Iranian Journal of Fisheries Sciences 21(4) 902-914 2022
DOI: 10.22092/ijfs.2022.127397

Research Article

Comparison of common carp (*Cyprinus carpio* L.) fecundity in two provinces of the southern part of the Caspian Sea in relation to the genetic variations

Andarz B.[1]; Kamali A.[2]; Avakh Keysami M.[3*]; Rajabi Islami H.[1*]

Received: December 2020 Accepted: March 2022

Abstract

To compare the fecundity of common carp in relation to their genetic variations in Guilan and Mazandaran provinces, sixty fish samples with 4-5 maturity stages cached from Guilan and Mazandaran provinces, 30 samples obtained from each interval during February 2018 to November 2018. DNA was extracted by phenol-chloroform method and investigated for 11 microsatellites loci. The mean of body weight, total length and age for Guilan and Mazandaran samples were 4.22±0.99 and 3.13±0.30 kg, 57.33±4.68 and 60.93±3.90 cm, 3.80±0.38 and 3.50±0.51 years, respectively. The mean absolute and relative fecundity of Guilan and Mazandaran samples were 239900±57921.67 and 139900±23008.76 as well as 57629.31±5583.58 and 43568.639±2129.45, respectively. The mean body height and eggs diameters measured were 17.50±2.06 and 14.60±1.42 cm as well as 0.759±0.11682 and 0.640±0.4291 mm, respectively. The mean of fecundity increased with increment of fish fork length, body weight and age. There were significant differences in fecundity rate between Guilan and Mazandaran samples. The results showed that the range of allele's number, expected and observed heterozygosity were 11-18, 63%-86%, respectively. The Fst and Rst values were significantly different between fish populations in Guilan and Mazandaran provinces. These differences may be due to broodstocks transportation by farmers. Tree investigated loci showed significant deviations from Hardy-Weinberg Equilibrium (p<0.05), mostly due to the excess of heterozygosity of Mazandaran samples. The Fst value and Gen flow due to number of alleles were 14.5 and 2.11. So it can be concluded that there were significant differences between number of alleles and genetic diversity in Guilan and Mazandaran.

Keywords: Common carp, Fecundity, Guilan, Mazandaran, Genetic Diversity, Microsatellite.

1-Department of Fisheries, Science and Research Branch, Islamic Azad University, P.O. Box: 14515-775, Tehran, Iran
2-Department of Food Industry, Kherad Institute of Higher Education, Bushehr, Iran
3-Mirza Koochak Khan Institute of Higher Education and Applied Science, Agricultural Research, Education and Extension Organization, Rasht, Iran
*Corresponding author's Email: dr.keysami@gmail.com; rajabi.h@srbiau.ac.ir

Introduction

Carp are numerous species of oily freshwater fish from the family Cyprinidae, a very large group of fish natively distributed in Europe and Asia. While carp is consumed in many parts of the world (Xu *et al.,* 2019). The common carp or European carp (*Cyprinus carpio*) is a widespread freshwater fish of eutrophic waters in lakes and large rivers in Europe and Asia (Piria *et al.,* 2016). Carp are a group of aquatic animals as the great importance to world aquaculture. Most carp species are fast growing and tend to occupy lower levels in the food web (Miao and Yuan, 2007).

In commercial fish farming the evaluation of reproductive ability is of interest to increase the efficiency of artificial propagation. High individual variations of carp broodstocks are frequently reported (Tóth *et al*., 2022). Genetic Variations and morphometric characteristics and fecundity of farmed common carp in the southern cost of the Caspian Sea are altered by wiled common carp and environment. Due to the fast growth and low feed cost in comparison to indigenous carp, genetically domesticated common carp have become popular in recent years (Balon, 2004; Chen et al., 2022). Today domesticated common carp is one of the most important species in freshwater fish culture mostly raised for human consumption, especially in Asia (FAO, 2012).

Fecundity of common carp broodstocks is a measure of the ability of fecundity to successfully reproductive ability, one of the most common parameters employed to study genetic diversity (Tóth *et al*., 2020). The fish farming industry has been more focused on the quality and quantity of eggs or larvae rather than that of genetic diversity, even though the genetic diversity may affect fertilization success and larval survival (Rurangwa *et al.,* 2004). Understanding the egg quality of different ages of broodstocks is a prerequisite for hatchery management. Female's gamete quality in fish has concentrated on relating egg characteristics to fertilization rate and offspring success. For example, egg size has been positively correlated with fertilizing capacity (Doğu *et al.,* 2022) and to larval size; (Marteinsdottir and Able, 1992).

