CJES
Caspian Journal of Environmental Sciences
Online ISSN: 1735-3866
Print ISSN: 1735-3033

Ovarian histological and gene expression analysis of two *Rutilus kutum* (Kamensky 1901) races during spawning migration

Saman Farasati[1], Majidreza Khoshkholgh[1*], Sajad Nazari[2]

1. Department of Fisheries, Faculty of Natural Resources, University of Guilan, P.O. Box 1144, Sowmehsara, Iran
2. Shahid Motahary Cold-water Fishes Genetic and Breeding Research Center, Iranian Fisheries Sciences Research Institute, Agricultural Research, Education and Extension Organization (AREEO), Yasouj, Iran

** Corresponding author's Email: majidreza@guilan.ac.ir*

ABSTRACT
Caspian kutum, *Rutilus kutum* is the most common species of the Cyprinidae family in the Iranian coastal waters of the Caspian Sea and comprises, on average, more than 70% of the annual commercial bony fish catch. This species consists of two migratory forms, the spring race (SR) and autumn race (AR), which show morphological identities. The morphological differences are classified as phenotypic markers and cannot be a reliable method for distinguishing individuals and populations. Therefore, we performed histological analysis concerning ovarians, as well as gene expression profiling differences, to gain more knowledge about the differential migratory behavior of the races. Ovary and liver samples were collected to observe the reproductive performance based on ovarian histology and related gene expression using real-time quantitative polymerase chain reaction (qRT-PCR). Histological study of the AR and the SR broodstocks showed that the ovaries were at vitellogenic (oocyte diameter = 978.4 ± 32.3 µm) and maturation (oocyte diameter = 1225 ± 27.6 µm) stages, respectively. The steps identified were predictable because the ARs needed a hibernation after migration for spawning, while the SRs were ready for spawning after moving into the spawning grounds. Additionally, we performed qRT-PCR to confirm the genes involved in the vitellogenic and maturation stages of ovarian development in *R. kutm*. Given the lack of information about the population aspects of this species, histological and gene profiling analyses in the current study showed evidence of different migratory behaviors in the two races. These valuable results can be used in fishery management programs.

Keywords: Caspian kutum, Ovarian histology, Gene expression.
Article type: Research Article.

INTRODUCTION
Rutilus kutum is an anadromous native fish species in the Caspian Sea that forms up to 70% of bony fish products in North Iran. This species is mainly distributed along the southern and southwestern coastline of the Caspian Sea (Valipour & Khanipour 2009). On the Iranian shores, *R. kutum* consists of two different migratory forms: the autumn race (AR) and the spring race (SR). The AR adults migrate into the Anzali wetland from the end of September to mid-December, spend the winter in deep water parts, and spawn on flooded terrestrial vegetation in spring (Mashhor *et al.* 2011). However, SR populations migrate from the end of March until the end of April in the main spawning rivers and breed on gravel beds (Shikhshabekov 1979). Information about AR populations is rare, and less than 2% of stocks belong to this race (Valipour *et al.* 2010). The SR populations comprised the main stocks of this species, accounting for 98% of the total catches. The populations of the two races are in high danger because of various problems, including climate change, destruction of spawning grounds, and human manipulation (Fazli *et al.* 2012). For instance, only two genetic pools were found in eight populations of SR using SNP markers, indicating the high homogeneity of the explored populations (Rahbar *et al.* 2023). The Iranian Fisheries Organization (IFO) is mainly focused on restocking SR populations, and there is little information about

Caspian Journal of Environmental Sciences, Vol. 23 No. 1 pp. 147-154 Received: May 20, 2024 Revised: Aug. 09, 2024 Accepted: Oct. 14, 2024
DOI: 10.22124/CJES.2025.8567
© The Author(s)
CC BY NC
Publisher: University of Guilan

