste buds in teleosts. *Z. mikrosk.-anat. Forsch.* 104: 529-560.
Kapoor BG, Evans HE, Pevzner RA (1975) The gustatory system in fish. *Advanches marine biology* 13: 53-108.
Kasumyan AO (1997) Gustatory reception and feeding behavior in fish. *J. Ichthyology* 37: 72-86.
Kasumyan AO (1999) Olfaction and taste in sturgeon behaviour. *J. App. Ichthyology* 15: 228-232.
Kasumyan AO, Døving KB (2003) Taste preferences in fish. *Fish and Fisheries* 4:289-347.
Kasumyan AO, Morsy AMH (1996) Taste sensitivity of common carp Cyprinus carpio to free amino acids and classical taste substances. *J. of Ichthyology* 36: 391-403.
Kasumyan AO, Sidorov SS (1995) Gustatory properties of free amino acids in Caspian trout Salmo trutta caspius. *J. of Ichthyology* 35: 8-20.
Kasumyan AO, Sidorov SS, Kazhlaev AA, Pashchenko NI (1995) Behavioral responses of young stellate sturgeon to smell and taste of artificial feeds and their compents. In: *Proceeding of International Symposium on Acipenserids* (eds. Gershanovich AD, Smith TIJ). VNIRO Publishing, Moscow: 278-288.
Mackie AM, Mitchell AI (1985) Identification of gustatory feeding stimulants for fish-applications in aquaculture. In: *Nutrition and feeding in fish* (eds. Cowey CB, Mackie AM, Bell JG). Academic Press, London: 177-189.
Papatryphon E, Soares JHJ (2000) The effect of dietary feeding stimulants on growth performance of striped bass, Morone saxatilis, fed a plant feedstuff-based diet. *Aquaculture* 185: 329-338.
Reutter K (1992) Sturcture of the peripheral gustatory organ, represented by the siluroid fish Plotosus lineatus (Thunberg). In: *Fish chemoreception* (ed. Hara TJ). Chapman and Hall, London: 60-78.
Takeda M, Takii K (1992) Gustation and nutrition in fishes: application to aquaculture. In: *Fish Chemoreception* (ed. Hara TJ). Chapman and Hall, London: 271-287.
Tandler A, Berg BA, Kissil GW, Mackie AM (1982) Effect of food attractants on appetite and growth rate of gilthead bream, Sparus aurata L. *J. of Fish Biology* 20: 673-681.
Toften H, Jorgensen EH, Jobling M (1995) The study of feeding preferences using radiography: oxytetracycline as a feeding deterren and squid extract as a stimulant in diets for Atlantic salmon. *Aquaculture Nutrition* 1: 145-149.

The Use of GIS Environment on Contamination Resources Hazardous Degree Assessment in Gilan Province

Homayoun Khoshravan
Head of Coastal Management Office,Caspian Sea Research & Study Center,Email: homayoun@umz.ac.ir ,Tel/Fax:(98)01512261405

Abstract
Gilan as an Iranian northern province surrounded between Caspian Sea and Alborz mountains chain has a special climate i.e. Khazari Mediterranean climate that gives potentials of agricultural and tourist attraction to the province. In addition, some parts of Caspian Sea watershed are located in this province
Growth of population, urban development, industrial and agricultural activities and obtaining natural sources applies environmental pollution risks on the valuable and sensitive ecosystem of the province.
This research assesses environmental pollution sources of the coastal province of Gilan, Iran. In fact, After collecting data on pollution sources such as industries, mines, agriculture and related activities, municipal and rural communities and installations, mineral springs, and hydrocarbon sources, a database of the sources developed.
A Universal Ranking System Model (URSM) created by defining indexes presenting characteristics of every kind of the sources as an environmental pollution risk source and assigning an importance number to every index and using Fuzzy theory to translating linguistic phrases to mathematical language.
Then, all data on the pollution sources introduced to a Geographic Information System i.e. ArcView GIS and by composing the GIS environment with MS Excel software computational environment, the ranking model of URSM applied on the data.
As a result, scale of relative risk of every pollution source in comparison with other sources of its kind and also other kinds determined. Then, by fractionating the province to 109 zones, zones with high pollution sources highlighted.
Finally, by composing the results of risk assessment with field surveys in addition to previous reported pollution monitors the needed conditions for the future pollution control programs obtained.

INTRODUCTION

Caspian Sea as the greatest lake of the world, surrounded between countries with different cultures and level of development, is subjected to environmental pollution sources located in its bed and coasts.
Gilan as an Iranian northern province surrounded between Caspian Sea and Alborz mountains chain, composes some parts of Caspian Sea watershed. Growth of population, urban development, industrial and agricultural activities and extraction of natural resources applies environmental pollution risks on the valuable and sensitive ecosystem of the province and Caspian Sea.
Several national and local organizations and universities of Iran have educated on environmental pollution sources of the province. From other hand, some international and regional organizations such as Caspian Environmental Program (CEP) are educating on several environmental features of Caspian Sea.
In order to identification and programming for removal or decreasing impacts of major pollution sources of Caspian Sea located in the province of Mazandaran, as case study area for entire of Caspian Sea watershed, this research educated pollution potentials in the province.
The research, by development and application of a Universal Ranking System Model (URSM), on Geographic Information System (GIS) of pollution sources of Gilan, identified programming priorities of the province future pollution prevention plans.

GEOGRAPHIC INFORMATION SYSTEM

Overlay is a traditional method in environmental assessment techniques. In this method information for an array of variables is collected for standard geographical units within the study area, and recorded on a series of maps, typically one for each variable. These maps are overlaid to produce a composite. The resulting composite maps characterize the area's physical, social, ecological, land use, and other relevant characteristics.

Traditionally, the overlay has been produced by hand. As a result of recent years developments Geographic Information Systems (GIS) became popular.

GISs are computer systems that can store, integrate, analyze and display data (Joao & Fonesca, 1996).

A significant application of GIS is the construction of real world models based on digital data. Modeling can analyze trends, identify factors that are causing them, reveal alternative paths to solving a given problem, and indicate the implication or consequences of decisions (Asian Development Bank, 1997).

In recent years GIS is sufficiently used as an environmental pollution assessment environment (Hession et. al. 2000).

FUZZY SETS

In this research, in development of raking model a mathematical theory i.e. theory of Fuzzy sets, is used.

A Fuzzy set in a universe of discourse U is characterized by a membership function $\mu_A(x)$ that takes values in the interval $[0,1]$.

Therefore, a fuzzy set is a generalization of a classical set by allowing the membership function to take any values in the interval $[0,1]$. In other words, the membership function of a classical set can only tack two values - zero and one, whereas the membership function of a Fuzzy set is a continuous function with range $[0,1]$.

UNIVERSAL RANKING SYSTEM MODEL

In order to cover all pollution sources required data on pollution sources gathered from related governmental organizations and offices. Two main conditions was enabled on the data gathering:

1- Validation
2- Reliability

Validation of the data means they must be compatible with the goals of the research, be universal and completely related. Reliability of the data means they must be accurate and be extracted or gotten from reliable references.

Data used in the research include data existing in databases and files of governmental organizations and information gathered from research reports of research centers and universities. Table 1 shows a classification of gathered data.

After gathering of the data it was necessary to arrange them so this great volume of digits, numbers, phrases sentences, graphs, and maps together and based on their importance and magnitude can be comparable. Thus, it was necessary to develop a universal system that include the information and make them comparable and assessable. So, a Universal Ranking System Model (URSM) developed that with inputting every pollution source in it, rank of that pollution sources could be obtained. In developing the URSM, for translation of all of data with several kinds (digits, maps, graphs, etc.), theory of Fuzzy sets that is a useful mathematical technique for translation of several kinds data to mathematical language (Zadeh, 1965) was used.

In the URSM for every kind of pollution sources some indexes were defined. The indexes could highlight the importance of the sources as an environmental pollution source. For example the indexes the indexes defined for the industrial factories and centers includes kinds of products, number of personnel, volume of discharged wastewater, having wastewater treatment plant or not, and kind of the environment that the wastewater or effluent discharge to it.

Then with a complete education on every index, an importance number assigned to every index. This importance number changes between 0 and 1. So must important index received importance number of 1 and the index without any importance (or effect) received 0.
Now, Fuzzy sets theory could be applied on the data so the indexes related to every kind of pollution source could be included in a Fuzzy set and the importance number of every index be the membership function of the index (μ) in that set. From other hand, importance numbers assigned to every kind of the pollution sources and they composed a second kind Fuzzy sets.

Table 1: Data Gathering

POLLUTION SOURCE		CONTAMINATIONS	STATE	LOCATION
Mines	- Metal - Non-Metal - Construction Materials	- Heavy Metals - Pesticides - Chemical Fertilizeres - Hydrocarbons - Other Chemicals - Oxygen Demandings - Radioactives - Sanitary & Industrial Wastewater - Municipal & Industrial Solid Waste	- Sea Side - Near to Water Volumes (Wetlandes, Small Reservoires, etc.) - Near to Rivers - Near to Streams - In Jungles - In Plains - In Urban Areas - In Suburbs	- Maps - Addresses - Positioning by Global Positioning Systems (GPS)
Industries	- Food - Mineral & Electrical - Textile & Leather - Chemical & Cellulose			
Agricultural Activities	- Without or With Low Limitation Agricultural Areas - With Limitation Agricultural Areas - Orchards - Mixed Orchard & Agriculture - Mixed Agriculture & Orchard - Animal Husbandry			
Municipal Activities	- Solid Waste - Sewage			
Natural Sources	- Hydrothermal Springs - Lithologic Unites -			
Hydrocarbon Sources	- Pipeline - Wells (Exploration & Extraction) - Refinery - Ports & Marine			
Marine Storm Surge				

For application of the URSM on data a software environment was needed that because of spatial data and information, Geographic Information Systems (GIS) selected. After evaluation of several GIS software, ArcView GIS 3.1 software environment because of compatibility with needs of the research selected.
All data introduced into the GIS environment and finally expect of layers of political boundaries, topography, and rivers network, 14 information layers presenting location and attributes of the

pollution sources created. In fact, database and GIS project of pollution sources of Caspian Sea located in the province of Mazandaran produced.
Now, it was possible to apply the URSM on the GIS environment. Of coarse, in this stage an information sharing between the GIS environment and MS Excel software computation environment was created. The information sharing made the complicated high volume computation of the ranks possible for the GIS environment.
In this stage risk rank of every pollution source relative to other pollution sources obtained.
Then, by fractionating the province into 109 zones (based on political deviations) and computing risk ranks for every zone by addition of the ranks of all existing pollution sources from same kind in that zone and dividing this number on area of the zone, relative distribution of every kind of pollution sources existence obtained for every zone. Also, by addition of relative distribution of all kind of the pollution sources, cumulative relative distribution of all kinds of pollution sources obtained for every zone.

RESULTS OF THE ASSESSMENT

Results of the research are as following:

1. Database of pollution sources of Caspian Sea located in the coastal province of Gilan, produced.
2. GIS maps of distribution of pollution sources of the watershed such as industrial centers, mines, agricultural activities, natural sources, storm surge, oil spills, and municipal sources, produced.
3. All pollution sources ranked in comparison to other pollution sources from same kind and from other kinds, too. For example a specific coalmine relative to other specific coalmine and also a specific textile factory became comparable. Figure 1 as a case example shows relative distribution of pollution load produced by application of chemical fertilizers and pesticides in agricultural area.
4. By fractionating the province into 109 zones, zones under impact of higher cumulative effect of existence of watershed pollution sources, highlighted. Figure 2 shows relative distribution of cumulative effect of existence of pollution sources in the province of Gilan.
5. As can be seen in figure 2, centeral zone have highest load of existence of watershed pollution sources. Most of zones that have higher levels of risk are located in eastern and central parts of the province.
6. Most important pollution sources of the watershed are the agricultural activities with high volumes of application of chemical pesticides and fertilizers.
7. Introduction and implementation of a universal environmental program for central of pollution sources in the province is necessary.

CONCLUSION

Gilan, a northern province of Iran, composes some parts of Caspian Sea southern watershed.
The research focusing on point and non-point-sources of Caspian Sea located in Gilan province ranked all of them relatively. Then by fractionating the province to 109 zones, distribution of load of every kind of pollution sources existence and cumulative effect of them, obtained. Assessment of results shows higher loads in the central and eastern parts of the province. As a result of high volume application of chemical pesticides and fertilizers, agricultural activities introduced as a major pollution source by this research.

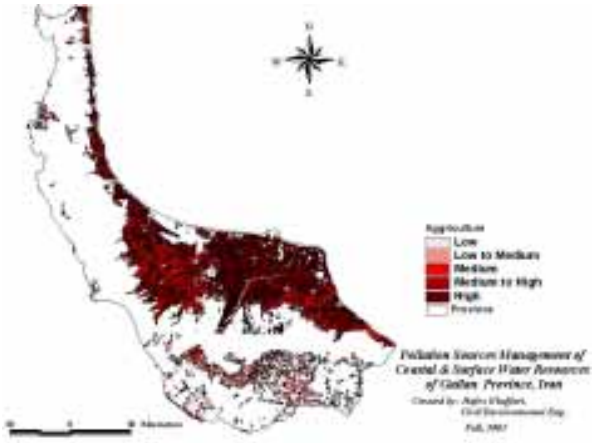


Fig 1 : relative distribution of pollution load produced by application of chemical fertilizers and pesticides in agricultural area.

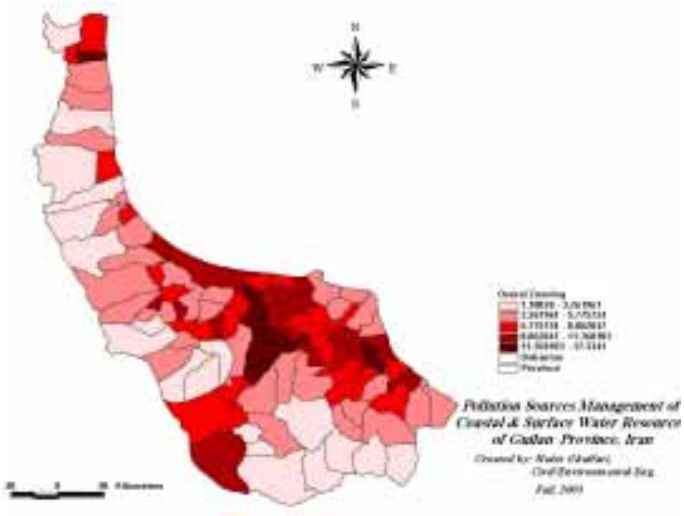


fig 2 : relative distribution of cumulative effect of existence of pollution sources in the province of Gilan.

ACKNOWLEDGMENT

The support of the National Caspian Sea Research and study Center in Sari, Iran, in providing support for this research and local governmental organizations and offices for providing needed data, is gratefully acknowledged.

REFERENCES

Asian Development Bank (1997), "EIA for developing countries", Chapter 3, Methodology of EIA, pp. 50-53

ESRI (1994), "Introducing ArcView", Environmental Systems Research Institute, Redlands, Calif., USA

Hession, W., C., Mc-Bride, M., Bennett, M. (2000), ”Statewide Non-Point-Source Pollution Assessment Methodology”, Journal of Water Resource Planning and Management, 126(3), ASCE, pp146-155

Joao, E. M., and Fonseca, A. (1996), “Current Use of Geographic Information Systems for Environmental Assessment: a discussion document”, Research Papers in Environmental and Spatial Analysis, No.36, Department of Geography, London School of Economics, London

Wang, Li-Xin, (1997), “A Course in Fuzzy Systems and Control”, International Edition, Prentice-Hall International, Inc., NJ, US, pp. 424

Zadeh, Loyfi, A. (1965), “Fuzzy Sets”, Information and Control, Vol. 8, pp. 338-353

Bacterial count in waters with depths of less than 10 meters in South Caspian Sea

Hesam Kiakojoory and Maryam Kamgar

Ecology Research Station of Caspian Sea, Nowshahr , Mazandaran province , IRAN, P.O.Box 498, Telefax :0192367 2657 ; Email: maryam_KMR@yahoo.com

Abstract

Bacterial count has been done in the framework of the large project designated as "hydrology and hydrobiology of southern part of Caspian Sea in the depths of less than 10 m" in 4 successive seasons from 1999 to 2000.

There were 10 sampling lines, which positioned perpendicular to seashore in Mazandaran and Golestan provinces. In every line, 5 stations were assigned and 12 samples were taken in every party and every line. The applied method was bacterial plate count with 5 dilutions 10^{-2},10^{-3},10^{-4},10^{-5},10^{-6} in nutrient agar prepared with seawater. Also warm blooded animal coliforms as representative organisms of pollution were detected.

Khazarabad and Tonekabon coastline had the most abundance bacteria and coliforms in all stations, the sediment counts were more than the count of the water column. More bacterial count were seen in stations far from the shore. Although sludge drainage is low in cold seasons, oppositely more bacterial count were observed in stations near the shore in warm seasons and this phenomenon is in compliance with the data obtained from "sediment sorting".

Keywords: Caspian Sea, bacterial count, coliforms.

Introduction

It may be reasoned that the numbers and types of bacteria recovered from water and sediment will reflect:

(a) The methods employed, i.e. direct versus plate count, the type of media used, temperature and duration of incubator, namely aerobic, enhanced carbon dioxide or anaerobic; and

(b) The physico-chemical conditions of the aquatic environment, i.e. temperature, pH, salinity and degree of eutrophication.

From the published literature, it is apparent that the bacterial numbers will be maximal during the height of summer and in highly eutrophic waters. Conversely, winter and clean or nutrient- deficient waters are conducive to low bacterial counts (Austin & Austin, 1989)

With direct counts such as by phase contrast or epifluorescence microscopy, there may be doubt about whether or not small objects are micro-organism or inanimate particles. Plate counts lack reliability in so far as there is no single technique, which permits the growth of bacteria. For example, aerobic incubation will preclude the development of colonies of anaerobes, and nutrient-rich media may inhibit the development of organisms, which prefer low nutrient concentration. Furthermore, colony counts on solid media preclude information about the precise whereabouts of the organisms in the aquatic environment (Austin & Austin, 1989)

This study is a part of the project hydrology and hydrobiology of south cousin of Caspian Sea in the depths of less than 10 m which has been done by Mazandaran research center In 1999-2000.

Material and methods

There are 10 lines perpendicular to shore whit 25-35 Km distance between each . For biological studies in Mazandaran and Golestan states . For bacterial study, 5 stations were choosed in every line according to depths (0m-1m-2m-5m-10m). In station 0 m, the only sample was sediment. In 1m station there were 2 samples, water surface and sediment. In 2m st. also 2 samples were caught (surface water and sediment). In 5m st. there were 3 samples: water surface, sediment and water of the bottom (water-sediment interface). In 10 m st. there were 4 samples in every tour, 3 ones like that of 5 m St., and the forth was from the water column of the layer 5m. Though in every season, 12 samples were caught in every line. At all, we examined 480 samples in one year. The water sampler was rottner and sediment sampler was grab. The samples were washed in every station by HCl 5%. Samples were put in 50 ml sterile bottles then were kept in ice container and carried to microbiology lab, as soon as possible. The samples were cultured after homogenization whit agitation. Then glycerin stock was prepared for long time preservation of samples (20days). Plate colony count was applied for total count. The medium was nutrient agar-seawater solution. The dilution factor was 10^{-1}–10^{-6} by sea water (B.Austin,1989). Coliform count was did by MPN method (standard methods, 1996) by some ratifications to EC medium. Statistics Methods were Mann Wittney and Krusckhalwallis.

Results

The results of total count (tables 1, 2, 3, 4) are arranged for comparing and discussion on different lines, seasons and layers of water. These lines could be classified in to east and west parts according to sediment texture, "grain size analysis" data (Salarvand, personal communication) the east part includes 3 lines: 16, 17, 18, and the rest lines, 9 Till 15 are included in west part. The mean of former lines is 96.23% clay and the mean of latter lines is 35.75% clay the difference is meaningful (t-Test). The arrange of high to low abundance of count in seasons is:

1-Summer 2-Spring 3-Winter 4-Fall. The difference of summer bacterial count with other seasons is meaningful ($p<0.05$). In different layers the arrange of plentiful ness is 1-Sediments 2-Surface 3-The bottom of 10m st. 4-The bottom of 5m st. The difference of sediment whit other layers is meaningful ($p<0.05$) but the difference among other layers is not meaningful ($p<0.05$). No meaningful difference was observed among sediment samples and also between surface water samples ($p<0.05$).

Discussion

In the summer, the lines 14 (Khazarabad), 9 (Tonekabon), 10 (Chaloos) and 13(Babolsar) had the most bacterial count that the major factor is release of the rivers Tajan , Tonekabon, Chaloos and Babolrood respectively. Farming (rice, oranges, vegetables, animals) is an essential rout of economy around two of these rivers: Tajan and Babolrood, which release to lines 14 and 13 respectively. Accordingly, at first a huge mass of organic matter and microorganism are washed from Alborz mountains, then these materials to streams increase due to streams through low land area and massive populations of bacteria release to these lines where many of them could be retained in low salinity of Caspian Sea (%1.3). the main cause of high count in lines 9 and 10 in summer is crowded urban and rural populations which increase with tourism around the rivers and swimming times in the sea.

Farming activities produce huge volume of organic matters. More over, fertilizers increase the concentration and variety of bacteria. Although the pesticides and herbicides decrease the variety of bacteria, but might increase the number of resistant bacteria in thousands or probably in million times. The mean of temperature in summer was 29.4°C that is one admission to multiplication of all of three thermal categories of bacteria .The fifth plentiful

line, 18 (Hasangholi) is crowded with bacteria due to fine texture of sediment which is more than %95 clay and this testifies previous study of author who stated more small the grain size of sediment is the better bed for bacterial growth.
In full, the lines 14, 13, 9, 8, had the highest count but the count of the line 10 (Chaloos) is decreased. As stated for summer ,the same reasons are present except rice plantation which was stopped till next spring in area related to lines 14,13 and 9, vegetation is economical in fall and winter however there are less rural economy around Chaloos (line10). In winter 2000,lines 14,17,16, had high count but lines 9 and 10 had the lowest counts. The reasons related to high count of line14 have been stated but the reasons of high counts of lines 16 and 17, and low counts of lines 9 and 10, now, are reversed. Weather Temperate during summer (29.4 °c) cultivates the saprophytes of woods, fields and rivers in a bed of organic matter but the cold of winter (10.5 °c) with the lock of rice fields and entirely moderate rate of production specific to cold seasons, lowered the count of bacteria however, much of microorganisms of the lines 16 and 17 are intrinsic to their habitat and during spring and summer have a good growth. In summer, entry of organic matter and micro- organism and other stimulators of temporal growth to lines 9 and 10 and a minor difference between summer and winter counts in lines 16 and 17 are not strange manners in argued lines.

Table1-numbers of bacteria ($ml^{-1} \times 10^3$) in water and sediment samples in summer 2000

	9	10	11	12	13	14	15	18	mean
0m sed	18870.0	11910.9	317.5	-	8350.0	29760.0	86.5	71.5	9966.6
1m sur	45.5	8.5	67.2	-	40.7	82.1	2.8	1.5	35.4
1m sed	594.4	28.9	872.4	-	139.6	334.5	0.1	-	328.3
2m sur	480.0	525.0	103.8	-	112.3	240.4	0.2	65.2	218.1
2m sed	835.6	228.9	1168.0	-	953.4	1246.4	287.0	2480.0	1028.4
5m sur	189.4	68.9	78.7	-	56.7	70.0	2.1	3.1	69766.9
5m bot	69.7	513.3	111.3	-	148.7	38.7	9.2	2.9	127.6
5m sed	560.3	122.5	179.4	-	516.7	6185.6	126.9	35.4	1103.8
10m sur	89.7	8.0	50.3	-	93.4	146.3	25.2	39.9	64.68
10mmid	30.8	31.4	45.3	-	15.5	40.4	1.0	5.3	24.2
10m bot	341.0	623.7	222.3	-	189.4	138.4	8.3	61.1	226.3
10m sed	58.0	23.7	206.3	-	150.0	2154.4	148.1	102.0	406.1
Mean	1847.0	1164.3	285.3	-	897.2	3369.8	58.1	297.1	

Average numbers of colonies from triplicate plates of seawater nutrient agar after incubation at (25-30 °c) for 4 days

Table2-numbers of bacteria ($ml^{-1} \times 10^3$) in water and sediment samples in fall 1999

	9	10	11	12	13	14	15	16	17	18	Mean
n sed	740.3	143.8	2.2	4560.0	334.1	5702.2	41.5	555.6	417.1	176.0	1267.2
n sur	1361.7	8.1	1.3	2.7	132.8	4212.0	41.0	13.4	215.6	185.6	617.3
n sed	1193.3	153.1	2.1	78.5	2450.0	25019.1	133.1	672.0	672.1	312.4	3068.5
n sur	627.3	19.9	1.1	38.7	1307.1	3016.7	76.7	212.1	311.4	13.0	562.4
n sed	631.0	185.0	175.1	21.2	6052.3	6280.1	491.7	182.7	223.7	4802.0	1904.2
n sur	810.0	10.0	4.5	44.2	6750.0	1661.3	4.1	16.7	116.7	9.1	942.6
n bot	259.7	443.2	55.7	69.8	305.7	11.7	4.6	-	76.4	1.0	136.3
n sed	72214.0	1152.0	471.0	381.2	41915.0	14552.0	364.0	837.3	213.0	192.8	9529.1
m sur	14.0	1.1	4.2	182.0	1.0	57.7	104.7	134.3	1275.	114.7	187.8

									0		
mmid	415.1	3.3	11.7	2.9	4.1	5.6	41.3	34.6	296.1	-	107.4
m bot	185.7	42.1	3.3	89.0	475.5	10.1	13.8	69.7	77.7	-	107.4
msed	1911.3	1352.2	283.7	629.9	3415.1	411.8	623.8	182.2	718.7	743.0	1027.1
nean	6697.1	292.8	84.4	508.3	2178.5	5078.3	161.6	264.3	384.4	654.7	

Average numbers of colonies from triplicate plates of seawater nutrient agar after incubation at (9-14°c) for 12 days

Table3-numbers of bacteria ($ml^{-1} \times 10^3$) in water and sediment samples in winter 2000

	9	10	12	13	14	15	16	17	18	mean
0m sed	211.2	173.0	143.8	542.2	2908.0	509.2	10650.0	9760.0	71.5	2945.3
1m sur	37.3	65.1	31.2	314.1	567.7	8.3	15.3	71.6	1.5	124.7
1m sed	323.1	116.7	125.5	619.4	1221.0	40.0	703.3	1300.0	-	628.8
2m sur	129.7	70.0	626.6	587.6	682.7	502.1	43.0	2.7	65.2	296.8
2m sed	713.3	300.0	307.8	2706.6	201.0	112.3	154.2	18001.1	2480.0	2800.2
5m sur	49.9	20.2	1092.4	890.3	512.2	1.1	6.7	1.8	3.1	288.4
5m bot	871.3	36.9	6.1	24.3	11.7	4.5	12.3	9.1	2.9	109.3
5m sed	729.1	1103.3	9450.1	150.7	70723.3	-	3612.8	3542.3	35.4	11279.2
10m sur	18.8	2.1	2.6	25.3	14.1	1.7	1.4	0	39.9	8.1
10mmid	5.1	9.4	330.0	27.6	4.1	7.3	11.5	1.0	5.3	44.7
10m bot	16.4	5.3	55.3	31.6	9.2	5.8	3.7	24.1	1.1	16.9
10msed	112.2	122.0	960.2	415.6	680.0	4132.2	821.0	4112.2	277.3	1292.5
mean	268.0	168.7	1094.3	527.9	6669.6	484.0	1336.2	1718.7	359.2	

Average numbers of colonies from triplicate plates of seawater nutrient agar after incubation at (9-14°c) for 12 days

Table4-numbers of bacteria ($ml^{-1} \times 10^3$) in water and sediment samples in spring 2000

