

Reproductive traits and annual hormonal cycles of Iranian brown trout, *Salmo trutta fario*

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ABSTRACT

There is significant variability in the reproductive organs of fish, making it challenging to establish a "universal" model for their basic function and structure. To address this, a study was conducted on *Salmo trutta fario* (STF), focusing on potential seasonal changes and histological effects. Five fish were captured during each season. After measuring the length and weight of each specimen, blood samples were collected and a histological evaluation of the reproductive organs was performed. Our findings revealed that hormone levels in both male and female STF peaked during the fall season. This conclusion was supported by measurements of seasonal hormone levels, physical characteristics, and histological examinations. Consequently, we confirmed that STF undergo seasonal changes, suggesting an adaptation of the gonads to meet physiological needs. So, it can be concluded that sex hormone levels increase significantly from summer, reach their peak in the fall, and then tend to decrease after mating.

Keywords: Reproductive system, Hormones, Histological examination, *Salmo trutta fario*, Iran

INTRODUCTION

Salmonids are a group of fish that are commonly known as salmon, trout, and char. They are renowned for their remarkable ability to navigate vast distances and challenging environments to complete their life cycle (Muhlfeld *et al.* 2019). Central to this cycle is the reproductive process, which plays a crucial role in the perpetuation and survival of these magnificent species (Fleming & Reynolds 2004). Salmonids possess both external and internal reproductive organs, each serving distinct functions in the reproductive process (Penney & Moffitt 2014). The most prominent external reproductive organs in male salmonids are the paired testes, which are responsible for producing sperm. These testes are often characterized by their distinct coloration and size, varying significantly among different species and individuals (Dziewulska & Domagala 2003). Conversely, female salmonids possess an external reproductive organ known as the ovipositor, which is responsible for the deposition of eggs. This specialized structure plays a pivotal role in the successful fertilization and development of offspring (Dodson *et al.* 2013). While the external reproductive organs offer striking visual cues, it is the internal reproductive organs of salmonids that truly reveal the complexity and intricate mechanisms at play during reproduction. In both male and female salmonids, the gonads are the primary internal organs responsible for producing gametes – sperm and eggs, respectively. These gametes are then released into the water during the spawning season, where they undergo fertilization, leading to the formation of new offspring (Fleming & Reynolds 2004; Birnie-Gauvin *et al.* 2021). Apart from the gonads, male salmonids also possess specialized structures known as the sperm ducts, which

transport sperm from the testes to the external environment. These ducts are typically characterized by their coiled and convoluted nature, facilitating the transportation of a large volume of sperm. On the other hand, female salmonids possess a unique set of internal reproductive organs, including the ovaries and oviducts. The ovaries function as the site of egg maturation and release, while the oviducts serve as conduits for the eggs until they are ready for fertilization (Fleming & Reynolds 2004).

STF, commonly known as brown trout, is a stunning species of freshwater fish that inhabits various rivers and streams in Iran. With its remarkable adaptability and intriguing reproductive characteristics, this species has served as a subject of intensive research, shedding light on its unique life cycle and hormonal fluctuations throughout the year (Lobón-Cerviá & Sanz 2017). Regarding reproductive characteristics, STF displays a complex reproductive cycle, characterized by distinct stages that are intricately controlled by various environmental and physiological factors. The reproductive cycle of this species can be broadly categorized into pre-spawning, spawning, and post-spawning phases, each marked by distinct behaviors and physiological changes. During the pre-spawning phase, individuals undergo physical transformations, such as increased body size and growth of secondary sexual characteristics (Brown 1994; ARSLAN & Aras 2007). For example, as the spawning season approaches, STF demonstrates intricate courtship rituals, including elaborate displays of colors and swimming patterns. The males establish and defend territories, engaging in fierce competition with other males for the opportunity to fertilize the eggs. The females, on the other hand, choose the most suitable mates based on their visual cues and courtship displays (Saraniemi *et al.* 2008).

Following successful fertilization, the post-spawning phase is marked by a decline in reproductive activity and physiological changes aimed at restoring energy reserves. The fish may undertake migration or remain resident in their respective habitats, as dictated by local environmental conditions. During a year, the reproductive success of STF is closely linked to the regulation of sexual hormones within their bodies. Hormonal changes play a pivotal role in stimulating the development of reproductive organs, controlling the timing of reproductive events, and ensuring successful breeding (Smialek *et al.* 2021; Santos *et al.* 2021). Consequently, STF experiences dynamic fluctuations in sexual hormone levels throughout the year. These fluctuations are influenced by various factors, including photoperiod, water temperature, and food availability. The precise interplay of these factors determines the onset and duration of each reproductive phase, ensuring optimal reproductive success for the species.

