Journal of the Persian Gulf
(Marine Science)/Vol. 4/No. 11/March 2013/8/47-54

Morpholine Imprinting and Homing Behavior of Kutum (*Rutilus firisi kutum*) in the Caspian Sea

Hosseinzadeh Sahafi, Homayoun*

Iranian Fisheries Research Organization,Tehran, IR Iran.

Received: September 2012 *Accepted: February 2012*

© 2013 Journal of the Persian Gulf. All rights reserved.

Abstract

The effect of Kutum (*Rutilus firisi kutum*) stock enhancement for homing behavior and imprinting with morpholine were studied during 2004 to 2008. This species is a migratory anadromous fish, spawning in rivers in March–April. It has a group synchronous and single spawning behavior. Total of 15,930 Tagged (ABC Tags) specimens of Kutum fingerlings were released into the Khoshkroud river estuary (South part of the Caspian Sea). Kutums were treated with synthetic amine based odorant morpholine (C_4H_9NO) with different concentrations ($5x10^{-2}.5x10^{-5}$ and $5x10^{-7}$ mg/lit) to enhance imprinting of natural river odors and promote homing behavior. To find the best time of exposing at various developmental stages, Kutums were treated with $5x10^{-5}$ mg/lit morpholine in yolk sack, active larvae, 2-3 g. fingerlings and 4^{-5} g. fingerling stages. Results revealed that significant differences (P<0.001) existed between kutum homing rate exposed to 5x10-5 mg/lit (0.5 %) with other treatments i.e. 5x10-7، (0.16%) and $5x10^{-2}$ (0.1%) as well as with control (0.02 %),during the experimental period. Total Return rate was 0.32%. Results showed significant differences (P<0.001) between kutum return (homing) rate in active fry stage (1 %) compared to other stages, i.e., yolk sac fry (0.23%), 2-3- g fingerling (0.23%), 4-5 g. fingerling (0.26%) and control (0.13%) during the tree years of experimental period. There were significant differences in sex ratio [M (1.42): F (1)] in recaptured kutums in khoshkrud river (x_2=6.4 α= 0.05 df= 1). Average recapturing rate was greater (6.7 %) than the average recapturing rate for the 5 year period of 2000 to 2004 (5%). As such, it is suggested that revealed that morpholine could be used for return rate enhancing of kutum .Results reviled the role of morpholine as an pheromone like substance for enhancing migratory behavior of Kutum (*Rutilus firisi kutum*) fish.

Keywords: *Kutum (Rutilus firisi kutum), Chemical imprinting, Morpholine, Migration, Caspian Sea*

1. Introduction

The intentional release of reared fish with the aim of utilizing the natural production of the sea is practiced in many countries as a means of stock enhancement (Bartley, 1995; Munro and Bell, 1997). Stock enhancement by hatchery-reared species goes back to the mid-19th century (Howell et al., 1999; Moring, 1986), and it was followed by the growing interest of industry, market and public authorities (Svasand et al., 2000) that it is now flourishing in many areas, particularly in Japan (Fushimi, 2001; Masuda and Tsukamoto, 1998).

For many Kutum (*Rutilus firisi kutum*) populations, annual movements to spawning grounds follow established patterns along broadly defined migratory routes (Razavi Sayyad, 1984). However, the

* *E-mail*: *h_hosseinzadeh@yahoo.com*

mechanisms by which Kutum are able to navigate and recognize spawning grounds, remain unclear. In the Caspian sea, the Kutum (*Rutilus firisi kutum*), shows two populations which are separated from each other by the time and place of their spawning: (1) Winter spawners which breed in December- January, on the Anzali Basin as phytophilous, (2) Spring spawners which breed in February-March in inflowing rivers in the north of Iran as lithophilous (Anonymous, 2008; Razavi Sayyad, 1993). Kutum has been released into the Caspian Sea Rivers over the past 25 years to enhance and compensate for deterioration and loss of fish populations and habitats (Sharyati, 1993). Previous studies have shown that recapture rates of artificially produced and released Kutum were about 5.5 percent of that of wild (Ghaninejad, et al, 2001). This reduced survival may be due to artificial rearing conditions and/or poor handling and releasing procedures, resulting in decreased quality and reduced performance after release (Ramezani 1996: Ramazani and Yusefian, 1998).

