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Evaluation of incubation temperatures on DNA damages of the Caspian trout (*Salmo trutta caspius* Kessler, 1877) single cells through developmental stages by Comet assay

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Abstract

Caspian trout (*Salmo trutta caspius* Kessler, 1877) as endemic migratory fish, possessing great ecological economic and biodiversity value. Among the habitat drivers, temperature related effects on the genome were considered in this research. During the embryonic developmental stages, genomic alterations of single cells were assessed by Comet assay. After artificial propagation of wild broodstocks, the batches of eggs, were transferred to incubators with constant temperatures, REDD water recycling, 3 stages water filtration, UV-C application on effluent of each incubator and DO–pH–temperature control. Samples were collected from normal (8°C), cold (4°C) and warm (12°C) treatments on eyed egg, alevin and larval stages. Single Cell Gel Electrophoresis were applied on samples for evaluation of Comet Tail length (TL), DNA in tail (DT) and Tail Moment (TM) indices as DNA damage markers. Comparison between mean values of Comet TL index indicated 85.19 ±9.2, 9.88 ±2.3 and 256.54±14.6 for 4, 8 and 12°C treatments, respectively. DT were 18.07±6.3, 9.72±1.9 and 31.47±2.8 and then TM were measured as 15.27± 4.1, 1.21±0.2 and 95.25±8.6 for 4, 8, and 12°C treatments, respectively. Significant difference between thermal treatments (p<0.05) observed. Warm treatments demonstrated greater DNA damage in compare with others. TM explained better information in comparison with TL and DT in all analyzed samples. In conclusion, temperatures out of the range of the optimal conditions could significantly affect Caspian trout genome which could be the subject of management considerations pertaining to Caspian trout stocks rehabilitation and captive rearing strategies.

Keywords: Caspian trout, *Salmo trutta caspius*, Temperature, DNA damage, Comet assay

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Introduction

Since 1967, Caspian trout (*Salmo trutta caspius* Kessler, 1877) has been one of the subjects of the fisheries natural stocks rehabilitation considerations for government authorities in Iran. Because of a couple of justifications such as ecologic-economic and biodiversity of this greatly valued endemic migratory subspecies (Jamshidi *et al*., 2006, Rajabi Islami *et al.*, 2016; Khara, 2016) in the Caspian Sea, critically endangered in the south Caspian Sea according to IUCN criteria (Jalali and Mojazi Amiri, 2009), considerable price in fish market which has the highest price between all fish protein sources (50 EURO Kg^{-1}, Autumn 2015, Tonekabon-Iran) and beliefs about health benefits supporting by local experiences, also aquaculture perspectives as a potential species besides rainbow trout (*Oncorhynchus mykiss*) in Iran coldwater aquaculture industry (Kalbassi *et al*., 2012) and a couple of reports pertaining to the importance of this subspecies (Vera *et al*., 2010; Kocabas *et al*., 2012), there was enough reasons for bring it inside the biodiversity-fisheries big subjects.

The Department of Environment (DOE) of Iran has included a penalty for the illegal catches and smuggling for this species. Also multibillions of Rails have been spent by the government authorities through the Iranian Fisheries Organization for stock protection and rehabilitation programs since 1967.

The south Caspian natural spawning grounds in freshwater habitats are highly degraded. Moreover climate change is occurring in Iran (Amiri and Eslamian, 2010) and the impacts of climate change on fisheries which was explained by Brander (2010) can be considered as potential adverse effects on the life cycle of the Caspian trout.

Gray outcomes in public observations stating further considerations is vital in order to save the fish, safe food providing, save national capital and sustainable use relevant to Caspian trout.

A numbers of research projects have been designed to provide help to natural stocks of Caspian trout, in parallel with artificial breeding and releasing parrs to the degraded and damaged habitats.