Our goal was to understanding the reproductive characteristics and hormonal changes in STF vital for the effective management and conservation of this species.

2. MATERIALS AND METHODS

2.1 Animals and sampling

First, Haraz River was chosen due to possessing suitable stocks of the desired fish. Five fish from each sex were captured seasonally by net fishing. To comply with the ethical principles, they were anesthetized and then euthanized and autopsied. Before sampling, anatomical characteristics including body weight and length were measured. Then, the blood samples were taken. Next, the fish were dissected and the reproductive tissues were separated using scissors and scalpel. The tissues were examined by an optical microscope (Mlaćo *et al.* 2014). Additionally, it should be noted that this study was approved by the Research Ethics Committees of Islamic Azad University Babol Branch, North Iran.

2.2 Serum markers

After blood sampling, the serum was separated by centrifuging the whole blood at 3000 RPM for 5 minutes. Then, total protein, calcium, and hormone levels were calculated by employing an autoanalyzer (Er & Dik 2014; Valaei *et al.* 2022).

2.3 Histological examination

Fresh tissue samples were fixed in a 10% buffered formalin solution and after tissues were processed, 10-micron slides were made. The prepared slides were stained by hematoxylin-eosin stain. In this type of staining, the nucleus of the cells is blue and the cytoplasm is red to pink. Finally, histological evaluation was performed using an optical microscope (Olympus, cx31; Salehi *et al.* 2024).

2.4 Data analysis

The quantitative results were analyzed by One-Way ANOVA and Duncan's post hoc tests using SPSS version 27 (Salehi *et al.* 2022a, Salehi *et al.* 2023). In terms of histological evaluation, each testicle was given a score of 0 to 4 for spermatozoa count and seminiferous tubules (0 was none, and 4 was the maximum score; Rippere-Lampe *et al.* 2001; Pavlov *et al.* 2020). A p -value < 0.05 was considered significant (Salehi *et al.* 2022b).

RESULTS

3.1 Weight change

Throughout the year, fish were weighed, and their liver tissues were biopsied and weighed. In the first three seasons, males were significantly heavier than females, though males were moderately lighter in winter. Females consistently had heavier livers than males, but this difference was not significant (Fig. 1).

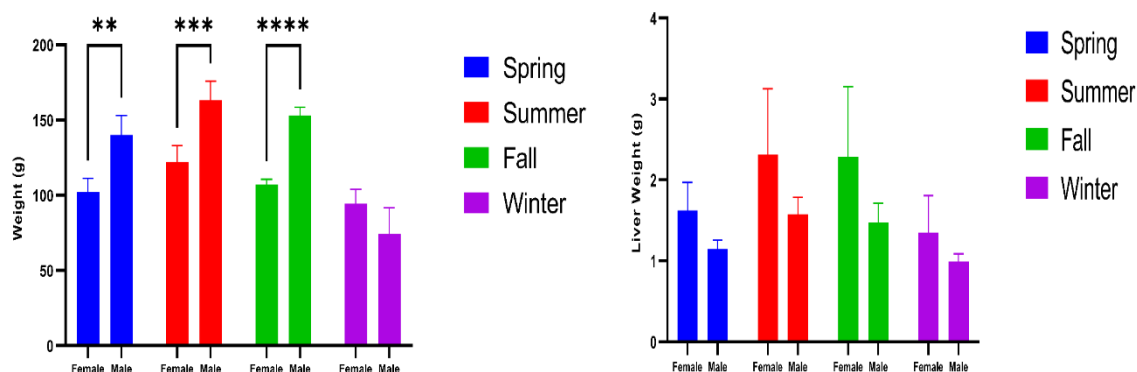


Fig. 1. Full body and liver weight alterations. Results are expressed as mean $\pm$ standard error. ** $p < 0.01$, *** $p < 0.001$, and **** $p < 0.0001$: significant difference between groups.

Reproductive tissue weight varied significantly across seasons. Male and female gonad weights increased from spring to fall, then significantly decreased in winter. The spring-to-summer increase in females was not significant (Fig. 2).