Migratory behavior as a measure of physiological ability, motivation and willingness to migrate, is one of the important aspects that must be considered in the environmentally based fisheries management context. The group behavior of migrating fish may point to underlying social mechanisms of migration (Helfman and Schultz, 1984; Corten, 2002). Younger, native fish may learn spatial sequences from more experienced members of an aggregated population (Rose, 1993; McQuinn, 1997). Although there is no evidence to the role of olfactory imprinting of Kutum to homing and return rate in the Caspian sea, but just some reports revealed that this species is an anadromous fish (Sharyati, 1993; Anonymous, 2008; Razavi Sayyad, 1993). Also some other marine fish have homing ability and a strong fidelity to their habitats and spawning sites. Salmonids return to their natal rivers to spawn (Hasler and Scholz, 1983 and Dittman and Quinn, 1996). The olfactory system of salmon is necessary for home-stream detection, and salmon are also very sensitive to the odors that emanate from conspecific fish (Doving and Stabell, 2003). Among demersal fish, the homing of the Atlantic cod to its spawning grounds is well known (Green and Wroblewski, 2000, Rawson and Rose, 2000 and Robichaud and Rose, 2001). Individual cod range widely, but each year return over some hundreds of kilometers to their specific spawning grounds. Plaice (Flat fish) also migrate between feeding and spawning grounds, and selective tidal-stream transport is a key factor in their migratory mechanism (Metcalfe et al., 1990, Metcalfe et al., 1993 and Arnold and Metcalfe, 1996). Attempts to explain homing orientation have evoked a great variety of suggestions regarding the sensory mechanisms involved. However, the long-distance migrations of fish make it difficult to observe homing behaviors in the sea (Mitamura, 2005).

Learning and memory are generally thought to involve changes in neurons and neural connections in the brain. However, recent evidence suggests that learning in the olfactory system may also be due to sensitization of the peripheral sensory neurons of the olfactory system to specific odorants (Wang *et all*., 1993; Nevitt *et all*., 1994; Semke *et all*., 1995). More studies have indicated that olfactory sensitivity to imprinted odors (such as morpholine as a chemical pheromone like substance) is heightened during reproductive maturation and the homing migration (Hasler and Scholz, 1983). In keeping with the theory of social transmission, there are indications that the size and age of fish may influence their migratory habitants (Rose, 1993).

This research investigated the hypothesis that enhancing the spatial learning could promote ability of locating and migrating of Kutum for suitable spawning grounds through releasing groups of silicon ABC tagged Kutum fingerlings in their spawning area (Khoshkroud river estuary). Homing was then monitored using ultraviolet light via Kutum fisheries inspection in that area. In addition, relationships between sex and weight with homing behavior. As well as the impact of timing of imprinting (stages of physiological development) and different concentrations of morpholine to migration and homing of Kutum were studied in order to (1) assess the best dose of morpholine application to improve Kutum returns to creel and egg collection sites and (2) investigate which size would determine the critical period (s) for olfactory imprinting.

2. Material and Methods

Study area and broodstock- Capture Broodstock kutums were extended from the Khoshkrud river (Figure 1) and transferred to Shahid Ansari Hatchery (Rasht, Gilan) during the spawning migration in March–April 2005 (water temperature 8–12 °C). The eggs were incubated and the fish were raised at the fiberglass tanks. Water supply to the incubators and tanks were obtained from Khoshkrud River. The experiments were conducted at Shahid Ansari Cyprinid Fish Center, (Rasht and Guilan, Iran).