	9	10	12	13	14	15	16	18	mean
0m sed	8462.3	10930.0	231.6	51.7	8359.3	71.6	158.7	122.3	3544.9
1m sur	66.7	53.8	169.7	171.5	223.3	43.3	2123.6	70.1	364.9
1m sed	3316.7	311.2	72.4	553.3	8835.6	15.5	583.7	5600.0	2411.7
2m sur	311.2	100.0	33.3	34.5	122.3	15.6	223.0	12.5	106.5
2m sed	1372.8	450.0	253.3	152.3	453.1	136.7	423.0	239.9	435.1
5m sur	253.4	3062.4	33.1	124.8	566.6	15.0	19.5	33.4	513.5
5m bot	112.2	110.0	25.5	68.3	523.0	22.1	33.2	43.8	117.3
5m sed	5132.5	833.3	184.5	529.3	783.0	51.1	382.2	650.0	1949.1
10m sur	111.3	1.1	81.2	1442.1	200.0	-	31.0	10.5	234.6
10mmid	72.1	21.0	25.2	11.2	712.0	31.9	22.5	33.7	116.2
10m bot	11.0	32.8	24.3	21.7	3501.4	38.7	11.5	12.4	456.7
10m sed	116.8	1250.0	130.5	350.0	155.6	22.0	251.0	96.7	296.6
mean	1603.5	603.2	105.2	292.5	2621.3	42.1	355.0	577.1	

Average numbers of colonies from triplicate plates of seawater nutrient agar after incubation at (23-28°c) for 6 days

9-Tonekabon 10-Chaloos 11-Hasanabad 12-Mahmoodabad 13-Babolsar
14-Khazarabad 15-Amirabad 16-Khagehnafas 17-Gomishan 18-Hasangholi

Table 5-Coliform count – MPN in summer 1999

	9	10	13	14	16	17	18
0m sed	1200	1200	1200	1200	28	1200	1200
1m sur	21	0	20	28	4	15	4
1m sed	1200	1200	1200	1200	28	28	15
2m sur	12	4	4	20	9	9	0
2m sed	1200	1200	1200	1200	4	20	0
5m sur	0	4	4	20	15	4	0
5m bot	0	4	9	0	21	20	20
5m sed	9	1200	9	1200	1200	28	0
10m sur	15	0	20	0	1200	28	0
10mmid	4	0	20	0	15	0	0
10m bot	4	0	15	0	0	0	0
10msed	20	20	15	1200	20	0	0

Number/100 ml water or 100 gr sediment
-Not examined

Table 6 -Coliform count – MPN in spring 2000

	9	10	13	14	16	17	18
0m sed	1200	1200	1200	1200	1200	1200	20
1m sur	28	21	4	21	4	28	4
1m sed	1200	1200	1200	1200	28	4	15
2m sur	28	21	9	4	9	9	0
2m sed	20	9	1200	1200	15	28	21
5m sur	21	15	21	20	21	4	0
5m bot	9	20	4	28	4	9	0
5m sed	15	4	1200	1200	28	28	20
10m sur	0	0	15	9	21	15	0
10mmid	0	9	20	0	0	0	0
10m bot	4	0	0	15	0	0	0
10msed	1200	1200	1200	1200	21	20	0

Number/100 ml water or 100 gr sediment
-Not examined

Reference

1-B.Austin and D.A.Austin , 1989,Bacterial fish pathogens , Department of Brewing and Biological sciences , Heriot – Watt university, Edinburgh

2- B.Austin , Methods in Aquatic Bacteriology 1989, Department of Brewing and Biological sciences , Heriot – Watt university, Edinburgh
3- Standard Methods , Microbiological examination, 1996

Features of osmotic and ionic regulation in Caspian Acipenserids

Lyudmila S. Krayushkina, Olga G. Semenova
Department of Hydrobiology and Ichthyology, Biological faculty, St. Petersburg State University, St. Petersburg, Russia.
Phone: 007 (812) 3213279. Fax: 007 (812) 4277310. E-mail: krayushkina@ mail.ru

Abstract
Characteristic features of the mechanisms for the support of osmotic and ionic homeostasis in Caspian acipenserids species in sea water (12.5‰, 403 mosm/l) after their transfer from fresh water were studied. In experiment conditions immature fish in 2+ age of old were investigated: secondary freshwater species – sterlet *Acipenser ruthenus* L. (from Middle Volga) and diadromous species-Russian sturgeon *A. gueldenstaedtii* Br., starred sturgeon *A. stellatus* Pall., great sturgeon *Huso huso* (L.). Species features of osmotic and ionic regulation were revealed on the strength of the analysis of the dynamics of the osmolarity and cation concentrations (Na^+, K^+, Ca^{2+}, Mg^{2+}) in blood serum, the urine and intestinal liquid, the level of a cortisol in blood serum. In the experiments, secondary freshwater sterlet is adapted to sea water 12.5‰ as a conformer. In sea water the diadromous acipenserids after their transfer from fresh water are capable to support blood serum osmolarity on relative constancy level in a result of the transition from hyperosmotic regulation to hypoosmotic one. However functional level of the mechanism of osmotic and ionic homeostasis is not equal in different species. This level rises in a number of acipenserids which rank as follow "Russian sturgeon→ great sturgeon→ starred sturgeon". Present data confirm the osmo- and ion regulatory systems of young fish in the same species, which were investigated before in the age of 1.5-2.5 months, (Krayushkina, 1983), are non-completed developed. The results of present investigation can be allowed for the practice of sturgeon rearing in fishery farms.

Keywords: acipenserids, osmoregulation, blood serum, intestine liquid, cortisol.

Introduction

At present time acipenserids are living in environment with different salinity including freshwater, diadromous brackish water and diadromous. Each of these groups has definite level of osmotic and ionic regulation that was developed in conformity with own biology. Caspian acipenserids include brackishwater diadromous species (Russian sturgeon, Persian sturgeon, starred sturgeon, great sturgeon, ship). In spite of similar biology and life in the same sea, diadromous species of Caspian acipenserids are differed in their saline tolerance. These distinctions were revealed in the larvae and young fish on early stages of the post embryogenesis-in the age from the hatchling to 1.5-2.5 months (Krayushkina, 1967, 1983; Kazemi, Bahmani, Krayushkina at al., 2003). At the same time, up to now the data on species peculiarities of osmotic and ionic regulation in the older young fish, that have definitive state of osmoregulatory and ionregulatory systems, are absent. The study of the development of these systems in the ontogenesis has practical significance in connection with the necessity to appreciate optimal duration for fish rearing in fishery farms and the place for the distribution of these fish in natural conditions. The study of peculiarities of osmotic and ionic regulation in Caspian acipenserids is important for the comparison with marine diadromous and freshwater species, forming a true notion about the evolution of these functions in a number of acipenserids.

The purpose of present investigation is the study of species peculiarities of osmotic and ionic regulation in Caspian acipenserids of older age, having developed osmoregulatory and ionregulatory systems, also the comparison of functional level of these systems with similar functions in young fish that were studied before. The choice of length-weight and age category of fish for experimental work was based on the data about the distribution of acipenserids in Caspian Sea.

It is known that adult acipenserids are found in all regions of Caspian Sea, from almost fresh water (3.6‰, 116mOsm/l) to typical sea water (14 ‰, 452 mOsm/l). General localization of Russian sturgeon is observed under the wide range of salinity – from 5.4 to 13.4‰. The congestion of starred sturgeon is usually observed in sea water of 11.75-12.96 ‰ salinity (Legeza, 1972). Young acipenserids, after their migration from river condition, are distributed in water of North Caspian Sea

with low salinity, and then they migrate in more saline water of Middle and South Caspian Sea. Young great sturgeons, having the length from 14 cm to 80 cm, are found in the water with 10.5-14.6‰ of salinity. Young Russian sturgeons, having the length 11-80cm, are found in 7-14‰ of salinity. Young starred sturgeons, having the length 7-80 cm, are found in the water with 6.3-14.5‰ of salinity (Silvestrova, 1973).

Materials and methods:

In conformity with these data, immature fish of the similar sizes in the age of 2+ were used for our experimental work. They are:

1. Russian sturgeon ***Acipenser gueldenstaedtii*** Brandt (length 63.0±1.0 cm, weight 302.4±20.2 g);
2. Starred sturgeon ***A. stellatus*** Pallas (length 45.5±1.2 cm, weight 175.2±11.4g);
3. Great sturgeon ***Huso huso*** (L.) (length 56.3±3.4 cm, weight 700.0±107.4g).
4. For the estimation of the level of the development of osmoregulatory and ionregulatory systems in diadromous species, comparatively with freshwater species, also immature sterlet ***A. ruthenus*** L. (length 39.0±0.6cm, weight 193.0±10.3g, age 2+) from Middle Volga River was investigated.

The species peculiarities of osmotic and ionic regulation was revealed on the strength of changes in the osmolarity and concentration of main cations (Na^+, K^+, Ca^{2+}, Mg^{2+}) in blood serum, the urine and intestine liquid in fish from fresh water (control) and after the adaptation to artificial sea water of 12.5‰ during 7 days after the transfer of fish from fresh water (experiment). Artificial water was close to average salinity of Caspian water (12.85‰) and had similar composition (Bruevich, 1937). Osmolarity of biological liquids and water were measured by cryoscopy method with microosmometer MT-2 (Russia). Sodium and potassium concentrations were measured with flame photometer AAS-1 (Germany), calcium and magnesium concentrations were measured with an absorption spectrophotometer "Flapho-4" (Germany).

Results and discussion:

In the experiment, secondary freshwater sterlet is adapted to sea water 12.5‰ as a conformer. In this case the increase of concentration of main cations (Na^+, Ca^{2+}, Mg^{2+}) is happened, compared to control, therefore the osmolarity increases on 55.8% and becomes isoosmotic to environment. Table 1. Sodium concentration in serum is found higher than in seawater. Table 2. The support of blood serum isoosmolarity (to the medium) and cation correlation in serum is possible as a result of the maintenance of high level of the diuresis and high cation concentrations, first of all Na^+, in the urine. Tables 1, 2. The kidneys fulfill a significant loading in the removal of surplus sodium from the organism.

In sea water the diadromous acipenserids after their transfer from fresh water have ability to support blood serum osmolarity lower environmental osmolarity (Table 1) as a result of the transition from hyperosmotic regulation to hypoosmotic one. However functional levels of the mechanism of osmotic and ionic homeostasis are not similar in different species. In a number of acipenserids, this level rises in the direction "Russian sturgeon → great sturgeon → starred sturgeon".. As a result, fluctuation range of the osmolarity, during the adaptation of these species to sea water, is reduced, accordingly 46.7 mOsm/l (17.8%) → 40.2 mOsm/l (14.4%) →33.5 mOsm/l (12.6%).The differences between the levels of the osmolarity as well as sodium concentration in blood serum and similar parameters in sea water increase: accordingly, 89.2 mOsm/l (21.8%) and 24.2 mEq/l (13.4%) in great sturgeon; 100 mOsm/l (24.4%) and 27.9 mEq/l (15.6%) in Russian sturgeon; 110.5 mOsm/l (27.1%) and 45.7 mEq/l (25.6%) in starred sturgeon.

It is important to note that the mechanism of hypoosmotic regulation attains the highest development in diadromous marine sturgeons, as shortnose sturgeon *A. medirostris* LeSueur and *A. oxyrhynchus* Mitchill that are living in ocean salinity to 33‰ (Krayushkina, 1998). This mechanism supports relatively constancy of serum osmolarity under wide fluctuation of salinity. When environmental salinity increases to 1000 mOsm/l, serum osmolarity increases only on 65-69 mOsm/l.

For the adaptation to hyperosmotic medium, investigated diadromous brackishwater species drink sea water for the compensation of water losses. The analysis of swallowed water passed through the intestine and taken from the posterior part of the spiral valve demonstrates that her composition

significantly differs from the composition of seawater. Table 2. The swallowed water losses a considerable part of sodium and potassium, but becomes enriched by magnesium, whose concentration becomes significantly higher than concentration in seawater.

Sodium concentration in intestine liquid is lower than in sea water on 41.9% - in Russian sturgeon, on 45.7% - in starred sturgeon (Table 2) whereas on 90.5% - in shortnose sturgeon (Krayushkina,1998). In intestine liquid of investigated diadromous species, the magnesium concentration was higher in 2-3.5 times than in sea water.

In all probability, the sterlet also can swallow some sea water, because the diuresis is enough high, although an existence of water in the stomach and spiral valve was not obvious. In sterlet the intestine liquid is differed from sea water only lesser ($p<0.01$) concentrations of sodium (on 17%) and potassium (on 35%), whereas concentrations of magnesium and calcium don't differ ($p>0.05$) from sea water. Table 2. Thus, in a number of acipenserids it is possible to discover the elevation of functional activity of the intestine that is directed on sodium absorption from swallowed sea water.

In fresh water acipenserids is produced hypoosmotic urine, but during the adaptation to sea water, the diuresis is reduced (Table 1) and urine osmolarity increases at the expense of the rise of ion concentration. The urine becomes isoosmotic to blood serum or close to it in the osmolarity. Tables 1, 2. In a number "sterlet → Russian sturgeon → starred sturgeon → great sturgeon" percentage content of sodium decreases.. This is 84.9% → 58.6% → 47.1% → 28.0%, accordingly. Percentage content of magnesium increases in this number. This is 6.2%→ 26.9% → 33.5% → 54.5%, accordingly. It is means that in this case sodium-absorption and magnesium-excretion increase. It is important to note that high sodium content in the urine is distinctive peculiarity if ionic regulation in acipenserids compared to teleosts.

The results of present work show that there is difference in hormonal regulation for supporting of osmotic and ionic homeostasis in different species. This level remains high after seven days of experiment. Table 3. It is means, on the one hand, that sterlet experiences strong stress loading and, the other hand, that the mechanism of the realization of hormonal signal for adaptive changes is absent. Perhaps, fermentative systems of this species have no ability to work effectively in hyperosmotic medium. In Russian sturgeon cortisol level decreases during the first 12 hours of the experiment. I can shows that there is the utilization of the hormone by the organ-targets and also that the utilization of the hormone predominates under its biosynthesis. But cortisol level is restored to control level (in fresh water) to the seventh day of the experiment. Table3.In starred sturgeon cortisol level remains without the changes during all period of its living in sea water. Perhaps, there is co-ordination between processes of hormonal biosynthesis and its utilization. Table 3.

Conclusion.

Thus, by contrast with secondary freshwater sterlet, diadromous species have mechanism of hypoosmotic regulation that is able to support the relative constancy of serum osmolarity and ion concentrations under the changes of environmental salinity.

Species peculiarities in the function of osmoregulatory and ionregulatory systems in diadromous acipenserids are observed. In great sturgeon and starred sturgeon, the mechanisms of osmotic and ionic homeostasis are developed in more level than in Russian sturgeon. Accordingly to some parameters in starred sturgeon these mechanisms are more developed than in great sturgeon. By comparison with diadromous marine species, Caspian diadromous species are possessed of less developed mechanisms, because they were formed in species, dwelling in lower environmental salinity. Comparison of morphological-functional state of osmo- and ion regulatory systems in young fish of the same species, which were investigated before in the age of 1.5-2.5 months (Krayushkina, 1967, 1983), with present data testifies to non-completed development of these systems. The results of present investigation can be allowed for the practice of sturgeon rearing in fishery farms.

References

Bruevich SV (1937) Hydrochemistry of the Middle and South Caspian Sea. Moscow-Leningrad. Press of Academia of Science of USSR. 350 pp. (in Russian).

Krayushkina LS (1967). Development of euryhalinity in different species and ecological forms of sturgeons during early stages of their ontogenesis. *Transactions of Central Research Institute of sturgeon fishery farm.* Moscow: Food Industry. 1:181-195 (in Russian).
Kazemi R, Bahmani M, Krayushkina LS, Pourkazemi M and Ogorzalek A (2003) Changes in blood serum osmolarity and ultrastructure of gill chloride cells in young Persian sturgeon *Acipenser persicus* (Borodin) of different sizes during adaptation to sea water. *Zoologica Poloniae* 48/1-4:5-30
Krayushkina LS (1983) Level of development of osmoregulatory system of young sturgeons depends on size and age. In: *Biological principles of sturgeon Fish-Farming.* (Barannikova, I.A., and Berdichevski, M.A., Eds.). Moscow: Science: 158-166 (in Russian).
Krayushkina LS (998) Characteristics of osmotic and ionic regulation in marine diadromous sturgeons *Acipenser brevirostrum* and *A. oxyrhynchus* (Acipenseridae). *J. Ichthyology* 38, 8:660-668.
Legeza MI (1972) Role of abiotic factors for distribution of acipenserids (fam. Acipenseridae, Pisces) in Caspian Sea. *Questions of Ichthyology* 12, 1(72):13-24 (in Russian).
Silvestrova NYa (1973) Distribution of young acipenserids in Caspian Sea. In: *New data in fish economic investigations of Azerbaijan.* Baku: 48-62 (in Russian).

Table 1. Osmolarity of blood serum , urine and intestine liquid in different species of Caspian sea in fresh water and after adaptation to sea water 12.5‰ (409±2) during 7 days.

Species and environmental medium	Osmolarity, mOsm/l			Diuresis, ml/h/100g	Na^+ excretion with urine, mEq/l/h/100g x 10^{-3}
	Blood serum	Urine	Intestine liquid		
A. ruthenus (n-5 in control, n-6 in experiment)					
in fresh water	263.6±1.2	410.7±4.0*	-	1.13±0.05(5)	17.68±2.63(5)
in sea water	50.3±2.7	398.8±7.4*	416.5±3.5	0.58±0.09(5)	100.95±13.21(4)
A. gueldenstaedtii (n-6 in control, n-5 in experiment)					
in fresh water	262.3±3.4	309.0±2.3**	-	0.82±0.08(6) [3]*	19.0. ±3.81(7)
in sea water	56.8±5.6	296.4±8.1**	308±8**	0.21±0.07(5)**	25.42±12.17(4)**
Huso huso (n-8 in control, n-6 in experiment)					
in fresh water	279.6±2.2	319.8±1.7*	-	-	-
in sea water	70.5±9.8	323.2±7.1*	300.4±4.3*	-	-
A. stellatus (n-8 in control, n-6 in experiment)					
in fresh water	265.3±2.6	302.3±3.2*	-	1.05±0.09(8)	14.72±1.82(7)
in sea water	61.2±16.0	300.0±4.1*	295.7±6.5	0.17±0.04(6)**	20.15±4.92(4)**

Note: n – the number of fish
*) p <0.001, **) p< 0.01 - compared to control (freshwater).
In columns for diuresis and Na^+ excretion: **) p< 0.01, [3]*) p< 0.05 – compared to *A. ruthenus*

Table 3. Concentration of cortisol in blood serum of acipenserids in fresh water and seawater of 12.5‰. salinity

Species	Cortisol, ng/ml			
	In fresh water	3 h in sea water	12 h in sea water	7 days in sea water
A. ruthenus	19.29±6.36(5)	75.13±12.98**(5)	66.54±15.96*(3)	79.82±30.90(4)
A. gueldenstaedtii	21.77±4.16(6)	8.26±1.31(5)*	5.56±0.95(5)**	37.18±7.39(6)
A. stellatus	16.89±5.68(8)	-	12.76±1.94(5)	17.04±4.19(5)

Note: *) p<0.05; **) p<0.01 –compared to control.
The number of fish is indicated in brackets

Table 2. Ion concentrations of blood serum, urine and intestinal liquid in different species of Caspian sturgeons

Species	In fresh water					After adaptation to 12.5‰				
	Ion concentrations, mEq/l					Ion concentrations, mEq/l				
	n	Na^+	K^+	Ca^{2+}	Mg^{2+}	n	Na^+	K^+	Ca^{2+}	Mg^{2+}
A. ruthenus										
Serum	5	113.6±0.4	1.68±0.20	3.20±0.12	1.16±0.32	6	200.3±4.4*	2.00±0.15	5.40±0.53*	3.48±0.66**
Urine	5	17.2±1.3	0.70±0.21	0.72±0.19	0.66±0.15	6	163.2±5.3*	3.38±0.23*	8.80±0.57*	12.00±1.41*
Intestinal liquid		-	-	-	-	6	146.7±5.4*	1.63±0.18**	28.80±5.91	124.00±48.04.
A. gueldenstaedtii										
Serum	6	124.2±4.5	1.73±0.11	4.83±0.43	0.69±0.11	6	150.8±0.4**	2.01 ± 0.04^{3}*	5.17±0.13	1.65±0.26*
Urine	6	22.3±3.4	1.46±0.17	1.77±0.07	0.88±0.06	5	110.8±9.8**	3.00±1.09	27.96±3.61**	53.55 ± 17.67^{3}*
Intestinal liquid		-	-	-	-	6	103.8±10.1*	1.30±0.11*	27.40±2.15*	135.00±31.05**
Huso huso										
Serum	10	136.3±1.95	2.65±0.06	5.62±0.34	0.43±0.04	6	156.7±2.6*	2.82±0.13	7.08±0.04*	2.08±0.41**
Urine	8	40.3±4.9	1.42±0.27	5.16±0.54	.93±0.40	6	70.7±12.2*	2.15±0.25**	43.00±4.78*	152.50±10.66
Intestinal liquid		-	-	-	-	5	83.4±5.3*	1.53±0.23**	40.80±3.14*	164.76±15.10*
A. stellatus (n-8 in control, n-6 in experiment)										
Serum	8	121.9±2.7	1.67±0.09	3.65±0.14	0.71±0.04	6	133.0 ± 3.0^{3}*	1.78±0.08	3.97±0.10	1.61±0.09*
Urine	8	14.8±1.9	0.45±0.13	1.92±0.17	1.53±0.15	5	90.0±7.3 *	6.02 ± 2.03^{3}*	31.30±5.32*	59.06±14.04**
Intestinal liquid		-	-	-	-	4	97.0±9.0*	1.37±0.24**	25.95±3.63	238.50±41.67**
Sea water		-	-	-	-	6	178.7±2.0	2.53±0.04	20.23±0.75	65.00±2.03

Note:
n – the number of fish
*) $p<0.001$, **) $p<0.01$, ^{3}*) $p<0.05$ compared to control

Studying the role of Caspian Sea on Precipitation condition in the shores of the north of Iran

Hamid Reza Moradi*
*Assistant professor of resource college of Tarbiat Modares (teacher training) university, watershed engineering group, Mazandaran. Noor, Imam Khomeini Street. Tel: 0122-6253101-3 Zip code: 46414 Fax: 0122-6253499
Email:Morady5hr@Yahoo.com

Abstract:
Caspian Sea, the largest lake of the word, has a special role in environment circumstances of its regions. In this research, we have considered the role of Caspian Sea in precipitation in the shores of the north of Iran country.
Results show that three kinds of air mass and pressure system affect precipitation in the south shores of Caspian Sea: Siberia high pressure, low- pressure systems and emigrant anticyclones. Between those factors, most precipitation originates from Siberia high pressure, while most and strangest precipitation originates from emigrant anticyclones. Evaporation that produce by Caspian Sea, provide moisture for precipitation that originated from Siberia high pressure, while moisture for low pressure and part of moisture for emigrant anticyclones, provide from black sea, Mediterranean and Atlantic Ocean.
Necessary condition for tacking place of density precipitation in area while provide when in the south shores of Caspian sea in the surface of the earth settle a high pressure ridge that resulted from cold air expansion and exit a trough in the middle and upper level of atmosphere. It should be point out that cold airflow from high latitude to the south shores of Caspian Sea cause in increase of air pressure in area. With due attention to season and high temperature of seawater, when cold air pass over sea. The lower part of it become warm and humid and consequently become unstable. This humid and unstable air will be driven to the south coasts of sea by a anticyclone airflow. The exit of Trough in the upper level support ascent of airflow.
So, Higher of pressure in the surface of the earth and deeper of the trough in the upper level lead to more expectation of more intensity precipitation.
Key words: Caspian Sea, Precipitation of The north coasts of Iran, Synoptic maps, Iceland emigrant low pressure and Siberia high pressure

Introduction:
The Caspian Sea with a surface area of 392000 square kilometer is extended in latitude of 36 to 47 to the north and longitude of 47 to 54 to the east. Its length from north to south is 1200 kilometers and its average width is 300 kilometers. Iran, Russia, Turkmenistan, Azerbaijan and Ghazaghestan share the Caspian Sea. The southern wing of this sea constitutes the northern coastal line of Iran.
With a brief look at the rainfall in regions around the Caspian sea (table 1) and comparing it with rainfall at southern coastal line of the Caspian sea (table2), we can observe clear differences in annual and monthly rainfall of these regions (8). While the annual mean rainfall in northern, western and eastern coastal lines of the Caspian sea associates with arid and semi-arid climate at the same time it could be seen that in the southern coastal line of this sea the annual rate of rainfall has a range of 1815 mm in Bandar Anzali to 600 mm in Gorgan. The same condition applies to the temperature conditions of these regions (table 3 and 4) while the annual mean rainfall over the Caspian Sea is about 196 mm.

The southern coastal line of the Caspian Sea in addition to following the pattern general circulation atmosphere is under influence of local factors (10). The presence of the Caspian Sea as the main source of humidity and Alborz Mountains in south of the coasts and north and northwest winds in the region, together created an ideal ecological (climatic) conditions in the region, which is unique in the world. The presence of fertile soil, good temperature and sufficient rainfall has prepared the region for growth of different plants. so that the region possess dense forests with high commercial value and is considered as the main agricultural region in Iran. Despite this, the locals of this region frequently suffer from the drought and shortage of water at most of the time and they face with destructive floods, which imposes financial damages as well as heavy losses of lives (9).

Therefore it is logical that by identifying the mechanism, the creation, reinforcement and systematic move and expansion of climatically conditions dominating the region to benefit from its positive impact and to avoid its damaging consequences or to minimize them (11).

The main aim in this research is to study the role of the Caspian Sea in the rainfall of northern coasts of Iran.

Method and data:

In this research the climatic parameters of meteorology synoptic stations of Astara, Bandar Anzali, Rasht, Ramsar, Noshahr, Babolsar and Gorgan from 1959-93 together with meteorology maps of daily and monthly rainfall during 1971-72 to 1988-89 have been collected and being studied. The reason for choosing this period (1971-89) was the accessibility and availability of suitable maps during this period at Meteorology Organization of Iran. The region under study was chosen at latitude of 10 to 90 to the north and longitude of west 90 to east 90. The reason for choosing this boundary is to study the source and the way the active pressure centers influence the climatic conditions of the region. Since the two main factors in the rainfall are humidity and the ascend of air (3), hence in this study the maps of earth surface as well as contours of 500 hectopascal have studied for determining the instability conditions (2). The reason for choosing the 500 Hecto Pascal is that most of water vapor present in the atmosphere (about 0.90) is located in the layer under the contour of 500 Hectopascal (6). On the other hand, the waves formation in this contour is the best representation for determining the stable or instable conditions of a region (4). In this way, the waves formation and position of atmospheric active systems in connection with atmospheric condition dominating the northern coasts of Iran and the patterns dominating the region at the time of rainfall have been identified.

Results and discussions:

The rainfall and temperature positions of the stations studied have been provided in tables 2 and 4. The table No.1 shows the annual and monthly mean rainfall country around Caspian sea and the table No.2 shows the annual and monthly mean rainfall in the northern coasts of Iran. The tables 3 and 4 show the monthly, annual mean temperature in these regions, respectively. As one could see in the table No.2, there is no arid month in the southern coasts of the Caspian Sea. In all stations except Gorgan station, the highest rainfall was in autumn and the least rainfall was in spring. In Gorgan, the rainfall in winter and autumn were identical and are considered as the highest rainfall seasons and the summer season is considered as the most arid season. The highest humidity month in all stations except the Gorgan's station is October. May, June and July are considered as the most dried months. At Gorgan's station, the highest rainfall happens in March.