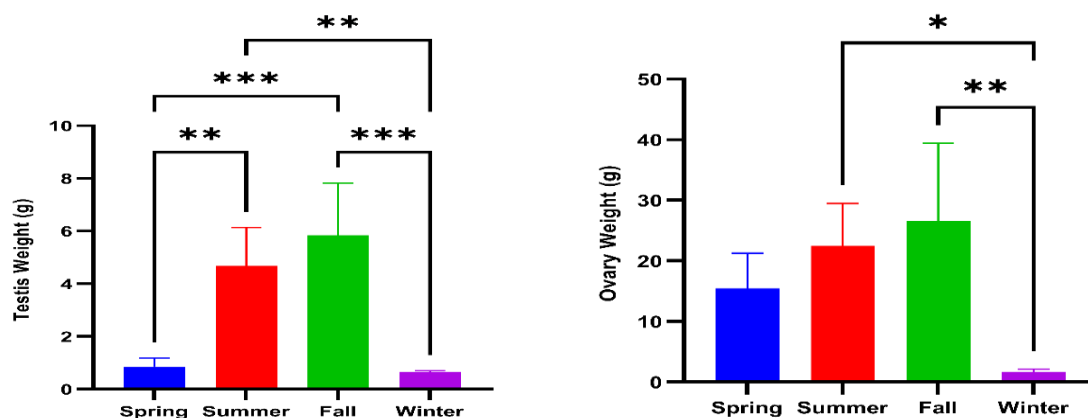


Fig. 2. Reproductive organ weight alterations. Results are expressed as mean $\pm$ standard error. * $p < 0.05$, ** $p < 0.01$, and *** $p < 0.001$: significant difference between groups.

3.2 Morphometric measurement

Male fish were longer than females in spring, summer, and fall, but this difference was only significant in the fall. In winter, females were slightly longer. These patterns in length are similar to those observed in weight (Fig. 3). Ovary length mirrored testis weight, increasing from spring to fall and decreasing in winter. Similarly, ovum counts rose in spring but significantly declined in winter (Fig. 3).