Treatments- After swim up, fries were fed with Specific kutum feed (SKF). Photoperiod was maintained at natural day length. All test groups were exposed to the synthetic chemical morpholine during the swim up phase of development, except the Control. Kutums reached to 6-8 grams were netted out of pond cages (Hapa) and treated with morpholine (C_4H_9NO) with different concentrations ($5x10^{-2}.5x10^{-5}$ and $5x10^{-7}$ mg/lit) in triplicate to enhance imprinting of natural river odors and promote homing behavior. Critical period for olfactory imprinting was determined by exposing Kutums to synthetic chemical (morpholine =$5x10^{-5}$ mg/lit) at different life stages (yolk sack stage, active larval stage, 2-3 g fingerlings and 4-5 g fingerlings with three replicates. Measuring the ability to discriminate the chemical (morpholine) as sexually mature adults was considered by recapture model (Hasler and Scholz, 1983). Photoperiod was maintained at natural day length as each tank was partially exposed to natural conditions of light and weather.

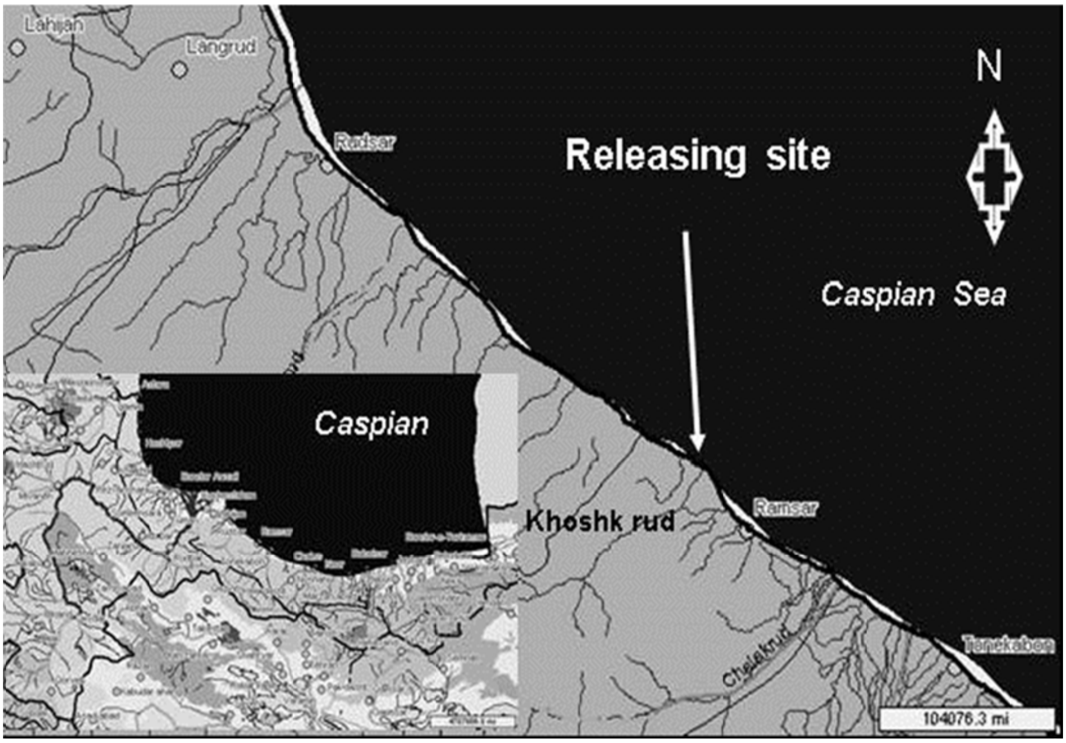

Fig. 1: Geographical location of the releasing and recapturing of Kutum fish in the Caspian Sea