In recent years, Iranian coldwater aquaculture farm owners have experienced rearing the Caspian trout in ponds and partially in sea cages as an alternative species to rainbow trout. Considering the life cycle of Caspian trout and natural adaptation from freshwater to brackish water habitats when they are close to the smolt stage, monitoring of incubation/rearing temperature which is mostly neglected by stakeholders could be very important and misconceptions developed by incorrect understanding of the smoltification process may not only lead scientists astray in developing new hypothesis and experimental designs, but of equal consequence, it can lead to incorrect practices by the aquaculture industry and those working on other aspects of natural resource management of salmonids (Bjornsson and Brandly, 2007).

Temperature has an important controlling influence on the spawning

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of salmonids and a direct effect on the survival of eggs and the rate at which alevins develop (Armstrong *et al*., 2003).

The upper and lower thermal limits to incubation for Spanish brown trout (*S. trutta*) was determined between 16 and 4 ^{0}C respectively (Ojanguren and Brana, 2003), and survival which was maximal at 8-10 ^{0}C, decreased at higher and lower temperatures. The upper thermal limit was firstly established to 13 ^{0}C (Elliott, 1981; Jungwirth and Winkler, 1984), but a later study (Humpesch, 1985) reported a constant temperature of 15.2 ^{0}C for egg incubation. Indeed researchers highly emphasize the fact that the conditions of the local habitat of the experimental species depending on geographical latitude should be taken into consideration (Jensen *et al*., 1989; Poxton, 1991).

The concept which supports the present research, assumes temperature as a key player in the targeted fish especially in the early life stages for the production high quality parrs based on the best available knowledge (Mitchelmore and Chipman, 1998 a; Samani and Kalbassi, 2015).

In order to detect the possible thermal damages to Caspian trout during natural or artificial rearing conditions, even immediately after fertilization, a couple of tools could provide quantification of non-observable alterations. Morphologic observations, malformations, hatching success, HSP_{70} gene expression, classic histology, karyological analysis and Comet assay could be applied to detect the effects of temperature on biological activities of aquatic organisms (Kalbassi *et al*., 2006; Dorafshan *et al*., 2008). Out of the various methods, Comet assay, which is subject of the present scientific research, could provide a powerful tool in order to detect damages at the cellular level (Esmaeilbeigi and Kalbassi, 2014).

In this study, Caspian trout was used for *in vitro* assessment of the level of genomic damage during cold, warm and normal temperatures of incubation. The three incubation facilities were similar and were equipped with the recycling system from REDD with water taken fresh from the Tonekabon-Dohezar-Daryasar upstream which is the natural spawning location of the Caspian trout. Only the temperature was the variable (Constant at 4, 8, 12°C) factor and the other factors were stable. Based on previous investigations related to north Spain Brown trout (Ojanguren and Brana, 2003) incubation temperatures were selected between the upper 14°C and the lower 4°C limits. To assess the level of DNA damage, Comet assay was used on samples of eyed eggs, alevins and larvae of the 3 incubation breeds.

Since little information regarding the structural effects of temperature on fishes is available, the main objective of this study was to investigate the effects of incubation temperatures on probable DNA damages of the Caspian trout, using groups of designed experiments and a new evaluation technique (Comet assay). Therefore green eggs of wild Caspian trout were loaded into 3 specific incubators kept at constant

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temperatures of 4, 8 and 12 ºC during the incubation procedure.

Materials and methods

Fish

Five pairs of wild broodstocks of Caspian trout (*S. trutta caspius* Kessler, 1877) were obtained for artificial fertilization. Ova and semen were mixed smoothly and stirred using a thin feather. REDD water was added to fertilized material and green eggs were produced.

Three thermal treatments, which were incubated at 4, 8 and 12 ^{0}C were used as cold stress (4 ^{0}C), normal (8 ^{0}C) and warm stress (12 ^{0}C) samples (Alp *et al*., 2010). Green eggs in the Cold, Normal and Warm treatments were incubated for 528, 672 and 720 degree/days, respectively up to the yolk sac absorption stage. Three developmental stages of Caspian trout including eyed eggs, alevins and larvae were considered for sampling.