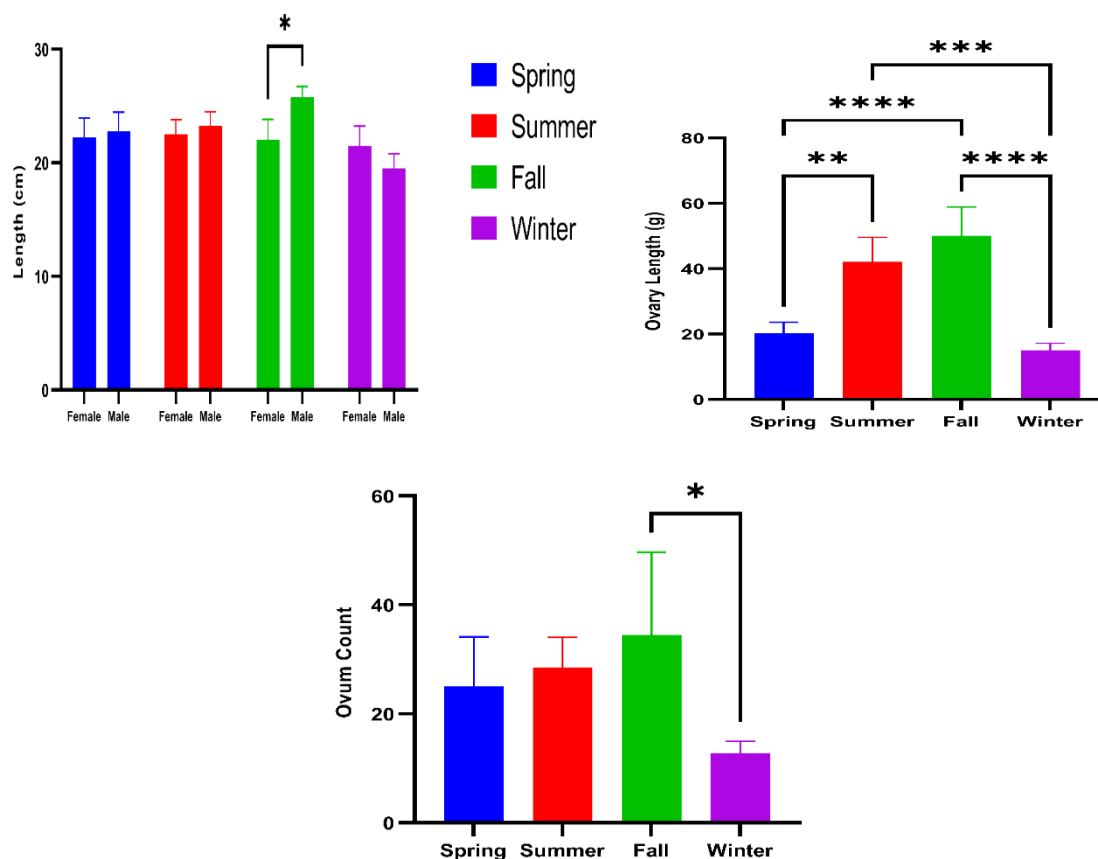


Fig. 3. Full length and ovary indices. Results are expressed as mean $\pm$ standard error. * $p < 0.05$, ** $p < 0.01$, *** $P < 0.001$, and **** $p < 0.0001$: significant difference between groups.

3.3. Biochemical evaluation

Serum total protein and calcium levels were measured from collected blood samples. Total protein levels varied annually but showed no significant difference between sexes. Females, however, consistently had higher calcium levels across all seasons, with this difference being significant in the first three seasons (Fig. 4).

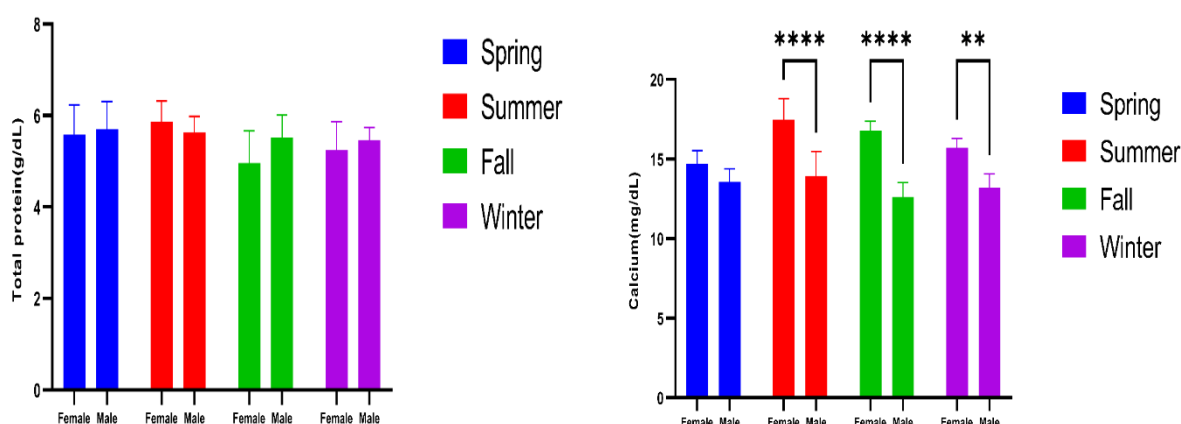


Fig. 4. Total protein and calcium levels. Results are expressed as mean $\pm$ standard error. ** $p < 0.01$ and **** $p < 0.0001$: significant difference between groups.

3.4 Hormonal changes

Estradiol and progesterone levels were predominantly higher in females, with significant differences observed in summer and fall for estradiol. Progesterone showed a similar trend, with minimal sex-based differences in spring

and winter. Female testosterone levels decreased from spring to summer, then significantly increased in fall before declining in winter. Male testosterone peaked in the fall (Fig. 5).