Good quality eggs are known as those which exhibit low levels of mortality at fertilization, hatching and first feeding and those which produce the fastest growing and healthiest fry (Brooks *et al.,* 1997). Usually, egg diameter, fertilization rate and hatching rate were used as indices of gamete quality. Number of eggs produced per brood fish, when it is sometimes referred to as absolute fecundity or more usually just as fecundity. Alternatively, fecundity may be expressed per unit body weight of post-stripped fish. A few studies showed that fish age influence quality of gametes such as common carp, *Cyprinus carpio* (Aliniya *et al.,* 2013) rainbow trout, *Oncorhynchus mykiss* (Risopatrón et al., 2018), turbot, *Scophthalmus*

maximus (Suquet *et al.*, 1998) and koi carp (Mordenti *et al.*, 2003). So far, genotype and fecundity differentiation in farm common carp has not been studied.

The aim of the present work was to investigate the effect of broodstocks genetic variation on quantity and quality of the broodstocks carp eggs. On other hand, polymorphic microsatellites were applied in the present study to study genetic variations among common carp population from Caspian Sea. Although suffering of overfishing and human impacts for many years in Caspian Sea, it is hope to preserve the gene bank of valuable fishes of this unique lake of the world including common carp.

Material and methods

Brood fish preparation

This study was conducted in a small commercial carp farm located in 15 far from Rasht city in Guilan, during farm carp spawning season, February to November 2018. Sampling stations were selected based on environmental variation and relation to fish farms (surrounded farms number, years of activity and environmental condition). Six fish farms located in Sari, Babolsar, Rammsar, Lahijan, Sangar and Somesara cities were selected as sampling stations. Totally 60 female fish samples, with 4-5 maturity stages, caught by cast net with almost 10 fish for each sampling station.

Thirty samples obtained from the east, center and west parts of each province. These mature females carp with three and four years old were labeled and stored in 3 earthen ponds with 2 ha surface and 2 m depth and were cultured under same conditions, respectively.

Female properties, biometry, eggs collection and fecundity assessment

Female properties including eggs diameters (mm), total and fork length and height (cm), body weight (kg) of brood fish, absolute fecundity and relative fecundity were measured. Fecundity was determined by weighing method (Hasan *et al.*, 2020) and egg size was determined by using a caliper (at 0.02 mm sensitivity). The relative fecundity was calculated by dividing the total egg number by the total body weight (Rizzo and Bazzoli, 2020). All measurements were made by the use of measurement tools such as scale, caliper and biometry board.

DNA extraction procedure

Caudal Fins of live fish clipped and immediately soaked in 95% ethanol then stored at -20°C until DNA extraction. For restocking millions of carp genomic DNA was extracted from ethanol-preserved fin using Proteinase-K digestion (Haji *et al.*, 2019).

Amplification of microsatellite loci

Eleven pairs of microsatellite marker with high allele count were selected (Thai *et al.*, 2007). Polymerase chain reaction (PCR) amplifications were performed in a 12.5 µL volume containing 10-50 ng DNA, 1×PCR buffer (10mmol.l tris–Cl pH 8.3, 1.5 mmol $MgCl_2$, 50 mmol L^{-1} KCl), 120

μmol L^{-1} dNtps, 0.15 μmol L^{-1} primers and 0.5 U taq DNA polymerase. The reaction was performed by thermal cycle and the cycle were as follows: a pre-denaturation at 94°C for 5 min; followed by 30 cycles of denaturation at 94°C for 0.5 min, annealing at proper temperature for 30 s and elongation at 72°C for 30 s and a final elongation at 72°C for 10 min. PCR products were separated on 7.5% non-denaturing polyacrylamide gels using TBE buffer in the gel and reservoirs at 200 V for 2–3 h according to alleles size, stained with ethidium bromide and visualized with ultraviolet (Tables 1 and 2).