Treatments

This research was carried out at the Coldwater Fishes Research Center (Tonekabon, Iran). Three incubators were designed and equipped to maintain constant temperatures, water recycling systems by REDD water, 3 stage water filtration (Silica+Active Coal+Zeolite), ultraviolet C (UVC) application on effluent of each incubator and digital controls to measure DO, pH and temperature. Temperature in each incubator was kept constant ±0.2 degree centigrade error with ENDA digital thermo-controller.

Green eggs of wild Caspian trout were distributed into the three experimental incubators. The batches of eggs (500 in number) were laid in baskets which were well aerated in REDD water. The structure of incubators were scaled down from California trays specifically designed and constructed for the present research with minor modifications in comparison with traditional California trays. The automatic incubators which introduced by Pourramazani and Kalbassi, (2008) in Tarbiat Modares University (Iran).

Samples were collected from Normal (8°C) treatments as well as from the Cold (4°C) and Warm (12°C) temperature exposed treatments and stored in liquid nitrogen until use. During stages of embryonic development, genomic alterations in the three thermal treatments (4, 8, 12 degree centigrade) were evaluated by detecting germ cell damage via Comet assay.

Comet assay

Single-Cell Gell (SCG)/Comet assay was used as a useful tool for identifying genotoxic damages of living organisms which was first developed by Ostling and Johanson, (1984) followed by Singh *et al*., (1988) through the detection of DNA breaks. The Comet assay method from Garcia *et al*. (2007) was modified with silver based dye, applied for staining and Tri Tek Comet Score freeware version 1.6.1.13 (Comet Score^{TM}) was used for analysis of Comet images (http://autocomet.com/TutorialCometScore.pdf).

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Clean Frosted slides (Catalogue No. 7105) were covered with 1.2 % Merck Normal Agarose (NA). Samples No: 5 (eyed eggs, alevin and larvae) were homogenized with PBS 1X ($NaCl+KCl+Na_2HPO_4+KH_2PO_4$). The upper dark phase (supernatant) contains individual cells of sample target tissue. After two rounds of centrifuge (1170 rpm for 10 min and 1130 rpm for 7 min) at 4 °C, the individual target cells were mixed with sigma low melting point agarose (LMPA) solution and loaded on pre-coated normal agarose (NA) frosted slides. This was followed by lysis (alkaline) exposure at pH=10, and alkaline electrophoresis (pH>13) for 20 minutes applied at 21 Volts, 250 milliamps. Slides were neutralized with Tris buffer for 2 minutes, and then rinsed smoothly with Double Distilled Water (DDW).

Silver based staining method which provides the capability of archiving slides, is less hazardous in comparison with fluorescence dyes, and gives the possibility of using normal light microscope against fluorescent microscope which is easily accessible in biology laboratories (Garcia *et al*., 2007; Samani and Kalbassi, 2015). Slides rested in the fixing solution (Trichloroacetic Acid, $ZnSO_4$, Glyserol, DDW), then were double rinsed using DDW, dried at 37°C and moistened with DDW for 5 minutes.

Cold (4°C) Staining solution A (Sodium carbonate) and B (Ammonium nitrate, Silicotungestic Acid, Formaldehyde, DDW) were mixed in the dark. The moistened slides were stained for 20 minutes in staining solution in the dark, without shaking, followed by double rinsing with DDW to remove dye particles from slides. Stop solution (1% Glacial Acetic Acid) was used to end the staining procedure. Stained – washed slides were set aside for 5 minutes at room temperature, and then observed using a normal light microscope, Nikon, at 100X magnification to capture images. Molecular biology grade chemicals from German Merck and LMP Agarose from Sigma-Aldrich Co (a part of Merck) were used.

Data analysis

Captured images of comets, analyzed by TriTek CometScore freeware version 1.6.1.13 (Comet Score ™). Comet Score outputs, were automatically transferred to the excel spreadsheet (Microsoft Office Excel 2007) and then analyzed using IBM SPSS Statistics Version 22.