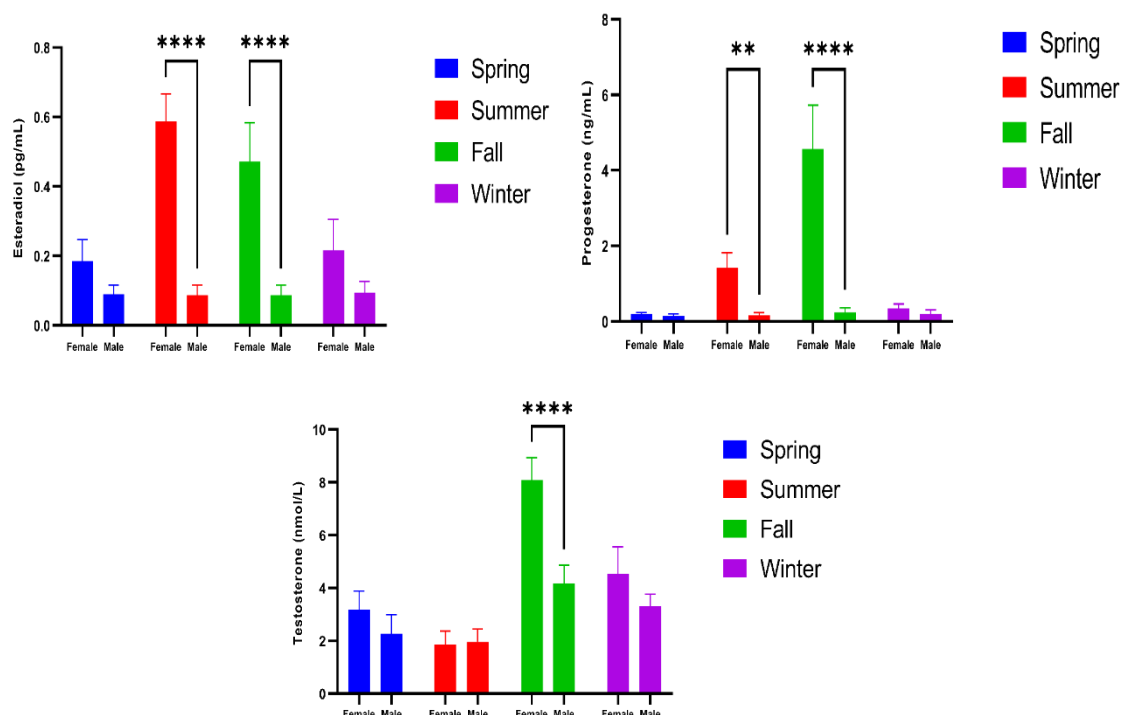


Fig. 5. Serum hormonal levels. Results are expressed as mean $\pm$ standard error. ** $p < 0.01$ and **** $p < 0.0001$: significant difference between groups.

Table 1. Significance (p -value) of the correlations between all variables.

	Seasons	Total Protein Female	Calcium Female	Estradiol Female	Progesterone Female	Testosterone Female	Total Protein Male	Calcium Male	Estradiol Male	Progesterone Male	Testosterone Male
Seasons	-	0.289	0.548	0.948	0.356	0.043*	0.665	0.464	0.802	0.174	0.026*
Total Protein Female	0.289	-	0.277	0.695	0.478	0.073	0.838	0.205	0.353	0.366	0.075
Calcium Female	0.548	0.277	-	0.035*	0.274	0.535	0.761	0.834	0.608	0.763	0.466
Estradiol Female	0.948	0.695	0.035*	-	0.034*	0.869	0.701	0.885	0.893	0.970	0.741
Progesterone Female	0.356	0.478	0.274	0.034*	-	0.001**	0.985	0.681	0.385	0.047*	0.012*
Testosterone Female	0.043*	0.073	0.535	0.869	0.001**	-	0.772	0.425	0.621	0.095	0.001**
Total Protein Male	0.665	0.838	0.761	0.701	0.985	0.772	-	0.026*	0.495	0.326	0.694
Calcium Male	0.464	0.205	0.834	0.885	0.681	0.425	0.026*	-	0.122	0.485	0.528
Estradiol Male	0.802	0.353	0.608	0.893	0.385	0.621	0.495	0.122	-	0.839	0.909
Progesterone Male	0.174	0.366	0.763	0.970	0.047*	0.095	0.326	0.485	0.839	-	0.061
Testosterone Male	0.026*	0.075	0.466	0.741	0.012*	0.001**	0.694	0.528	0.909	0.061	-

3.5 Correlation of indices

Correlations between serum markers were evaluated. In female fish, testosterone levels correlated significantly with seasons ($p < 0.05$). Additionally, significant correlations were found between calcium and estradiol levels,

and between estradiol and progesterone levels. Progesterone levels also correlated significantly with testosterone levels ($p < 0.001$). In male fish, a similar correlation was observed between testosterone levels and season. A significant correlation was also found between testosterone levels and sexes ($p < 0.001$; Table 1).