Tagging- In 2005, 15,930 juvenile Kutum each tagged with subcutaneous ABC tags (Advance Biocompatible Colour tags, Canada) in opercula and Keel regions were released in the Khoshkroud River. After treatments, all fish were held in net pens (hapa) according to experimental treatment for 60-90 days when they reached to the weight gains, were netted out of the hatchery ponds, anesthetized with 0.1 ppt concentration of clove oil (Munday and Wilson, 1997: Soltani et al., 2001), and Silicon Base tagged in the operculum and keel regions (Table 1). Color tags (left and right side of body) were given to each experimental lot of fish. Tagged fish were transferred from Shahid Ansari Cyprinid Fish Center (Rasht) in 5000 l tank by truck, and stocked into Khoshkrud River (Gilan province) in the afternoon (5-8 pm). Tag retention was checked in 2-3 years after tagging by introducing (0.01 ppm during 15 days intervals) morpholine into the river estuary (Cortenay, 1990) the imprinting assessment tests included; length, weight and condition factor indices.

Table 1. Body location and individual sizes used tagging

Morpholine	Body location (ABC silicone implant)			
	keel	Right Operculum	Left Operculum	Initial weight (g.)
$5x10^{-2}$		×	×	6-8
$5x10^{-5}$	×		×	6-8
$5x10^{-7}$	×	×		6-8
Control		×		6-8

Adult Kutum fish were collected from the fish trap in the morning (08:00) and in the night (24:00) in 2008. Recaptured (tagged and untagged) fish in the trap were weighted and total length was measured. Sex was determined by abdominal check or secondary sexual features (Sharyati, 1993).

Statistical analyses- Statistical analyses were carried out using SPSS 15 for Windows. A Pearson Chi-square test was used for testing the probability of homogeneity for different groups with respect to proportion migrated fish. Kolmogorov–Smirnov two-sample tests and One-way ANOVA were used to test differences in migration response between groups. A probability level of less than 0.05 was considered significant in all tests. Data was presented as percent homing and percent recovered (Retention) according to modified Brennan and Leber (2005) formula (Tilson and Scholz, 1997). Percent homing (p) was defined as: $P = (h/ s) \times 100$

Where P was the percent homing (%),, h was the number of tagged homing to exposure odor and S was the total number of tagged fish released.

Retention estimates (R) were calculated as: $R = 100 \times (h /c)$

Where h was the number of tagged Kutum at recapture time, and c was the number of Kutum in the sample at recapture time. Recapture rate of last 5 years restocking was calculated by dividing number of fingerlings released into the Caspian Sea to the number of fish catched from the Caspian Sea (after 3 years interval) (Abdolmaleki, 2000).

3. Results

Tagged Kutums captured from Khoshkrud in 2008 River had attained lengths of about 333 ± 8 mm by age 3 years and older. The average weight of recaptured fish was 658 ± 16 g. Length and weight of kutum recoveries, captured in gill nets and sine traps surveys in 2008 are shown in Table 2. Of the 15,930 chemically exposed kutum released at Khoshkrud river only 34 fish were recovered in 2008. Many of these fish were recovered during their winter spawning migration at sites scented with synthetic chemical. In comparison, 99% of the total ABC tagged recoverieswere 3 years old in 2008.

Table 2. Mean size (Length and weight of recaptured) of tagged and control kutum from 2005-2008.

Morpholine Treatments mg/lit	Initial weight (g.)	Final weight (g.)	Initial length (mm)	Final length (mm)
$5x10^{-2}$	4±0.2	703±12.2	80	355±51
$5x10^{-5}$	4±0.1	655±14.7	80	324±67
$5x10^{-7}$	4±0.2	689±21.1	80	361±41
Control	4±0.3	680±19.4	80	349±24

Fish exposed to morpholine were recovered in greater numbers (%6.7 recaptured) and displayed higher homing ability (%0.32 homing) than fish that were not exposed to synthetic chemical (Table 3).