Kolmogorov-Smirnov normality (test Z) descriptive test was applied prior to statistical analysis.

Independent T-test was used to assess data in order to explain the representation of samples for community. Correlation analyses were carried out using Spearman correlation test with significance level of $p<0.05$. One way ANOVA was used for comparison between parameter means and post hoc tests (Duncan) was applied for determination of differences between mean values.

Results

From the post hoc tests (Duncan), 3 distinct groups (a, b, c) were considered

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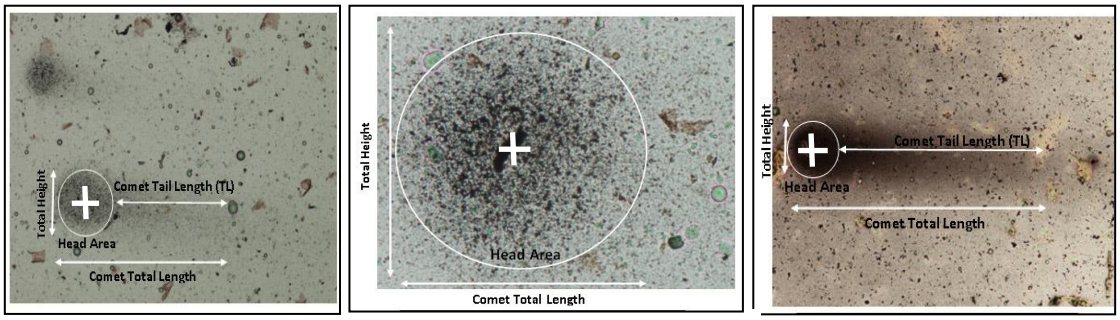
$p \leq 0.01$ for tail length (TL px), % DNA in tail (DT) and Tail Moment (TM) relevant to each of the thermal treatments (4, 8, and 12 °C).

Total means for thermal groups, indicated degrees of damage which affect Caspian trout genome distressed by incubation temperature (Figs. 1,2).



a: Cold incubator Comet b: Normal incubator (8°C). Comet c: Warm incubator (12°C) Comet

Figure 1: Demonstration of comets representatives detected for cold, normal and warm treatments of Caspian trout larva. Circles surrounded the position of single cell's nucleus in center and lines shows approximate limits of Comet factors. Measurements made by semiautomatic Comet score freeware. a: Caspian trout reared in Cold incubator (4°C), Larva single cell Comet by X100 magnification. b: Caspian trout reared in Normal incubator (8°C). Larva single cell Comet by X400 magnification. Comet tail unachieved by Comet Score Software. c: Caspian trout reared in Warm incubator (12°C). Larva single cell Comet by X100 magnification.

Mean values of Comet TL index indicated were 85.19±9.2, 9.88±2.3 and 256.54±14.6 for 4 °C, 8 °C, 12 °C treatments respectively. DT mean values were measured as 18.07±6.3, 9.72±1.9 and 31.47±2.8 and then TM mean values measured were 15.27±4.1, 1.21±0.2 and 95.25±8.6 for 4°C, 8°C, 12°C treatments, respectively.

Warm (12°C) treatments significantly demonstrated greater DNA damage in comparison with treatments at 4 and 8°C. Hatching success (P_h) was measured as 0.38, 0.91 and 0.73 for the cold, normal and warm incubation, respectively (Viavant, 1996). Degree days are provided in the chronology

Table 1: Achieved degree days of Caspian trout

Chronology	4°C degree day	8° degree day	12° degree day
Eyed egg	248	296	216
Hatching	388	448	648
End yolk sac absorption	528	672	720

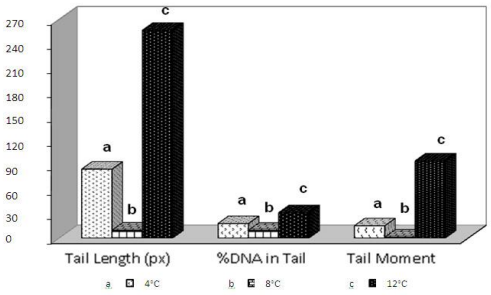


Figure 2: Caspian trout DNA damage description by total means for TL, DT & TM variables of Comet assay, 3 separated groups indicated by a (4°C), b (8°C), c (12°C).