restocking AR populations (Valipour *et al.* 2010). Various studies have been conducted on different aspects of SR populations, including genetic differences using molecular markers (Rezaei *et al.* 2010; Abdolhay *et al.* 2012; Safari 2016; Kashmiri *et al.* 2018), fingerling reproduction (Fazli & Daryanabard 2020), feeding regime strategies (Afraei Bandpei *et al.* 2013), salinity change effects (Gheisvandi *et al.* 2015), and trace element determination (Sattari *et al.* 2020). However, there are limited studies aiming to explore the differences between the two races, which show contradictory results (Chakmehdouz *et al.* 2009, Rezvani Ghilkolahi *et al.* 2012). Thus, for better monitoring of the different spawning behaviors of the two races, investigation of differences in ovarian histology and gene profiling may be useful.
Histology has been used for decades as a powerful tool for monitoring the reproductive health of many fish species. This method can be used for sex determination, sexual development stages, abnormality documentation, and atresia measurements. In addition, histology can be applied in more specific studies, including the analysis of vitelline thickness at different stages and yolk appearance (Blazer 2003). Ovarian histological examinations provide the most accurate and unbiased information about spawning periods and are crucial for identifying specific details of the reproductive cycle (Tingud-Sequeira *et al.* 2009). The *R. kutum* ovarian development is similar to that of other Cyprinidae family, such as common carp (Shirali *et al.* 2011), and exhibits asynchronous ovarian follicle development (Sivakumaran *et al.* 2003). Different internal and external factors can affect the reproductive performance of fish. Various studies have shown that photoperiod and temperature can regulate gonadal development (Takashima *et al.* 2008; Bapary & Fainulei 2009; Ghomi *et al.* 2011). Caspian kutum migration occurs in two spatiotemporal regimes that can significantly affect the sexual maturity of fish broodstocks. Gene expression quantification is a promising research approach to better understand the physiological differences between individuals and populations (Dekkers *et al.* 2012). Quantitative real-time PCR (qRT-PCR) is widely used in various studies and is characterized by its reproducibility, high-throughput capabilities, high specificity, sensitivity, low transcript level detection, and speed (Poon *et al.* 2009; Sudarshan *et al.* 2011). In recent years, this method has been widely used in aquaculture and fishery studies. For instance, in studies focusing on fish immunology (Yang *et al.* 2011), gene expression levels at embryonic developmental stages (Zheng & Sun 2011), stress conditions (Wang *et al.* 2016), and population genetics (Wong *et al.* 2022), RT-qPCR has been employed to analyze expression changes in selected fish. Caspian kutum is listed in the IUCN with the "Conservation dependent organisms" tag (Esmaeili *et al.* 2015). Thus, it can be concluded that the populations of this valuable species are in danger and need to be treated. In addition, studies showing accurate differences between the two races are scarce. Therefore, we performed ovarian histology in conjunction with gene expression analysis of the female Caspian kutum to provide a better understanding of the different migratory behaviors of both races during their reproductive cycle and spawning migration.

MATERIALS AND METHODS

Ethics statement

All experiments were performed in compliance with the National Institute of Health's Guide for the Care and Use of Laboratory Animals and were approved by the Research Ethics Committee at the University of Guilan in Rasht, Iran (approval ID: IR.GUILAN.REC.1401.066).

Collection and acclimatization of broodstock

Sampling was performed in mid-December for AR and mid-February for SR. Three mature females of AR and SR (in total six specimens) were caught by trapping dams from Anzali Wetland and Havigh River, the SW Caspian Sea respectively. To reduce stress and stabilization, the specimens were kept in 100-L tanks available in the breading facilities of Faculty of Natural Resources, University of Guilan, Sowmehsara, Iran. Subsequently, the specimens were euthanized with clove powder extract and dissected, then the ovaries and liver were extracted. For histological investigation, the ovary parts fixed in 10% formalin were washed in running water and stored in 70% ethanol until processing. Liver tissues were immediately frozen in liquid nitrogen for qRT-PCR, and the middle part of each ovary was fixed in 10% formalin for histological analysis.

Histological analysis

In total, six mature female gonads were histologically examined. To initiate histology, fixed tissues were washed with distilled water and stored in 70% alcohol. The center part of each tissue was then cross-sectioned, fixed in paraffin, and sectioned transversely to 5 μm with a microtome (Leica, Wetzler, Germany). Distilled water was used to fix the sections on glass slides, and the hematoxylin and eosin (H&E) method was applied to stain the

selected tissues (Culling *et al.* 1985; Roberts, 2001). We used the terminology proposed by West (1990) and Wallace & Selman (1981) to detect the stages of ovarian development. Finally, stained slides from tissue samples were viewed through a light compound microscope photomicrographs using an Olympus CX41 bright field light microscope (Olympus, Tokyo, Japan).