It could be said that the rainfalls in southeast of the Caspian Sea in some extent follow the regime of internal rainfall of Iran plain.

For comparing the rainfall position at southern coasts of the Caspian Sea with other coastal regions of this sea, the monthly and annual mean rainfall of some of these stations have been provided in table No.1. As it can be seen the rate of rainfall at the Caspian Sea region and its neighboring surroundings is very little and most of the time the average rainfall is less than 200mm. By averaging the 119-year statistics that presented by A.D.Doberolski, A.N. Kosario and O.K Leontif it was observed that the average rate of rainfall on the Caspian Sea is equal to 192 mm (9).

In analyzing synoptic meteorology maps the following results were obtained:

The northern coasts of Iran are under the influence of Mediterranean low pressures that reaches the Caspian sea via Turkey and black sea or it is directly influenced by migratory Island low pressure system that causes the invasion of the Caspian sea by cold weather via polar front or Arctic and would create plenty of rainfall, particularly in autumn, over Northern coasts of Iran by removal of humidity and warming during its passage over the warm water of the Caspian sea. When the migratory Island low-pressure system goes toward the southern regions and stabilizes over Eastern Europe. By expansion of cold front this low-pressure center goes toward the southern region, and the high-pressure ridge of northern Europe would settle over the Caspian Sea and gradually the north-northwest currents would settle over the Caspian Sea (5). With emergence of these currents, the cold weather of arctic would pass through the Caspian Sea and by absorbing sufficient heat and humidity from the sea become instable and particularly in western region of southern coasts of the Caspian Sea causes considerable rainfall. This rainfall would be more severe when the difference in temperature and water level is high and sometimes in some regions the rainfall would reach more than 300 mm in 24 hours (8). These rainfalls would continue until such time that the high pressure have not yet settle along the Caspian sea or on the other hand the currents are located in North-Northeast (figures No 1 and 2).

In other words, the severe rainfalls in southern coasts of the Caspian sea occurs when the earth surface is under the influence of high pressure ridge from cold type and the center of this high pressure is normally would settle in northwest of black sea (in eastern Europe). In front of this high pressure is a low pressure system, which is of Mediterranean origin, and it reaches south of Caspian Sea via Turkey and black sea and it causes the invasion of cold weather to the Caspian region via polar front (figure No 2). In contour of 500 Hectopascal, the occurrence of rainfall in the region together with settlement of ridge over the black sea, east to center of Europe and east of Mediterranean sea would cause a deep trough to be settled in the east of black sea in around eastern longitude of 40 to 50(figure No 3). When the center of this trough would be positioned around north pole and along the eastern Europe then by expansion of its cold front to the south, the high pressure ridge in the eastern Europe would settle over the Caspian sea and gradually with emergence of North-Northwest currents over the Caspian sea, the cold weather of arctic would pass over the Caspian sea and by absorbing heat and sufficient humidity becomes instable and particularly in western region of northern coasts of Iran would cause considerable rainfall. Therefore for the rainfall over the southern coasts of the Caspian sea is that to have trough in middle and upper atmosphere and on earth surface we would have front (specially cold front). More pressure on the earth surface the more would be the rate of rainfall, if other conditions would hold (such as humidity at different contours, presence of weather front on earth surface, presence of trough in upper contours and etc).

Reference

1- Angell, J.K., and J. Korshover, 1992."Relation between 300-mb north polar vortex and equatorial SST,QBO,and sunspot number and the record contraction of the vortex in 1988-89".J.Climate ,5,22-29
2- Divis, R.E.,and S.R.Benkovic,1992:"Climatological variations in the northern hemisphere circumpolar vortex in January". Theor.Appl. Climatol., 46, 63-74.
3- Ghaemi, H.1996, general meteorology. Samt Organization.
4- Harvey, V.L., and M .H. Hitchman, 1996;"a climatology of the Aleutian High"
. J.of the atmospheric sciences. Vol.53.No.14.
5- Kerang L.and A.Makarau.1994. Drought and desertification. Report series. World climate programme,WCASP (28) WMO/TD.No605,pp286.
6- Khairandish, M, 1984. Synoptic meteorology. Iran meteorology organization.
7- McIntyre, M.E., 1988: "how well do we understand the dynamics of stratospheric arming?" J.Meteorol.Soc.Jpn.60, 37-65. W
8- Morady,H.R, 2002. The polar vortex and Precipitations on the Southern Coast of the Caspian Sea
9- Morady,H.R, 2003. Synoptic Analysis of the Precipitation on the Southern Coast of the Caspian Sea. the Caspian Sea international conference.p.157.
10- Nash, Eric .R. and A. Newman 1996:"An objective determination of the polar vortex using Ertels potential vorticity ". J.Geop.Res.Vol.101.No.D5.9471-9478.
11- W.M.O/td 1990:"program on long-range forecasting research" (Nanjing, China,8-12 October 1990) No.362 .

Table1: The annual and monthly mean rainfall rate in stations around the Caspian Sea

Year	D	N	O	S	A	J	J	M	A	M	F	J	Month / Station
203.8	25.2	30	22.2	14.3	3.2	7.9	2.4	13.2	17.4	29	12.4	15.6	Hassanghly
143.1	13.7	20.1	15.9	5.5	3.5	5.6	2.7	14.6	16.9	23.2	13.4	14	krasnodesk
157.9	13.4	15.2	17.7	11.2	11	15.4	26	12.3	12.3	12.6	89	10.1	ghoriov
209.6	16.2	12.8	20.2	22.5	16.3	14.2	25.5	20.7	19.8	15.3	11.6	13.1	astarakhan
321.8	26.4	27.3	32.8	37.1	25.9	25.7	26.8	23.6	19.9	22.5	29.7	24.1	mkachkla

Table2: The annual and monthly mean rainfall rate in stations Southern Coast of Caspian Sea in duration 1959-1993

Year	D	N	O	S	A	J	J	M	A	M	F	J	Month / Station
1219.5	96	140.9	281.5	212	65.4	28.8	43.4	50.6	48.1	96.9	72.4	83.5	astara
1815.	219.	292.	367.	207	107.	41.	44.	50.	49.	112.	136.	187.	anzaly

7	9	6	4		3	2	1	6	9	2	2	2	
1416.9	173.3	202.2	236.1	138.3	73.6	39.6	39.6	58.6	56.7	116.5	130.1	150.2	rasht
1248.9	119.3	161.7	309.8	161.8	69.4	49.7	49.7	50.4	40.5	89.2	78.6	84.4	ramsar
1326.6	140.1	206.9	242.1	153.8	79.5	52.6	52.6	48.2	47.8	92.4	109.1	114.4	noshar
913.4	131.8	131.8	166.3	73.3	67.1	16.4	16.4	23.7	31.3	70.5	79	99.2	babolsar
599.8	64.2	60.3	69.2	37.9	31.5	30.6	30.6	46.8	45.2	76.9	63.7	55.2	gorgan

Table3: The annual and monthly mean temperature rate in stations around the Caspian Sea

Year	D	N	O	S	A	J	J	M	A	M	F	J	Month / Station
15.9	6.7	11	17.3	23.7	27.1	26.8	23.9	20.2	14.9	8.9	5.6	4.5	Hassangholy
14.5	5.2	9.2	14.6	21.7	27.1	27.5	23.9	19.7	13.5	6.8	2.8	2.2	krasnodesk
9.4	-3.9	1.8	7.8	17.5	25.2	26.1	23.3	11.9	11.2	-0.3	-8.3	-7.3	ghoriov
9.7	-1.5	6.3	8.7	17.5	24.3	25.5	22.3	18.4	11.2	0.9	-5.2	-5.5	astarakhan
12.1	3.5	7.9	13.1	19.8	23.8	24.6	21.5	14.4	10.2	3.7	0.5	0.3	Mkachkla
-0.4	-13.4	-7.8	-0.8	7	12.4	15.7	13.1	7.8	0.9	-7.5	-15.7	-16.6	Bayser

Table4: The annual and monthly mean temperature in stations Southern Coast of Caspian Sea in duration 1959-1993

Year	D	N	O	S	A	J	J	M	A	M	F	J	Month / Station
15.1	7.7	12	16.6	21.5	24.7	25.6	22.5	18.1	12.8	8.5	5.5	5.7	astara
16.1	9.9	13.5	17.8	22.5	25.4	25.2	23.4	18.8	13.1	8.6	6.7	7.1	anzaly
15.7	8.7	12.6	17.1	22	24.8	25.2	23	19	14.1	8.8	6.6	6.7	rasht
15.8	9.3	13.2	17.5	22.4	24.8	25.6	22.8	18.6	13.6	8.8	7	7.1	ramsar
15.9	9.6	13.4	18	22.6	24.9	25	22.4	18.3	13.4	8.9	7.1	7.3	noshar
16.9	9.8	14.1	18.6	23.7	26	26.5	23.9	19.7	14.7	10	7.9	7.8	babolsar

17.6	9.8	13.6	19.1	23.9	27.3	27.9	25.5	12.2	16.3	10.8	8.2	8	gorgan

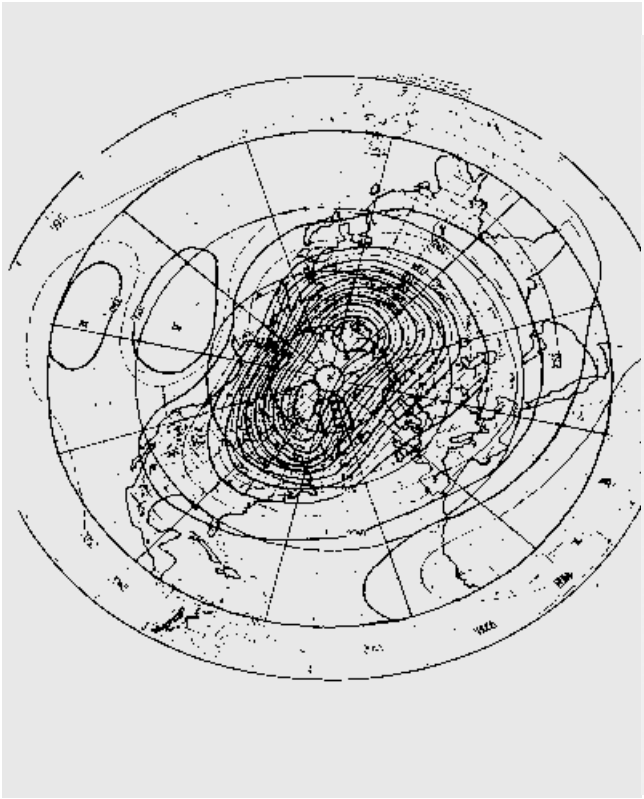


Figure 1: the synoptic map level 50 hp
Of the January 1972

Figure 4: the mean sea surface
Temperature Caspian Sea

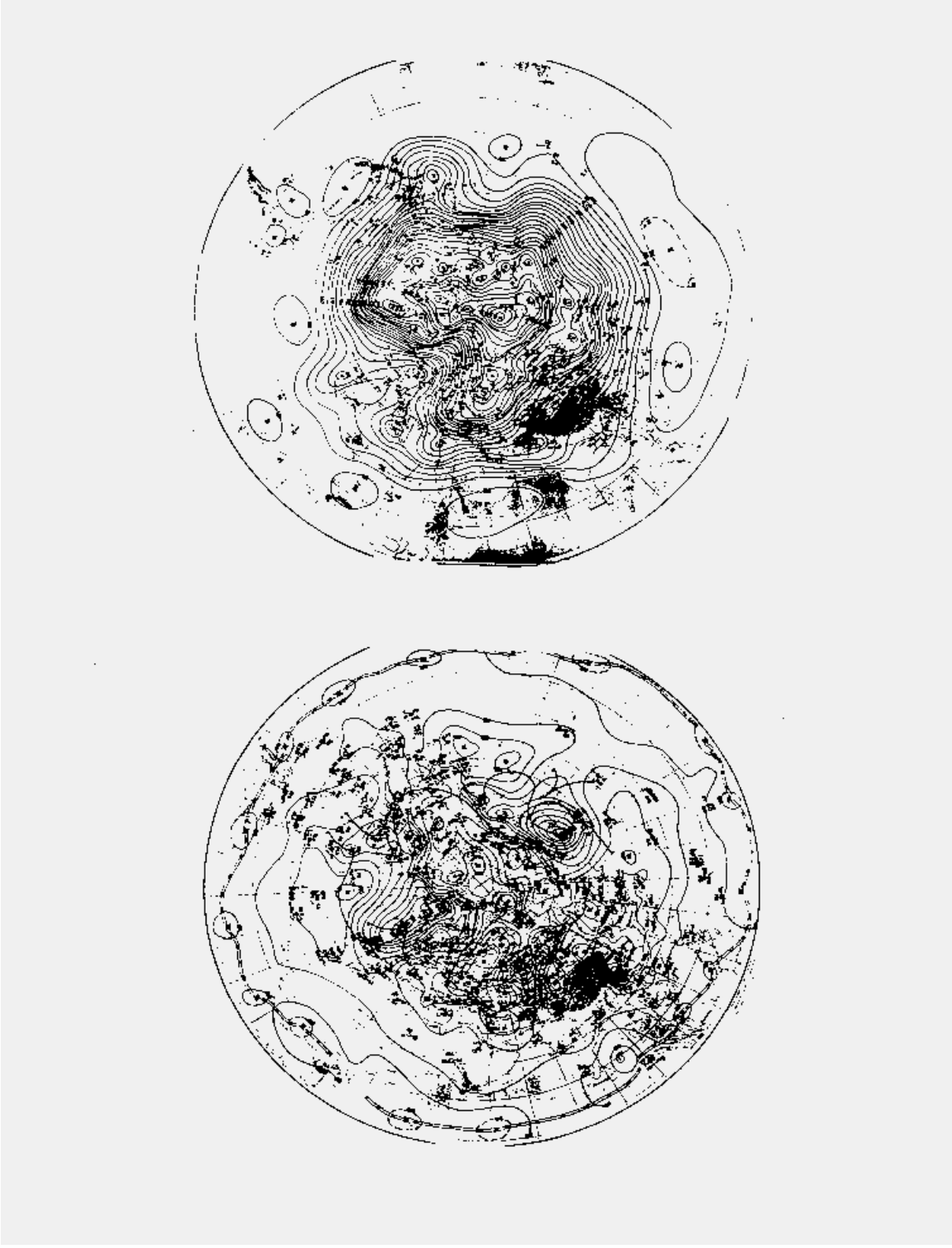


Figure 2(above): the synoptic map level 500 hp 2/1/1989
Figure 3(below): the synoptic map earth surface 30/11/1984

The survey of invasion effects of Cetenophora (*Mnemiopsis Leidyi*) on ecosystem of southern caspian sea.

Hamid Ramzani
Aquaculture Department, Ecological Academy of the caspian sea, Sari , Iran, P. O.Box 961. Phone: 0098- 0152- 346- 2498, 9. Fax: 0098- 0152- 346- 2495; E-mail: Hamid _ ramzani @ yahoo. Com

Abstract:
Caspian sea is the largest lake in the world that its extent is 438000 km. Sqr. Caspian sea takes into acount the largest lake in the world. It is located between Iran, Russian Federal Republic, Azarbaijan, Turkamanstan and ghzaghestan countries. Caspian sea has 114 species, 63 subspecies and 114 fish race. This ecosystem is one of rare aquatic ecosystem in the world. In the recent years, this ecosystem is exposed to threat by invasion of *Mnemiopsis Leidyi* (Ctenophora). Despite planktons are inportant in food chain of aquatic ecosystem. The planktonic survey carried out by ten tonnes vessel in less than 10m and 2500 tonnes vessel (gilan ship) in more than 10 m in the caspian sea. This survey took place before entrance years of cetenophore (1994-1996) and after entrance of ctenophore (1999-2000). The comparison of this survey indicated that the main changes of densites percentage and biomass of planktons observed. Before 1997 in phytoplankton groups crysophyto phylum has been dominant phytoplankton in the southern caspian sea where as 1999-2000 years, pyrophyto phylum has shown large increase in summer and autum seasons. Also, the zooplankton changes showed in coastal areas of caspian sea in 1991. The vertical and horizontal distribution of zooplankton varied in depths. The majority of zooplankton contain arthropoda phylom, copepoda order, naupli, cladocera order and rotiforia phylum. In 2001, during sampling from 50m depth, the marked decreasing in the percentage of zooplankton population observed. The general of eurytemora, calanipeda, podon, polyphemus and synchaeta in zooplankton population of caspian sea were not observed. After entrance years of cetenophora the survey showed the decrease of biomass in copepoda order. This decreasing is synchronous with increasing of cetenophora biomasss in many coastal areas, Before entrance of cetenophora, kilka catch reached from 90000 up to 95000 tonnes, but after entrance of cetenophora, in 1998 –1999 this figure was decreased, this figure reached to 45000 tonnes in 2001, annuanlly, the decreasing had social and economical effects. The cooperation of mariginal countries in the caspian sea, policy markers and university community want to biological challenge against cetenophora of caspian sea.

Key word: Caspian sea, *Mnemiopsis Leidyi*, Phytoplankton, Zooplankton, Kilka.

Introduction:

Caspian sea, with the extent of 438000 km^2 and having 114 pspecies, 63 subspecies,and 114 fishrace, isthelargst lake in the world. It's located between Iran, Federative Republic ofRussian, Azarbaijan, Torkamanestan and Ghazaghestan. Cetenophoras are a group of aquatic animals which are distributed throughout the world oceans. The species M. Leidyi (cetenephora) which lives in the coastal areas is a native in the waters of the northe oceans and the coasts of Florida and has a distribution in these areas, that is entered from the mentioned coasts to Black (1980) and Marmareh (1982) Sea, by the balanced ship carring grains, and then entered the caspian sea, through Volga- Don river. (Ivano, 2000, Dumont, 1995). Although it has a distribution in the coasts of five exploitative countries, but the ecological conditions in the southern caspian sea are more appropriate for this species to live. The studies of phytoplankton, Zooplankton, and kilka, before and after the entrance of cetenophora to the caspian sea show that marked changes in dispersion and distribution of phyto, zoo, and also decrease in kilka catch, have been occurred, kilkas are one of the native

and valuable fishes in the caspian sea, which play an important role in the economics of the marginal countries of the caspian sea such as, Iran. Since, M.L. has a common food chain with kilka, its resourses are exposed to the serious threat. The totals of the directand in direct employmees, in a didition to their families, in kilka catch industry, fish powder and conserve industry, are about 20.000 persons. In addition to the economical problems and social subordinations the continuation of the process, with respect to 40 percent decrease in kilka catch in 2001 have affected the resources of the other species, such as, caviare and bony fishes, that eventually will have dangerous social economical effects and ask the serious challenge for the researches managers and planning directors.

Matterials and Methods:

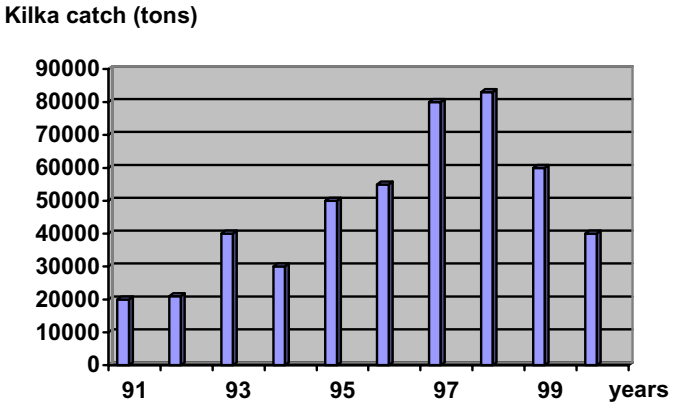
For sampling, less than 10m in depth, a 10 tonnes vessel and more than the depth of 10m a 250 tonnes ship (Gilan) was used. The number of the stations before the entrance of cetenophora, was at first,, 69 semilines perpendicular to the coast from Astara to Hosseingholi border, Then it decreased by 32 semilines. The number of these stations, regarding thegathered in for mations, decreased by 18 semilines after the entrance of cetenophora. The sampling was accomplished in a manner of marine full round in every season. The sampling in evry line, under the depth of 10m, by o, 5, and 10m, and in depth from 10m to 100m and more than it, was done, phyto's samples and the physical and chemical samples in the water was accomplished by Routener, but 200 samples was cought by a plankton catch net with 100 micron length and 36 cm^2 nozzle. Plankton samples were fixed in a 4 percent formalin and transfered in to the laboratory. (Newell, 1977). After the recognition of plankton species, by a revers Microscope in the volumes of 1, 0. (2 repetitions), and 10 ml,the samples with three- fold repetitions, was analysed quantitiatively and qualitatively in the laboratory. The statistical calendar of iran fisheries (92- 2001), has been used in analysing the changes of kilka catch, during the years after and before the entrance of cetenophora in the caspian sea.

Results:

Five main species in phytoplanktons at a depth of 10m are as follow: chrysophyto, pyrophyta, cyanophyta, Chlorophyta, and Euglenophyta, have been studied. The results showed that the main changes of the densities percentage and biomass of planktons oberved during 94- 2001. Sothat, in phytoplankton groups, chrysophyta, has been dominant, in the southern caspian sea, whereas, phrophyta phylum showed large increase in summer and autumn seasons. Also zoo plankton changes at a depth of 10m in 94- 95, 96-99 and 2001 showed the large decrease in zooplankton population in summer 2001, so that their abundance was about 1450 n/m^2 and their biomass was about 12.93 ml/ m^2, which is 26 percent less than the last years, and decrease has an effect on caoepoda populatiions, and the others species. Particularly, Meroplanktons in souther caspian sea. Also, the study of zooplanktons at a depth of 50m, show that the vertical and horizontal distribution of them, isl varied in depth in the coastal areas of the caspian sea, so that most zooplanktons pertain to Aqurthropoda phylum copepoda and Naupli order, and cladocera order. And also Rotatoria phylum but, we have observed a marked decrease in the percentage of zooplankton population, during sampling at as depth of 50m, in 2001, so that the genuses Eurytemora, carinipeda, podon, polyphemus and syncheta were not obserred in the zooplankton, population of the caspian sea. The results are the same, in 2002. These studies showed a marked decrease of biomass in copepoda order, and it was synchronized with the increase of cetenophora biomass in most coastal areas of the caspian sea. Figure-1 shows kilka's catch process during the last decade, in which it increased from 20.000 tonnes in 92 and 93 to 4000 tonnes in 94, and is decreased by 30.000t, but in 96, it reached to 5000t, tin 97, 55000t in 98, 90.000t, and in 99, 95000t, arisen from the increase of

the fishing activities, or in another words, the increase of fishing vessels, but Kilka. Catch decreased by 75000ts in 99 and 45000ts, in 2001. Planctonic changes and the decrease of Kilka catch were synchronized with the increase of cetenophora biomass, M.L., in the caspian sea.

Figure 1: Catch of the Kilka in the south of caspian sea (1991-2000)

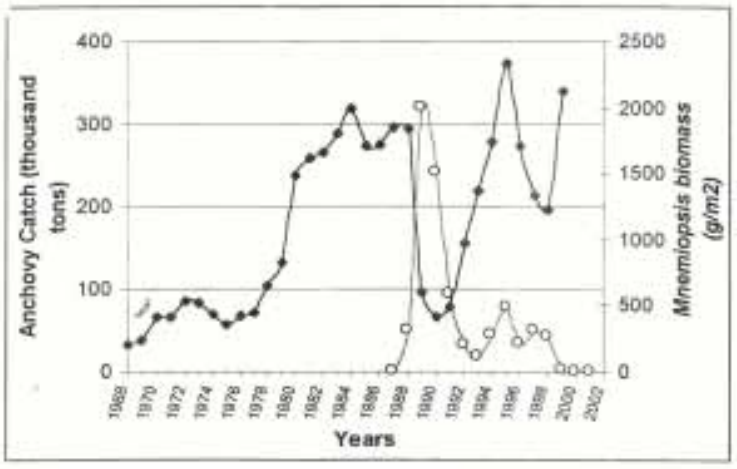


Discussion:

Mnemiopsis Leidyi, is a native in the waters of the northoceans, and the coasts of Florida, which lives in the waters of the northoceans, and the coasts of Florida, which lives in the coastal areas and has a distribution in these areas that is entered from the mentioned coasts to the Black (1980) and Marmareh (1982) sea, by the balanced ship carring grains. The entrance of cetenophora indicated 88% decrease in kilka catch, after 8 years. Figure-2 shows that the catch rate, was equaled 30.000 tonnes in 1968- 1970, and about 70.000 ts in 1972- 73, and 1976 reached 90.000 tonnes, but it indicated a decrease in 1976. Developing the industrial catch (fishing), this figure has shown an increasing growth, from 1977 to 1986, in Black sea, so that it reached from 50.000 tonnes to 10.000ts in 1986, but it decreased by 60.000ts in 1990, and synchronized with the increase of M.L. biomass. Facing the increase of the catch process, in 1990, they campaigned against cetenphora, biologically. The entrance of the jelly hunter to the caspian sea, through volga- Don channel is reported, in 1995, that is a threat. (Dlumant, 1995). The first report was recorded by M.L. first entrance to the coasts of Ghazaghestan in autumn 1999. Late september, the lake was full of M.Leidyi, and it merged the southern caspian sea, and volga- Don, because of the excessive saltiness of the Northern oceasns. (sharifi, 2002). Since, phytoplanktons, are important in the food chain of the aquatic ecosystems, these changes result in a change in phytoplankton, population, thus, planktonic survey, carried out by ten tonnes vessels, less than 10m in depth and 2500 tonnes vessels (Gilan ship), belonging to the Ecological Academy of the caspian sea, before and after the entrance of cetenophora to the caspian sea, sothat, the main changes in the density percentage and biomass of phytoplanktons, were observed, and it's because of the environmental ecosystem, changes and the entrance and the in crease of M.leidyi in the caspian sea. (Tahami, 2002). Since M.L. has a common food chain with kilka (nourished by zooplankton), the study of zooplankton, after and before the entrance of cetenophora was compared and

showed that zooplanktons, Population in the caspian sea affected by cetenophora invasion, so that a decreae in the varieties was observed, (Rohi Tabari 2001). The study of 68 samples cought from the coastal waters of Torkamanestan, Iran, Azarbaijan, and Russian, showed that the regional and vertical distribution of M. leidyi, in these arease, concentrated on the majority of the biomass of this migratory species, with 48 percent in the coastal waters of Iran, and then Azarbaijan, Torkamanestan and Russian, with 37, 14 and 1 percent, (Rohi, 2002) So the southern caspian sea has a better ecological conditions to multiply M.leidyi. There has been an increase in kilka catch in the caspian sea, in 90s. because of its valuable resources that reached from 20.000 tonnes in 1992 to more than 95000 tonnes, in 1999, but was decreased by 75000 tonnes, after the entrance of M.L. in 2001, and it reached 45000 tonnes in 2001, that indicates and annual decrease. By 30 percent in this species, (Ramezani, 2002). The increase of Kilka consumption by human, on the other hand, indicates an annual increase of 15% in the recentyears, (salmani, 2000). The total number of the vessels, catching kilka, are amounted to 183 vessels in the southern coasts of the caspian sea, with a capacity from 600 tonnes to 700 tonnes a year on the average. According to the statistical studies, based on catching 45000 tonnes in 2001, the catch average in these years, was estimated to be about 250 tonnes, the annual income of avessel, calculating 1000 Rials, each kilo, was estimated to be 25 million tomans, which equals the depreciation of the vessel's annual asset and the current expense, consequently, this level remains unchanged in 2001, since, the fisherman does not profit, it should not be expected that the vessels, practically continue their navigation. On the other hand, if the average cost of the vessels was 70.000.000 tomans, 10.000.000 tomans of it, is depreciating, (Ramezani, 2002). Also, according to these surveys, 40, factories, producing fish powder were exploited, with a production capacity of 56000 ts per unit. (equivlent to 250.000 to 280.000 tonnes kilka in Mazandaran units). The studies show that the factories have made use of one- third (1/3) of the capacity, in spite of the maximum range of kilka catch in 1999. If we take kilka catch into consideration used in the powder production, the maximum range of kilka catch is sufficient for 3 units of 2000 tonners or 6 units of 1000 tonner in the fish powder factory, If it remains unchanged, we won's profit from the investment in 34 units (equivalent to 45 milliard tomans in Mazandaran and Gilan province) and the expected
Employment in those units, so, it has injurious economical- social effects.