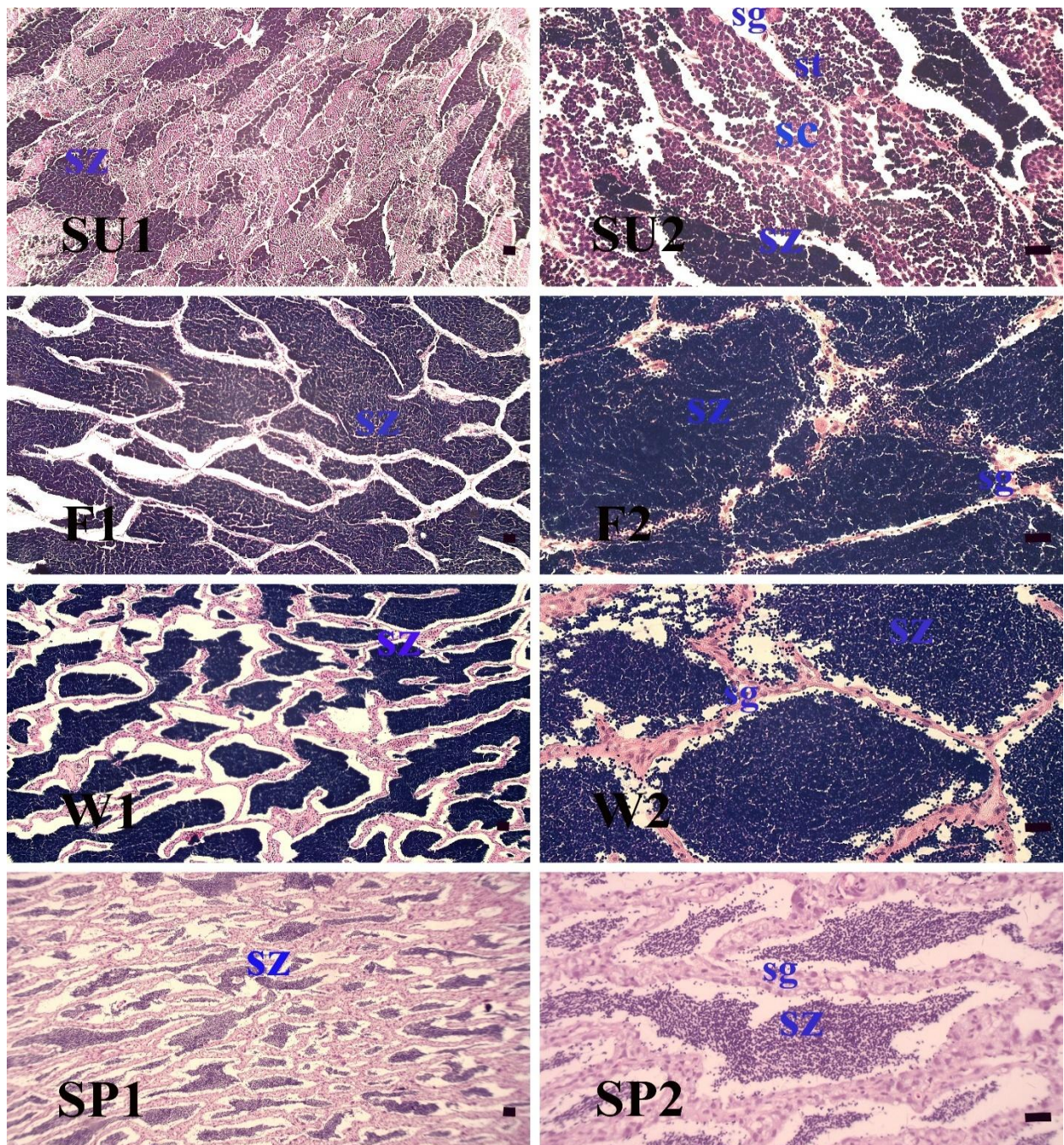


Figure 6. Testicle tissue. Summer (SU), Fall (F), Winter (W), Spring (SP). Spermatid (st), Spermatozoa (SZ), Spermatogonia (sg), Spermatocyte (sc). X10 and X40 magnification. H & E staining. Bar scale: 40 μm.