Table 1: Characteristics of microsatellite markers in common carp

Loci	Number of allele	Primer sequences	Annealing temperature C	Reference
MFW2	22	F: CACACCGGGCTACTGCAGAG R: GTGCAGTGCAGGCAGTTTGC	64	(Baerwald and May, 2004)
MFW7	22	F: TACTTTGCTCAGGACGGATGC R: ATCACCTGCACATGGCCACTC	62	(Baerwald and May, 2004)
MFW13	17	F: ATGATGAGAACATTGTTTACAG R: TGAGAGAACAATGTGGATGAC	56	(Baerwald and May, 2004)
MFW16	18	F: GTCCATTGTGTCAAGATAGAG R: TCTTCATTTCAGGCTGCAAAG	57	(Baerwald and May, 2004)
MFW17	22	F: CTCAACTACAGAGAAATTTCA R: GAAATGGTACATGACCTCAAG	57	(Baerwald and May, 2004)
MFW20	19	F: CAGTGAGACGATTACCTTGG R: GTGAGCAGCCCACATTGAAC	60	(Baerwald and May, 2004)
MFW26	16	F: CCCTGAGATAGAAACCACTG R: CACCATGCTTGGATGCAAAAG	60	(Baerwald and May, 2004)
LCypG2	14	F: CTGCCGCATCAGAGATAAACACT R: TGGCGGTAAGGGTAGACCAC	158	(Baerwald and May, 2004)
Syp4	13	F: CACACCGGGCTACTGCAGAG R: GTGCAGTGCAGGCAGTTTGC	58	(Crooijmans *et al*., 1997)
HLJ809	12	F: ATCATCACAGCCAAAGAAGT R: TACGGACATAGTGCAGACAA	54	(Liu *et al.*, 2007)
LOC5	14	F-TTACACAGCCAAGACTATGT R-CAAGTGATTTTGCTTACTGC	58	(Baerwald and May, 2004)

Table 2: Cycle number, Time (min) and temperature (^{0}C) used in PCR.

Cycle number	Stage	Time (min)	temperature(^{0}C)
1	Denaturation	5	94
5	Denaturation	0.5	94
	Annealing	0.5	5
	Extension	0.5	72
32	Denaturation	0.5	94
	Annealing	0.5	64-56
	Extension	0.5	72
1	Final extension	10	72

Statistical analysis

Data in female properties and fecundity assessment were statistically analyzed using Student-pair tests. All statistical analyses were performed using the statistical program SPSS 20.0. Data are presented as mean±SD. The 11 highly variable microsatellite loci were used to investigate genetic variations and population structure of common carp of Guilan and Mazandaran provinces.

The indices of genetic diversity for the populations, e.g. the observed number of alleles (A), effective number of alleles frequency (Ne), allele frequency (P), observed heterozygosity (Ho), expected heterozygosity (He), gene diversity, were calculated and deviations from Hardy–Weinberg Equilibrium (HWE) was estimated X 2 test, Fst and Nm between populations were given by the software of Gene Alex.

Alleles range in size from 124 to 464 bp were found over 11 loci. The number of allele ranged from 5 at LidII to 13 at Ca3.4. The highest allelic frequency was 0.382 in female spawners at Loc5 loci and syp4 loci.

Genetic variations within population in experimental group (Guilan and Mazandaran) for each locus were tested and average observed heterozygosity ranged from 0.412 to 1.

Results

The females' morphological and biological properties of two provinces groups during spawning season are presented in Table 3.

Table 3: The mean±SD of some properties of female carp.

Parameters	Mazandaran broodstocks	Guilan broodstocks
Age (yr)	3.50 ± 0.51	3.8 ± 0.38
Body weight (kg)	3.13 ± 0.30 [a]	4.22 ± 0.99 [b]
Total length (cm)	60.93 ± 3.90 [b]	57.33 ± 4.68 [a]
Fork length (cm)	55.26 ± 4.15	52.96± 4.61
Standard length (cm)	49.20 ± . 4.45 [b]	47.66 ± 5.19[a]
Body height (cm)	14.60± 1.42 [a]	17.50± 2.06 [b]
Egg diameter (mm)	*0*.640± 04291 [b]	0.759±• .11682 [a]
Absolute fecundity (No.)	139900 ± 23008.76 [a]	239900 ± 57921.67 [b]
Relative fecundity (No.)	*639* 43568.± 21229.45[a]	57629.31 ± 55843.58 [b]

The values with different letters are significantly different (p< 0.05; n= 3).

Among evaluated parameters just female fish age didn't show significant differences between two provinces. The body weight, age, body height, egg diameter of Guilan broodstock were 4.22±0.99 kg., 57.33±4.68 cm., 3.80±0.38 yr. 17.50±2.06 and 0.759±0.11682 mm respectively. But body weight, age, body height, egg diameter in Mazandaran broodstock were 3.13±0.30 kg., 60.93±3.90, 3.50±0.51 yr., 14.60±1.42 and 0.640±0.4291 mm respectively. The mean absolute and relative fecundity of