RNA extraction

Total RNA was isolated from liver samples from both strains of *R. kutum* using TRIzol reagent (Invitrogen; www.invitrogen.com). PrimerScript RT Reagent Kit with gDNA Eraser (TaKaRa; www.takarabio.com) was used to synthesize the first-strand complementary (c) DNA according to the manufacturer's instructions. The purified RNA was dissolved in RNase-free water, and genomic DNA contamination was removed using DNase I (Invitrogen). RNA concentration and purity were measured using the NanoDrop spectrophotometer (ND- 1000, Nanodrop Technologies), and RNA integrity was checked using agarose gel electrophoresis and BioAnalyzer measurements. Only high-quality RNA samples (OD260/OD280 ranged from 1.8 to 2.2, RIN ≥8.0) were used to construct the sequencing library. The RNA of each strain's livers was pooled into three samples for later sequencing.

qRT-PCR

In this paper, six candidate genes including gonadotropin-releasing hormone 1 (*gnrh1*), gtH II beta subunit (*gth 2*), luteinizing hormone beta subunit (*lhb*), cytochrome P450 A polypeptide 1b (*cyp19a1b*), 5-hydroxytryptamine receptor 2A (*htr2a*) and nuclear receptor subfamily 2 group C member 2 (*Nrs2*) were selected for quantitative real-time polymerase chain reaction (qRT-PCR) analysis.

Referring to the full cDNA sequence of candidate DEGs, specific qRT-PCR primers were designed by Primer Premier 6.0 package (Biosoft, USA). Forward and reverse primers and amplicon size for each amplified gene are shown in Table 1. Annealing temperatures were optimized using a temperature-gradient program. Quantitative RT–PCR experiments were carried out on a 7500 real-time PCR system (Applied Biosystems, USA). To determine the detection range, linearity and real-time qPCR amplification efficiency, a standard curve of each primer pair for qPCR was applied. This value is estimated based on the threshold cycle (Ct) to the logarithm of cDNA input by a 10-fold serial dilution series. The total RNA was reverse transcribed using the PrimeScriptRT reagent Kit (Takara). Each 10 µL reaction volume contained 1 µL cDNA, 0.2 µL each sense and reverse primers, 5 µL SYBR Green Master Mix (TaKaRa), and 3.6 µL RNase-free H_2O in triplicate reaction.

Table 1. Primers used in Real-time PCR confirmation of DEGs

Gene	Forward primer	Reverse primer	Product length
Gonadotropin-releasing hormone 1	GTATGCGCCTACCGGAAGC	TGCTCTCACGCCTGTCACAA	168
Luteinizing hormone beta subunit	ATCGGTAGTCGTGCGAGTGAC	GTCTCGATGCACGTGACTCGAT	212
5-hydroxytryptamine receptor 2A	ACGCGCTCGATGATAGCGAG	CTACTCATCTAGACACGACTCG	196
GtH II beta subunit	GTAGGCTCTACGCTGCCGATG	GATGCATACTCGCACTATCTA	208
Nuclear receptor subfamily 2 group C member 2	TACGACGTGTCGTGACTAGC	TATGCCCTCATTACGGCT	164
Cytochrome P450 family 19 subfamily A polypeptide 1b	GTGTCCAGGATCGCCACAGCT	GTGTCGAGTCAGTATCAGTCGT	218

Template-minus negative controls were run for each subsample and all qPCR subsamples were performed in triplicate. The qRT-PCR reaction was performed with a specific program: 95 °C for 10 s, followed by 40 cycles of 95 °C for 5 s, 60 °C for 10 s and 72 °C for 35 s. For internal standardization to normalize the Ct values to conduct the $2^{-\Delta\Delta Ct}$ method (Livak and Schmittgen, 2001), expression of β-actin primers (GenBank No. AJ438158.1) was used as a reference.

RESULTS AND DISCUSSION

The specimens selected for this study were mature females that migrated to fresh water for spawning. Therefore, the microscopy-observed sections allowed us to classify the ovaries into two developmental stages based on the

results of West (1990) and Wallace & Selman (1981). Compared to all the groups, all three AR female broodstocks were in the vitellogenin stage (Fig. 1). In contrast, all three SR females were at a higher developmental stage or mature oocyte stage (Fig. 2). In the vitellogenin stage, the follicular and granulosa layers became thicker and the cortical alveoli were displaced with yolk globules. At the end of this stage, the germinal vesicle initiated migration to the animal pole. Oocyte diameter was 978.4 ± 32.3 µm in this stage. In the mature oocyte stage, germinal vesicle migration was completed after oocyte hydration, the theca layer became more visible, the follicular layer developed in an irregular pattern, and the yolk globules covered the entire cytoplasm. Oocyte diameter was 1225 ± 27.6 µm in this stage.