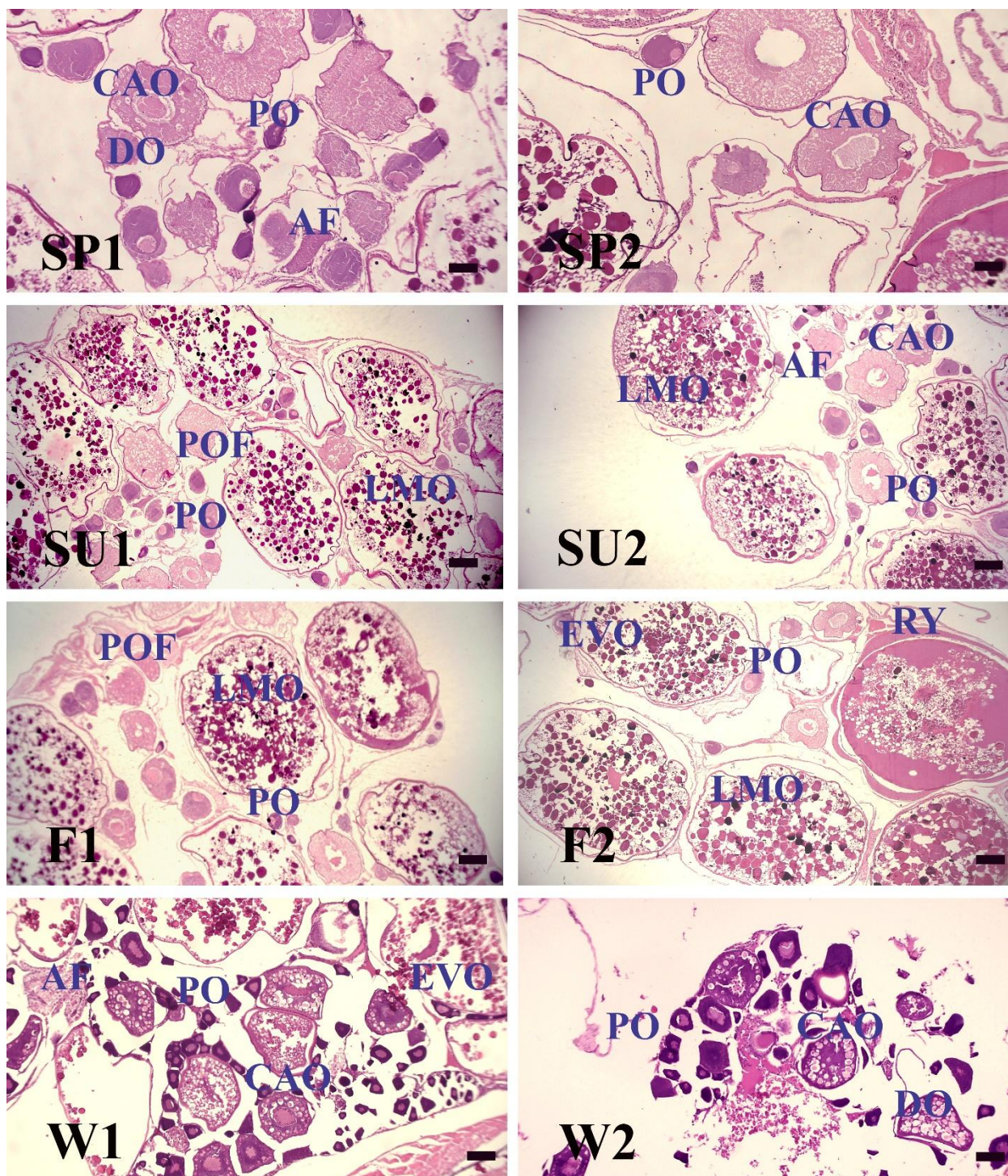


Fig. 7. Ovarian tissue. Summer (SU), Fall (F), Winter (W), Spring (SP). Perinuclear oocyte (PO), cortical alveolar oocyte (CAO), early vitellogenic oocyte (EVO), late and mature oocyte (LMO), postovulatory follicles (POF), atretic follicles (AF), degenerating oocyte (DO), and reabsorb the yolk (RY). X10 magnification. H & E staining. Bar scale: 40 μ m.

3.6 Histological evaluation

In male organ histological examination, the number of seminiferous tubules reached the peak in fall and the lowest amounts in spring and summer. The population of spermatozoa followed the same trend and peaked in the fall and then started to reduce afterward (Table 2).

Table 2. Quantitative indices in testicular tissue. Each testicle was given a score of 0 to 4 for spermatozoa count, and seminiferous tubules (0 was none, and 4 was the maximum score).

	Spermatozoa population	Number of seminiferous tubules
Summer	++	++
Fall	++++	++++
Winter	+++	+++
Spring	++	++

Male tissue (spermatid, spermatozoa, spermatogonia, and spermatocyte) consistently showed normal conditions (Fig. 6). Female organ indices varied seasonally (Fig. 7). While overall tissue remained normal, postovulatory follicles, cortical alveolar oocytes, atretic follicles, and degenerating oocytes decreased in fall. Conversely, fall marked peak levels for early vitellogenic, late, and mature oocytes, postovulatory follicles, and reabsorbed yolk (Table 3).