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Discussion

According to the advantage of alkaline microelectrophoresis, fragments of DNA containing negative charge migrate towards the anode in the frame of the comets (Singh *et al*., 1988). By increasing DNA damages, more DNA fragments move into the tail region of the Comet and were quantified in terms of either increased Comet cloud in the tail region (percentage DNA in the tail), tail length, or a product of these two measurements called as tail moment (Mitchelmore *et al*., 1998b).

For the adaptive application of the Comet assay, previous attempts and several modifications were considered since 1984 (Singh *et al*., 1988; Mitchelmore *et al*., 1998b; Rojas *et al*., 1999; Kopjar and Garaj-Vrhovac2001; Dhawan *et al*., 2003; Kirilova *et al*., 2005; Frenzilli *et al*., 2009; Liao *et al*., 2009; Speit *et al*., 2009; Pandey *et al*., 2011; Zhang *et al*., 2011; Collins, 2014), following its first introduction (Ostling and Johanson 1984). Two main staining methods used DNA-binding fluorescent (propidium iodide or ethidium bromide) and non fluorescent dye (Silver based) for visualization of Comets. The fluorescent staining methods using potential carcinogens dyes, require a high quality microscope with epiflourescent optics, 100-W mercury lamp, sensitive CCD camera and sophisticated image analysis software. The inexpensive non fluorescent silver staining method allows preservation of slides for long periods of time and their analysis with conventional light microscope, which is available in most laboratories (Nadin *et al*., 2001; Samani and Kalbassi, 2015). In the present analysis, Silver based dye (Merril, 1990) afterwards modified and applied on alkaline Comet assay (Nadin *et al*., 2001; Garcia *et al*., 2004, 2007) was considered with minor modifications.

Pourramazani and Kalbassi (2008) reported that by increasing incubation temperature by 2°C between 8 and 10 °C, incubation was shortened by 20 days between green egg and hatching stages without the occurrence of malformations and other morphologic indications, up to the end of yolk sac absorption in Caspian trout. Ojanguren and Brana (2003) also stated the lower and upper incubation thermal limits for Brown trout during developmental stages to be between 4 and 14°C. In both reports fish treatments were alive without observable morphologic damage until the start of exogenous feeding, but there were no documents about their larval genomic health. Ojanguren and Brana (2003) mentioned embryos incubated at lower temperatures were larger at 50% hatching which is assumed as fiber muscle differences.

Use of warmer incubation temperatures resulted in faster embryonic and larval development (Alp *et al*., 2010). Awareness of what would be happening with live morphologically healthy larva which apparently passed incubation procedure and hatched successfully, led the present research to address some possible invisible signs. Investigation of genomic alterations directed the research to look at DNA health using a cellular-molecular tool.

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Alkaline Comet assay/Silver based staining which was used as a powerful screening tool in this research provided 17 parameters of each detected single cell comets as output of Cometscore freeware. Out of 17 measured parameters of detected comets, Comet tail length (TL), DNA in tail (DT) and tail moment (TM) indices were selected as DNA damage markers (Kopjar and Garaj-Vrhovac, 2001).

The results of the present investigation demonstrated significant differences between thermal treatments (p<0.05) relevant to DNA damage in Caspian trout single cells. Warm (12°C) treatments demonstrated greater DNA damage in comparison with 4°C and 8 °C treatments.