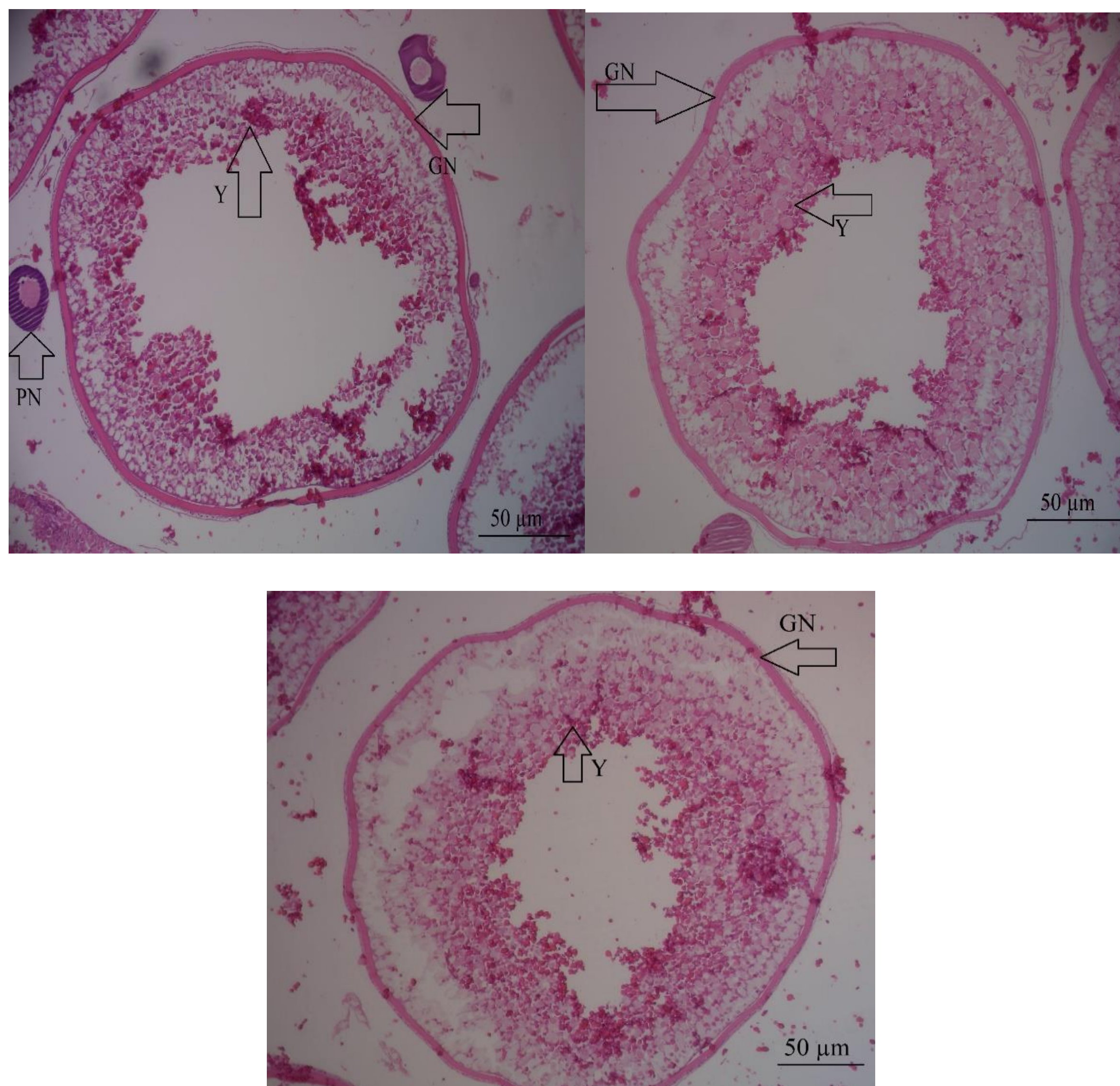

Fig. 1. Ovarian histology of *R. kutum* autumn race indicating vitellogenic stage (H & E 10x10x). GN: Granulosa cells, Y: Yolk, PN: Peri nucleolus stage oocyte.

The relative changes were calculated with the fold changes ($2^{-\Delta\Delta C_T}$) of genes determined by qRT-PCR and the *β-actin* gene was amplified as the reference gene. The columns represent gene expression based on the log2 ratio (fragments per kilobase of transcript per million mapped reads; FPKM) of different tissues. All genes selected for qRT-PCR analysis were analyzed in three biological replicates. The results showed that the mean relative expression of each gene obtained by RT-qPCR was congruent with the mean FPKM value of each group obtained by transcriptome sequencing. In Fig. 4, the FPKM from the relative qRT-PCR expression level is shown.