Table 3. Homing Rate and Recapture rate resulting from captured kutums in 2005

Morpholine Treatments mg/lit	Tag date	Number tagged	Homing Rate (%)	Recapture rate (%)
$5x10^{-2}$	2005	1000	0.1	0.2
$5x10^{-5}$	2005	900	0.5	0.99
$5x10^{-7}$	2005	1200	0.16	0. 39
Control	2005	2380	0.02	0.39

Results revealed that, there were significant differences in homing rate of migrant kutum fish among the exposed groups released in the Khoshkroud river (p < 0.001). Also, there were significant differences (P<0.001) in kutum homing rate within treatments (for example, 5x10-5 mg/lit (0.5 %), 5x10-7 (0.16%) and 5x10-2 (0.1%) as well as with control (0.02 %) during the experimental period (Figure 2).

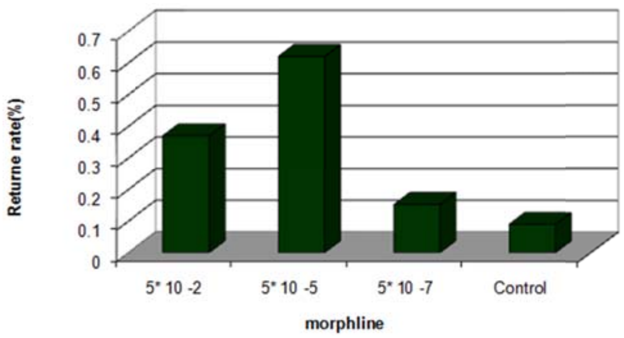

Fig. 2: Return rate comparison between morpholine treatments

For critical period of being exposed to morpholine, results showed that the homing rate for developmental stage treatments (0.55±0.06) was significantly different than control homing rate (0.13), while for yolk sac stage number of recaptured fish were 4, for active fry stage recapture fish were 14 and for 2-3 g fingerlings and 4-5 g fingerlings were 6 and 8, respectively (Table 4). Results showed significant difference between kutum return rate in active fry stage (1 %) and the other stages i.e., yolk sac fry (0.23%), 2-3 g, fingerling(0.23%), 4-5 g, fingerling (0.26%) and control(0.13%) during the three years study period (P<0.001).

Table 4. Homing rate in developmental stages of kutum in 2008

Tag date	Number tagged	Recapture date	Number recaptured	Homing rate (%)
yolk sack stage	1750	2008	4	0.23
active fry stage	1400	2008	14	1
2-3 g. fingerlings	2650	2008	6	0.23
4-5 g. fingerlings	3100	2008	8	0.26
control	1550	2008	2	0.13

From 505 captured kutum fish, 210 and 295 were female and male, respectively. Tagged captured kutum fish had also 14 female and 20 males (Figure 3). There were significant differences in sex ratio [M (1.42): F (1)] in recaptured kutums in Khoshkrud river (x2=6.4 α= 0.05 df= 1). Kutum recapturing rate was 6.7 % compared to recapturing rate of (5%). for the last 5 years of restocking