Figure 2: The frequency of Anchovy catch and Mnemiopsis Liedyi in the black sea



Recommendations:

1-The fishermen and the relevant convertible industries which are certainly considered as legal persons, Can claim for damage, sustained by the countries that are interfered in the entrance of cetenophora, by IRI government.

2-The catch of Kilka, should be stopped for 3 years in order to revive its resources.

References:

1-M.Mirbozorgi, A glance to the Caspian sea- Mazandaran fisheries. 1991.

2-A.Taghavi- The social- economical effects on the decrease of kilka catch, by the invasion of cetenophora- The first national conference of cetenophora in the caspian sea- The Ecological Academy of the caspian sea- 2000.

3-M.Sharifi- some of the ecological charactristics of M.L. in the caspian sea- The first national conference of cetenophora in the caspian sea- The Ecological Academy of the caspian sea- 2002.

4-A. Salmani. J., The Study of developing fish powder industries in Mazandaran province- The thesis of master of science, The ministry of performing Jihaj, I mam khomeiny center of sublime education, 2000.

5-F,. Tahami, The study of phytoplanktons change in southern caspian sea, after and before the entrance of M.L., The first national conference of cetenophora in the caspian sea, The Ecological Academy of the Caspian sea, 2001.

6-A. Rohi, An introduction to the Ecology of cetenophora in the caspian sea, 2000.

7-H. Ramezani, the stuey of M.L. invasion social economical effects on the southern coast of the Caspian sea, The first national conference of caspian sea, The first national conference of cetenophora in the caspian sea- The Ecological Academy of the caspian sea, 2002.

8-M.Roshan Tabari, the effect of M.L. on Zooplankton direction in the southern caspian sea. (lom in depth)- the first Conference of cetenophora in the caspian sea, The ecological Academy of the caspian sea, 2002.

9-Ivano PI , Am. Kama kin, VB ushivtzew, T. shiganova, O.Zhukoa, N. Aladin, SI. Wilson, GR harbison, HJ. Dumont 2000, Invasion of caspian sea by the comb Jelly fish mnemiopsis leidyi (Ctenophora), Biological In vasions 2; 955 –958.

10-Dumont H.J. 1995 , Ecocide in the caspian sea Nature 377 . 673-674.

The comparison of some important hematological and serum biochemical parameters in healthy and diseased Golden grey mullet *(Lisa auratus)* fishes in southern coasts of Caspian sea.

Ali Asghar Saeedi [1] , Hossein Ali Khoshbavr Rostami [1], Maryam Kamgar[1] , Ziba Rezvani [1] , Mohammad E. Jalil Zorriehzahra [2] , Issa Sharifpour [2] ,
[1] Ecology Research Station of Caspian Sea , Nowshahr , Mazandaran province , IRAN, P.O.Box 498, Telefax :0192367 2657 [2] Aquatic Animal Health & Diseases Dept., Iranian Fisheries Research Institute, (IFRO) , Tehran, Iran. E.mail: Maryam_KMR@yahoo.com

Abstract
Unknown acute morbidity has recently been occurred in Golden grey mullet fish of Caspian Sea. First announcement of the morbidity was reported from *Lisa auratus* species in Ziba-kenar beach of Guilan province in Feb.2004.
The affected fish weighted 200-250 g and clinical signs of the moribund fishes were erratic swimming behavior such as; spiral and belly-up at rest, lethargic appearance without any surface erosion. The post-mortem findings in affected fish which observed were gas accumulation and high distention in swim bladder, yellowish liver, liquefaction of gall in gall bladder, presence of excess micro sand accumulation in ceacum with hyper anemia of intestine . In order to investigate the etiology of the mortality, some hematological parameters include erythrocyte and Leukocyte count, hemoglobin, packed cell volume(PCV), mean corpuscular volume(M.C.V),mean corpuscular hemoglobin (M.C.H),mean corpuscular hemoglobin concentration(M.C.H.C), leukocyte differential count and biochemical parameter of serum such as serum glutamic oxaloacetic transaminase(S.G.O.T) were examined .
About 60 blood samples from *Lisa auratus* fish (30 diseased and 30 healthy)were obtained and compared. This comparison revealed that erythrocyte count, hemoglobin and packed cell volume were highly decreased in diseased fishes. So this differences in compare with healthy fishes were significant ($p<0.05$), but the amount of M.C.V , M.C.H and M.C.H.C were natural . The results showed that the diseased fishes faced a normocytic normochoromic anemia.
The number of leukocyte (W.B.C) was highly decreased in diseased fishes .In this case the diseased fish had leukocopenia. On the other side the enzyme amount (S.G.O.T) was highly increased in diseased fishes . Among the leukocytes and S.G.O.T there was significant differences in two groups of *Lisa auratus* fishes. The normocytic normochoromic anemia , leukopenia and increase of S.G.O.T in diseased fishes could be considered as an indicator of presence of probably a viral agent in the affected fishes. More investigation and comprehensive research project would be necessary to clarify and define the causative agent of the disease in the affected fishes

Key words: Caspian sea- Liza- Leukocyte- Erythrocyte- Hemoglobin- Haematocrit- Index of blood – transaminas Enzyme – disease

Introduction

The application of hematology science in the field of aquatic animals is seriously under development because they live in natural environment and can not be easily catch also there are different hematological parameters in various fish species . On the other side some factors like glucose and cortisole are changing regularly in the fish . This science started development since 1980 .

In Iran the aquaculture specially fish diseases in fish farms is going to be under development . Now days in order to releasing the sturgeon fish into the rivers that is necessary to measure the hematological factors. For instance the amount of hemoglobin protein fraction (Amany , 2003) are called as a typical factors .
There are various data bank regarding hematology of aquatic animals and these data belong to salmon and carp fishes with the aims of control production . In Iran in the field of hematological responses to environmental factors (temperature and salinity) including sturgeon fish (saeedi. 1998) has been well studied . In connection to cold water fishes (salmon) the picture of natural hematology were also well studied(Jamalzadeh , 2002). Also in Norvey the Bio- chemical factors (sendens, 1988) and the changes of hematological parameters of rain bow trout by fungicide toxic (tri- phenyl acetate) , in Germany (schwaiger. 1996) and also the amount of leukocyte , Erythrocyte and index of blood by viral necrotic Erythrocyte in U.S.A (Haley , 1992) has been also well studied .
The references and information indicates that the study of hematology in aquatic animals is depended directly proportional to physiological conditions . The changes of hematological and Biochemical parameters in fish diseases have not been studied enough. Our studied done was depended upon the
Etiological mortality regarding Mugil fish in south of Caspian sea .

Materials and methods

In order to sampling , the blood has been taken from the behind portion of anus in healthy and diseased Mugil fish by the use of syringe 5 ^{cc} .
Each blood sample was divided into two parts . Initially we added anticoagulant heparin In one of the samples (1 drop + 1^{cc} of blood) . This has been done for measuring the amount of W.B.C count R.B.C count . hemoglobin , haematocrit and index of blood (mean corpuscular volume M.C.V , (mean corpuscular hemoglobin M.C.H.C , mean corpuscular hemoglobin concentration M.C.H.C) and differential of blood count . we did not add anticoagulant heparin to other part of blood because we needed serum for measuring the transaminase enzyme (serum glutamic oxalacetic transaminase S.G.O.T and serum glutamic phosphate transaminase S.G.P.T).
1-The number of leukocyte were counted by the used of fluid dilution (Raice) , slide counter , Melangure white pipette with dilution of $\frac{1}{20}$ (count / mm^3) .
2- The number of Erythrocyte were counted by the used of normal saline dilution , melangure white pipette with dilution of $\frac{1}{200}$ (count / mm^3)
3- Hemoglobin was measured by the use of cyanomet hemoglobin , spectrophotometer with wave length 540 nanometer (gr / dl) .
4- Haematocrit was measured by the use of centrifuge of micro haematocrit, micro pipette(%).
5- Differential of W.B.C count was counted by the use of preparation of blood smear and then fixed by methanol , then stained of smear by Giemsa .

Results

Table 1 : statistical analysis of some important hematological parameters in healthy Mugil fish .

Parameters of hematologic al	R.B.C count$\times 10^4$ /mm^3	P.C.V %	Hb g/dl	M.C.V F l	M.C.H pg	M.C.H.C %	W.B.C count/ mm^3
Mean	406.8	45.5	11	115.2	27.9	24	48033.3
Std. Error of mean	21.5	1.3	0.49	517	115	0.516	4591
Std .Deviation	83.3	5.4	1.91	22.4	6.1	2	17780.8
Variance	6947.6	29.2	3.66	501.7	37.6	4	3.2+08
Minimum	201	37	8.5	91	22	2	21000
Maximum	537	53	15.4	184	45	29	86000

Table 1 : statistical analysis of some important hematological parameters in disease Mugil fish .

Parameters of hematological	R.B.C count$\times 10^4$ /mm^3	P.C.V %	Hb g/dl	M.C.V F l	M.C.H pg	M.C.H.C %	W.B.C Count/ mm^3
mean	298.3	33.1	9.1	111	30/5	27.2	19375
Std. Error of mean	24.9	28	0.97	4.1	2	1.08	2156
Std. Deviation	70.6	8.4	217	1117	5.6	3.05	5956.6
Variance	4987. 9	71.5	7.6	138.2	32	9.3	3.5+07
Minimum	148	16	4.1	95	26	24	8000
Maximum	368	42	13.7	134	44	33	27000

Discussion

The comparison of hematological parameters in two groups of healthy and diseases Mugil fish showed that the amount of Erythrocyte , hemoglobin and haematocrite in diseases Mugil fish were highly decreased but the amount of blood index including mean corpuscular volume M.C.V , mean corpuscular hemoglobin M.C.H, mean corpuscular hemoglobin concentration M.C.H.C have not been changed . we observed that the shape and size of erythrocytes were quite normal . The results showed that the diseases mugil fishes were faced by anemia .

This kind of anemia was as normocytic normochromic . on the other side we observed that the amount of W.B.C were highly decreased in diseased Mugil fish (Leukopenia) . The difference between the amount of leukocyte in two groups of healthy and diseased fish were significant ($P< 0.05$ t . test) but the amount of enzyme (S .G.O.T) was increased in diseased Mugil fish. We think that the tissues of liver , heart and kidney were facing disorder function . In this connection the study of pathology of mentioned tissues proved that situation .

The etiology of mentioned symptoms of paraclinical may be as follows :

1-The factor of haemorrhgia can not be caused by above mentioned symptoms and kidney tissues are not able to compensate the anemia .

2- toxicity probably is not an agent regarding above mentioned symptoms .

3- Leukopenia or decrease of leukocytes showed that the viral agent is a main factor for creation of diseases . The separation of viral from brain tissues proved this condition .

Reference

1-Austin .A , Austin D.A 1987 Bacterial fish pathogens disease in farmed wild fish.
2- Amani. G 2003 standard weight and age of sturgeon young fish for releasing . In Persian .
3- Haley .P. J .weiser , M.G 1985 Erythrocyte volume distribution in rain bow trout Vet- Res. 46(10) ; 2210-2212
4- Sandes . K , Lie .C . waagoboe .R.1988 normal ranges of some blood chemistry parameters in adult formed Atlantic salmon (salmon-salar) fish biology 32(1) –129-136
5- Jamlzadeh .H.R 2001 assessment of hematological parameters of Caspian salmo trutta .In persian.
6-saeedi. A.A and co-operations 1998 comarision of the W.B.C count and their differential count in sturgeon fishes. In Persian .
7-Schwaiger, Fent . Stecher.H ferling .H . Negele , R.V ,1966 effect of sublethal concentration of triphengl linacetate (TPTAC) on Raein bow trout (onchorhynchus-mykiss) Eviron.30(3): 327-334
8-Vosoghy . G 1997 hematological assessment on ordinary carp fish . In Persian.
9- Vosyliene . M.Z. 1990 physiological indicas of rain bow trout (onchorhynchus- mykiss) during short term exposure to copper Ecology 3:12-18

Investigation on Food Competition between Mullets (*Liza spp*) and Kutum Roach (*Rutilus frisii kutum*) along Mazandaran Coast of the Caspian Sea

Jafar Seyfabadi[1], Hossein Negarestan[2] and Nemat Naeech[3]
1- Marine Biology Dpartment, Marine Resources Faculty, Tarbiat Modarres University, Iran. Phone: +98-9122182335, Email: seyfabadi@hotmail.com; 2- Fisheries Research Organization; 3- Graduate student

Abstract
The possibility of feeding competition between two groups of commercially important fishes, mullets and kutum roach in the southern coast of the Caspian Sea along Mazandaran province was investigated using 213 randomly collected fish from three major beach seining sites. Feeding regimes were determined using numerical method, followed gravimetric method of the gut contents for further accuracy. Analysis of data was done using ANOVA and S.N.K. method. Emptiness or fullness of guts, food properties, frequency of prey occurrence index (Fp) was also determined.
Results showed that feed priority for roach included barnacle, bivalves and crabs, while for mullets included foraminifers, ostracods, bivalves and gastropods. Fp for roach was found to include barnacle, bivalves and crab, of which barnacle constituted about 61.7% of the gut contents; for mullets Fp included foraminifers, ostracods, bivalves and gastropods, of which foraminifers constituted about 64.2% of the gut contents. The investigation showed no feeding competition for the main items (benthos) in these fishes.

Keywords: Caspian Sea, Food competition, *Rutilus frisii, Liza spp.*

Introduction
Mullets*, Liza spp*. (successfully introduced into the Caspian Sea during 1930-1934, Berg, 1949) and kutum roach, *Rutilus frisii kutum* (an indigenous species), are considered as the most commercially valuable bony fishes along the Iranian coasts of the Caspian Sea, constituting about 83% of total beach-seining catch in 2003 (SHILAT, 2004).
Feeding habits of these fishes have been studied separately (Razavi, 1995; Zarrinkamar, 1996; Zarbalieva, 1995; Holcik, 1995; Ghadirnjad, 1996). Considering both species are benthic feeders and their annual catch composition rates fluctuate (sometime significantly), this study was conducted to find out whether food competition (in other words, the food resources availability) could be a contributing factor to these fluctuations along Mazandaran coast of the Caspian Sea.

Materials and Methods
1- Area
Three major beach seining sites were selected along Mazandaran coast for sampling: Khazarabad of Sari (53º 17' E & 36º 52' N) with sandy bed as the eastern- most site; Noor (52º 38' E & 36º 43' N) with sandy bed as the middle site, and mostly rocky shore of Tonekabon (51º 31' E & 36º 40' N) as the western- most site**.**

2- Sampling
Totally 213 fish samples (107 roach & 106 mullet) were randomly collected from the freshly caught fish that included various available sizes of both sexes. The samples' length & weight were recorded at collection sites and their digestive tracts were immediately removed and placed into separate containers containing 4% formalin to be analyzed for their contents in

laboratory. Sexes were determined through secondary sexual characteristics and observation of their gonads as well. Scales were taken from each sample for age determination.

3- Laboratory work

In the laboratory, factors such as length & weight of empty and full intestines and stomachs and weight of gonads were determined using 0.01 gram sensitive scale. The fish age was also determined using dermal scales (Bagenal, 1987).

For observation of eaten feed particles, the stomach and intestinal contents were emptied into Petri dishes and observed under microscope; planktonic particles were observed using Bogarov depression slide under invert microscope of 200 x or 400 x magnification. Identification of feed particles was done using identification guides of the Caspian organisms (Brishtina *et al.*, 1968; Broshkina *et al.*, 1968). For analysis of gut contents, numerical and gravimetrical methods were adopted (Biswas, 1993).

Results

1-sex & age

Females constituted 60% of roach and 67% of mullet samples. Majority of all samples were in age groups of **3+** & **4+** and 1+& 7+ constituted the least.

2-Degree of tract fullness

The degree of digestive tract fullness is shown in **Table 1.**

Table 1: Tract fullness (%) of roach (Ro) and mullet (Mul) samples (individuals) in various sampling months

Sampling Months / Degree of fullness (%)	Jan-Feb		Feb-Mar		Aug-Sep		Sep-Oct		Oct-Nov		Nov-Dec		Total	
	Ro	Mul	Ro	Mul	Ro	Mul	Ro	Mul	Ro	Mul	Ro	Mul	Ro	Mul
0	1	0	0	3	0	1	0	0	2	0	0	0	3	4
25	6	14	14	4	12	4	6	9	4	10	9	10	51	48
50	9	0	2	7	5	9	10	6	9	6	6	6	41	38
75	1	1	2	0	1	4	2	3	3	2	3	2	12	11
100	0	1	0	4	0	0	0	0	0	0	0	0	0	5

Comparing the index of fullness (IF), the highest and lowest intensity of feeding for roach was observed in Nov-Dec and Feb-Mar, respectively, while those for mullet were observed in Oct-Nov and Feb-Mar, respectively.

3- Feeding priority

Among benthic organisms*, Balanus sp*, *Cerastoderm lamarcki*, *Abra ovata, Mytilaster lineatus, and Dreissena sp.* were found to have the highest priority in roach feeding, while the highest priority for mullet was found in Foraminifers, Ostracods, *Cerastoderm lamarcki*, and Gastropods

Discussion

Various methods such as numerical, gravimetrical and volumetric ones have been employed in determination of fish feeding regime. In this work numerical method mainly and gravimetrical method to limited extent was employed. Although these methods reveal somehow similar results, employing numerical method alone would not be enough, besides being defective in certain respect to get the desirable result. For example, although the weight of shell or sand content of the digestive tract is far greater than the planktonic content, nutritive values of the former could be far inferior to the latter one. For the very same reason,

determination of feeding regime through numerical method alone appeared to be doubtful and, therefore, we also employed gravimetrical at the end. Numerical method can, however, be good enough method in determining Food Priority and Frequency of Prey Occurrence (Fp) indices.

Benthos and detrital residues constituted more than 99.9% of the tract content of both roach and mullet, which indicated the negligible role of plankton. The planktonic item was, most probably, devoured non-selectively.

ANOVA & SNK test of collected samples from three sites revealed significant difference ($P<0.05$) in the main food items and degree of tract fullness in various months for roach and mullet. No significant difference ($P>0.05$) in food items in various collected size groups and sexes of both roach and mullet was observed.

Conclusion

The investigation showed no feeding competition for the main items (benthos) in these fishes. However, more work employing various methods is required to come to more accurate results.

References:

Biswas, S. P. 1993. Manual of Methods in Fish Biology. South Asian Publisher. New Delhi, India.

Berg, L. S. 1949. Freshwater Fishes of USSR & Adjacent Countries. Israel Program for Scientific Translation. Vol.3. New Delhi.

Bagenal.T.1987. Methods for Assessment of Fish Production in Freshwater. Blackwell Scientific Publications. Oxford .UK

Brishtina, Y. E. *et al.* 1998. Atlas of the Caspian Sea Invertebrates (Translated into Persian).

Ghadirnejad, H. 1996. Population Dynamics of Grey Mullet Species (*Liza aurata* and *L. saliens*) in the Southern Caspian Sea. Ph.D. Thesis.

Holcik, J. 1995. New Data on Ecology of *Rutilus frisii kutum* from the Caspian Sea. J. Ecol. Fresh.Fish.Vol. 4, no 4. pp.175-179.

Razavi, S. B. 1995. Whitefish, *Rutilus frisii kutum,* (in Persian). Fisheries Research Institute, Ministry of Jahad-e- Sazandegi, Tehran, Iran

Zarbalieva, T.S. 1987. Feeding of *Rutilus frisii kutum* off the Western coast of the South Caspian Sea. J. Ichthyo. Vol. 27, no. 2, pp.347-350.

Zarrinkamar, H. 1996. Physiology and Feeding Habits of *Rutilus frisii kutum* in Bandar Anzali Aera. M.Sc, Thesis (in Persian). Islamic Azad University, North TehranUnit

The ecology of the Caspian sea & the present status of sturgeon stocks in this ecosystem

Aboulgasem shakiati
Member of board of Academy Mirza Kochak Khan Higher Fisheries Education Centre, mirzaedu@yahoo.com

Abstract
Caspian sea is the largest landlocked lake of the world lactated in southeastern Europe bordering Asian continent. The lake is 1039 – 1200 Kms in length & 435km in width & occupies a surface area of 378 – 390 thousands square kilometers with a total water volume of 76000 – 78000km3 comprising approximately 44% of the whole lake waters on the planet earth.
In terms of maximum depth, (1025m), the Caspian sea matches with a number of open seas such as Baltic sea, Black sea, & yellow sea & is larger in size than Adriatic sea, sea of Azof, Aral sea & Aegean sea. (GOR – S – Zonn 1999) Tab. No1
Table one clearly shows the Vast expense of the Caspian Sea in Comparison to other major lakes in the world.
The area & water volume of the Caspian Sea and the periodic fluctuation force of the Surface water are considerably variable. The water shed area of the Caspian sea covers 3.1-3.5 million square kilometers, comprising nearly 10% of the total surface areas of the worlds landlocked lakes. (Golubev 1997) which stretches some 2500 kilometers in length from north to south with an average breadth of 2000 kilometers from west to east. The watershed area of the Caspian Sea is approximately 127. 0f
The Caspian Sea coasts are now scattered along 5 countries namely as Russia, Iran, Azarbaijan, Turkmenia & kazakstan whereas the watershed areas include 9 countries altogether including Georgia, Uzbekistan, Armeinia & Turkey .
Except for its being cut off from the Oceans of the world which occurred some 6 million years ago, the Caspian Sea enjoys all the marine characteristics of the open Seas which have been left since the geological era.
The Caspian Sea level is currently 27 meters below the Ocean level & the Sea is divided into three distinct hydro biological zones: the northern zone, the middle zone & the southern zone. In terms of surface area, the northern middle & southern zone occupy 25%, 36% & 39% respectively.
The maximum depth of these three zones are 27m,788m, & 1025m and the average depth are 4.4 , 192, & 345 meters respectively.
Some 20%. of the northern zone is less than one meter deep. (Fig 1.2)
The natural characteristics of such an immense ecosystem as the Caspian are dependant upon the physico – geographical conditions. The determinant hydrabiological , hydrchemical & biological products & marine plants are liable to natural climatic conditions, which result in the production of biogenetic material & movement of organic substances.
The northern zone of the Sea which let in 90% of the incoming river waters are mainly shallow) (the maximum depth in areas bordering middle zone is 25 – 30 meters). In this zone the maximum annual temperature (July) is 25 – 26 centigrades whereas in shallow zone the temperature is 28 – 29 centigrades. The horizontal distribution of temperature appears monothermically & it is only in the middle zone that high temperature thermocling appears.
(Kosarev 1975). The northern Caspian Sea with its lower salinity water gets warm in summer time & during this period the water become rich in nutrients & nutritional materials which are consumed by young fish and adult fish (i.e. sturgeons, shad fish carps etc.)
which are commercially valuable.

Ichtyophauna of the sea includes more than 124 kinds and subspecies, and 30 fish species have commercial importance while in marine part there are less than 5-6 kinds of fish. At the same time, it is unique in the world as a pool, where the genenec diversity of six kinds of sturgeon fishes was kept. On the food qualities and cost, both on internal, and in the international markets the sturgeons and specially their caviar fall into the most valuable fish goods, their role in economics of a number of basins is extremely great.

In view of large value of sturgeons ancient times they served as an object of catch. The history of sturgeon catch in the Astrakhan area and in the Caspian sea is very great it began more than 2500 years ago. Caspian sturgeons are mentioned by Arabian writer Ibn-Fakih, European.

Travelers in particular Marco polo and Olearius. Russians in 15-16 cc caught sturgeons in Volga and in influxes. The history of the organized marine fish catch on a Caspian shore began from time of the Astrakhan coast conquest by Russians.

As shown in the table 1 after disintegration of USSR sturgeon catch fell sharply on the Caspian sea. Our country of Iran has about 80 years of history of sturgeon catch in the Caspian sea.

After disintegration of USSR sturgeon catch also came to a low level each year, it seems that the sturgeon reserves are in a position of disappearance.

This process of sturgeon catch reduction was unprecedented in all coast of the Caspian sea.

Though it is impossible to see the poachers in this sea, it is possible to say, that the volumes of it is great and even more, than volume of official sturgeon catch of all Caspian states. At all events, the process of sturgeon catch reduction displays the decay of reserves of these

valuable fishes and is the warning of disappearance of this living fossil. Reasons of reduction of sturgeon reserves are these:

1.Disintegration of USSR

Disintegration of the soviet Union and as a corollary the destruction of the centralized management system of bioresources of the sea, absence of legal fundamentals of international regulation of fishery, considerable reduction of a volume of marine researches in the last years have complicated solution of a problem of preservation of fish reserves on the Caspian.

2.Poaching

After disintegration of USSR the capability of sturgeon catch regulation and control have practically vanished. The reduction of sturgeon in Caspian basin 1990s was called by wide area illegal poaching. Owing to broad development of marine and river harpoon fishing the number of sturgeons is permanently reduced and now there is an actual hazard of their full annihilation.

3. Contamination

Increase of anthropogenic press on the ecosystem of the Caspian sea – contamination by pesticides, petroleum, heavy metals – first of all perniciously render harm to sturgeons.

Besides industrial, agricultural and domestic waste run into the rivers and then in the sea.

4.The aggravation of conditions of natural sturgeon reproduction in the majority of the running rivers in the Caspian sea was caused by construction of dambs which display negative effect on sturgeon migration and their natural reproduction. Annually many sturgeons perish in power stations, channels and water pumps, which exist in the rivers.

5. Reduction of artificial propagation

Before disintegration of the Soviet Union they have annually let out above 100 millions fry in the Caspian sea, especially on Volga. After disintegration of the Soviet Union this quantity decreased up to 40 millions fry.
But the artificial propagation of sturgeons in Iran had heightened the process and last years Iran lets out more than 25 millions fry in the Caspian sea.
6. The irregularity of official catch of Caspian countries Despite various meetings and conferences and conclusion of the agreements on sturgeon catch in the sea, unfortunately now sturgeon sea catch Bbiiob goes on and this is a large step to disappearance of these valuable fishes.

The conclusion and the proposals
In view of above-stated presentations, all Caspian states should know, that if they do not solve without delay these problems of sturgeon resources conservation as a living fossil of the world, in the near future we shall lose this divine gift and it will remain only to regret and repenting. Thus there are indispensable steps for the coordination and cooperation of the.
Caspian states for Fulfillment of the following proposals;

1. Preservation and permanent support of Caspian sea and river water animals;
2. Recovery of natural spawning sit for sturgeons by the Caspian countries;
3. Containment of input of agricultural , industrial and household waste and specially that from oil mining;
4. Special attention of the Caspian states should be concentrated on artificial propagation and increase of fry volume for release in the Caspian Sea;
5. Observation of the principle rules of sturgeon catch by Caspian countries and containment of unripe fish catch.

If we abide by the above-stated proposals, we can restore the reserves of these valuable fishes, but if we shall not get good outcomes , it is necessary completely to prohibit sturgeon catch in the Caspian Sea and in its rivers.
References :
1-Bagdanov.M.E The degeneration & death of Russian sturgeon 1990. " Ecological issues ". Moscow publications inc.
2- Berg. A.L.S . The climate of USSR 1938.
Moscow Publication inc.
3- Graskin .P.L & Katonin O.N. The fish & oil of the Caspian Sea. 1996.
No: 4 P.46. Moscow publications inc.
4- Kasimov . A. 1996. The ecology of the Caspian Sea. 1996.Translated by .A. Shariati.
5- A. Shariati .2004. The Caspian Sea fishes & it's watershed areas Mehr Publication inc.
6- 2nd & 3rd International Conference of sturgeons – Russian 2002-2004.