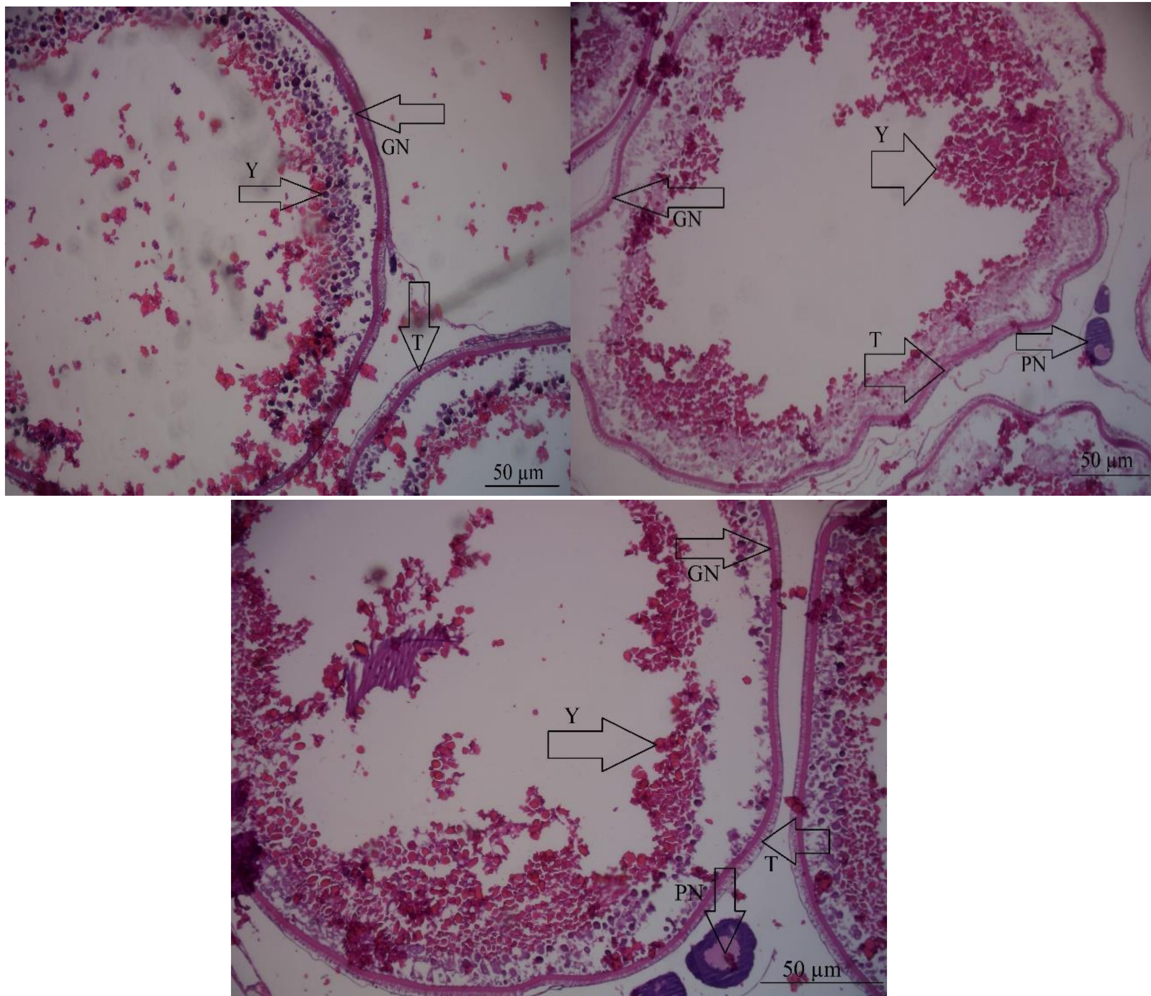

Fig. 2. Ovarian histology of *R. kutum* spring race with irregular follicular layer indicating mature oocyte stage. (H & E 10x10x). T: Techa cells, GN: Granulosa cells, Y: Yolk, PN: Peri nucleolus stage oocyte.