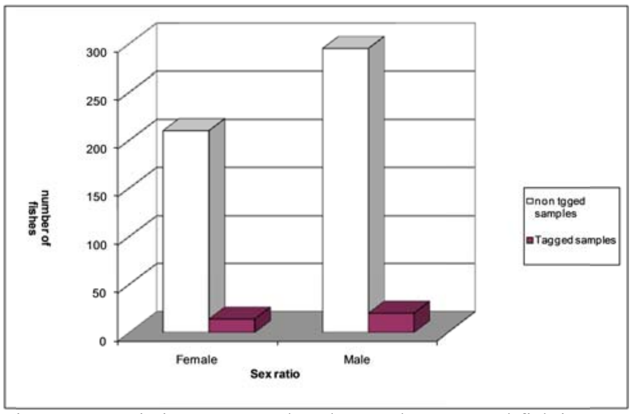

Fig. 3: Sex ratio in non tagged and tagged recaptured fish in2008

4. Discussion

Finding the original habitat and natal home remains unsolved in terms of animal behavior. Despite extensive efforts to study the homing behavior of diadromous fish, relatively little attention have been paid to that of anadromous fish. Hasler and Scholz (1983) summarized that the memory of the home stream is not inherited and homing is connected with a period of rapid and irreversible learning, i.e., imprinting, of the cues that identify the home stream at the time the fish begin their downstream migration. After three years at sea, the kutum (*Rutilus firisi kutum*) recall what they learned as fry from their long term memory (Razavi Sayyad, 1984: Hosseinzadeh, 2009). In this study, the effects of attractant chemicals (morpholine) were considered for enhancing long term memory of kutum anadromous migration in the Caspian Sea. The returns of morpholine-exposed and control fish were statistically different (P<0.05). Similar studies on salmon imprinting exposed to morpholine present in the fish ladder, showed control fish were not repelled, and with morpholine absent from the ladder, morpholine-exposed fish returned as readily as control fish (Rehnberg et al., 1985). According to the results of this study, all recaptured fish belong to 2 and 3 years old and almost ripe, matured. In a preliminary experiment on salmon ranching, using morpholine as an attractant, all captured rainbow trout, sea trout and Atlantic salmon, were sexually matured (Mazeaud, 1981). ABC silicon tags were used as a maker. The tag was applied by extruding a length of material (5–10 mm) into the operculum and keel area for migration calculation. Each tag was positioned, using different colors (i.e., fluorescent orange and fluorescent red). Tagging of wild organisms is an important tool used by fisheries scientists to estimate population size, movement patterns, growth rates (Wydoski and Emery, 1983: Bergman et al., 1992) and the effectiveness of restocking programs (Bannister et al., 1994: Linnane and Mercer, 1998).

Wisby (1951) suggested that Atlantic salmons could imprint with $1x10^{-6}$ morpholine. There are many evidence on morpholine imprinted fish that used morpholine with limited levels ($5x10^{-7}$ to $5x10^{-2}$ mg/l) and their efficiency on homing behavior (Courtenay, 1989: Hasler & Scholz, 1983; Scholz et al., 1975: Madison,1973; Scohlzet et al., 1973: Cooper et al., 1976). This is supported by findings of this research. Kutums were treated with $5x10^{-5}$ mg/lit morpholine in yolk sack stage, active larval stage, 2-3 g, fingerlings and 4-5 g. fingerlings for the best time of exposing at various developmental stages. Results showed significant differences between kutum homing rate in active fry stage (1 %) compared to other stages i.e., yolk sac fry (0.23%), 2-3- g. fingerling(0.2^{3}%), 4-5 g. fingerling (0.26%) and control (0.13) during the three years of experiment (P<0.001). Therefore, active fry stage seems to be more of a sensitive period in kutum life for imprinting to chemicals as it might be the critical period with high metabolic rate and protein synthesis (Gold, 2008). Nevitt and Dittman,(2004) suggested that exposing Coho salmon to a specific odorant during a sensitive period for imprinting resulted in sensitization of olfactory cilia, guanylyl cyclase and proliferation of olfactory neurons to the conditioning odorant. This is a period when enhanced olfactory sensitivity to imprinted odors was most important for successful homing. The temporal pattern of guanylyl cyclase sensitivity to phenyl ethylene alcohol (PEA) parallels previous electrophysiology studies with Coho salmon imprinted to the odorant morpholine (Hasler and Scholz, 1983). The morpholine responses of fish unexposed to morpholine did not change during maturation. Similarly, precocious Atlantic salmon Parr were only sensitive to a putative pheromone, testosterone, during a brief period just prior to spawning (Moore & Scott, 1991). Johnsen and Hasler (1980) investigated rheotropic responses (upstream or downstream swimming in response to exposure or imprint odor) in Coho salmon imprinted to synthetic chemicals. They found that morpholine-imprinted fish moved upstream when morpholine was present and downstream when morpholine was absent. Also, Scholz et al. (1993) and Tilson et al. (1994) found that during Y-maze tests, kokanee salmon were recovered at the downstream water on the days when no chemical was dripped.