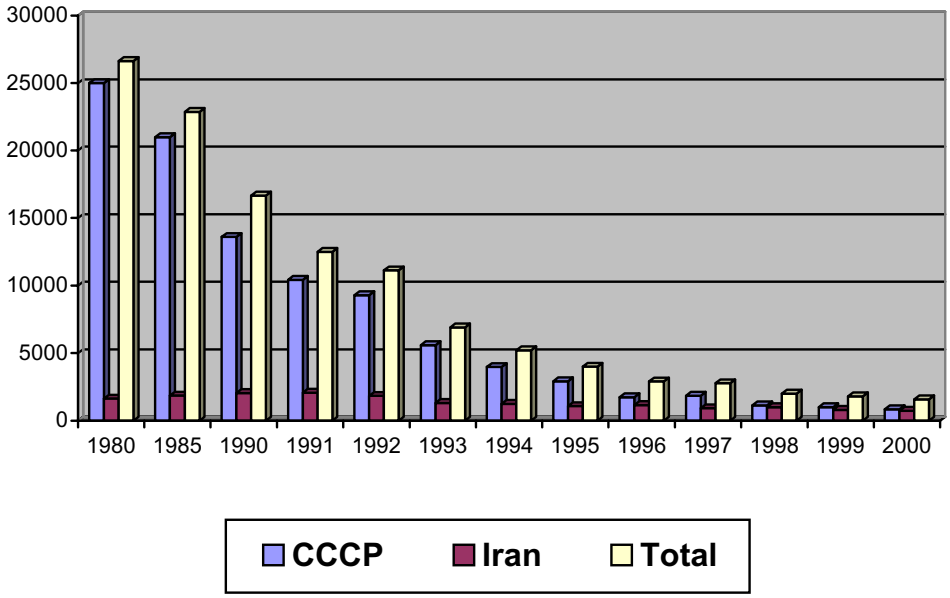
1. Caspian Sea versus other open Seas

	Sea	Area km2	Maximum Depth	Average depth	Volume of water km3
1	Caspian Sea	37100	1025	197	75000
2	Black Sea	423000	2245	1271	537000
3	Baltic's	39700	450	86	22300
4	Barents Sea	11 + 05000	1000	229	232000

5	Persian Gulf	239000	280	20	47800
6	White Sea	90000	400	60	5400
7	Aral Sea	64000	68	15	960
8	Sea of Azof	37600	13.5	7/2	320

The table 2. Sturgeon catch in the Caspian sea in 1980 – 2000,tons

Years	Of the state		Total on caspian
	USSR(Russia, Kazakhstan,Azerbaijan, Tyrkmenistan)	Iran	
1980	25000	1640	26640
1985	21000	1865	22865
1990	13600	2052	16652
1991	10423	2072	12495
1992	9289	1844	11123
1993	5587	1317	6904
1994	3975	1244	5219
1995	2920	1084	4004
1996	1784	1165	2913
1997	1845	928	2773
1998	1137	1003	2010
1999	1000	810	1810
2000	846	733	1579



Toxicity and LC50 of phenol and naphthol in *Abramis brama* and *Rutilus frisii kutum* fingerlings

Shariati, F[1]., A. Esmaili Sari[2] and M. Piri[3]
1-Faculty of Islamic Azad University, Lahidjan, P.O.BOX :1616,Tel: (0141)2229081-4, E-mail: shariat_20@yahoo.com; 2- Faculty of Natural Resources and Marine Sciences, Tarbiat Modarres University, P.O.Box: 46414-356 Noor,Iran; 3- Golestan Fisheries Research Center, P.O.Box: 130 Gorgan, Iran

Abstract
Acute toxicity of phenol and 1-naphthol compounds in vitro were studied in the bream Abramis brama orientalis and the carp Rutilus frisii kutum. Tests were conducted in static system for a period of 96 hours. Important physical and chemical parameters in water such as temperature, pH, dissolved oxygen, hardness and electrical conductivity were monitored during the experiment. Tests were conducted using 5 different treatments and 3 replicates were run for each treatment. Results obtained were analyzed statistically using computer-based software including statgraph, Quatro pro and Probit Analysis.
LC50 96h for phenol and 1-naphthol were 2.1593 and 2.5188 mg l^{-1}, respectively in R. frisii kutum and 25.1880 and 2.8490 mg l^{-1}, respectively in A. brama orientalis. The Maximum Allowable Concentration (MAC) for phenol was 2.1593 mg l^{-1} in R. frisii kutum and 2.5188 mg l^{-1} in A. brama orientalis, whereas that for 1-naphthol was 0.2154 mg l^{-1} in R. frisii kutum and 0.2849 mg l^{-1} in A. brama orientalis. It is evident from the results obtained that R. frisii kutum is more vulnerable to phenol and 1-naphthol toxicity as compared to A. brama and 1-naphthol compounds are more toxic than phenolic compounds for both species.
Key words: Abramis brama orientalis, acute toxicity, LC50, 1-naphthol, Phenol, Rutilus frisii kutum,

Introduction
Significant amounts of oil enter the sea every year as a result of communication and transport of oil tankers, river effluents, atmospheric precipitations, natural seepage, domestic effluents and accidental release. At present the number of oil tankers commuting annually has exceeded the limit by thousands and it is predicted that the use of oil resources will show a marked increase in the coming years. Phenolic compounds are among the several compounds found in oil and sewage of refineries that may be mono functional (phenol, kresol, naphthol and xylenol) or poly functional (pirokatcol, resocine, hydroxine, pyrogalol and floroglucin). Apart from this phenol is also used in the manufacture of several aromatic compounds including explosives, chemical fertilizers, illuminating gases, dyes, tires made from asbestos, detergents, dyes, synthetic resins, substances to protect wood, textiles, drugs, perfumes, rubber (with derivatives) other plastic materials such as phenol and formaldehyde. Phenol is also used in the industries such as leather, paper, toys, tanning, dyeing, painting and agriculture(Sanayee,1977).Sewage from aluminum and automobile factories, manufacturers of organic chemicals, steel mills and oil refineries and sewage from hospitals also contain phenolic compounds (Khorshidi Rad, 1985; Ramezani Gorabi, 1990).
These compounds are considered the most toxic compounds to aquatic organisms. On the basis of available literature the amounts of these substances in some regions of the Caspian Sea have increased as an effect of exploration of oil resources and also the inflow of rivers in the drainage basin. Thus the toxic effects of phenol and 1-naphthol were studied on two commercially valuable species, *Rutilus frisii kutum* and *Abramis brama orientalis,* of the Caspian Sea (Bazrafshan, 1994; Hossein Ziyabari, 1995; Taghipour, 1998 and Shariati, 1999). In another study the LC50 96h for phenol for marine fish species has been reported in the range of 5.6 to 30.6 mg l^{-1} (Kondaiah et al., 1994). According to earlier studies the LC50 96h for phenol in rainbow trout, *Onchorhynchus mykiss* was 10 mg l^{-1} in a static test, while

that for bighead minnow Pimephales promelas was 24.9 mg l^{-1} in another study (Anderson et al., 1979 & De Graeve et al., 1980). Similarly the LC50 96h for 1-naphthol in *Channa punctata* was reported as 2.99 mg l^{-1} (Tilak, 1981).
R. frisii kutum, the stocks of which are replenished through artificial breeding, is one of the most commercially important bony fishes in the southern shores of the Caspian Sea. Every year more than hundreds of millions of fry are produced and a large percentage of them are released into the outlets of the Anzali Lagoon. *A. brama* is also a commercial species of the Caspian Sea. Catch amounts for this species have plunged in the recent years mainly as a consequence of water pollution (Piri & Ordog, 1998).

Material and Methods
Tests were carried out using extra pure compounds of phenol and 1-naphthol. *R. frisii kutum* and *A. brama* fingerlings weighing 0.5-2.5 g each were obtained from the Shahid Ansari hatchery and transferred to the laboratory where they were acclimatized to laboratory conditions for at least one week. Five treatments were used and 3 replicates run for each treatment. Totally 16 aquaria each filled with 20 L water were used for the tests. To remove chlorine, water in the aquaria was aerated for 24 h prior to stocking them with fingerlings. This resulted in lowering the water hardness as well. Different concentrations of the toxins, determined logarithmically, were prepared using distill water. Ten fingerlings were then introduced to each aquarium. The stocking density of fingerlings was maintained at 1 g l^{-1} (TRC, 1984). Tests were repeated several to obtain a true value.
Important physical an chemical parameters of the water in the aquaria such as water temperature, dissolved oxygen concentration, pH, hardness and electrical conductivity were monitored on a daily basis (several times a day for some parameters) throughout the different experimental phases. Central aeration was supplied to the aquaria and dissolved oxygen concentrations were maintained at higher that 5 mg l^{-1}. Mortality of fishes was recorded at the end of each day, dead fishes were examined and any kind of external symptoms were recorded. Experiments were conducted for a period of 96 h following OECD methods (TRC, 1984). All results obtained were analysed using computer based software such as statgraphics, Quatro Pro and probit analysis an the experimental doses for 24, 48, 72 and 96 h were calculated for *R. frisii kutum* and *A. brama orientalis.*
The experimental doses for phenol were 20.1, 21.9, 24.0, 26.4 and 28.8 mg/l for *R. frisii kutum* and 21.0, 23.10, 24.9, 27.3 and 29.7 mg/l for *A. brama orientalis*. Experimental doses used for 1-naphthol were 2.0, 2.2, 2.45, 2.7 and 3 mg/l for R. frisii kutum and 5.0, 6.0, 7.0, 8.3 and 10.0 mg/l for *A. brama orientalis*. The sensitivity of the two species to the two compounds was calculated. Fishes under study were not fed during the experimental period and all experiments were carried out at 25 ± 2 ºC.

Results
Phenol:
The morphological effects of phenol were similar in *R. frisii kutum* and *A. brama orientalis* fingerlings. It was observed that at low concentrations fishes belonging to both species exhibited rapid swimming and abnormal behavior. In addition to hyperacivity and erratic swimming, fishes also showed loss of equilibrium. At higher doses fishes exhibited backward and vertical swimming movements and after prolonged exposure the color at the body surface turned light. Protrusion of eyes (exophthalmy), increased mucous on body surface, blood patches around eyes and congestion of gills were among the other symptoms observed in some fishes.
The LC90, LC50, and LC10 at 24 h, 48 h, 72 h and 96 h for phenol were calculated from the data obtained from the experiments for *R. frisii kutum* and *A. brama orientalis.* The results

obtained are presented in Table 1. It is evident from the results obtained that highest mortalities caused by phenol occurred in the first 24 h. The Lowest Observed Effective Concentration (LOEC) and the Non-Observed Effect Concentration (NOEC) and Maximum Allowable Concentration (MAC) were calculated from the LC values obtained (Table 2).

1-Naphthol:

Loss of equilibrium and lethargic movements were observed in fishes. After being exposed to 1-naphthol for sometime the color on the body surface turns light and mucous secretions increase on dorsal surface. Congestion of gills and protrusion of eyes was also observed and recorded in some of the fishes studied. Chemical parameters such as pH, hardness and electric conductivity were not influenced by the addition of 1-naphthol to aquaria.

The LC50 96 h for 1-naphthol in *R. frisii kutum* and *A. brama orientalis* are presented in Table 3. The LOEC, NOEC and MAC for 1-naphthol are shown in Table 4.

Discussion

It is evident from the results obtained that toxicity for 1-naphthol is greater than that for phenol in *R. frisii kutum* and *A. brama orientalis*. These findings correlate with those of other researchers conducted on other fishes (Shene and Lonning, 1981; Chen and Rong, 1991; Noga, 2000; Crookes and Howe, 1996; Tilak, 1982; Dang and Masurekar, 1985; Mason, 1996; Svobodova, 1993).

R. frisii kutum is more sensitive to phenol than *A. brama orientalis*. The sensitivity factor is calculated using the formula:

$$\text{Sensitivity factor} = \frac{\text{LC50 for } \textit{A. brama orientalis}}{\text{LC50 for } \textit{R. frisii kutum}} = \frac{25.1888}{21.593} = 1.17$$

R. frisii kutum is 1.32 times more sensitive to 1-naphthol as compared to *A. brama orientalis*.

On the basis of the results obtained from the present study LC50 96 h for phenol for *R. frisii kutum* and *A. brama orientalis* are 21.593 and 25.188 mg L^{-1}, respectively. These values are very close to those obtained by other researchers and are also within the range reported by Berton et al (Anderson et al., 1979; De Graeve et al., 1980; Berton et al, 1998). The LC50 96 h for 1-naphthol for *R. frisii kutum* and *A. brama orientalis* were 2.154 and 2.849 mg L^{-1}, respectively that are very close to values reported by Tilak for the same species (Tilak, 1982).

Phenolic compounds found in the south Caspian Sea basin have been determined in several studies. Comparison of results obtained from the present study with the values obtained in previous studies indicated that in all cases the amount of phenol present was lower than the toxic levels determined in the tests (Taghi, 1998; Bazrafshan, 1994, Tamaskani Esphahankelayeh, 1998; Hasani Ziyabari, 2000).

However as a result of oil extraction activities in the Caspian Sea, and increase in domestic and industrial sewage that may contain phenolic compounds these values are likely to increase. Thus the need for necessary measures to be taken is highly felt e.g. treating domestic and industrial sewage to decrease their toxicity before they are discharged into natural environments.

It is also recommended that toxicity tests be conducted in aquatic organisms using flow through systems because simulation of natural environments is possible in such systems. It is also suggested that toxicity tests be carried out using combination of toxins found in aquatic systems such as organochlorine and organophosphate pesticides, nitrogen and phosphorous fertilizers and heavy metals in order to study synergism and antagonism as well as interacting effects of these compounds that naturally occur in the ecosystems.

Comparison of toxicity caused by phenol and 1-naphthol in different fishes show that 1-naphthol is about 10 times more toxic as compared to phenol. This may be attributed to the two-ring characteristic of 1-naphthol as compared to phenol which has a single ring because more the number of rings in aromatic compounds the more toxic they are known to be.

References

Anderson, P., P.D. Spear, S. Apollina, S. Perry and J. Dick,(1979) Alberta Oil Sands. Environmental Research Program. Report 79.

Bazrafshan, A.A., (1994) Study of physical-chemical parameters and oil pollution in the southeast Caspian Sea.. M.Sc. thesis in the field of Marine Pollution. Islamic Azad University, North Tehran Branch 176 p.

Berton, R., (1998) Supporting document for the environmental assessment of the priority substances phenol. Canadian Environmental Protection Act., Environment Canada, Government Canada.

Chen, Y. and P. Rong,(1990) Effect of no. 2 fuel oil, phenol and sodium sulphide on growth, reproduction and chlorophyll-a content of *Nitzschia closterium*. Oceanol. Limnol. Sin. Haiyang. Yu. Huzhao, vol. 22, no. 1, pp. 21-28.

Crookes, M.J. and P. Howe, (1996) Environmental Hazard assessment: Phenol, toxic substance. The Division Department of Environment, London, TDS/25, 98 p.

Dang, A.D. and V.B. Masurekar, (1985) Toxicity of petroleum hydrocarbons to the estuarine fish *Therapon jarba* and the estuarine clam *Katelysia opima*. Proceedings of the symposium on coastal aquaculture held at Cochio from January 12-18, 1980. Part 3-Finfish culture, India, 1985. No. 6, pp. 828-832.

De Graeve, G.M. et al.(1980) Acute and embryo-larval of phenolic compounds. Arch. Environ. Contam. Toxicol. 9: 557-568.

Hasani Ziyabari, S.E.,(2000) Qualitative examination of PAHs in water in the Bandar Anzali fishery-commercial port. M.Sc. thesis in the field of Marine Pollution. Islamic Azad University, North Tehran Branch.

Khorshidi Rad, F., F. Azari, Study industrial pollution in water and soil. Department of Environment, Gilan Province. Pp. 58-106.

Mason, C.F., (1996) Biology of freshwater pollution. London. Pp. 23, 27, 34-35, 71.

Noga, E.J.,(2000) Fish disease diagnosis and treatment. Iowa State University Press. 234 pp.

Piri, M. and V. Ordog,(1998) Effects of diazinon, malathion, machiti and satern on mortality in *Rutilis frisii kutum* fingerlings. Iranian Fisheries Scientific Journal. Seventh year, No. 4, pp. 9-18.

Ramezani, Gorabi, B.,(1999) Polluting sources in water and soil. Planning and Budget Organization. Planning and Research Dept. 42 p.

Sanaie, G., (1997)Industrial Toxicology. Vol. 2. Tehran University Publications. Pp. 61-95.

Shariati, A.,(1999) Ecology of Caspian Sea. Iranian Fisheries Research Organization. Pp. 171-208.

Stene, A. and S. Lonning, (1985) Effects of short time exposure to naphthalenic, methyl and hydroxynaphthalenes on two different embryonic stages of cod (*Gadus morhua*). Sarsia, vol. 70, no. 4, pp. 279-285.

Svobodova, Z.R. and L.J. Machova, (1983) Water quality and fish health. FAO, pp. 27-28.

Taghipour, V.,(1998) Estimating pollution load in the south Caspian Sea in terms of phenolic compounds. M.Sc. thesis in the field of Marine Pollution. Islamic Azad University, North Tehran Branch. 94 p.

Tamaskani Esphahankelateh, (1997) Using spectrophotometeric methods in toxicology and to determine phenolic compounds present in water in the Gorgan Bay. Pp. 42-50.

Tilak, K.S., (1982) Relative toxicity of carbaryl, 1-naphthol and three hydrocarbons to the estuarine fish Channa punctata. Malaysia no. 8, pp. 15-17.

TRC, (1984) OECD guideline for testing of chemicals. Section 2. Effects on biotic systems.

Table 1 Phenol toxicity in *R. frisii kutum* and *A. brama orientalis*

	LC10(mg /L)				LC50 (mg/l)				LC90 (mg/L)			
	24 h	48 h	72 h	96 h	24 h	48 h	72 h	96 h	24 h	48 h	72 h	96 h
R. frisii kutum	20.1814	18.1666	18.1666	18.1666	24.2339	21.5928	21.5928	21.5928	31.549	25.6652	25.6652	25.6652
A. brama orientalis	22.8551	22.1755	21.9937	21.9937	26.2750	25.3281	25.1880	25.1880	30.2081	28.9289	28.8464	28.8462

Table 2 LOEC, NOEC and MAC values for phenol in *R. frisii kutum* and *A. brama orientalis*

	LOEC (mg/L)	NOEC (mg/L)	MAC (mg/L)
R. frisii kutum	18.1666	2.1593	2.1593
A. brama orientalis	21.9937	2.5188	2.5188

Table 3 1-naphthol toxicity in *R. frisii kutum* and *A. brama orientalis*

	LC10(mg /L)				LC50 (mg/l)				LC90 (mg/L)			
	24 h	48 h	72 h	96 h	24 h	48 h	72 h	96 h	24 h	48 h	72 h	96 h
R. frisii kutum	2.4836	1.7939	1.5949	1.8301	3.1552	2.6615	2.3276	2.1544	2.008	3.9288	23.3966	2.5362
A. brama orientalis	2.7797	2.5736	2.4652	2.4562	3.6324	3.6322	3.0771	2.849	4.7467	4.3925	3.8565	3.3047

Table 4 LOEC, NOEC and MAC values for 1-naphthol in *R. frisii kutum* and *A. brama orientalis*

	LOEC (mg/L)	NOEC (mg/L)	MAC (mg/L)
R. frisii kutum	1.8301	0.2152	0.2154
A. brama orientalis	2.4562	0.2849	0.2849

The pattern of Caspian Sea penetration into the Anzali Wetland, Iran

Mozafar Sharifi[1]
1- Department of Biology, Faculty of Science, Razi University, Kermanshah, Iran. Phone: +98-831-4274545 Email: sharifimozafar@hotmail.com

Abstract
Long term fluctuation of the Caspian Sea level and increase in pollution loads in the Anzali basin have dramatically influenced water quality in the Anzali wetland by increasing the extent of salt water penetration and increasing anaerobic condition at the bottom of the wetland. To understand the extent of Caspian Sea penetration into the Anzali wetland, the water salinity was measured at different depths along six transacts at the mouth of the wetland and along five tributaries connecting the wetland to the sea. The results demonstrated a depth-dependent salinity gradient extending up to 10 kilometers into the wetland. The pattern of saltwater and freshwater interface presented in this study indicates that greatest loss of water quality in the wetland occurs where the input of polluted effluent coincides with the penetration of the saline water.

Keywords: Anzali Wetland, Caspian Sea, salinity gradient.

Introduction

Although Caspian Sea water is much less saline than open sea but it is some 50 times more saline than some of the tributaries which feed Anzali Wetland. The Caspian's low salinity is due to freshwater input (Kosarev and Yablonskaya, 1994). The Volga River contributes up to 82 per cent of the inflow with the rest supplied by some 130 other rivers, principally Ural, Kura and Atrak (Dumont, 1995). The high salinity of water in some water courses in Anzali Wetland especially on stormy is been a familiar phenomenon. Early this century, Rabino the French council at Rasht reported salt water penetration in Mianposhteh (Shantia, 1988). However, a complete picture of Caspian Sea penetration into Anzali Wetland has never been reported in a scientific literature. The pattern and chemistry of the interface has shown to dramatically influence water quality (Sharifi, 1990) and nutrient cycling (Sharifi, 1989) in some parts of Anzali Wetland. Recent rise of Caspian Sea and increased pollution load in many water courses in Anzali Wetland, once more, draw attention toward the understanding and monitoring of the extent of the penetration and possible role of this phenomenon in developing increased sedimentation and enhancing anaerobic conditions created in polluted areas.

Materials and Methods

A modified Irwin water sampler was used in order to collect water at desired depth. The salinity was measured at place on a small boat using a Harris conductivity meter. The samplings were conducted at three points along six transacts at the Shipping Channel and continued along the watercourses (Fig.2) as far as the salinity at surface became similar to that of the salinity at bottom. At any sampling site the water samples were obtained from surface, bottom and mid distance between the two.

The Anzali wetland is located at south-western corner of the Caspian Sea beside the delta of the River Sepidrood. The catchments area of the wetland is about 3740 km^2, of which more than 2000 km^2 are flat terrain and the rest consists of rolling hills and

mountains. The area is located between 48°46' and 49°43' E longitude and 36°54' to 37°34' Latitude. The lowland of the basin is intensively cultivated for rice and the natural cover of the upland is temperate-deciduous forest.

The area benefits from considerable precipitation and does not has a dry season. Therefore, the wetland, although experiencing some sea water penetration, is a freshwater ecosystem. The waterlogged area of the wetland varies considerably with time, and is strongly influenced by the well-known fluctuation of the Caspian Sea as dictated by the hydraulic gradient between sea and wetland. Recent evaluations indicate that since 1929 the extent of the Anzali wetland has been decreased from 300 to 100 km2 (Shantia, 1988). A recent rise of Caspian Sea level has expanded the wetland well beyond the pervious circumference, around which considerable land has been drained and cultivated during the last two decades.

Results

Fig.1 demonstrates the variation in electrical conductivity of water at three different depth on the five tributaries (Shanbeh Bazar Roga, Nahang Roga, Rassteh Khaleh Roga, Pir Bazar Roga and Ssossar Roga) of Anzali Wetland. Fig.2 provides the extent of salt water penetration into Anzali wetland on the basis of salinity values at bottom. Values obtained for conductivity of water at surface in all sampling sites showed small variations extending from 1000 micromhos in most remote sections of the wetland to 5000 micromhos at the end of the Shipping Channel which is in close proximity to the sea water. In contrast to the surface water, variation in values obtained from various depth at different sampling sites were considerable. The extent of the variation is caused mainly by depth of the water courses rather than by distance from the sea. The pattern of saltwater penetration into Anzali Wetland indicates that although a salinity gradient persists in Anzali Wetland but the stratification between waters with different salinity fade out as one moves from the sea into the wetland. With the diffusion of the sea water driven partly by difference in density of salt water along the watercourses and by the decline of wave action, the differences between salinity decreases until becomes equal at all depths. Differences between salinity at surface and in depth increase until the end of the diffusion zone in which salinity values decline sharply and finally become alike.

Discussion

Low level of water discharge from the water courses together with low salinity level of Caspian Sea are the main reasons for absence of typical rigid halocline as is normally seen in some lakes or estuaries. Moreover, although powerful waves action are partially controlled by long weirs build at the sea shore but the waves can disturb any salinity gradient far into the water courses. This is especially significant on stormy days. In one such situation at Shipping Channel the salinity at surface water was measured to be 7000 micromhos some 60 per cent higher than average values in a normal day.

Relationships between depth and salinity are shown in Fig.3. This figure is based on information obtained on all sampling sites. However, information obtained from the Shipping Channel which is common in all watercourses is considered here once in order to prevent overrepresentation of the data. The general relationship between depth and salinity does not follow a linear pattern because other factors such as distance from sea,

direction toward the sea and topography at water course bed are to some extent influential to the amount of salinity. For example Shanbeh Bazar water course which is the closest water course to the sea has a linear relationship between salinity and depth with higher coefficient of variation. Similarly, Sossar water course which is farthest of all water course to the sea has a very weak correlation between depth and salinity.

It has been reported that the rate of sedimentation in Anzali wetland is unusually high (Shantia, 1989). This has been inferred on the basis of rapid filling of sediment traps constructed in the inner parts of the wetland (Shantia, 1989). It has been shown that salt water may influence the rate of sedimentation by various mechanisms. In a dilute colloidal suspension the suspended solids in water remain in complete dispersion. In this situation, the particles tend to repel each other, permitting each particle to act independently of the others. Dispersion of particles is encouraged by higher pH values and the size and nature of the colloidal particles. Moreover, the amount of multi charged ions, more tightly adsorbed by a micelle together with contraction of micelle, and temperature are other factors which can control electro negativity in a solution.

Although highly mono valent ions such as Na+ which are not very tightly held by the micelle, are dominant in sea water, but constant collision of saltwater and freshwater has a significant role in flocculation of suspended solids coming from basin to the sea. Overbick (1990) showed that sedimentation at the mouth of estuaries is to large extent due to flocculation of suspended materials under influence of sea water electrolytes. The presence of a saltwater layer beneath the freshwater in Anzali Wetland may have encouraged sedimentation in some parts of Anzali Wetland. The catchments area of Anzali Basin is relatively small (3470 km2) and total suspended solids in various part of the wetland are not characteristically high (Shantia, 1989) yet there are reports of accelerated sedimentation in the wetland. Further investigation is necessary for clarification of the sedimentation under influence of different waters with various salinity.

The ways Caspian Sea penetrate Anzali wetland differ in many respects to that of estuaries. Absence of the tidal fluctuation characterises the interface between freshwater and sea water. In estuaries the tidal fluctuations are Important ecological factors in providing energy, nutrient and reducing the community respiration. In wetlands that interface with open sea the easy access to nutrient and energy from sea water together with the regular fluctuation in the physical conditions provides a environment in which an orderly process of community development is not possible. Therefore the system remains more or less ecologically young and simple. Certainly, prolong condition governing the estuarine environment has evolutionary significance and causes peculiar adaptations for life in the tidal zone. Generally, plants and animals living in these environment have broad ranges of tolerance to extreme physical conditions.