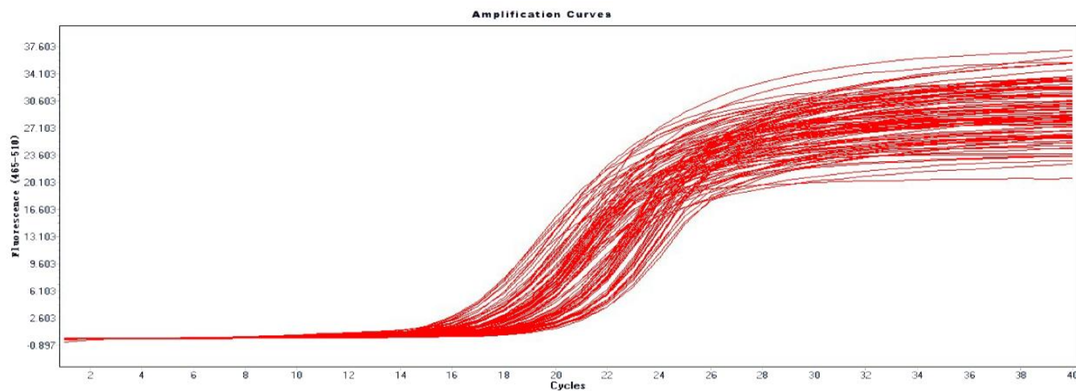

Fig. 3. Amplification curve of fluorescence quantitative PCR.

Previous studies indicated that the reproductive features of fish species differ according to different conditions including the physical, chemistry, and hydrographic properties of the water systems in their habitats (Dubost *et al.* 1997). Detection of the stages of ovarian development was used to inspect reproductive characteristics as breeding period and fecundity facilitates the collection of data on the reproductive success of a fish species (Minos *et al.* 2010). Ovarian histology is commonly used to examine gonadal development and the reproduction phase during the reproduction cycle (Brown-Peterson *et al.* 2011). In the current study, in the female *R. kutum*, there were similar relative gene expression levels including cytochrome P450 A polypeptide 1b (*cyp19a1b*), 5-hydroxytryptamine receptor 2A (*htr2a*) and nuclear receptor subfamily 2 group C member 2 (*Nrs2*) during the reproductive cycle between both races. The relative (*gnrh1*), gtH II beta subunit (*gth 2*), and luteinizing hormone

beta subunit (*lhb*), gene expression started to increase significantly ($p < 0.05$). The relative cytochrome P450 A polypeptide 1b (*cyp19a1b*) gene expression levels did not differ significantly between spring and autumn races. These genes were expressed throughout the observation periods and concomitantly with ovarian histology observation. Increased relative *gnrh1* and *gth 2* gene expression levels occurred in the developing phase for the spring strain and *gth 2* peaked at the beginning of the spawning-capable phase. Pearson correlation (r) analysis showed moderate, positive correlations between the relative *gnrh1* and *gth 2* gene expression and the histology results during spawning migration (r = 0.821, $p < 0.01$ and r = 0.718, $p < 0.01$, respectively). It seemed that significantly higher relative *gnrh1* and *gth 2* gene expression levels from the spring strain were related to the higher numbers of vitellogenic oocytes.

Fig. 4. Gene expression levels were measured by qRT-PCR. The relative changes were calculated with the fold changes ($2^{-\Delta\Delta C_T}$) of genes determined by qRT-PCR and the *β-actin* gene was amplified as the reference gene. Line plots represent gene expression according to the log2 ratio (fragments per kilobase of transcript per million mapped reads (FPKM) of different tissues determined by RNA-Seq. All genes selected for qRT-PCR analysis were analyzed in three biological replicates. Gonadotropin-releasing hormone 1 (*gnrh1*), luteinizing hormone beta subunit (*lhb*), gtH II beta subunit (*gth 2*), cytochrome P450 A polypeptide 1b (*cyp19a1b*), 5-hydroxytryptamine receptor 2A (*htr2a*), nuclear receptor subfamily 2 group C member 2 (*Nrs2*).

ACKNOWLEDGMENTS

We would like to the University of Guilan and the Iran National Science Foundation (INSF) for supporting the research and financing. We also appreciate our colleagues in the College of Fisheries Science.

Funding information

Funding was provided by Iran National Science Foundation (INSF) research fellowship to M. Khoshkholgh (INSF-4013197).

REFERENCES

Abdolhay, H, Daud, SK, Rezvani, S, Pourkazemi, M, Siraj, SS, Laloei, F, Javanmard, A & Hassanzadeh Saber, M, 2012, Population genetic structure of Mahi Sefid (*Rutilus frisii kutum*) in the of South Caspian Sea: Implications for fishery management. *Iranian Journal of Animal Biosystematics*, 8(1).

Afraei Bandpei, MA, Solaimani Roudi, A and Janbaz, AA, 2012, Feeding regime of Kutum, *Rutilus frisii kutum* (Kamensky 1901) at the various ages in the southern Caspian Sea (Mazandaran waters) in survey. *Aquaculture Sciences*, *1*(1), pp.47-61.