Guilan and Mazandaran samples were 239900.0±57921.6, 139900.0±23008.7, 57629.3±5583.5, and 43568.6±2129.4, respectively. Results indicated that there were significant differences between Guilan and Mazandaran in mean absolute and relative fecundity, body weight, body height, egg diameter, total length and fork length (p<0.05). The results (Table 4) showed that the range of allele's number, expected and observed heterozygosity were 11-18, 63%-86%, respectively. The analysis of molecular variance did not show high genetic diversity (93%) within populations, but the Fst and Rst values were significantly different between Guilan and Mazandaran fish populations. The Fst value was 0.011 which indicated the low genetic differentiation between the studied sites. Tree investigated loci showed significant deviation from Hardy-Weinberg Equilibrium (p<0.05), mostly due to the excess of heterozygosity of Mazandaran samples. The Fst value and Gen flow due to number of alleles were 14.5 and 2.11, respectively. UPEMA cluster analysis based on Nei genetic distance showed that there are two different populations. Based on the results of this study it can be concluded that there were significant differences between number of alleles and genetic diversity in Guilan and Mazandaran.

Table 4: Allele frequencies at each locus and the expected (He) and observed heterozygosity (Ho) at 11 microsatellite loci in two groups of common carp.

Loci		MFW2	MFW7	Syp4	MFW13	MFW16	HLJ809	MFW17	MFW20	LOC_5	MFW26	GyPG24
Area	**Parameter**											
Mazandaran	N_a	14	10	12	13	13	17	20	16	13	12	13
	N_e	12.23	8.07	9.21	9.26	11.17	11.15	11.22	12.33	11.22	11.08	6.11
	H_o	0.86	0.86	0.86	0.86	0.86	0.86	0.86	0.86	0.86	0.86	0.86
	H_e	0.72	0.66	0.63	0.65	0.69	0.68	0.69	0.72	0.69	0.69	0.65
Guilan	N_a	11	14	11	14	11	13	12	13	2	11	7
	N_e	10.21	10.23	10.26	9.16	5.22	8.36	7.1	11.21	7.23	7.23	6.24
	H_o	0.86	0.86	0.86	0.86	0.86	0.86	0.86	0.86	0.86	0.86	0.86
	H_e	0.39	0.33	0.37	0.38	0.28	0.33	0.27	0.39	0.38	0.34	0.38

Totally 60 adult common carp (*Cyprinus carpio* L.) were sampled from warm water fish farm. In overall, genotyped giving 24 in average alleles over all loci. The mean number of alleles, per locus in population's ranges from 11.0 to 13.0 and the mean observed heterozygosity at the 11 loci was 0.86. An analysis of genetic variations indicated the range of He was 0.27-0.72 that the lowest was in MFW17. Highly significant deviation from Hardy–Weinberg, mostly due to deficits of heterozygote based on 2-test, showed no deviation from HWE (Tables 5 and 6).

Table 5: The estimated Fst and Nm between the 11 loci.

GyPG24	MFW26	LOC5	MFW20	MFW17	HLJ809	MFW16	MFW13	Syp4	MFW7	MFW2	Loci
10.15	26.63	17.11	29.14	7.12	17.16	17.37	16.43	11.62	15.11	22.17	Nm
0.027	0.015	0.017	0.006	0.046	0.017	0.016	0.011	0.025	0.015	0.018	F_{ST}

Table 6: Homogeneity and heterogeneity of different common carp variation matrix (the number above table diameter is homogeneity and the number under table diameter is heterogeneity).

Area	Guilan	Mazandaran
Guilan		0.63
Mazandaran	0.37	

Discussion

Results of this study, indicated that there were significant differences between Guilan and Mazandaran in mean absolute and relative fecundity, body weight, body height, egg diameter, total length and fork length (p<0.05).

In the current study, morphological and biological parameters results showed that there are some differences in farm carp population between two provinces. Since, farm common carp in Guilan and Mazandaran have a similar origin, this may be due to a change in biological and morphological characteristics of these populations as a result of adaptation to local environments or hybridization with wild carp populations (Balon, 1995).

Commercial fish farming has been more focused on the fecundity and quality of eggs. The differences in fecundity results of this study may be due to feeding conditions, husbandry procedures, age, environmental factors, spawning time or dilution ratio (Aliniya *et al.,* 2013). In recent years the importance of broodstock fish has been known and it has also been understood that good quality breeders mean good quality eggs and production. Therefore, these have made it inevitable that brood fish are selected more carefully and raised in more suitable conditions (Bromage, 1998). The egg diameter has a good impact on the fertilization rate and improvement of egg incubation. In this study, Guilan females produced bigger eggs. Most researchers have stated that when brood fish size and age rise, egg size will be increased (Bromage *et al.,* 1992; Fujihara *et al*., 2022). In our study, the number of eggs per gr was higher in Mazandaran females compared to Guilan individuals. This could be due to the smaller size of eggs in Mazandaran females than in Guilan females. Fish fecundity is known to increase with the age and size of breeders (Barry *et al*., 2022). Total fecundity and egg size increase with age and size, while contrary relative fecundity decreases with age (Springate *et al.,* 1984). The