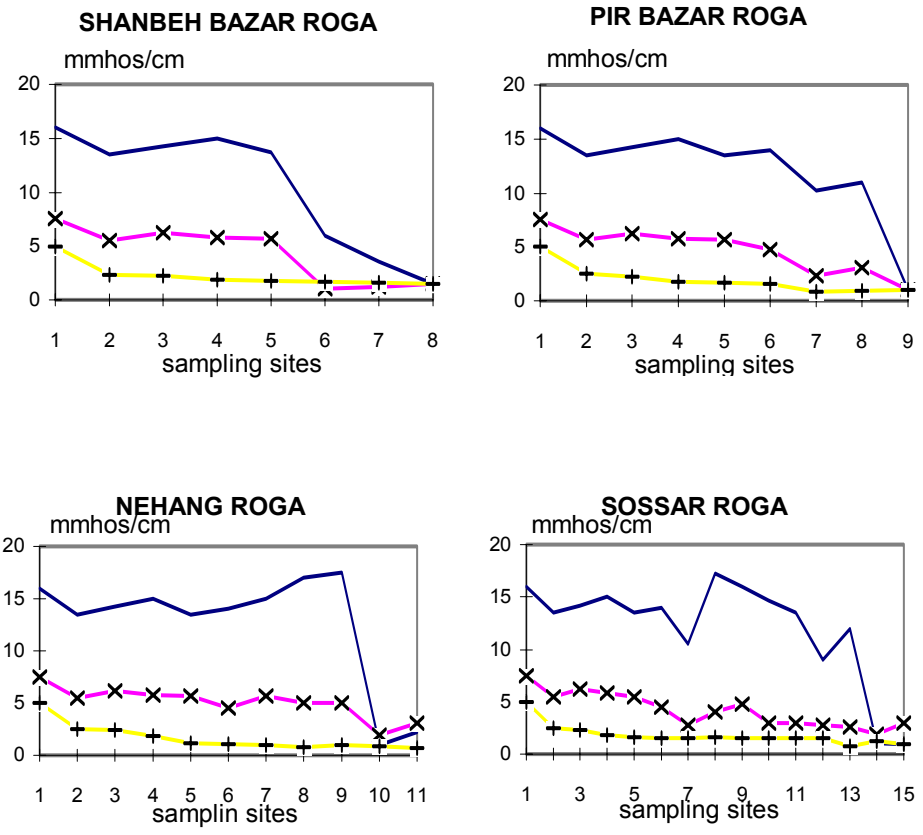
Tidal fluctuation in the Caspian Sea is not sensible and probably is biologically insignificant. In the absence of a turbulent interface and the tidal zone the Caspian Sea interface is characterise by a consistent halocline which remains calmly beneath the freshwater. The presence of a constant layer of saline water in parts of Anzali wetland may cause some nutrient being transported to the wetland but unlike open sea can't help to reduce the community respiration by removing unwanted material to the sea.

The halocline in Anzali wetland is under influence of wave actions, long term fluctuation of the Caspian Sea and the annual cycles in freshwater discharge from the Anzali basin. There in no evidence supporting the development of any character rising under the selection values along the halocline. It is more probable that factor

compensation takes place along the halocline in the form of species composition change. Compensation along the salinity gradient may also involve development of genetic races or ecotypes. Further investigation for recognition of community characteristics and variation in individuals are recommended in and around the interface.

Reference

Dumont H. (1995) Ecocide in the Caspian Sea. Nature. Vol 377.
Kosarev AN and Yablonskaya EA.(1994). The Caspian Sea SPB Academic, The Netherlands.
Mason CF (1994) Biology of Freshwater Pollution. Longman Scientific & Technical.
Rodionov SN (1994) Global and Regional Climate Interaction: The Caspian Sea Experience. Kluwer, The Netherlands.
Shantia H (1989) Effects of Caspian sea level on Anzali wetland ecosystem (in Farsi). Proc. Caspian Sea Level Expansion Seminar. Ramsar, July 1989.
Sharifi M (1989). Summer Release of Phosphate in Anzali Wetland. In the proceedings of the sixth Chemistry and Chemical Engineering conference, Rasht, Iran.
Sharifi M (1990) Assessment of Surface Water Quality by an Index System in Anzali Basin. In The Hydrological Basis for Water Resources Management. Ed Shamir, U., and Jiaqi, C. IAHS Press. Institute of Hydrology, Wallingford, Oxfordshire, UK.



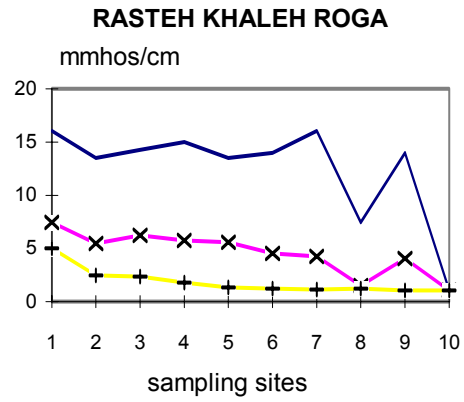


Fig.1. Electrical conductivity of water at surface (-), bottom (×) and mid-depth (+). Distance between each sampling sites is roughly 400 metres.

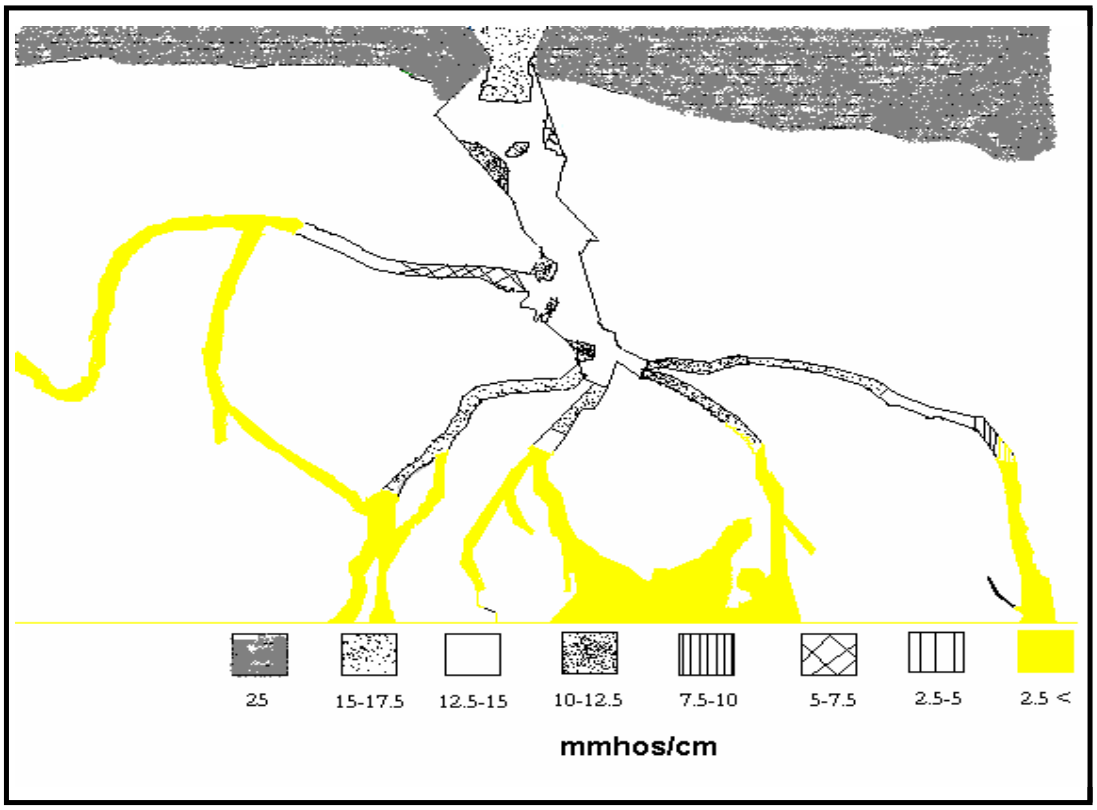


Fig 2. A pattern of salt water penetration into Anzali wetland on the basis of the salinity measured at bottom.

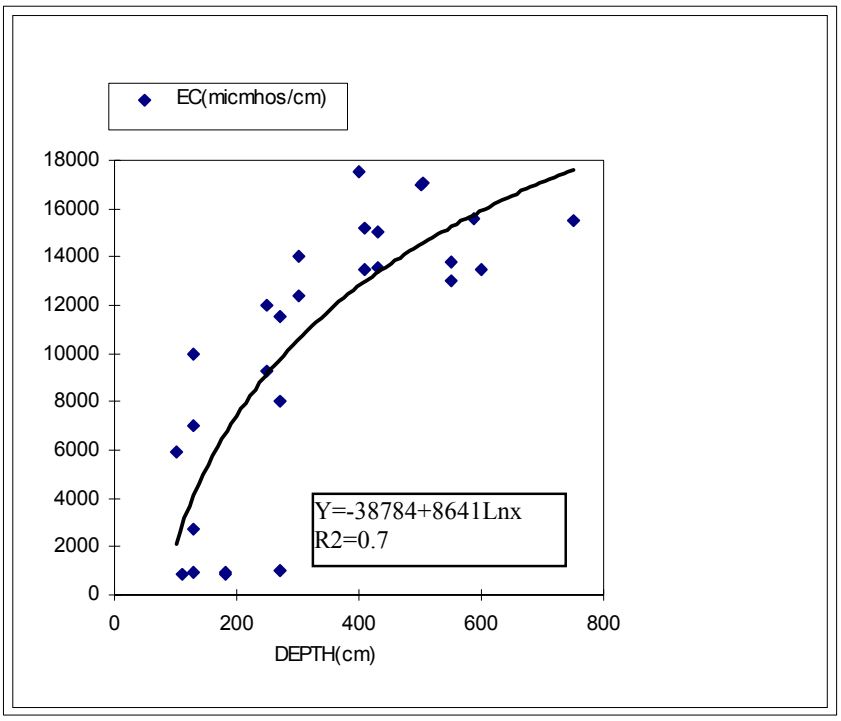


Fig.3. A regression analysis between depth and salinity at depth at various sampling site in Anzali Wetland.

Virology, molecular biology and histopathology investigations on mortality of wild golden grey mullet (*Liza-auratus*) in Iranian waters of Caspian Sea

Mohammad Jalil Zorriehzahra[1*]; Dennis Kaw Gomez [2]; Toshihiro Nakai [2]; Issa Sharifpour [3]; Chi Shau-Chi [4]; Mehdi Soltani [5;] Mostafa Sharif Rohani[6] and Ali Asghar Saidi [7]

[1, 3, 6] Aquatic Animal Health & Diseases Dept., Iranian Fisheries Research Institute, (IFRO),Tehran, Iran
[2] Graduate School of Biosphere Science, Hiroshima University, Higashihiroshima 739-8528, Japan
[4] Dept. of Life Science, National Taiwan University, Taipei, Taiwan
[5] Dept. of Aquatic Animal Health, Faculty of Veterinary Medicine, University of Tehran, Tehran, Iran
[7] Mazandaran Fisheries Research Center, Sari (Khazar –abad), Iran
*Corresponding author: Dr Mohammad Jalil Zorriehzahra, Dept. of Aquatic Animal Health & Diseases, Iranian Fisheries Research Institute, (IFRO), No 297, West Fatemi St.,Tehran, Iran; E-mail: zorrieh@yahoo.com Tel: 009821-6919152 Mobile: 0098912-107-5728 Fax: 009821-6420732

Abstract
Unknown acute mortality occurred in wild golden gray mullet *Lisa auratus* in Iranian Caspian Sea in February, 2004. Clinical signs of the moribund fish were erratic swimming behavior such as spiral and belly-up at rest and high distention of swimming bladder. Histological examination of the brain revealed hyperaemia, degeneration and necrosis in external granular layers as well as focal extension. In virological examinations, the brains of affected golden grey mullet were positive in the reverse transcriptase-polymerase chain reaction test for piscine nodavirus (*Nodaviridae*, *Betanodavirus*), the causative agent of viral nervous necrosis (VNN) in a variety of cultured marine fish worldwide. Nucleotide sequence of the PCR amplicons is closely related with the coat protein gene of piscine nodaviruses, particularly redspotted grouper nervous necrosis virus (RGNNV). Although, It was not succeeded to isolate the virus, experimental infection with the brain homogenates of the affected fish in sevenband grouper (*Epinephelus septemfasciatus)*, which is highly susceptible to RGNNV, produced neurological abnormality followed by mortality. These results suggest that the present mortality of golden grey mullet in Caspian Sea is associated with piscine nodavirus infection.

Keywords: Caspian Sea - Golden gray mullet- Iran - Viral nervous necrosis - Nodavirus – PCR.

Introduction
Unknown acute morbidity occurred in wild golden grey mullet *(Lisa auratus)* of Caspian Sea in the Guilan province, Iran, in February, 2004. First announcement of the morbidity was reported in Ziba-Kenar region of Rasht city, in 10.4-10.8^{o}C water temperature and fish size of 15-20 cm. A similar morbidity was also found in *Lisa auratus in* Babolsar region of Mazandaran province in February, 2002 and a considerable amount of the affected fishes were died. The increase of mortality rate during recent years, could be affected by ecological changes in Caspian Sea .In this regard, invasion of the comb jelly *(Mnemiopsis leidyi*) and it's impacts on eggs and larvae stocks of Clupeidae fishes, could be considered. The affected fish has no gross pathological signs on the body surface exception distention of the belly. The affected fishes were also characterized by erratic swimming behavior. One of the diseases which has similar gross signs is reported as Viral nervous necrosis (VNN).

Viral nervous necrosis (VNN) or viral encephalopathy and retinopathy (VER) caused by piscine nodavirus (*Nodaviridae*). It is a worldwide disease that affecting many species of marine fish, and causing high mortalities of affected larvae and juveniles (Munday *et al.*1992; Nakai *et al* .1995; Chi. *et al.* 1997; Breton *et al.* 1997; Munday *et al*.1997). VNN was first described by Yoshikoshi & Inoue (1990) in Japanese parrotfish (*Opelegnathus fasciatus*) in Japan. Disease has been reported as a serious viral disease of larval and juvenile and sometimes older marine fish that occurs worldwide except for Africa (Office International des Epizootices 2003). VNN disease has spread to 30 or more marine fish species of 14 families in the Indo- pacific region, the Mediterranean region, Scandinavia and North America (Munday *et al*. 2002).

In order to determine the cause of the mortality, virological, molecular biological and histopathological examinations were carried out.

Material & Methods

Affected fish weighting 200-250 g were collected for examinations. Moribund fish were then examined clinically and paraclinically.

Tissue samples from liver, kidney, intestine, stomach, gill, skin and muscle, gall bladder, gonads and brain were taken and fixed in %10 buffer formalin. After dehydration and clearing, samples were embedded into paraffin wax using automatic tissue processor. Paraffinated blocks containing tissues were then sectioned using rotary microtome to prepare 5 micron sections. The prepared microscopic sections were stained by haematoxylin and eosin (H&E) staining method and studied by light microscope.

Necropsy was done in aseptic condition on fish frozen at -20^{o}C. Tissues such as liver, kidney, spleen, eye, and brain, were removed, homogenized with PBS, and centrifuged at 2,000 rpm for 10 minutes. The supernatant was passed through 0.45 μm membrane filter. These supernatants were examined in Hiroshima University (O.I.E. Reference Laboratory for "Viral Encephalopathy and Retinopathy" or "Viral Nervous Necrosis"), Japan and National Taiwan University where virological examinations targeting on piscine nodaviruses are carried out according to O.I.E. Manual of Diagnostic Tests for Aquatic Animals (O.I.E. 2003) with some modifications. New primer sets (Nakai, unpublished) were used for reverse transcriptase-polymerase chain reaction (RT-PCR) and nested PCR. The sequence data obtained was compared with representative coat protein gene sequences of piscine nodaviruses which belonged to different genogroups; striped jack nervous necrosis virus (SJNNV), tiger puffer nervous necrosis virus (TPNNV), barfin flounder nervous necrosis virus (BFNNV) and redspotted grouper nervous necrosis virus (RGNNV) (Nishizawa *et al.* 1997, Iwamoto *et al.* 2001). For the pathogenicity test, sevenband grouper (*Epinephelus septemfasciatus*) weighting average 45 g was challenged with the filtered homogenate of pooled brains of the diseased golden grey mullet (water temperature: 21˚C). Sevenband grouper is highly susceptible to RGNNV (Tanaka *et al.* 1998).

Also, sea water, sediments, nutrients materials and biomass of Caspian Sea were examined to find environmental factors for the mortality using standard procedures.

Results

Clinical signs and macroscopic observations:

Clinical signs of moribund fish were erratic swimming behavior such as spiral and belly-up at rest, lethargic appearance without any surface erosion. The gross pathological changes were characterized by gas accumulation and high distention in swim bladder, yellowish liver,

liquefaction of gall in gall bladder, presence of excess micro sands accumulation in caecum with hyperaemia of intestine (Fig. 1).

Fig. 1- Clinical and gross pathological signs of moribund fishes

Histopathological findings:

Examination of brain sections revealed hyperemia, mild degeneration and necrosis in external granular layers as well as some focal vacuolation. However, these damages in the brain were not severe. Hyperemia was observed in the swim bladder. There were no notifiable pathological changes in the other organs.

Examinations for piscine nodavirus:

The coat protein gene of piscine nodavirus was detected in all 8 brain homogenates of diseased golden grey mullet by RT-PCR and nested PCR, though the RT-PCR amplicons weakly appeared in the agarose gel electrophoresis. The sequence analysis on the nested PCR products (177 bases) indicates that the present virus is closely related to reported piscine nodaviruses. Mortality at 100% was produced in sevenband grouper by intravitreous injection of the filtrate of pooled brain homogenates. Fish lost balance and died 4-6 days after injection. The brains of the dead sevenband grouper were strongly positive in RT-PCR test. The sequence analysis of the RT-PCR amplicon (345 bases) revealed again genetic relatedness between the present virus and RGNNV (Table 1, Fig. 2). However, nodavirus antigens were not demonstrated by indirect fluorescent antibody technique (IFAT) using rabbit polyclonal antibodies (anti-SJNNV) in the

brains of the affected golden grey mullet or sevenband grouper. Moreover, trials to isolate viruses using E-11 cells (Iwamoto *et al.* 2000) have not succeeded.

Fig. 2. Molecular phylogenetic tree deduced from analysis of the nucleotide sequences of known betanodaviruses and GMNNV

Table 1. Nucleotide sequence similarities of the coat protein genes of known betanodaviruses and GMNNV*

	GMNNV	BFNNV	RGNNV	SJNNV	TPNNV
GMNNV	100	72	93	64	62
BFNNV		100	77	64	69
RGNNV			100	66	66
SJNNV				100	73
TPNNV					100

*present virus from diseased golden grey mullet

Ecological findings:

The average of biomass was 2.124 g/m^2 in the region which is equivalent to that in 1992 (less 4 g/m^2) (Table 2). There were no significant differences in physico-chemical parameters between pre-outbreak and post-outbreak of the mortality (Table 3).

Table 2- Specification of biomass in Ziba-Kenar beach (Feb.2004)

Specimen name	**No./ m^2**	**Weight/m^2**
Nereidae	50	2.075
Tubificidae	25	0.033
Amphipoda	8	0.016
Totals	83	2.124

Discussion

The present study was conducted to determine the cause of recent mortality in golden grey mullet in Caspian Sea in Iran. The disease was characterized by erratic swimming behavior and hyperinflation of swim bladder, but no more clinical signs was observed. These signs are different from abdominal swelling, water belly, dropsy, gas bubble disease or swim bladder stress syndrome (Woo, PTK. & Bruno, DE. 1999) and have not been previously reported in wild fish in Caspian Sea so far.

The nutrient materials in the region consist of *Ostracoda, Mysidae, Balanus, bivalva* and larvae of *Nematod* and *Gastropoda* revealed that diets of golden grey mullet were normal without any quantitative changes. Water analysis records also showed no changes in comparison with the last sampling. It was concluded that no environmental parameters could be engaged in the incidence of the disease.

At present, piscine nodavirus detected in the affected brains is the only suspect for the present mortality in golden grey mullet, though viral etiology was not fully demonstrated. Piscine nodavirus is well known as the agent of viral nervous necrosis (VNN) or viral encephalopathy and retinopathy (VER) in a variety of marine fish species worldwide (Munday and Nakai 1997, Munday *et al.* 2002, O.I.E. 2003). The disease was characterized by a variety of neural abnormalities. In addition to the similarity of clinical signs, RT-PCR with primers specific to piscine nodaviruses identified the coat protein gene in the affected brains of golden grey mullet and the nucleotide sequence analysis revealed that the present mullet virus is genetically most related with RGNNV among known 4 betanodavirus genogroups. However, the amino acid sequence similarity with RGNNV was only 80%, suggesting that the present virus belongs to a new genogroup. This difference in the amino acid sequence probably leads to antigenic difference of the coat protein since the affected brains were negative in IFAT test with anti-SJNNV rabbit serum, with which all reported betanodaviruses reacted (Mori *et al.* 2003). Meanwhile, the brain homogenate from diseased golden grey mullet produced mortality in sevenband grouper. Therefore, it is concluded that RGNNV-like betanodavirus is associated with severe mortality of recent golden grey mullet in Iranian Caspian Sea. To fulfill Koch's postulates, experimental infection in golden grey mullet will be required to know the susceptibility to the present golden grey mullet betanodavirus, although it is difficult to keep this fish species under experimental conditions.

In most case, RGNNV-type of betanodaviruses has been isolated from warm water fish species and has higher optimum growth temperature in vitro and in vivo (25-30˚C) (Iwamoto *et al.* 2000, Chi *et al*. 2003). Although the present mortality occurred when water temperature was low (10-11˚C), more severe mortality could be expected in Caspian Sea in summer if it is due to betanodavirus infection. VNN has long been limited to marine fish species but recently reported in some reared freshwater species such as; European eel (*Anguilla anguilla)* and Chinese catfish *(Parasilurus asotus)* indicating that salinity is obviously not limiting factor in VNN transmission (Chi *et al.* 2003). Therefore, VNN outbreaks in Caspian Sea could be a potent hazard for freshwater fishes and marine fishes in north of Iran. Based on the results of this study, it is very important to monitor the spread of NNV in Caspian Sea carefully, because this disease always can cause very high rates mortality and leads to severe economic losses. Intensive investigation for the VNN must be undertaken for all fish species in Caspian Sea in order to prevent further spread of this disease.

Acknowledgments:

The authors would like to thank the staff of the Guilan Research Center of IFRO for providing sample fishes used in this study. We also thank staff of Aquatic Animal Health & Diseases Lab. in Veterinary Faculty of Tehran University. Special thanks also go to Dr. Igor Schelchonov for his valuable advice and Ms. Shiva Shams for her interest and Mr. H. Bagheri and Mr. Saydanlou for their efforts in this study.

References

Chi S.C., Shieh J.R., Lin S.J.,(2003).Genetic and antigenic analysis of betanodaviruses isolated from aquatic organism in Taiwan . *J .Disease of Aquatic Organisms*, **55**,221-228.

Chi S.C., Lin S.C., Su H.M. & Hu W.W. (1999).Temperature effect on nervous necrosis virus infection in grouper cell line and in grouper larvae. *J .Virus Research*, **63**,107-114.

Iwamoto T., Mise K., Mori K., Arimoto M., Nakai T. & Okuno T. (2001). Establishment of an infectious RNA transcription system for *striped jack* nervous necrosis virus, the type species of the *Betanodavirus ,J.Gen Virol*., **82**, 2653-2662.

Iwamoto, T., Nakai T., Mori K., Arimoto M. & Furusawa I. (2000). Cloning of fish cell line SSN-1 for piscine Nodaviruses. *Dis. Aquat. Org.*, **43**, 81-89.

Munday B.L., Kwang J. & Moody N. (2002) Betanodavirus infections of teleost fish: a review. *J. Fish Dis.* **25**, 127-142.

Munday B.L & Nakai T., (1997). Special topic review: Nodaviruses as pathogens in larval and juvenile marine finfish. *World Journal of Microbiology and Biotechnology*, **13**, 357-381.

Nishizawa T., Furuhashi M., Nagai T., Nakai T. & Muroga K. (1997) Genomic classification of fish nodaviruses by molecular phylogenetic analysis of the coat protein gene. *Applied and Environmental Microbiology* **63**, 1633-1636.

Office International des Epizooties OIE (2003) Viral encephalopathy and retinopathy. In: Manual of Diagnostic Tests for Aquatic Animals, pp.135-141. OIE, Paris.

Tanaka S., Aoki H. & Nakai T. (1998) Pathogenicity of the nodavirus detected from diseased sevenband grouper *Epinephelus septemfasciatus*. *Fish Pathol.* **33**, 31-36.

Woo PTK, Bruno DE (eds) (1999). Fish diseases and disorders, volume 3, viral,
Bacterial and fungal infections, oxon, UK.

Table 3 - Physico-chemical analysis of Caspian Sea water in Ziba kenar beach

Date weather time	Station	Transparency cm	Air Temperature o^c	Water Temperature o^c	CO_2	CO_3	EC Ms/cm	Salinity ppt	pH	SiO_2	PO_4	NH_4	NO_2	NO_3	O_2
Jan. 26, 2004 cloudy 16:15	Surface	250	15	11.4	-	12	13.90	11.10	8.51	0.462	0.053	0.033	0.005	0.024	11.04
	5 meter depth	250	15	10.2	-	12	14	11.20	8.51	0.381	0.082	0.025	0.003	0.012	I0.09
	12 meter depth	250	15	10.4	-	12	14.60	11.58	8.51	0.088	0.059	0.026	0.001	0.020	I0.70
Feb. 3 2004 sunny 10:45	Surface	200	11	10.4	-	5	14.70	11.05	8.45	0.370	0.023	0.021	0.003	0.041	10.54
	18 meter depth	200	11	10.8	-	8	15.62	11.84	8.50	0.084	0.022	0.018	0.002	0.023	I0.54

The study of day-night effects of temperature on growing *Asepencer baeri* species

Yazdani Sadati M. A.[1], Vlasov V. A.[2]
1-P.O. Box 127550, № 45, Timiriazevskaya St., Department of Aquaculture, Moscow Agricultural Science Academy named after Timiriazev, E-mail: yazdanisadati2004@yahoo.com, tell: +7(916)3030991
2-P.O. Box 127550, № 45, Timiriazevskaya St., Department of Aquaculture, Moscow Agricultural Science Academy named after Timiriazev, tell: +7(095)9760009

Abstract
Asepenceridae family with the long history of life is one of the most valuable caviar fishes because of the importance of meat and caviar in global trademark. Their main survey is extended in Kaspian Sea with up to 80% of global caching. Unfortunately, because of ecological changes created in their life environment and also the human disturbing effects such as over-caching, the increase of industrial and agricultural pollutions have been resulted to decrease their total population. Thus, IUCN and Also CITES have limited the traditional caching to special rules. One of the effective ways to protect the species and to avoid over-caching is the cultivation in artificial conditions in fishery pools aimed to obtain meat and caviar. *Asepencer baeri* species has high cultivation ability in artificial conditions and matures within 6-7 years to produce caviar, while in natural conditions 12-16 years is necessary. In this study with investigating and obtaining biotechnical aspects necessary to the cultivation, were studied the day-night effects of temperature on its growth. The obtained results showed that temperature changes were directly correlated with respiration measure and was resulted to decrease consumed energy wastes necessary for the life actions, which ultimately was resulted to increase in the fish growth.
Key words: *Asepencer baeri*, aquaculture, temperature mode

Introduction

The growing of caviar fish in Russia steel is on the early way and now there is not a high biological potential for the growing species and the produced caviar is not capable to meet the demand of consumers because of being one of the most expensive products obtained from growing fish.
There are three basic aspects in fishery of caviar: pasture, semi-extensive and extensive. The effectiveness of later is related to the providing of regulated and controlled conditions for growing the fish. One of the basic factors as a controlling factor of consumed food volume and its digestion is termed as temperature mode.
It is well known that the use of temperature mode increases the growth speed and decreases the breath severity. As a result, the decrease of consumed energy is resulted to allocation of the excess energy to growth phase of fish (Konstantinov, Zadanovich, 1986).
The growing caviar fishes in industrial conditions is suggested a stable temperature mode. However, the existence of organisms in non-stable conditions is a biological norm, but in the stable and constant conditions this biological norm is infringed (Pooshkarev et al, 1999).
The objective of current study is to investigate the effects of stable and non-stable temperatures on the growth of caviar *Asepencer baeri* species.