Blazer, VS 2002, Histopathological assessment of gonadal tissue in wild fishes. *Fish Physiology and Biochemistry*, *26*, pp.85-101.

Bapary, MAJ, Fainuulelei, P & Takemura, A, 2009, Environmental control of gonadal development in the tropical damselfish Chrysiptera cyanea. *Marine Biology Research*, 5: 462-469.

Brown-Peterson, NJ, Wyanski, DM, Saborido-rey, F, Macewicz, BJ, Lowerre-Barbieri, SK 2011, A standardized terminology for describing reproductive development in fishes. *Marine and Coastal Fisheries*, 3: 52-70. doi.org/10.1080/19425120.2011.555724.

Chakmehdouz, GF, Pourkazemi, M, Zamini, A, Yarmohammadi, M, Baradaran, NS, Hasanzadeh, SM, Rezvani, S & Azizzadeh, L 2009, Genetic analysis of spring and autumn races of Caspian Sea kutum (*Rutilus frisii kutum*) using microsatellite markers. *Iranian Journal of Animal Biosystematics* (IJAB) 5: 1-8, ISSN: 1735-434X.

Culling, CFA, Allison, RT and Barr, WT 2014, *Cellular Pathology Technique*. Elsevier.

Dekkers, BJ, Willems, L, Bassel, GW, van Bolderen-Veldkamp, RP, Ligterink, W, Hilhorst, HW & Bentsink, L 2012, Identification of reference genes for RT–qPCR expression analysis in Arabidopsis and tomato seeds. *Plant and Cell Physiology*, 53: 28-37.

Esmaeili, HR, Babai, S, Gholamifard, A, Pazira, A & Gholamhosseini, A 2015, Fishes of the Persis region of Iran: an updated checklist and ichthyogeography. *Iranian Journal of Ichthyology*, 2: 201-223.

Fazli, H, Daryanabard, G, Salmanmahiny, A, Abdolmaleki, S, Bandani, G & Bandpei, MAA 2012, Fingerling release program, biomass trend and evolution of the condition factor of Caspian Kutum during the 1991-2011 period/Programme d'alevinage et evolution de la biomasse et du coefficient de condition du kutum de la mer Caspienne au cours de la periode 1991-2011. Cybium, *International Journal of Ichthyology*, 36: 545-551.

Gheisvandi, N, Hajimoradloo, A, Ghorbani, R & Hoseinifar, SH 2015, The effects of gradual or abrupt changes in salinity on digestive enzymes activity of Caspian kutum, *Rutilus kutum* (Kamensky, 1901) larvae. *Journal of Applied Ichthyology*, 31: 1107-1112.

Ghomi, MR, Zarei, M & Sohrabnejad, M 2011, Effect of photoperiod on growth and feed conversion of juvenile wild carp, *Cyprinus carpio*. *Acta Biologica Hungarica*, *62*, pp.215-218.

Kashiri, H, Shabani, A, Gorgin, S, Rezaii, M & Jabale, AR 2018, Microsatellite DNA Marker Analysis of Genetic Variation in Wild and Hatchery Populations of Caspian Kutum (*Rutilus kutum*). *Turkish Journal of Fisheries and Aquatic Sciences,* 18: 1101-1111.

Mashhor, MAM, Abdolmaleki, SH, Najafpour, SH, Bani, A, Pourgholam, R, Fazli, H, Nasrolahzadeh, H & Janbaz, AA 2011, The environmental effect on spawning time, length at maturity and fecundity of Kutum (*Rutilus frisii* kutum, Kamensky, 1901) in southern part of Caspian Sea, Iran. *Iranian (Iranica) Journal of Energy & Environment,* 2(4).

Poon, LL, Chan, KH, Smith, GJ, Leung, CSW, Guan, Y, Yuen, KY & Peiris, JSM, 2009, Molecular detection of a novel human influenza (H1N1) of pandemic potential by conventional and real-time quantitative RT-PCR assays. *Clinical Chemistry*, 55:1555-1558.

Rahbar, M, Khoshkholgh, M & Nazari, S 2023, Population structure of Caspian Kutum (*Rutilus frisii*, Nordmann, 1840) in the southern coast of Caspian Sea using genome-wide single nucleotide polymorphism markers. *Fisheries Research,* 257: 106499.

Rezaei, M, Shabani, A, Shabanpour, B & Kashiri, H 2010, Microsatellite diversity and population genetic structure of *Rutilus frisii* kutum in Mazandaran coasts. *Iranian Journal of Biology*, pp. 548–558.