same researches have been demonstrated these results, depending on fish age and fish size increase. Relative fecundity has been expected to decline with age and size (Barrett *et al*., 2022). In our experiment, similar trends were observed in absolute fecundity and relative fecundity with size. Broodstock size has an effective influence on development stages after fertilization. The present study demonstrated how the size of brood fish can affect egg diameter. The crossing Guilan region fish with different sizes can be used as a simple procedure for achieving better results of fecundity in carp hatcheries. Since there were no significant differences between age of female fish between Guilan and Mazandaran, the higher fecundity of Guilan farm carp can be due to the higher size of Guilan female fish. Although it is well recognized that the egg size of fish shows considerable intra- and inter-specific variation. Even parental fish of the same strain, weight and length have eggs that in different size (Weber and Lee, 2014).

The results of this study showed there were significant differences between number of alleles and genetic diversity in Guilan and Mazandaran.

There are several reports of analyzing the microsatellite variation in common carp (Desvignes *et al.,* 2001; Bártfai *et al.,* 2003; Kohlmann *et al.,* 2003; Liao *et al.,* 2006). In most of these studies, there have limitations, due to the sampling of a restricted number of populations or the use of small sample sizes such as the present study. Therefore, the levels of variation detected are broadly similar to the results of this study. Loss of variations in closed hatchery populations can occur during establishment (founder effects) and over subsequent generations though genetic drift arising from low effective broodstocks number (Allendorf and Phelps, 1980). The large reduction in genetic variability in the experimental lines observed in this study, because of the differentiation between farm carp and wild carp samples sources. Thai *et al*. (2006) indicated the potential negative impact of captive breeding on domesticated common carp stocks in Vietnam and elsewhere. Thus, the low levels of genetic variations most likely reflect the difficulties in genetic management of broodstock leading to low Ne. Recently, wild common carp are extremely endangered or already extinct in many areas of their nature range because of loss of habitats, overfishing, pollution and hybridization with domesticated carp (Fallahbagheri *et al.,* 2013). It is important that the observed heterozygosis was relatively low, which are represented by the significant departure from HWE at maturity of microsatellite loci. Actually several factors will lead to deviation from HWE (Castric *et al.,* 2002); Ecosystems in the Caspian Sea region have been heavily modified by anthropogenic activities, mainly as a result of changes in the water flow and degradation of the water quality in the ecosystems (Barannik *et al.,* 2004). Based on the results of this study it can be concluded

that there were significant differences between the number of alleles and genetic diversity in Guilan and Mazandaran. Higher heterozygosity in Mazandaran may be due to hybridization with Wild common carp. Wild common carp may enter into the farms and hybridize with farm species thereby altering the genetic-variations of domesticated common carp. (Hansen *et al.*, 1993; Naylor *et al.*, 2000). Such genetic alteration might change the spawning behavior of the fish which are genetically adapted to their natural spawning ground (Naylor *et al.*, 2000).

The hybridization also may result in loss of genetic variation or homozygosity which is associated with the deterioration of important production characteristics such as fecundity, survival and growth (Araki *et al.*, 2007; Matsuzaki *et al.*, 2009). Differences in domesticated carp morphological and biological characteristics are appearing throughout the southern coast of the Caspian Sea farms. Environmental condition and hybridization with wild carp appear to be the main reason for this variation. The purity of domesticated carp, that genetically modified may change through crossing with wild carp populations. This condition can degrade the genetic variations of domesticated carp thereby posing a threat to farm common carp population. In Mazandaran, the impact of wild carp is more obvious due to the geographical situation. This condition can lead to the domination of wild carp in farms. Conservation of farm carp population is urgently necessary and therefore strict measures should be taken to avoid any release of wild carp into the farms.

The results of this study might act as the base information of the genetic variations and population structure of farm common carp, use in future for carp aquaculture and farming. It is also useful for illustration of the effect of restocking on the genetic structure of common carp in Guilan and Mazandaran.

Acknowledgement

The authors would like to thank Mr. Fazelli for sample collection and technical assistance and the staff of Mirza Koochak Khan institute of higher education and applied science, Rash, Iran for their help in providing laboratory facilities and valuable contribution.