Materials and Methods

The current study was performed in the aquaculture department of Moscow agricultural Academy. Anneal *Asepencer baeri* with the average weight 0f 130 g. was grown in the 500 l aquaria with capacity of 40 pieces / M^3. The temperature mode was consisted from 4 treatments as following (table1):

1) Day-night constant temperature (22±1 ^{o}C)
2) Day-night temperature next to natural temperatures (19-25 ^{o}C)
3) On the contrary of the second treatment (25- 19 ^{o}C)
4) two treatments of the increase (19-25 ^{o}C) and decrease (25- 19 ^{o}C) of water temperature.

For the study of growth characters in different treatments, the fishes were cached and were conducted biometrical studies on them. The day-night absolute growth was calculated as the following:

$$W_2- W_1/ t_2-t_1 \text{ (g/day-night)}$$

And relatively growth speed was calculated as following:

$$C1= [10(\log W_2- \log W_1)/ t_2-t_1-1] \times 100 \text{ (Vinberg, 1956)}$$

Results and Discussion

The results showed that maximum growth was obtained for second treatment (increase of water temperature from 8 a.m. to 16 p.m.) and in a lower extent, was 4th treatment (increase of water temperature in the day time and night time). For 3th treatment the absolute growth was in the least level (table2).

Also data showed that for second treatment was obtained the highest growth speed (1.9-2.6%), indicating that dietary conversion coefficient was 0.8 U, so that for this variant was obtained the highest group weight (261.6 gr/ fish) and with the use of the least food consume (table3).

Therefore, on the basis of the results from this study, we can conclude that:

1. *Asepencer baeri* in aquaculture conditions can obtain a high growth so that relatively growth speed in temperature mode from 19 to 25 ^{o}C can reach to >2.6%.
2. the more optimal temperature mode of growth is the increase in water temperature from 19 to 25 in a period of 8 a.m. to 16 p.m. (second treatment).

References

1. Konstantinov AC, Zadanovich VV, Tikhomirov DG (1986) The effects of acceleration of temperature on growth and energetic of young Karp. *Thesis paper* : 27
2. Vinberg FF (1956) Breath severity and food digestion in fish. *Minsk University Pub*.: 24
3. Pooshkarev VI, Zadanovich VV (1999) Optimization of temperature mode in aquaculture. *Krasnadar University Pub*. : 84-85

Tables Legends

Table1. the schema of treatments

Parameter	Treatment			
	1 (control)	2	3	4
Temperature mode	22 ^{o}C for 24 hours	from 19 to 25^{o}C (from 8 a.m. to 16p.m.), from 25 to 19^{o}C (from 16 p.m. to 8a.m.)	from 19 to 25^{o}C (from 8 a.m. to 16p.m.), from 25 to 19^{o}C (from 16 p.m. to 8a.m.)	from 19 to 25^{o}C(from 8 a.m. to 16p.m.), from 25 to 19^{o}C (from 16 p.m. to 8a.m.)
Aquarium volume (L)	500	500	500	500
Early weight of fish (gr)	130	130	130	130
Number of fishes (piece/ M^3)	40	40	40	40

Table2. the growth of fish in different treatments

Date	I		II		III		IV	
	M±m	g/d	M±m	g/d	M±m	g/d	M±m	g/d
25.02	132.8±4.4	-	130.1±4.6	-	126.0±4.4	-	131.5±4.1	-
8.03	152.3±5.5	1.6	151.8±4.5	1.8	141.1±7.0	1.3	152.5±4.7	1.7
19.03	188.1±10.0	3.3	203.6±10.8	4.7	182.4±10.3	3.7	201.2±6.9	4.4
2.04	196.2±12.1	-	224.6±14.1	-	184.6±14.0	-	219.6±11.9	-
21.04	235.8±12.7	2.1	261.6±17.6	2.0	221.9±19.4	2.0	252.8±11.8	1.8
Total	-	2.5	-	3.1	-	2.3	-	2.9

Table3. total results obtained from growing *Asepencer baeri*

Date	parametr	I		II		III		IV	
		culture	added weight	culture	added weight	culture	added weight	culture	added weight
25.02 -8.03	average weight (g)	132.8	152.3	130.1	151.8	126.0	141.1	131.5	152.5
	total weight	2565	3046	2862	3340	2645	2963	2630	3050
	growth	-	390	-	478	-	318	-	420
	food consumption (kg/kg)	-	0.84	-	0.69	-	1.03	-	0.78
9.03-19.03	average weight (g)	149.4	188.1	151.8	203.7	143.9	182.7	152.5	201.2
	total weight	3434	4327	3037	4073	2878	3647	3050	4024
	growth	-	893	-	1036	-	769	-	974
	food consumption (kg/kg)	-	0.81	-	0.70	-	0.94	-	0.75
2.04-021.04	average weight (g)	196.1	235.8	224.5	261.6	184.6	221.9	215.1	252.8
	total weight	3922	4716	4491	5232	3692	4438	4301	5055
	growth	-	794	-	741	-	746	-	754
	food consumption (kg/kg)	-	1.09	-	1.17	-	1.16	-	1.15
25.02 - 21.04	average weight (g)	132.8	235.8	130.1	261.6	126.0	221.9	131.5	252.8
	total weight	2656	4733	2862	5117	2645	4478	2630	4778
	growth	-	2077	-	2255	-	1833	-	214
	food consumption (kg/kg)	-	0.92	-	0.86	-	1.05	-	0.8

Virology, molecular biology and histopathology investigations on mortality of wild golden grey mullet (*Liza-auratus*) in Iranian waters of Caspian Sea

Mohammad Jalil Zorriehzahra[1*]; Dennis Kaw Gomez [2]; Toshihiro Nakai [2]; Issa Sharifpour [3]; Chi Shau-Chi [4]; Mehdi Soltani [5;] Mostafa Sharif Rohani[6] and Ali Asghar Saidi [7]

[1, 3, 6] Aquatic Animal Health & Diseases Dept., Iranian Fisheries Research Institute, (IFRO),Tehran, Iran
[2] Graduate School of Biosphere Science, Hiroshima University, Higashihiroshima 739-8528, Japan
[4] Dept. of Life Science, National Taiwan University, Taipei, Taiwan
[5] Dept. of Aquatic Animal Health, Faculty of Veterinary Medicine, University of Tehran, Tehran, Iran
[7] Mazandaran Fisheries Research Center, Sari (Khazar –abad), Iran
*Corresponding author: Dr Mohammad Jalil Zorriehzahra, Dept. of Aquatic Animal Health & Diseases, Iranian Fisheries Research Institute, (IFRO), No 297, West Fatemi St.,Tehran, Iran; E-mail: zorrieh@yahoo.com Tel: 009821-6919152 Mobile: 0098912-107-5728 Fax: 009821-6420732

ABSTRACT
Unknown acute mortality occurred in wild golden grey mullet *Lisa auratus* in Iranian Caspian Sea in February, 2004. Clinical signs of the moribund fish were erratic swimming behavior such as spiral and belly-up at rest and high distention of swimming bladder. Histological examination of the brain revealed hyperaemia, degeneration and necrosis in external granular layers as well as focal extension. In virological examinations, the brains of affected golden grey mullet were positive in the reverse transcriptase-polymerase chain reaction test for piscine nodavirus (*Nodaviridae*, *Betanodavirus*), the causative agent of viral nervous necrosis (VNN) in a variety of cultured marine fish worldwide. Nucleotide sequence of the PCR amplicons is closely related with the coat protein gene of piscine nodaviruses, particularly redspotted grouper nervous necrosis virus (RGNNV). Although, It was not succeeded to isolate the virus, experimental infection with the brain homogenates of the affected fish in sevenband grouper (*Epinepherus septemfasciatus)*, which is highly susceptible to RGNNV, produced neurological abnormality followed by mortality. These results suggest that the present mortality of golden grey mullet in Caspian Sea is associated with piscine nodavirus infection.

Key words: Caspian Sea - Golden grey mullet- Iran - Viral nervous necrosis - Nodavirus - PCR

Introduction
Unknown acute morbidity occurred in wild golden grey mullet *(Lisa auratus)* of Caspian Sea in the Guilan province, Iran, in February, 2004. First announcement of the morbidity was reported in Ziba-Kenar region of Rasht city, in 10.4-10.8 ^{oC} water temperature and fish size of 15-20 cm. A similar morbidity was also found in *Lisa auratus in* Babolsar region of Mazandaran province in February, 2002 and a considerable amount of the affected fishes were died.The increase of mortality rate during recent years, could be affected by ecological changes in Caspian Sea .In this regard, invasion of the comb jelly *(Mnemiopsis leidyi*) and it's impacts on eggs and larvae stocks of Clupeidae fishes, could be considered.The affected fish has no gross pathological signs on the body surface exception

distention of the belly.The affected fishes were also characterized by erratic swimming behavior.One of the diseases which has similar gross signs is reported as Viral nervous necrosis (VNN).

Viral nervous necrosis (VNN) or viral encephalopathy and retinopathy (VER) caused by piscine nodavirus (*Nodaviridae*).It is a worldwide disease that affecting many species of marine fish, and causing high mortalities of affected larvae and juveniles (Munday *et al.*1992; Nakai *et al* .1995; Chi. *et al.* 1997; Breton *et al.* 1997; Munday *et al*.1997).VNN was first described by Yoshikoshi & Inoue (1990) in Japanese parrotfish (*Opelegnathus fasciatus*) in Japan. Disease has been reported as a serious viral disease of larval and juvenile and sometimes older marine fish that occurs worldwide except for Africa (Office International des Epizootices 2003).VNN disease has spread to 30 or more marine fish species of 14 families in the Indo- pacific region, the Mediterranean region, Scandinavia and North America (Munday *et al*. 2002).

In order to determine the cause of the mortality, virological, molecular biological and histopathological examinations were carried out.

Material & Methods

Affected fish weighting 200-250 g were collected for examinations. Moribund fish were then examined clinically and paraclinically.

Tissue samples from liver, kidney, intestine, stomach, gill, skin and muscle, gall bladder, gonads and brain were taken and fixed in %10 buffer formalin. After dehydration and clearing, samples were embedded into paraffin wax using automatic tissue processor. Paraffinated blocks containing tissues were then sectioned using rotary microtome to prepare 5 micron sections. The prepared microscopic sections were stained by haematoxylin and eosin (H&E) staining method and studied by light microscope.

Necropsy was done in aseptic condition on fish frozen at -20^{o}C. Tissues such as liver, kidney, spleen, eye, and brain, were removed, homogenized with PBS, and centrifuged at 2,000 rpm for 10 minutes. The supernatant was passed through 0.45 μm membrane filter. These supernatants were examined in Hiroshima University (O.I.E. Reference Laboratory for “Viral Encephalopathy and Retinopathy” or “Viral Nervous Necrosis”), Japan and National Taiwan University where virological examinations targeting on piscine nodaviruses are carried out according to O.I.E. Manual of Diagnostic Tests for Aquatic Animals (O.I.E. 2003) with some modifications. New primer sets (Nakai, unpublished) were used for reverse transcriptase-polymerase chain reaction (RT-PCR) and nested PCR. The sequence data obtained was compared with representative coat protein gene sequences of piscine nodaviruses which belonged to different genogroups; striped jack nervous necrosis virus (SJNNV), tiger puffer nervous necrosis virus (TPNNV), barfin flounder nervous necrosis virus (BFNNV) and redspotted grouper nervous necrosis virus (RGNNV) (Nishizawa *et al.* 1997, Iwamoto *et al.* 2001). For the pathogenicity test, sevenband grouper (*Epinephelus septemfasciatus*) weighting average 45 g was challenged with the filtered homogenate of pooled brains of the diseased golden grey mullet (water temperature: 21˚C). Sevenband grouper is highly susceptible to RGNNV (Tanaka *et al.* 1998).

Also, sea water, sediments, nutrients materials and biomass of Caspian Sea were examined to find environmental factors for the mortality using standard procedures.

Results

Clinical signs and macroscopic observations:

Clinical signs of moribund fish were erratic swimming behavior such as spiral and belly-up at rest, lethargic appearance without any surface erosion. The gross pathological changes were characterized by gas accumulation and high distention in swim bladder, yellowish liver, liquefaction of gall in gall bladder, presence of excess micro sands accumulation in caecum with hyperaemia of intestine (Fig. 1).

Fig. 1- Clinical and gross pathological signs of moribund fishes

Histopathological findings:

Examination of brain sections revealed hyperemia, mild degeneration and necrosis in external granular layers as well as some focal vacuolation. However, these damages in the brain were not severe. Hyperemia was observed in the swim bladder. There were no notifiable pathological changes in the other organs.

Examinations for piscine nodavirus:

The coat protein gene of piscine nodavirus was detected in all 8 brain homogenates of diseased golden grey mullet by RT-PCR and nested PCR, though the RT-PCR amplicons weakly appeared in the agarose gel electrophoresis. The sequence analysis on the nested PCR products (177 bases) indicates that the present virus is closely related to reported piscine nodaviruses. Mortality at 100% was produced in sevenband grouper by intravitreous injection of the filtrate of pooled brain homogenates. Fish lost balance and died 4-6 days after injection. The brains of the dead sevenband grouper were strongly

positive in RT-PCR test. The sequence analysis of the RT-PCR amplicon (345 bases) revealed again genetic relatedness between the present virus and RGNNV (Table 1, Fig. 2). However, nodavirus antigens were not demonstrated by indirect fluorescent antibody technique (IFAT) using rabbit polyclonal antibodies (anti-SJNNV) in the brains of the affected golden grey mullet or sevenband grouper. Moreover, trials to isolate viruses using E-11 cells (Iwamoto *et al.* 2000) have not succeeded.

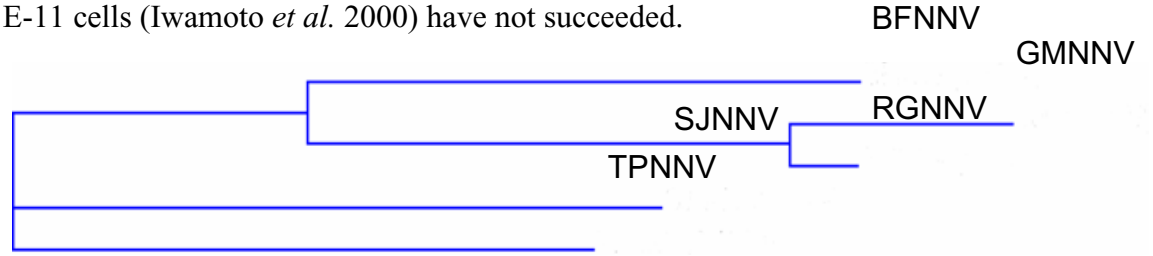


Fig. 2. Molecular phylogenetic tree deduced from analysis of the nucleotide sequences of known betanodaviruses and GMNNV

Table 1. Nucleotide sequence similarities of the coat protein genes of known betanodaviruses and GMNNV*

	GMNNV	BFNNV	RGNNV	SJNNV	TPNNV
GMNNV	100	72	93	64	62
BFNNV		100	77	64	69
RGNNV			100	66	66
SJNNV				100	73
TPNNV					100

*present virus from diseased golden grey mullet

Ecological findings:

The average of biomass was 2.124 g/m^2 in the region which is equivalent to that in 1992 (less 4 g/m^2) (Table 2). There were no significant differences in physico-chemical parameters between pre-outbreak and post-outbreak of the mortality (Table 3).

Table 2- Specification of biomass in Ziba-Kenar beach (Feb.2004)

Specimen name	**No./ m^2**	**Weight/m^2**
Nereidae	50	2.075
Tubificidae	25	0.033
Amphipoda	8	0.016

Totals	83	2.124

Discussion

The present study was conducted to determine the cause of recent mortality in golden grey mullet in Caspian Sea in Iran. The disease was characterized by erratic swimming behavior and hyperinflation of swim bladder, but no more clinical signs were observed. These signs are different from abdominal swelling, water belly, dropsy, gas bubble disease or swim bladder stress syndrome (Woo, PTK. & Bruno, DE. 1999) and have not been previously reported in wild fish in Caspian Sea so far.

The nutrient materials in the region consist of *Ostracoda, Mysidae, Balanus, bivalva* and larvae of *Nematod* and *Gastropoda* revealed that diets of golden grey mullet were normal without any quantitative changes. Water analysis records also showed no changes in comparison with the last sampling. It was concluded that no environmental parameters could be engaged in the incidence of the disease.

At present, piscine nodavirus detected in the affected brains is the only suspect for the present mortality in golden grey mullet, though viral etiology was not fully demonstrated. Piscine nodavirus is well known as the agent of viral nervous necrosis (VNN) or viral encephalopathy and retinopathy (VER) in a variety of marine fish species worldwide (Munday and Nakai 1997, Munday *et al.* 2002, O.I.E. 2003). The disease was characterized by a variety of neural abnormalities. In addition to the similarity of clinical signs, RT-PCR with primers specific to piscine nodaviruses identified the coat protein gene in the affected brains of golden grey mullet and the nucleotide sequence analysis revealed that the present mullet virus is genetically most related with RGNNV among known 4 betanodavirus genogroups. However, the amino acid sequence similarity with RGNNV was only 80%, suggesting that the present virus belongs to a new genogroup. This difference in the amino acid sequence probably leads to antigenic difference of the coat protein since the affected brains were negative in IFAT test with anti-SJNNV rabbit serum, with which all reported betanodaviruses reacted (Mori *et al.* 2003).Meanwhile, the brain homogenate from diseased golden grey mullet produced mortality in sevenband grouper. Therefore, it is concluded that RGNNV-like betanodavirus is associated with severe mortality of recent golden grey mullet in Iranian Caspian Sea. To fulfill Koch's postulates, experimental infection in golden grey mullet will be required to know the susceptibility to the present golden grey mullet betanodavirus, although it is difficult to keep this fish species under experimental conditions.

In most case, RGNNV-type of betanodaviruses has been isolated from warm water fish species and has higher optimum growth temperature in vitro and in vivo (25-30˚C) (Iwamoto *et al.* 2000, Chi *et al*. 2003). Although the present mortality occurred when water temperarure was low (10-11˚C), more severe mortality could be expected in Caspian Sea in summer if it is due to betanodavirus infection. VNN has long been limited to marine fish species but recently reported in some reared freshwater species such as; European eel (*Anguilla anguilla)* and Chinese catfish *(Parasilurus asotus)* indicating that salinity is obviously not limiting factor in VNN transmission (Chi *et al.* 2003). Therefore, VNN outbreaks in Caspian Sea could be a potent hazard for freshwater fishes and marine fishes

in north of Iran. Based on the results of this study, it is very important to monitor the spread of NNV in Caspian Sea carefully, because this disease always can cause a very high rates mortality and leads to severe economic losses. Intensive investigation for the VNN must be undertaken for all fish species in Caspian Sea in order to prevent further spread of this disease.

Acknowledgments:

The authors would like to thank the staff of the Guilan Research Center of IFRO for providing sample fishes used in this study. We also thank staff of Aquatic Animal Health & Diseases Lab. in Veterinary Faculty of Tehran University. Special thanks also go to Dr. Igor Schelchonov for his valuable advice and Ms. Shiva Shams for her interest and Mr. H. Bagheri and Mr. Saydanlou for their efforts in this study.

References

Chi S.C., Shieh J.R., Lin S.J.,(2003).Genetic and antigenic analysis of betanodaviruses isolated from aquatic organism in Taiwan . *J .Disease of Aquatic Organisms*, **55**,221-228.

Chi S.C., Lin S.C., Su H.M. & Hu W.W. (1999).Temperature effect on nervous necrosis virus infection in grouper cell line and in grouper larvae. *J .Virus Research*, **63**,107-114.

Iwamoto T., Mise K., Mori K., Arimoto M., Nakai T. & Okuno T. (2001). Establishment of an infectious RNA transcription system for *striped jack* nervous necrosis virus, the type species of the *Betanodavirus ,J.Gen Virol*., **82**, 2653-2662.

Iwamoto, T., Nakai T., Mori K., Arimoto M. & Furusawa I. (2000). Cloning of fish cell line SSN-1 for piscine Nodaviruses. *Dis. Aquat. Org.*, **43**, 81-89.

Munday B.L., Kwang J. & Moody N. (2002) Betanodavirus infections of teleost fish: a review. *J. Fish Dis.* **25**, 127-142.

Munday B.L & Nakai T., (1997). Special topic review: Nodaviruses as pathogens in larval and juvenile marine finfish. *World Journal of Microbiology and Biotechnology*, **13**, 357-381.

Nishizawa T., Furuhashi M., Nagai T., Nakai T. & Muroga K. (1997) Genomic classification of fish nodaviruses by molecular phylogenetic analysis of the coat protein gene. *Applied and Environmental Microbiology* **63**, 1633-1636.

Office International des Epizooties OIE (2003) Viral encephalopathy and retinopathy. In: Manual of Diagnostic Tests for Aquatic Animals, pp.135-141. OIE, Paris.

Tanaka S., Aoki H. & Nakai T. (1998) Pathogenicity of the nodavirus detected from diseased sevenband grouper *Epinephelus septemfasciatus*. *Fish Pathol.* **33**, 31-36.

Woo PTK, Bruno DE (eds) (1999). Fish diseases and disorders, volume 3, viral, Bacterial and fungal infections, oxon, UK.

Table 3 - Physico-chemical analysis of Caspian Sea water in Ziba kenar beach

Date weather time	Station	Transparency cm	Air Temperature o^c	Water Temperature o^c	CO_2	CO_3	EC Ms/cm	Salinity ppt	pH	SiO_2	PO_4	NH_4	NO_2	NO_3	O_2
Jan. 26, 2004 cloudy 16:15	Surface	250	15	11.4	-	12	13.90	11.10	8.51	0.462	0.053	0.033	0.005	0.024	11.04
	5 meter depth	250	15	10.2	-	12	14	11.20	8.51	0.381	0.082	0.025	0.003	0.012	I0.09
	12 meter depth	250	15	10.4	-	12	14.60	11.58	8.51	0.088	0.059	0.026	0.001	0.020	I0.70
Feb. 3 2004 sunny 10:45	Surface	200	11	10.4	-	5	14.70	11.05	8.45	0.370	0.023	0.021	0.003	0.041	10.54
	18 meter depth	200	11	10.8	-	8	15.62	11.84	8.50	0.084	0.022	0.018	0.002	0.023	I0.54

Managing watershed area: tools for the Caspian ecological sustainability

H. Alizade Lahijani
Iranian National Center for Oceanography ;Tehran, POBox 14155-4781,hamidakl@yahoo.com

Abstract:
The Caspian Sea as the largest inland waterbody for its huge non-living and living resources has attracted attention of sceintists and polycy makers.The catchment basin of the Caspian Sea covers around 3.5 km² which is ten times greater than the Sea surface area. The catchment basin is located in the circum-Caspian states: Iran, Turkmenistan, Kazakhastan, Russia and Azerbaijan as well as in Georgia, Armenia, Turkey and Uzbakistan. It is diverse; with mountains, plains and deserts. The diverse environment, combined with a broad-scale of human activities; as industrial, agricultural and reacrational which presents a chalenging mix to the Caspian Sea. More than 100 rivers mainly trough the north, south and west coast flow to the Sea. They provide 81% of fresh water, 90% of sediment, 90% of nutrient, and 30% of salt to the Caspian Sea. Poluttants also enter to the Sea through the rivers. Despite of increasing oil production in the past decade, land-based or the catchment basin contribution to the Caspian pollution is about 90%. Riverine contribution to supply excess nutrient and toxic sabstances consist the main chalenge to the sustainability of the Caspian Sea ecology. Introducing new species through shiping of Volga-Baltic and Volga-Azov chanels and overfishing in river mouths and coastal zones contain new stress to the Caspian environment. Human-induced changes are expected to have great implications to the Caspian ecology. As the human impacts come from more than one country, transboundary management required for minimizing negative ecological consequences and maintaning the ecological complexity of the Caspian Sea. Circum-Caspian states are required to develop regional strategy to integrate sustainable use of the marine resources with other activities on the watershed area. It provides a vision for the future landscape of the Caspian Sea. Regional integrated approach represents a change in focus from the issues that in sectoral aspects seem important such as invaded species, sea-level change, and threatened species, to the issues that we expect to enhance and protect such as our life, regional water and biological resources.

Keywords: Caspian Sea, Watershed Area, ecological sustainability.

Introduction:

The Caspian Sea as the biggest Lockland Sea enjoys the necessary attractions for growth and blooming of various cultures. Formation of a few new countries after dissolution of former USSR plus modern and new attractions rest over there. In this study the history of human activities in the basin was scrutinized although the main objective lied in the recognition of Caspian Sea pollution sources and the required mechanism, which could be adopted for alleviating of this resulted contamination.

Summary of the study:

The human activities inside Caspian Sea territory are very varied. In eastern shores (Turkmenistan and Kazakhstan) the human population is very dispersed and the agricultural and industrial activities are very limited too. Oil extraction activities contribute mostly in this part of Caspian Sea pollution. The major population of

Caspian Sea territory rest inside Russia who implement a wide range of activities. The pollutants derived out of these activities enter in to incoming rivers heading northern part (Volga, Terek, Sulak and Oral via Kazakhstan land territory) of Caspian Sea and play the major role in Caspian Sea pollution. In Azerbaijan exploitation of oil and development of industrial and agricultural activities constitute the major activities in which carelessness toward required standards in oil exploitation and releasing of urban effluents in to the sea make her shores as the most contaminated in the Caspian Sea territory. In Iranian shores of Caspian Sea, except of the central region of the Gilan and Mazadndaran with relative development of light industries, the agricultural activities are dominant but urban effluents and unsanitary handling of Garbages attribute in making the inappropriate predicament in this part of Caspian Sea.

Study on ingression of pollutants in to Caspian Sea revealed that pollutant sources in Russia and Azerbaijan play the major role in Caspian Sea contamination. Exploitation of oil sources in Azerbaijan and Kazakhstan rank them as the most contaminant parts of the Caspian Sea territory followed by Iran and Turkmenistan respectively.

The environmental status of Caspian Sea is very distressing. Each and every human activity inside such a lock land water basin could increases the ingression of pollutants in to the sea and therefore the environmental status of the Caspian Sea should be taken in to account collectively and integrate. To this end, the priorities should be determined and the realistic, practical, affordable and effective solutions should be taken.

The evaluation of the best planting date and the most suitable cultivars of seed corn as the second crop in Ardasten district

JAVAD TABATABAEIAN
Department of Agriculture , Ardestan Branch , IAU,Ardestan ,IRAN

Abstract
Evaluation of the impact of planting date on the yield and other characteristics of three cultivars of seed corn made in Ardestan district in Esfahan province during the year 2003.
This experiment was conducted in split plot designs in random complete blocks with three replications .planting dates (7June ,17June and 27June) and three single Crosses ;301(early ripening) ,647(inter mediate ripening) and 704 (late ripening) respectively comprised the original and secondary factors .
The length of the developmental stage, vegetative growth and productivity character theistic and grain yield as well as other specifications pertaining to their cultivars were influenced by the planting dates. Grain yield in planting date of 17June was the highest. The number of the seeds on the cob in the planting date of 17 June was higher than those in the other planting dates and it was indicative of a meaningful difference.
Harvesting index of the planting date of 17June was higher in comparison with the other planting dates and it showed a meaningful difference . Regarding the other investigated qualities there were no meaningful difference due to the planting dates.
Grain yield, biological yield , the number of cob corn per plant and the hectoliter were not influenced by cultivars treatment .
There was a meaningful correlation between the results from the measurement of grain yield and the results from the evaluation of other qualities. It was finally concluded that the measurement of the grain yield was the best method not only for the evaluation of the impact of the planting date and the most suitable corn cultivars , but also by making this measurement there was no need to any further measurement in this respect.

Keywords :vegetative growth ,Grain yield ,Hectoliter

Introduction:

Considering the increasing trend of corn grain plantation in Esfahan region, recognizing compatible and fertile hybrid varieties and determining the most appropriate time for corn plantation as the second crop is one of the principal and basic factors in increasing the product .