It seems that TM explained better information in comparison with TL & DT in all analyzed samples. Short term adverse effects of temperature could appear as mortality when very cold (<4°C) or very warm (>12°C) temperatures were used during incubation, otherwise temperatures between limits resulting in probable hidden damages in live larvae of Caspian trout may occur subsequently in the future. Simulation advantages of incubation in 4°C and 12°C treatments in comparison with normal (8°C) treatment, expressed empirical information regarding the importance of appropriate incubation temperature to natural physiological requirement of the species.

The temperature in situ REDD for Caspian trout is 6-8 °C during incubation in natural habitats. However in coldwater aquaculture systems which were designed for rainbow trout, Caspian trout green eggs were produced for stock rehabilitation projects since 1967 by FAO and later by Russian advisors. The incubation temperature was not considered as a key player for production of healthy parrs of Caspian trout. Also recently the requests for Caspian trout presmolts is increasing for stocking in cage culture systems floating in the South Caspian Sea. Indeed more fish fry is needed from hatcheries that could support these developing aquaculture stakeholders. Besides in recent years, Iranian coldwater aquaculture private owners have experiences regarding rearing Caspian trout in ponds and partially in sea cages. Whether the release of parrs is for highly expensive projects of stock rehabilitation or in response to cage culture and race way farms, the consideration of incubation temperature is the key to Caspian trout fisheries/aquaculture sustainability.

The information obtained in the present attempt demonstrated considerable damages to genomic materials of Caspian trout when temperature is deviated by normal incubation. Indeed study on long term adverse effects of non- lethal temperatures could be the proposed subject of future experiments.

In Conclusion**,** temperatures out of optimal thermal conditions could significantly affect Caspian trout genome which could be subject of management considerations pertaining to Caspian trout stock rehabilitation and captive rearing strategies.

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References

Alp, A., Erer, M. and Kamalak, A., 2010. Eggs incubation, early development and growth in frys of brown trout (*Salmo trutta macrostigma*) and Black Sea trout (*Salmo trutta labrax*). *Turkish Journal of Fisheries and Aquatic Sciences*, 10, 387-394.

Amiri, M.J. and Eslamian, S.S. 2010. Investigation of climate change in Iran. *Journal of Environmental Science and Technology*, 3(**4**), 208-216.

Armstrong, J.D., Kemp, P.S., Kennedy, G.J.A., Ladle, M. and Milner, M.J. 2003. Habitat requirements of Atlantic salmon and brown trout in rivers and streams. *Fisheries Research*, 62, 143-170.

Bjornsson, B.T. and Brandly, M., 2007. Epilogue: Past successes, present misconceptions and future milestones in salmon smoltification research. *Aquaculture*, 273, 384-391.

Brander, K., 2010. Impacts of climate change on fisheries. *Journal of Marine Systems*, 79, 389-402.

Collins, A.R., 2014. Measuring oxidative damage to DNA and its repair with the Comet assay. *Biochimica et Biophysica Acta*. 1840, 794-800.

Comet Score Tutorial. http://autocomet.com (accessed 26 December 2015)

Dhawan, A., Bajpayee, M., Pandey, A.K. and Parmar, D., 2003. Protocol for the single cell gel electrophoresis/Comet assay for rapid genotoxicity assessment. Development Toxicity Division. Industrial Toxicity Research Centre, India. 10 P.

Dorafshan S., Kalbassi M.R., Pourkazemi M., Amiri, B. and Soltankarimi, S., 2008. Effects of triploidy on the Caspian salmon *Salmo trutta caspius* haematology. *Journal of Fish Physiology and Biochemistry*, 34(**3**), 195-200

Elliott, J.M., 1981. Some aspects of thermal stress on freshwater teleosts. In: Stress and fish (Pickering, A. D., ed.). London Academic Press. pp. 209-245.

Esmaeilbeigi M. and Kalbassi M.R., 2014. Optimization of single-cell gel electrophoresis (SCGE) method for study of liver single cell DNA in

[Downloaded from jifro.ir on 2026-11-19]

[DOR: 20.1001.1.15622916.2019.18.1.4.9]

Caspian kutum (*Rutilus frisii kutum*). *Aquatic Physiology and Biotechnology*, 2(**3**), 13-30.