References

Aliniya, M., Khara, H., Noveiri, S.B. and Dadras, H., 2013. Influence of age of common carp (*Cyprinus carpio*) broodstock on reproductive traits and fertilization. *Turkish Journal of Fisheries and Aquatic Sciences*, 13(**1**), 19-25. DOI: 10.4194/1303-2712-v13_1_02

Allendorf, F.W. and Phelps, S.R., 1980. Loss of genetic variation in a hatchery stock of cutthroat trout. *Transactions of the American Fisheries Society*, 109(**5**), 537-543. DOI: 10.1577/1548-8659(1980)109<537:LOGVIA>2.0.CO;2

Araki, H., Cooper, B. and Blouin, M.S., 2007. Genetic effects of captive breeding cause a rapid, cumulative fitness decline in the wild. *Science*, 318, 100-103. DOI: 10.1126/science.1145621

Baerwald, M.R. and May, B., 2004. Characterization of microsatellite loci for five members of the minnow family Cyprinidae found in the Sacramento-San Joaquin Delta and its tributaries. *Molecular Ecology Notes*, 4, 385-390. DOI: 10.1111/j.1471-8286.2004.00661.x

Balon, E.K., 1995. Origin and domestication of the wild carp, *Cyprinus carpio*: From Roman gourmets to the swimming flowers. *Aquaculture*, 129, 3-48. DOI: 10.1016/0044-8486(94)00227-F

Balon, E.K., 2004. About the oldest domesticates among fishes. *Journal of Fish Biology*, 65, 1-27. DOI: 10.1111/j.0022-1112.2004.00563.x

Barannik, V., Borysova, O. and Stolberg, F., 2004. The Caspian Sea region: environmental change. *AMBIO: A Journal of the Human Environment*, 33(**1**), 45-52. DOI: 10.1579/0044-7447-33.1.45

Barrett, T.J., Hordyk, A.R., Barrett, M.A. and van den Heuvel, M.R., 2022. Spatial and temporal differences in fecundity of Atlantic herring (*Clupea harengus*) off Nova Scotia and consequences for biological reference points. *Canadian Journal of Fisheries and Aquatic Sciences*, 99(**999**), 1-11. DOI: 10.1139/cjfas-2021-0269

Barry, P., Broquet, T. and Gagnaire, P.A., 2022. Age-specific survivorship and fecundity shape genetic diversity in marine fishes. *Evolution letters*, 6(**1**), 46-62. DOI: 10.1002/evl3.265

Bártfai, R., Egedi, S., Yue, G.H., Kovács, B., Urbányi, B., Tamás. and G., Orbán, L., 2003. Genetic analysis of two common carp broodstocks by RAPD and microsatellite markers. *Aquaculture*, 219 (**1-4**), 157-167. DOI: 10.1016/S0044-8486(02)00571-9

Bromage, N., Jones, J., Randall, C., Thrush, M., Davies, B., Springate, J. and Barker, G., 1992. Broodstock management, fecundity, egg quality and the timing of egg production in the rainbow trout (*Oncorhynchus mykiss*). *Aquaculture*, 100(**1-3**), 141-166. DOI: 10.1016/0044-8486(92)90355-O

Bromage, N.R., 1998. Broodstock management and the optimisation of seed supplies. *Aquaculture Science*, 46(**3**), 395-401. DOI: 10.11233/aquaculturesci1953.46.395

Brooks, S., Tyler, C.R. and Sumpter, J.P., 1997. Egg quality in fish: what makes a good egg? *Reviews in Fish Biology and fisheries*, 7(**4**), 387-416. DOI: 10.1023/A:1018400130692

Castric, V., Bernatchez, L., Belkhir, K. and Bonhomme, F., 2002. Heterozygote deficiencies in small lacustrine populations of brook charr *Salvelinus fontinalis* Mitchill (Pisces, Salmonidae): a test of alternative hypotheses. *Heredity*, 89,

27-35. DOI: 10.1038/sj.hdy.6800089

Chen, L., Xu, J., Sun, X. and Xu, P., 2022. Research advances and future perspectives of genomics and genetic improvement in allotetraploid common carp. *Reviews in Aquaculture*, 14(**2**), pp.957-978. DOI: doi.org/10.1111/raq.12636

Crooijmans, R.P.M.A., Bierbooms, V.A.F., Komen, J., Van der poal, J.J. and Groenen, M.A.M., 1997. Microsatellite markers in common carp (*Cyprinus carpio* L.). *Animal Genetics*. 28:129-134. DOI: 10.1111/j.1365-2052.1997.00097.x