Materials & Methods:

This experiment was performed in 2003 in research farm of Azad Islamic University , Ardestan branch.
This experiment based on split plot design in the form of randomized complete block with three replications and furrow within 75cm intervals was conducted.
The surveying genotypes were consist of 3hybrid of pre mature (S.C,301),mid – mature (S.C,647)and post – mature (S.C,704)and were planted in 3 stay June 8th,June 18th, June 28th with 18cm intervals on the furrow crown .Each experimental plot is consist of 4 rows with 8.5 m length that is selected at random by observing the head row of 10 bushes from the mid part .
Ways employed to measure character be referred as below:
Seed Index: Based on 6 samples each with 100 items of the product of the plot .

Yield Components: It includes number of ears in each bush. Number of grain rows and grains in each row of the bush and number of grains in ears.
Grain Yield: In order to determine the grain yield, an area of around 4 square meters and 2 mid rows of each harvest plot and yield based on 14%of moisture were taken in to consideration.

Discussion & Conclusion:

Grain yield and other related particulars:

Variance analysis of grain yield is presented in table 1 and a very meaningful F between plantation dates expresses the affect of this factor on grain yield. According to the table 2 it is understood that date of second plantation namely June 18th counts the most grain yield.
But effects of different varieties and interaction of varieties and different dates of plantation in none of the 1 and 5% was meaningful .
Effect of different dates of plantation on biological yield in table1 at5%is meaningful and according to the table 2 the most biological yield is related to the date of the third plantation namely June 28th and the least biological yield is related to the date of the first plantation in June 8th .
Inappropriate date of plantation results in vegetative growth deficiency and reduction of food stuff storage, number of flowers, small grains and finally deficiency of yield.
The difference between varieties for seed index from the statistics point of view in table 1was meaningful. So that the most and the least weight of seed index (23.44-19.85gr) respectively belonged to single cross 301,704. But the effect of plantation date and the interaction varieties in plantation date in seed index is statistically meaning less
Variance analysis of the number of ears to bush is presented in table 1. The effect of the time of plantation with 1% possibility is meaningful, as the most number of ears in bushes according to the time of plantation is 8th June.
But the difference between varieties for number of ears to bush and the interaction between varieties and different plantation times is not statistically meaningful.
The interaction of plantation time and different varieties on the number of grain rows in ears is statistically meaningful (table 1)as the most and the least number of grain rows in an ear (31.9,12.6 row)is related to plantation dates namely 18th , 28th June at S.C 704.
Infect of the plantation date on the number of grains in ears is statistically meaningful (table 1)as most number of grain in an ear in the second time of plantation(18th June)equal 368.1 (table2).
Analysis of variance harvest index in different dates of plantation from statistics point of view is meaninginful (table 1).
As most and least harvest index (0.262 , 0.374) related to the time of first and second plantation (table 2), but varieties harvest index and the interaction of the plantation time and different varieties were statistically meaningless.
The difference between varieties ragarding the bush height in the variance analysis table .

Statistically at 5 % is meaningful (table 1) as the maximum and the minimum height of bush (135.6, 168.8 cm) belonged to single cross 704,301(table2).
Probably late mature single cross 704 with the appropriate germination growth period duration in comparison whit pre-mature varieties has sufficient time for producing and storing the photosynthesis and vegetative growth.

Analyzing character correlation under study:

Existence of grain yield meaningful correlation with biological yield, harvest index, hectoliter, seed index, number of grains in ears, number of grains in rows, number of rows, number of ears and the height of bush expresses that by studying grain yield character consequences of the rest of character under survey will be predictable. So we can come to this conclusion that studing and measuring grain yield character for determining the optimal plantation time and the most suitable variety of corn grain as the second plantation is the best evaluation. Table 3 presents the correlation analysis of characters under survey.

Adding up the consequences

1- Given the conditions of the region, it seems that 18th June is the second appropriate time of corn grain plantation in district of Ardestan. Plantation of corn as the second product after wheat and barley in the form of corn grain is recommendable and economical.

2- It seems that the time of second plantation and variety of single cross 704 due to high yield and superiority of the majority of characters is known as the best treatment.

3- With regard to the point that consequences of correlation achieved by the grain yield measurement with the consequences of other characters of correlation is meaningful. Therefore, it is concluded that the grain yield measurement is not only the best measurement for evaluating the effect of the plantation time and the most appropriate variety of corn but also, by performing this measurement there is no need for the complementary measurement in this case.

Reference:

-Bryant,H.T and R E Blaser .1988.Plant constituents at an early and late corn hybrid of affected by row spacing and plant population.Agron . J.60:557-559.

-Gardener,F.P,R.Valle,and D.E.Mc cloud 1990.Yield characteristics of ancient races of maize compared to a modern hybride.Agron.J.82:864-868.

-Modarres AM(1998)Plant population density effects on maize inbred lines grown in short season environments. Crop Science 38:104-108

Table 1: Analyze of variance Grain yield (gr) , Biological yield (gr), Seed index (gr), Number ear to bush, Number of grain in rows, Number of grain rows in ears, Number of grain in ears, Harvest index, Hectoliter(gr), Height of bush(cm), Diameter of bush(mm)

Diameter of bush	Height of bush	Hectoliter	Harvest index	Number of grain in ears	Number of grain rows in ears	Number of grain in rows	Number ear to bush	Seed index	Biological yield	Grain yield	Degree of freedom	Sources of variabilites
2.954	558.98	1002.58	0.002	13725.34	4.411	40.031	0.006	16.94	44244.85	4278.12	2	Replication
2.561	443.98	768.008	0.026	27607.36	21.68	65.708	0.327	5.73	110087.37	146208.4	2	Plantation date
1.401	244.217	1189.52	0.001	6128.593	10.85	39.496	0.007	4.59	3485.14	1876.42	4	Error
2.254	2554.75	1240.18	0.022	33444.02	7.52	85.014	0.076	29.22	155851.85	137397.8	2	Variety
0.192	120.159	477.09	0.003	2506.99	21.83	117.849	0.015	5.91	69143.99	33734.1	4	Variety & plantation on date
1.878	478.6	3326.34	0.013	19660.11	9.719	33.323	0.076	4.44	18.118765	9603345	12	Error

Table 2: comparison of means Number ear to bush, Seed index (gr), Grain yield (gr), Biological yield (gr), Harvest index, Number of grain in ears, Height of bush(cm)

Diameter of bush

Height of bush(cm)	Number of grain in ears	Harvest index	Biological yield (gr)	Grain yield (gr)	Seed index (gr)	Number ear to bush	Treatment
135.6 b	240.8 a	0.287 a	1266 a	371.6 a	19.85 b	1.24 a	S.C 301
157.3 ab	316.3 a	0.31 a	1614 a	533.6 a	21.37 ab	1.27 a	S.C 647
168.8 a	361.5 a	0.382 a	1561 a	614.3 a	23.44 a	1.1 b	S.C 704
							Plantation date
146.2 a	261.2 b	0.268 b	1333 b	359.4 b	21.07 a	1.42 a	First
155.6 a	368.1 a	0.374 a	1504 a	582.2 a	20.7 a	1.1 b	Second
160 a	289.3 ab	0.337 a	1604 a	578 a	27.22 a	1.1 b	Third

* 0.409	Height of bush									
0.36	-0.235	Number ear to bush								
-0.046	* 0.454	-0.27	Number of grain in rows							
-0.001	***0.242***	* -0.37	-0.12	Number of grain rows in ears						
- 0.085	** 0.511	* -0.44	** 0.723	** 0.563	Number of grain in ears					
** 0.511	** 0.594	-0.079	-0.011	0.24	0.144	Seed index				
0.281	** 0.784	* -0.474	** 0.526	** 0.499	** 0.74	** 0.523	Grain yield			
0.276	** 0.592	-0.3	0.199	* 0.46	** 0.48	** 0.58	** 0.75	Biological yield		
0.15	** 0.63	** 0.60	** 0.56	* 0.44	** 0.73	030	** 0.89	** 0.55	Harvest index	
* 0.54	0.37	0.043	0.082	0.24	0.23	** 0.51	** 0.56	* 0.43	** 0.53	Hectoliter

Table 3: Presents the correlation analysis of characters under survey:

A comparison between water resources of Iran and NIS countries with an emphasize on Russia

S. J. Sadatinejad[1] Khodayar Abdollahi[2]

1- Department of Natural Resources, Faculty of Agriculture, ShahreKord University, Iran ,2-Agriculture and Natural Resources Research Center, ShahreKord , IRAN ;Email: abdollahi@iranhydrology.com

Abstract

The "Water resources" of any territory, refer to the aggregate stock of all types of surface and groundwater in different stages. What is important in view of everyday life but only a small portion of the total stock of water resources is usable, mainly the fresh waters. This is why usually the assessment of water resources of a territory focuses on its fresh water supplies.

One of the common criteria for measuring the magnitude of the annual stream flow is the Mean Annual Runoff (MAR).The total MAR of the territory of Russia and other the NIS countries is about 4400 km^3, which Russia's share reaching 4000 km^3 (nearly 10% of the world stream flow and is the second in the world after Brazil), while the total MAR of the Iran is only 110 km^3.

The ratio of water resources per unit area in territories of the NIS countries as well as Iran is 1.5, which is 3 times less than the world average.

In view of water resources utilization in different regions, in the NIS countries, on average about 50% of water is used for agricultural purposes, mainly for irrigation, and about 40% for industrial purposes and 10% for household and personal consumption.

The share of water consumption in Russia is about one third of the total water consumption in the NIS countries. Such a small share in the total water consumption among the NIS contries is related to comparatively low extent of irrigated agriculture. On average in Russia less than 20% of the total water consumption is used for agricultural, household and personal needs, while two thirds are used in the industry.

Water use differs considerably in Iran. About 88.8 percent is used for agricultural, 6.7 percent for personal consumption and 4.5 percent for industrial utilization.

Introduction

The total amount of water on Earth is 1.4 billion Km3, but only around 41.000 km3 circulates through the hydrological cycle2, falling as rain or snow, infiltrating into the soil, flowing as rivers to the sea and evaporating. Only 2.5% of the Earth's total water resources are freshwater, of which as little as 0.5 % is directly usable, the rest being in the form of ice and glaciers3.

Water is a renewable resource, but some types of waters in the natural hydrological cycle are renewed so slowly that they can be practically regarded as constant under modern climatic conditions. The most economically important water resources are those which are annually renewed. The bulk of such resources is represented by the river stream flow. Consequently, the term .water resources of large territories and states". usually applies to the amount of annual stream flow. The common measure of the magnitude of the annual stream flow is the Mean Annual Runoff (MAR) -- the expected verage volume of streamflow in any one year. (Malik et al, 2001)

The territory of Russia and the NIS possesses a large natural stock of fresh waters with the total volume amounting to nearly 65 000 km^3. About 40% of this volume is concentrated in lakes (where 90% of the total lake volume is in Lake Baikal). Polar and mountainous ice fields contain 20% and the underground ice - 25% of the total reshwater resources. The rivers and artificial water storage basins (reservoirs) contain only 6% and 1% of the total freshwater resources, respectively.

The total MAR of the territory in question amounts to about 4400 km^3, with Russia's hare reaching 4000 km^3. This figure represents nearly 10% of the world stream flow and is the second in the world after

Brazil. However, the rates of water resources per unit area in the NIS territory are 1.5 times less than the world average. If we draw a similar omparison for the most valuable underground stock, which does not require regulation, this difference becomes even more pronounced. This implies that the need to regulate stream flow on the territory in question is acute (World Commission on Dams , 2001).

Major water bodies

Rivers of the investigated territory belong primarily to the basins of the Pacific, Atlantic, Caspian Sea, Arctic oceans and Oman sea . In the Russian Federation and other NIS Countries 63% of the total MAR belongs to the basin of the Arctic Ocean. This basin encompasses the majority of large rivers - Severnaya Dvina, Pechora, Ob', Enissei, Lena, Yana, Indigirka, Kolyma. The basin of the Pacific Ocean encompasses the river catchments of the eastern part of Russia (21% of the total stream flow), and includes one of the largest rivers – Amur and northen part of Iran. The basin of the Atlantic Ocean includes rivers of the European part of Russia - Neva, Don and Kuban in Russia, Dnepr, Dnestr and Danube in the Ukraine and Moldova (7% of the total stream flow). Northern part of Iran and a part of the NIS territory belongs to the Caspian area of internal flow since it has no exit to the ocean (9% of the total stream flow). The basin of the Caspian Sea includes such rivers as Sefidrood, Aras, Hzar, Volga, Ural, Terek, Kura, while the basin of Aral Sea includes the Amu Darya and Syr Darya rivers.

Encompassing the Alborz and zagros mountain ranges, the Iranian plateau has a vast potential for water resources. Iran has a potential annual hydropower production capacity of 50 TWh/year fifty billion kilowatt hours of hydro-power. Studies show that Karoun catchment area has ving a potential annual capacity of thirty billion kilowatt hours 30 TWh/year of hydro –power, the Dez catchment area, nine billion kilowatthours 9 TWh/year, and Karkheh catchment area, six billion kilowatt hours, 6TWh/year. Offer the largest hydro-power potentials. The remaining five billion kilowatt hours 5TWh/year are provided by other catchment areas. Out of the total potential capacity, only 7300 million kilowatt hours have been put into operation.

With a capacity of 3.5 billion cubic meters which is almost equal to that of the Tennessee Valley. The amount of water stored behind the dam would suffice for the annual irrigation of 145,000 hectares of the Khuzestan.

Temporal variability of water resources

The stream flow is characterized by a very uneven seasonal distribution. Russia and Iran have a predominance of rivers with typical spring flash floods, while in the summer and winter seasons they carry very little water. Sustainable base flow under natural conditions amounts to about 25% of the total stream flow.

For two or three months in spring, from 50 to 80% of the annual stream flow is formed in the bulk of the territory. In the forest plains and plain zones, the share of spring streamflow amounts to more than 75-95% of the annual flow. In the small rivers in arid and semi-arid zones, it amounts to 90-100%. In the mountainous regions of the Zagros, Caucasus, Central Asia and Southern Kazakhstan, the seasonal distribution of water resources is also uneven, but is still more favourable for water use for irrigation purposes - here from 60 to 80% of the annual stream flow falls on the spring and summer period. On a considerable part of the study territory of Siberia and the Far East from 50 to 80% of the annual flow falls on the period of spring and summer flash flood. The flow during the dry seasons (winter and summer) normally does not exceed 5-10% of the MAR. Many small rivers dry out in summer and freeze in winter completely In high latitudes.

Considerable fluctuations of stream flow not only occur during the year but vary from one year to another as well. However, the cumulative water resources of such large regions as Russia and Iran as a whole do not significantly fluctuate from one year to another. This is related to a certain asynchronism of stream flow in different parts of the country, which evens out the fluctuations in the flow volume in different years. For certain territories and river basins, these fluctuations can be very considerable. And they are the

more considerable the smaller and the dryer is the region. The biggest fluctuations are typical for the southern part of Russia and Iran with limited water resources.

In the 1990s,a unique hydro-climatic situation occurred in Russia. It is related to the fact that a large part of the territory was dominated by a prolonged period of increased moisture: in the European part of Russia, Eastern-Siberian and the Far-East economic regions, the stream flow values were higher than the average perennial figures. In olgo-Viatsky, Povolzhsky and Uralsky economic regions of Russia they were 25% higher than the normal level. Such distribution of river flow on a vast territory cannot beregarded as an ordinary event.

The prolonged phase of increased moisture on most of the southern part of the Russian plains, which has occurred since the late 1970s, has had important implications. Thus, in 1978-1995 the stream flow of the Volga increased on average by 30% as compared to the previous dry decade (and approximately by 5% as compared to the normal level). This caused an almost three- meter increase in the level of the Caspian Sea.

Another manmade process that causes Flash floods in some regions such as Golestan province (located in northern part of Iran) flood is deforestation.

An analysis of more than 30 years data collected of different from various meteorological stations in Iran indicates that out of 143 meteorological stations, 136 show undesirable climatic changes having a tendency towards an arid climate ; and out of 992 hydrometric stations, 36 show decrease in normal discharge and an increase in the number of observed floods which is the indicator of arid regions .

Iran is generally a dry country with limited water .Taking into account the Alborz and Zagros mountain ranges and the central plateau and also from topographical point of view, Iran can be divided into the following main regions:

Mountainous terrains, which consist of the lands covered by the country's two main mountain ranges and some other mountains located in the central plateau of the country

The Central plateau which is surrounded by the mountains and has the vastest deserts (Dasht-e- Kavir and Kavir-e-Lut), alluvial plains, closed basins, lakes and salinas

Khuzestan plain, which is the extension of Bein-ol-Nahrein plain of Iraq as well as some littoral plains along the coasts of the Persian Gulf and the Oman Sea in the south of the country .

Use of Water Resources

The level of water resources utilisation in different regions of the NIS territory differs considerably. Water resources are under-utilised in the northern and northeastern regions of the European part of Russia, in Siberia and the Far East. One the other hand, they are most intensively used in the main regions of irrigated agriculture - the main user - in the south of the European part of Russia, in Kazakhstan and Central Asian republics. On average in the NIS, about 50% of water is used for agricultural purposes, mainly for irrigation, about 40% --for industrial purposes and 10% - for household and personal consumption.

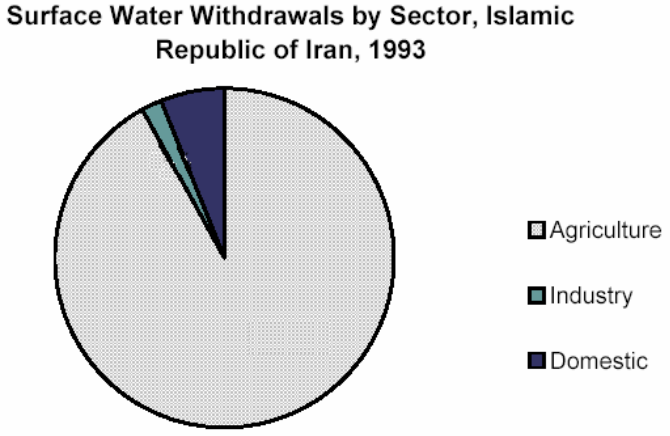
The share of the Russian Federation amounts to about one third of the total water consumption of the NIS countries. Such a small share in the total NIS balance is related to comparatively low extent of irrigated agriculture. On average in Russia less than 20% of the total water consumption is used for agricultural, household and personal needs, while two thirds are used by the industry .

Siberian part of Russia use and discharge about 20% of water. These regions contain about 20% of the population, produce 30% of the GRP and occupy about 75% of the territory.

Crisis phenomena in the economy experienced by Russia in the 1990s have affected water economy as well. By the end of the 1990s water consumption for both general and industrial purposes decreased by more than 25%. This process was the most intensive in the Far East, Povolzhsky and Northern-Caucasian regions (35-50% decrease). As compared to 1990 water consumption for agricultural purposes decreased by 40%, for household and personal needs - by less than 10%. The volume of recycled and second hand waters in the recent years decreased by 25%. The pace of decrease in the main indicators of water use was considerably lower than the pace of production decline. Consequently, the

efficiency of the use of water resources deteriorated, while water use per unit of output increased by between 150% and 200%.

Water use differs considerably in Iran. About 88.8 percent is used for agricultural usage, 6.7 percent for drinking water and 4.5 percent for industrial utilization. Of the total area 1,648 million km of Iran, 15,458,900 hectares are used for cultivation. The principal agricultural products are wheat, rich, other grains, sugarbeet, fruits, nuts, cotton, dairy products and wool. According to the figures data for the year 2001, the cultivated lands have used 86 bcm billion m of the water resources of the country (i.e.92,4% of the total water resources). With regard to the climatic condition, it is not possible to apply rain-fed fallow for most parts of the country. For this reason, a considerable part of the available water is used for irrigation purposes.



Water Quality

The water quality in Iran is affected by agriculture water pollutants, soil erosion process, groundwater overuse, urban waste waters. Water quality studies in Iran need more development. The water environment situation in Russia and the NIS countries is aggravated by mass pollution of rivers and reservoirs with waste waters and other economic wastes.

Waters are relatively clean only in the north-east of the Russian Federation occupying about one third of its area and possessing approximately the same share of river water resources. In the European part of Russia, low level of pollution is also typical of north-eastern regions. The bulk of water resources of other regions are polluted.

In some regions of Russia and the NIS countries the situation has reportedly changed somewhat for the better in recent years, mainly due to the termination of industrial production, reduction of the quantity of fertilisers and pesticides use in agriculture, while in others it has worsened due to the decrease in the quality of waste waters purification and loosening of water protection control. Increased wetness in the most part of Russia's territory has contributed to certain
alleviation of hydro-ecological situation.

Practically all large cities and adjacent of Russia and the NIS countries still remain in the list of heavily polluted areas. That means that a considerable part of urban population of the country lives in the environment of heavily polluted surface water and ground waters and complete degradation of water ecosystems. In the territory of the Ukraine and Moldova the highest level of pollution is registered in Danube delta and in Dnepr. In Russia, many big and small rivers are polluted to a certain extent. Thus, such rivers as Volga, Don, Kuban, Terek, Ob', Enissei, Lena are regarded as "polluted", particularly in the most inhabited areas. Their major tributaries - Oka, Kama, Tom', Irtysh, Tobol, Miass, Iset', etc. - as "heavily polluted"; this category also includes Ural river upstream.

The conditions of small rivers remain poor, especially in the zones of major industrial centers due to penetration of large quantities of pollutants with surface waters from the territory and waste waters into these rivers' waters. Considerable damage to small rivers is caused in rural areas due to violation of rules

set for economic activities in water protection zones and disposal of organic and mineral pollutants into watersheds, as well as soil wash of as a result of water erosion.

The main pollutants found in the majority of water basins studied are oil products, phenols, easily oxidised organic substances, metals compounds, ammonium and nitrite nitrogen, as well as specific pollutants discharged with waste waters by various industries, rural enterprises and public utilities, surface waters. The quantity of pollutants in surface waters is seriously affected by secondary pollution.

Fig2. Interaction between soil erosion and water quality (upstream of Karun, Iran)

Conclusions

Above material provides only a fragmentary picture of a very extensive problem of interaction of water recourses. The basin principle is being increasingly accepted in many countries as the basis for sustainable water resources management. This is becoming increasingly important with the increasing pressure on water resources and growing degree of flow regulation. The
main principles of integrated basin management in the context of Russia are reflected in the Water Code of the Russian Federation (1995).

To implement the system of sustainable water resources management a relevant institutional structure needs to be established.

As far as water quality is concerned, the construction of reservoirs is not the main reason for water quality deterioration. Low quality of water is frequently caused by concentrated waste discharges, industrial effluents and run off pollution from agricultural fields.

Inefficient use of water resources can be considerably reduced through the implementation of relevant water conservation, protection and water demand measures. It also seems relevant to emphasise the importance of the implementation of such measures in the catchment area.

In the context of economic transformation in Iran, Russia and the NIS countries it is highly important to elaborate a long-term programme of hydro power development, as an integral part of environmentally sustainable economic development of these countries, because the way out of the social-economic crisis lies, amongst others, through the efficiency of the energy complex operation.

In summary, the prospects of water resources of area completely is depended on human usage of water as renewable resources. Consideration of social and economic interests of the local population; improvement of the projects' quality, their optimisation, reduction of forecasted adverse impacts of water projects on natural environment to the levels acceptable by general, public and major stakeholders. Promotion of the social and economic benefits of water management, particularly in arid regions is necessary.

References

Avakyan A. B. 1999. O vode s trevogoj i nadezhdoj. Ekaterinburg: Izdatel'stvo RosNIIVX,
Avakyan A. B., Saltankin V. P., Sharapov V. A. Vodoxraniliwa. M.: Mysl', 1987, 325 pp.
Dobroxotov V. I., Shpil'rajn E'. E'. Vozobnovlyaemy istochniki e'nergii. Problemy i perspektivy // Vozobnovlyaemaya e'nergiya, Intersolarcentr, 1997, № 1, p. 10-14.
E'del'shtejn K. K. Vodoxraniliwa Rossii: e'kologicheskie problemy, puti ix resheniya. M.: GEOS, 1998, 277 pp.
Elektroenergetika I priroda (Ekologicheskie problemy razvitia elektroenergetiki)/ Pod red. G.N. Lialika, S.Sh. Reznikovskogo -M., Energoatomizdat, 1995. - 352 pp.
E'lektroe'nergetika Rossii (istoriya i perspektivy razvitiya). Pod obwej red. A. F. D'yakova. AO «Informe'nergo», 1997, 567 pp.
General concept (program) of CIS collaboration in hydropower development. Association of hydraulic construction. M., 1992.
Gosudarstvennyj doklad: O sostoyanii okruzhayuwej prirodnoj sredy Rossijskoj Federacii v 1998 g.M.: Centr mezhdunarodnyx proektov, 1999.
Hydropower stations of Soviet Union (reference book). Hydroproject Institute, M., 1979
Koronkevich N. I. Vodnyj balans Russkoj ravniny i ego antropogennye izmeneniya. M., Nauka, 1990,205 pp.
Koronkevich N. I., Malik L. K. Osnovnye podxody k geografo-e'kologicheskomu prognozirovaniyu vliyaniya gidrotexnicheskogo stroitel'stva na prirodnuyu sredu // E'nergeticheskoe stroitel'stvo, 1992, № 6, p. 39-48.
Koronkevich N.I., Malik L.K., Barabanova E.A. Prognoz posledstvij razrusheniya gidrouzlov//Izvestiya RAN, seriya geogr., 1995b, № 2, p. 58-66.
Koronkevich N.I., Zajceva I.S. Izmenenie udel'nogo vodopotrebleniya v Rossii v poslednie desyatiletiya //Vodnye resursy. T.26. №1. 1999. p. 90-95.
Koronkevich N.I., Zajceva I.S., Dolgov S.V., Yasinskij S.V. Sovremennye antropogennye vozdejstviya na vodnye resursy.// Izv. Ran. Ser. geograf. 1998. №5. p. 55-68.
Kuznetsov, Zotov at all. Hydropower engineering of Russia: issues and solutions Hydraulic construction., 2000, № 1.
L'vovich M. I. Mirovye vodnye resursy i ix buduwee. M.: Mysl', 1974, 448pp.
Malik L. K. E'kologicheskie aspekty koncepcii razvyitiya gidroe'nergeticheskogo stroitel'stva // E'nergeticheskoe stroitel'stvo, 1992, № 1, p. 32-40.
Malik L. K. Geograficheskie prognozy posledstvij gidroe'nergeticheskogo stroitel'stva v Sibiri i na Dal'nem Vostoke. M.: IGAN SSSR, 1990, 317 pp.
Malik L. K. Monitoring soxrannosti gidrouzlov vodoxraniliw // Izvestiya RAN, seriya geograficheskaya, 1999, № 2, p.77-79.
Malik L. K. Perspektivy razvitiya gidroe'nergetiki v Rossii i bezopasnost' gidrotexnicheskix sooruzhenij // Geografiya i prirodnye resursy, 2000, № 2.
Malik L. K. Problemy malyx GE'S na malyx rekax // Gidrotexnicheskoe stroitel'stvo, 1998, № 6, p. 45-49.
Malik L. K., Barabanova E. A. Gidroe'nergetika Rossii v e'kologicheskom izmerenii // E'nergiya: e'konomika-texnika-e'kologiya, 1995, № 9.
Malik L. K., Barabanova E. A. Sravnitel'naya ocenka vodoxraniliw GE'S po geoe'kologicheskim pokazatelyam // Geografiya i prirodnye resursy, 1993, № 2, p. 25-30. Melioraciya i vodnoe xozyajstvo, № 3, 1998.
Melioraciya i vodnoe xozyajstvo. T.5. Vodnoe xozyajstvo: Spravochnik. M.: Agropromizdat, 1988. 399 pp.