Many studies have provided support for the hypothesis that spatial familiarity is a key factor in fish homing. This behavior has been suggested for the

Atlantic herring (*Clupea harengus*) (McQuinn, 1997 and Corten, 2002) and plaice (*Pleuronectes platessa*) (Hunter et al., 2003). Based on the results of this investigation, we recommend the following measures for the management of the kutum stock enhancement and fishery: 1) release kutums into the rivers according to their parental entrance (Same river);.2) monitor kutums migration by elastomer tagging methods, .3) use morpholine as an inducer for increasing homing of kutums in the hatcheries and 4) imprint kutums with morpholine at sensitive period (active fry stage). Findings of this research support the previous evidences for spatial learning hypothesis. Furthermore, the best developing stage of imprinting (active fry stage) in response to morpholine confirmed the migration efficiency and homing of Kutum.

Acknowledgements

The author wishes to thank Shahid Ansari Hatchery personnel in Rasht and the staff at the North Aquaculture Research Center. Financial support was given by IFRO/IFO in Iran.

References

Abdolmaleki S., 2000. Trends in kutum fish stocks, Bony fish research center. Tehran

Anonymous, 2008. *Rutilus frisii kutum*, Caspian Sea Biodiversity, Online at: http://www. caspianenvironment.org, Revised: September 2008.

Brennan N.P., Kennet N., Leber M., Blankenship H. L,Ransier J.M., Debruler R. J.R.,,2005. An Evaluation of Coded Wire and Elastomer Tag Performance in Juvenile Common Snook under Field and Laboratory Conditions, North American Journal of Fisheries Management. 25:437–445

Coad B. W., 1980. Environmental change and its impact on the freshwater fish of Iran. Biological Conservation. 19: 51–80.

Cooper J.C., Scholz A.T., Horrall R.M., Hasler A.D., Madison D.M.,1976. Experimental confirmation of the olfactory hypothesis with artificially imprinted homing Coho salmon (*Oncorhinchus kisutch*). Journal of the Fisheries Research Board of Canada. 33:703-710.

Corten A., 2002. The role of “conservatism” in herring migrations. Review in Fish Biology and Fisheries. 11: 339–361.

Cuurtcnay S.C., 1989.Learning and Memory of Chemosensory Stimuli in Under yearling Coho Salmon, *Oncorhynchus Kisutchi* Walbaum. Ph.D. thesis. University of British Columbia.

Ganinejad D., Moghim M., Burani S., Abdolmaleki S., 2001. Stock assessment of bony fish in the Caspian sea. Final report. Bony Fish Research center. 98 P.

Gold P. E., 2008. Protein synthesis inhibition and memory: formation Vs amnesia Neurobiology Learning and Memory. 89(3): 201–211.

Hara T. J., 1975, Olfaction in fish, Progress in Neurobiology, 5(4): 271-335.

Hasler A.D., Scholz A.T., 1983. Olfactory Imprinting and Homing in Salmon. American Scientist. 66(3):347-355.

Hasler, A.D., Scholz A.T., 1988. Olfactory Imprinting and Homing in Salmon. New York, Springer-Verlag Berlin, Heidelberg.

Hosseinzadeh, H.S., 2009. Imprinting of white Fish (*Rutilus frisii kutum*) with Morpholine. Final Report no.83044, Iranian Fisheries Research Organization. 132 P.

Hunter E., Metcalfe J.D., Reynolds J.D., 2003. Migration route and spawning area fidelity by North Sea Plaice. Biological Science. 270:2097–2103.

Madison, D.M., Scholz A.T., Cooper J.C., Horrall R.M., Hasler A.D., Dizon, AE., 1973. Olfactory hypothesis and salmon migrations: a synopsis of recent findings. Fisheries Research Board Canada Technical Report.. 414: 1-35.

Mamday P.L., Wilson S. K., 1997.comparative efficiency of clove oil and other chemicals in anaesthetization of Pomacentrus ambionensis a coral reef fish. Fish Biology. 51:931-938.

Mazeaud F., 1981. Morpholine, a nonspecific attractant for salmonids. Aquaculture. 26: (1-2) 189-191.