Desvignes, J.F., Laroche, J., Durand, J.D. and Bouvet, Y., 2001. Genetic variability in reared stocks of common carp (*Cyprinus carpio* L) based on allozymes and microsatellites. *Aquaculture*, 194(**3-4**), 291-301. DOI: 10.1016/S0044-8486(00)00534-2

Doğu, Z., Aral, F. and Şahinöz, E., 2022. The effect of cryopreservative extender developed for Mesopotamian spiny eel (*Mastacembelus mastacembelus* Banks & Solander, 1794) on sperm motility and fertilization rate after thawing. *Iranian Journal of Fisheries Sciences*, 21(**2**), 619-633. DOI: 10.22092/ijfs.2022.126499

Fallahbagheri, F., Dorafshan, S., Pourkazemi, M., Keivany, Y. and Chakmedouz Qasemi, F., 2013. Genetic analysis of wild common carp, *Cyprinus carpio* L. in the Anzali wetland, the Caspian Sea. *Iranian Journal of Fisheries Sciences*, 12(**1**), 1-11.

FAO, 2012. *Cyprinus carpio* (Linnaeus, 1758). Cultured Aquatic Species Information Programme. Food and Agriculture Organization of the United Nations, Rome, Italy, 17 P.

Fujihara, R., Katayama, N., Sadaie, S., Miwa, M., Sanchez Matias, G.A., Ichida, K., Fujii, W., Naito, K., Hayashi, M. and Yoshizaki, G., 2022. Production of germ cell-less rainbow trout by dead end gene knockout and their use as recipients for germ cell transplantation. *Marine Biotechnology*, 24(**2**), 417-429. DOI: 10.1007/s10126-022-10128-w

Haji, M., Zafar, I., Muhammad Usman, I., Tayyaba, Y. and Qamar, B., 2019. An efficient method of dna extraction from fish fin. *Pakistan*. *Journal of Agricultural Sciences*, 53(**4**), 843-850. DOI: 10.21162/PAKJAS/16.3998

Hansen, P., Jacobsen, J.A. and Und, R.A., 1993. High number of farmed Atlantic salmon, *Salmo salar*, observed in oceanic water north of the Faroe Islands. *Aquaculture and Fishery Management*, 24, 777-781. DOI: 10.1111/j.1365-2109.1993.tb00657.x

Hasan, M., Hosen, M.H.A., Miah, M.I., Ahmed, Z.F., Chhanda, M.S. and Shahriar, S.I.M., 2020. Fecundity, length at maturity and gonadal development indices of river catfish (*Clupisoma garua*) of the old Brahmaputra River in Bangladesh. *The Egyptian Journal of Aquatic Research*, 46(**3**), 259-263. DOI:

10.1016/j.ejar.2020.08.003

Kohlmann, K., Gross, R., Murakaeva, A. and Kersten, P., 2003. Genetic variability and structure of common carp (*Cyprinus carpio*) populations throughout the distribution range inferred from allozyme, microsatellite and mitochondrial DNA markers. *Aquatic Living Resources*, 16(**5**), 421-431. DOI: 10.1016/S0990-7440(03)00082-2

Liao, X., Yu, X. and Tong, J., 2006. Genetic diversity of common carp from two largest Chinese lakes and the Yangtze River revealed by microsatellite markers. *Hydrobiologia*, 568(**1**), 445-453. DOI: 10.1007/s10750-006-0222-0

Liu, Z., 2007. Concept of Genome and Genomic, Aquaculture genome technologies,1st, Oxford, Blackwell Publishing, Iowa, USA, 584 P.

Marteinsdottir, G. and Able, K.W., 1992. Influence of egg size on embryos and larvae of *Fundulus heteroclitus* (L). *Journal of Fish Biology*, 41(**6**), 883-896. DOI: 10.1111/j.1095-8649.1992.tb02717.x

Matsuzaki, S.S., Mabuchi, K., Takamura, N., Nishida, M. and Washitani., I., 2009. Behavioral and morphological differences between feral and domesticated strains of common carp *Cyprinus carpio*. *Journal of Fish Biology*, 75, 1206-1220. DOI: 10.1111/j.1095-8649.2009.02345.

Miao, W. and Yuan, X., 2007. The carp farming industry in China - an overview. In: Leung, P., Lee, C.S. and O'Bryen, P.J. (eds.) Species and system selection for sustainable aquaculture.Blackwell Publishing, Iowa, USA.