Frenzilli, G., Nigro, M. and Lyons, B.P., 2009. The Comet assay for the evaluation of genotoxic impact in aquatic environments. Mutation research/reviews in mutation research. pp. 80-92.

Garcia, O., Mandina, T., Lamadrid, A.I., Diaz, A., Remigio, A., Gonzalez, Y., Piloto, J., Gonzalez, J.E. and Alvarez, A., 2004. Sensitivity and variability of visual scoring in the Comet assay results of an inter-laboratory scoring exercise with the use of silver staining. *Mutation Research*, 556, 25-34.

Garcia, O., Romero, I., Gonzalez, J.E. and Mandina, T., 2007. Measurements of DNA damage on silver stained Comets using free Internet software. *Mutation Research*, 627, 186-190.

Humpesch, U.H., 1985. Inter and intra-specific variation in hatching success and embryonic development of five species of Salmonids and *Thymallus thymallus*. *Archiv fur Hydrobiologie.* 104, 129-144.

Jalali, M.A. and Mojazi Amiri, B., 2009. Threatened fishes of the world: *Salmo trutta caspius* (Kessler, 1877) (Salmoniforms: Salmonidae). *Environmental Biology of Fishes*, 86(**3**), 375-376.

Jamshidi, Sh., Kalbassi. M.R., 2011. Conspecific relation between two seasonal migratory forms of endangered Caspian trout, *Salmo trutta caspius* Kessler, 1877, revealed by RAPD markers. Iranian Journal of Fisheries Sciences. 10 (3). 437-446.

Jensen, A.J., Johnsen, B.O. and Saksgard, L., 1989. Temperature requirements in Atlantic salmon (*Salmo salar*), brown trout (*Salmo trutta*), and Arctic char (*Salvelinus alpinus*) from hatching to initial feeding compared with geographic distribution. *Canadian Journal of Fisheries and Aquatic Sciences*, 46, 786–789.

Jungwirth, M. and Winkler, H., 1984. The temperature dependence of embryonic development of grayling (*Thymallus thymallus*), Danube salmon (*Hucho hucho*), Arctic char (*Salvelinus alpinus*) and brown trout (*Salmo trutta fario*). *Aquaculture.* 38, 315-327.

Kalbassi, M.R., Dorafshan, S., Tavakolian, T., Khazab, M. and Abdolhay, H., 2006. Karyological analysis on endangered Caspian salmon, *Salmo trutta caspius***.** *Aquaculture Research*, 37(**13**), 1341-1347.

Kalbassi, M.R., Abdollahzadeh, E. and Salari-Joo, H., 2012. A review on aquaculture development in Iran. *Journal of Ecopersia*, 1(**2**), 159-178.

Khara H., 2016. Effect of successive milt collection on sperm quality and reproduction of wild and cultured endangered Caspian Brown trout, *Salmo trutta* . IJFS. 15 (1) :31-38.

Kirilova, M., Ivanov, R. and Miloshev, G., 2005. A novel parameter in Comet assay

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[DOR: 20.1001.1.15622916.2019.18.1.4.9]

measurements. *Genetika*, 37(**2**), 93-101.

Kocabas, M., Bascinar, N., Sahin, S.A. and Kutluyer, F., 2012. Hatching performances and yolk sac absorptions of Caspian brown trout (*Salmo trutta caspius*, 1954). *The Journal of Animal and Plant Sciences*, 22(**1**), 88-92.

Kopjar, N. and Garaj-Vrhovac, V., 2001. Application of the alkaline Comet assay in human biomonitoring for genotoxicity: a study on Croatian medical personnel handling antineoplastic drugs. *Mutagenesis*. 16(**1**), 71-78.