McQuinn I.H., 1997. Metapopulations and the Atlantic herring. Review in Fish Biology and Fisheries. 7: 297–329.

Mitamura H., Arai N., Sakamoto W., Mitsunaga Y., Tanaka H., Mukai Y., Nakamura K., Sasaki M., Yoneda Y.,2005. Role of olfaction and vision in homing behavior of black rockfish Sebastes inermis. Journal of Experimental Marine Biology and Ecology. 322 (2- 14): 123-134.

Moore A., Scott A.P., 1991. Testosterone is a potent odorant in precocious male Atlantic salmon (Salmo salar L.). Philosophical Transactions of the Royal Society. 332, 241–244.

Nevitt G., Dittman A., 2004. Olfactory imprinting in Salmon, New model and Approaches, In: Emde G., Mogdalce J., Kapoor, B.G., The scene of fish Adaptations, Norsa Publish House. 108-128.

Nevitt G.A, Dittman A.H., Quinn T.P., Moody W.J., 1994. Evidence for a peripheral olfactory memory in imprinted salmon, Proc. Natl. Acad. Sci. USA. 91: 4288–4292.

Ramazani H.M., 1996. Restocking of fingerlings on the rivers of Mazandran Province. Mission Report. Mazandaran Research Center. Tehran(in Persian).

Ramazani, H.M., Yousefian M., 1998.Study on sturgeon fingerlings releasing from Shahid Rajaee Hatchery. Final report.51 p.

Razavi Sayad, B., 1993. Racing of Kutum fish by electrophoresis, MSC Thesis. Islamic Azad Univ.Tehran.

Razavi Sayyad B., 1984. Mahi Sefid, *Rutilus frisii kutum*, Iranian Fisheries Research Organization. 32–36

Rehnberg B. G., Curtis L. C., Schreck B., 1985. Homing of Coho salmon (*Oncorhynchus kisutch*) exposed to morpholine . Aquaculture. 44 (3):253-255.

Scholz A.T., Horrah R.M., Cooper J.C., Hasler A.D., 1975. Imprinting to chemical cues; the basis for home stream selection in salmon, Science. 196: 1247-9.

Scholz AT., Cooper J.C., Madison D.M., Horra., RM., Hasler A.D., Dizon AE., Poff R.J.,1973. Olfactory imprinting in Coho salmon: behavioral and electrophysiological evidence. Proceedings of the Conference on Great Lakes Research. 16:143-53.

Semke E., Distel H., Hudson R., 1995. Specific enhancement of olfactory receptor sensitivity associated with fetal learning of food odors in the rabbit. Naturwissenschaften. 82:148–149.

Sharyati A., 1993. Fish of the Caspian Sea Region, Iranian Fisheries Company. Iran.77–79.

Soltani M., Omidbeigi R., Rezvani S., Mehrabi M.R., Chitsaz H.,2001. Study of anesthetic effects induced by clove flower(*Eugina caryophillata*) on rainbow trout under various water quality conditions. Journal of Faculty of Veterinary Medicine .56(4): 85-89.

Suboski M. D., Bain A S., Carty E., McQuoid L. M., Seelen M.I., Seifert M.,1990. Alarm Reaction in Acquisition and Social Transmission of Simulated-Predator Recognition by Zebra Danio Fish (*Brachydanio rerio*). Journal of Comparative Psychology. 104 (1): 101-112.

Vossoughi G., Mostajeer B., 1994. Fresh Water Fish (Second edition). Tehran University Publication. 227–229.

Wang H., Ysocki C.J. W., Gold G.H., 1993. Induction of olfactory receptor sensitivity in mice. Science. 260: 998–1000.

Wisby, W.J. ,1952. Olfactory responses of fish as related to parent stream behavior. Unpublished Ph.D. Thesis. University of Wisconsin. Madison.

Wydoski R.,Emery L.,1983.Tagging and marking, In: Nielsen L.A., Johnson D.L., (Ed), Fisheries Techniques. American Fisheries Society. Bethesda. Maryland. 215–237.