Mordenti, O., Roncarati, A., Dees, A., Bonaldo, A. and Melotti, P., 2003. Influence of broodstock age on reproductive performance in koi carp (*Cyprinus carpio* L). *Italian Journal of Animal Science*, 2, 640-642. DOI: 10.4081/ijas.2003.s1.640

Naylor, R.L., Goldburg, R.J., Primavera, J.H., Kautsky, N. and Beveridge, M.C.M., Caly, J.W., Folke, C., Lubchenco, J., Mooney, H. and Troell, M., 2000. Effect of aquaculture on world fish supplies. *Nature*, 405, 1017-1024. DOI: 10.1038/35016500

Piria, M., Tomljanović, T., Tomislav, T., Roman, S. and Matulić, A.I.D., 2016. The common carp *Cyprinus carpio* in Croatia (Danube and Adriatic basins): a historical review. *Aquaculture International*, 24(**6**), 1527-1541. DOI: 10.1007/s10499-016-0029-6

Risopatrón, J., Merino, O., Cheuquemán, C., Figueroa, E., Sánchez, R., Farías, J.G. and Valdebenito, I., 2018. Effect of the age of broodstock males on sperm function during cold storage in the trout (*Oncorhynchus mykiss*). *Andrologia*. 50(**2**), e12857. DOI: 10.1111/and.12857

Rizzo, E. and Bazzoli, N. 2020. Chapter 13 - Reproduction and embryogenesis. In: Baldisserotto, B., Urbinati, E.C. and Cyrino, J.E.P. (eds.) Biology and Physiology of

Freshwater Neotropical Fish. Academic Press, NY. DOI: 10.1016/B978-0-12-815872-2.00013-0

Rurangwa, E., Kime, D.E., Ollevier, F. and Nash, J.P., 2004. The measurement of sperm motility and factors affecting sperm quality in cultured fish. *Aquaculture*, 234(**1-4**), 1-28. DOI: 10.1016/j.aquaculture.2003.12.006

Springate, J.R.C., Bromage, N.R., Elliott, J.A.K. and Hudson, D.L., 1984. The timing of ovulation and stripping and their effects on the rates of fertilization and survival to eying, hatch and swim-up in the rainbow trout (*Salmo gairdneri* R). *Aquaculture*, 43(**1-3**), 313-322. DOI: 10.1016/0044-8486(84)90032-2

Suquet, M., Dreanno, C., Dorange G., Normant, Y., Quemener, L., Gaignon, J.L. and Billard, R., 1998. The ageing phenomenon of turbot spermatozoa: effects on morphology, motility and concentration, intracellular ATP content, fertilization, and storage capacities. *Journal of Fish Biology*, 52(**1**), 31-41. DOI: 10.1111/j.1095-8649.1998.tb01550.x

Thai, B.T., Pham, T.A. and Austin, C.M., 2006. Genetic diversity of common carp in Vietnam using direct sequencing and SSCP analysis of the mitochondrial DNA control region. *Aquaculture*, 258(**1-4**), 228-240. DOI: 10.1016/j.aquaculture.2006.03.025

Thai, B.T., Burridge, C.P. and Austin, C.M., 2007. Genetic diversity of common carp (*Cyprinus carpio* L) in Vietnam using four microsatellites. *Aquaculture*, 269(**1-4**), 174-186. DOI: 10.1016/j.aquaculture.2007.05.017

Tóth, B., Khosravi, R., Ashrafzadeh, M.R., Bagi, Z., Fehér, M., Bársony, P., Kovács, G. and Kusza, S., 2020. Genetic diversity and structure of common carp (Cyprinus carpio L.) in the Centre of Carpathian Basin: Implications for conservation. *Genes*, 11(11), 1268. DOI: 10.3390/genes11111268

Tóth, B., Ashrafzadeh, M.R., Khosravi, R., Bagi, Z., Fehér, M., Bársony, P., Kovács, G. and Kusza, S., 2022. Insights into mitochondrial DNA variation of common carp Cyprinus carpio strains in the Centre of Carpathian Basin. *Aquaculture*, 554, p.738116. DOI: 10.1016/j.aquaculture.2022.738116

Weber, G. and Lee, C.S., 2014. Current and Future Assisted Reproductive Technologies for Fish Species. In: Lamb, G., DiLorenzo, N. (eds.) Current and Future Reproductive Technologies and World Food Production. Advances in Experimental Medicine and Biology, vol 752. Springer, NY. DOI: 10.1007/978-1-4614-8887-3_3

Xu, J., Zhao, Z., Hanyuan, P., Wenzhu, F., Jianxin, D., Chuanju, C., Baohua, T. and Ruyu, P., 2019. Patterns of Geographical and Potential Adaptive Divergence in the Genome of the Common Carp (*Cyprinus carpio*). *Frontiers in Genetics*, 10, 660. DOI: 10.3389/fgene.2019.00660