Rezvani, GS, Kavan, L & Safari, R 2012, A study of genetic structure of *Rutilus frisii kutum* in Anzali Lagoon, using microsatellite markers.

Roberts, RJ 2003, Nutritional pathology. In: Fish Nutrition Academic Press, pp. 453-504.

Safari, R 2016, Population structure of *Rutilus frisii kutum* in Iranian coastline of the Caspian Sea using microsatellite markers. *Environmental Resources Research*, 4: 65–74.

Sattari, M, Imanpour Namin, J, Bibak, M, Forouhar Vajargah, M, Bakhshalizadeh, S, Faggio, C 2020, Determination of trace element accumulation in gonads of *Rutilus kutum* (Kamensky, 1901) from the south Caspian Sea trace element contaminations in gonads. Proceedings of the National Academy of Sciences, India Section B: Biological Sciences, 90: 777–784.

Shikhshabekov, MM 1979, The reproductive biology of kutum, *Rutilus frisii kutum*, the Asp, *Aspius aspius*, Vimba, *Vimba vimba persa*, and the Rudd, *Scardinus erythrophthalmus*, in the water of Daghestan. *Ichthyology*, 19: 98-105.

Sivakumaran. KP 2003, Maturation and reproductive biology of female wild carp, *Cyprinus carpio*, in Victoria, Australia. *Environmental Biology of Fishes*, *68*, pp.321-332.

Shirali, S, Erfani, MN, Mesbah, M and Seifiabad, SM 2011 Histological and histometrical study of common carp ovarian development during breeding season in Khouzestan province in Iran.

Sudarshan, M, Weirather, JL, Wilson, ME & Sundar, S 2011, Study of parasite kinetics with antileishmanial drugs using real-time quantitative PCR in Indian visceral leishmaniasis. *Journal of Antimicrobial Chemotherapy*, 66: 1751-1755.

Takashima, M, Kobayashi, M, Shiota, T, Higuchi, T & Soyano, T 2008, Effects of temperature on oocyte development and regression of mudskipper (*Periophathal musmudestus*). *Cybium*, *32*, p.196.

Tingaud-Sequeira, A, Chauvigné, F, Lozano, J, Agulleiro, MJ, Asensio, E & Cerdà, J 2009, New insights into molecular pathways associated with flatfish ovarian development and atresia revealed by transcriptional analysis. *BMC genomics*, 10: 1-25.

Valipour, AR & Khanipour, AA 2009, Kutum: jewel of the Caspian Sea. Iranian Fisheries Research Organization, Tehran, Iran, 7 p.

Valipour, AR, Karimzadeh, K, Talebi Haghighi, D, Fallahi, M, Vatandoost, M, Behmanesh, S, Mosavi, SA, Nosrati, M, Khaval, A, Ramezani, MR & Mehdizadeh, G 2010 Artificial propagation and Culture of *Rutilus frisii kutum* of Autumn form for restocking.

Wang, H, Wen, H, Li, Y, Zhang, K & Liu, Y 2018, Evaluation of potential reference genes for quantitative RT-PCR analysis in spotted sea bass (*Lateolabrax maculatus*) under normal and salinity stress conditions. *PeerJ*, *6*, p.e5631.

Wallace, RA & Selman, K 1981, Cellular and dynamic aspects of oocyte growth in teleosts. *American Zoologist*, 21: 325-343.

West, G 1990. Methods of assessing ovarian development in fishes: a review. *Marine and Freshwater Research*, 41: 199-222.

Wong, ML & Medrano, JF 2005, Real-time PCR for mRNA quantitation. *Biotechniques*, 39: 75-85.

Yang, CG, Wang, XL, Wang, L, Zhang, B & Chen, SL 2011, A new Akirin1 gene in turbot (*Scophthalmus maximus*): molecular cloning, characterization and expression analysis in response to bacterial and viral immunological challenge. *Fish & Shellfish Immunology*, 30: 1031-1041.

Zheng, WJ & Sun, L 2011, Evaluation of housekeeping genes as references for quantitative real time RT-PCR analysis of gene expression in Japanese flounder (*Paralichthys olivaceus*). *Fish & shellfish immunology*, 30: 638-645.

Bibliographic information of this paper for citing:

Farasati, S, Khoshkholgh, M, Nazari, S 2025, Ovarian histological and gene expression analysis of two *Rutilus kutum* (Kamensky 1901) races during spawning migration, Caspian Journal of Environmental Sciences, 23: 147-154.

Copyright © 2025