Liao, W., McNutt, M.A. and Zhu, W.G., 2009. The Comet assay: A sensitive method for detecting DNA damage in individual cells. *Methods*, 48, 46-53.

Merril, C.R., 1990. Silver staining of proteins and DNA. *Nature*, 343, 779-780.

Mitchelmore, C.L. and Chipman, J.K., 1998a. Detection of DNA strand breaks in brown trout (*Salmo trutta*) hepatocytes and blood cells using the single cell gel electrophoresis (Comet) assay. *Aquatic Toxicology*, 41, 161-182.

Mitchelmore, C.L. and Chipman, J.K., 1998b. DNA strand breakage in aquatic organisms and potential value of the Comet assay in environmental monitoring. *Mutation Research*, 399, 135-147.

Nadin, S.B., Vargas-Roig, L.M. and Ciocca, D.R., 2001. A silver staining method for single-cell gel assay. *The Journal of Histochemistry and Cytochemistry*, 49(**9**), 1183-1186.

Ojanguren, A.F. and Brana, F., 2003. Thermal dependence of embryonic growth and development in brown trout. *Journal of Fish Biology*, 62, 580-590.

Ostling, O. and Johanson, K.J., 1984. Microelectrophoresis study of radiation-induced DNA damages in individual mammalian cells. *Biochemistry Biophysics Research Communications*, 123, 291-298.

Pandey, A.K., Nagpure, N.S., Trivedi, S.P., Kumar, R. and Kushwaha, B., 2011. Profenofos induced DNA damage in freshwater fish, *Channa punctatus* (Bloch) using alkaline single cell gel electrophoresis. *Mutation research/Genetic Toxicity and Environmental Mutagenesis*, 726, 209-214.

Pourramazani, M. and Kalbassi, M.R., 2008. Hatching acceleration of Caspian brown trout (*Salmo trutta caspius*) in an experimental incubator and its effect on some larval quality parameters. Tarbiat Modares Univ. M.Sc Dissertation. 97 P.

Rajabi Islami H, Arab N, Assare R, Rastravan M, Ebtekari R., 2016. Blood parameters of Caspian brown trout (Salmo trutta caspius) fingerlings affected by dietary L-ascorbyl-2-polyphosphate. IJFS; 15 (3) :1167-1186

Rojas, E., Lopez, M.C. and Valverde, M., 1999. Single cell gel electrophoresis assay: methodology and applications. *Journal of Chromatography*, 722(**1-2**),225–254.

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[DOR: 20.1001.1.15622916.2019.18.1.4.9]

Samani, P. and Kalbassi, M.R., 2015. Assessment of dose and time dependant DNA damage induced by ultraviolet -B and C radiation on egg and larvae of rainbow trout (*Oncorhynchus mykiss*) by alkaline Comet assay. *Modern Genetics*, 10(**2**), 187-196.

Singh, N.P., McCoy, M.T., Tice, R.R. and Schneider, E.L., 1988. A simple technique for quantitation of low levels of DNA damage in individual cells. *Experimental Cell Research*, 175(**1**), 184-191.

Speit, G., Vasquez, M. and Hartmann, A., 2009. The Comet assay as an indicator test for germ cell genotoxicity. *Mutation Research/Reviews in Mutation Research*, 681(**1**), 3-12.

Vera, **M., Sourinejad, I., Bouza, C., Vilas, R., Pino-Querido, A., Kalbassi, M.R. and Martínez, P., 2010.** Phylogeography, genetic structure, and conservation of the endangered Caspian brown trout, *Salmo trutta caspius* (Kessler, 1877), from Iran. *Hydrobiologia*, 664 (**1**), 51-67.

Zhang, L.J., Jia, J.F., Hao, J.G., Cen, J.R. and Li T.K., 2011. A modified protocol for the Comet assay allowing the processing of multiple samples. *Mutation Research/Genetic Toxicology and Environmental Mutagenesis*. 721, 153–156.

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[DOR: 20.1001.1.15622916.2019.18.1.4.9]