Table 3. Histological indices of ovarian tissue. Perinuclear oocyte (PO), cortical alveolar oocyte (CAO), early vitellogenic oocyte (EVO), late and mature oocyte (LMO), postovulatory follicles (POF), atretic follicles (AF), degenerating oocyte (DO), and reabsorb the yolk (RY). Results are expressed as mean

	PO	CAO	EVO	LMO	POF	AF	DO	RY
Spring	8.33	3.17	2.17	2.33	1.50	0.83	0.50	0.17
Summer	6.67	2.67	2.33	3.50	2.33	0.67	0.33	0.17
Fall	4.17	2.00	2.83	4.17	3.33	0.50	0.17	0.33
Winter	10.33	4.17	2.00	1.83	0.83	1.83	0.67	0.17

DISCUSSION

In order to better comprehend the intricacies of salmonid reproduction, it is essential to evaluate the hormonal and histological alterations during the complete cycle because they could vary between different fishes (Mukhopadhyay & Sinha 1986). It was previously observed that environmental factors, such as season, can change the expression of sexual hormones and other indices in fish (Fatima *et al.* 2018; Santos *et al.* 2021). Since changes in body size and growth of secondary sexual characteristics are crucial for attracting mates and establishing dominance within the population, this study aimed to investigate the underlying changes in different seasons of a year during one reproduction cycle. Briefly, it was observed that STF length and overall weight were significantly different between sexes in fall. Moreover, reproductive organ weight peaked at this season, too. Additionally, a surge in progesterone and testosterone levels was observed in this season. Backed up by histological findings, it could be proposed that STF is more sexually active in the fall season due to the rise of related hormones (Billard 1987; Caputo *et al.* 2010).

In terms of weight changes, it was observed that male fish were significantly heavier, which could be due to their higher energy requirement (Gross & Sargent 1985). Since male needs more energy for mating, it is proposed that this rise in weight is correlated with the reproductive characteristic of male fish. Also, female fish had more liver mass but no significant difference was noticed.

Since fish need more energy for mating in the fall, testis and ovary tissue weight peaked in the fall and then reduced substantially in winter. This was in line with our previous findings, which demonstrated overall weight alterations. Moreover, it showed that one of the major factors of weight loss in fish could be seasonal manners, mostly related to sexual behaviors and mating. In addition, the length of male fish was significantly higher than that of females, which could be due to its physical characteristics caused by sexual hormones in the fall season.

Looking at the serum level of total protein, no significant alteration was observed between seasons or sexes. However, the rise of calcium levels and significant difference between sexes were interesting. Since calcium contributes to the formation of eggshells, spawning behavior, and the development of healthy juveniles, it is expected to rise in the reproduction season, especially in females. Additionally, calcium is involved in the regulation of hormones and enzymes that control fish reproduction (Hossain & Yoshimatsu 2014).

Regarding hormone levels, a significant surge of estradiol was observed in female fish, starting from summer and lasting until fall. This could be the result of the pre-phase of getting ready for mating (Fregeneda-Grandes *et al.* 2013). For example, a rise of the T₃ hormone was previously observed in STF in the latter stage of the sexual development cycle (Mylonas *et al.* 1994). As found in our study, male fish showed no significant change in estradiol levels throughout the year. Meanwhile, progesterone levels followed the same trend in male fish.

However, it started to grow in summer and peaked in fall in females. These findings were in line with other sexual hormones, which both are indicators of getting ready for mating. In the case of testosterone, the level of it was increased in male fish moderately, while females experienced a massive surge of testosterone in the fall. Since it was previously observed that testosterone can improve sexual activity and reproduction of female fish (Liu *et al.* 2021), this rise in testosterone could be a sign of getting ready for mating season in STF. Additionally, in histological examination, a rise in tissue activity was observed in fall in both sexes. This observation was in line with our previous findings, which showed a rise in serum hormone levels. The number of spermatozoa increased in the fall and lasted for only one season, similar to the results obtained by Billard (1983). The fall season is considered the spawning phase in fish (Chaves-Pozo *et al.* 2005), when we observed testicle tissue development (Fig. 6).

CONCLUSION

It can be concluded that STF shows a nearly predictable sexual manner. The sex-related hormone levels mostly begin to rise in summer and peak in fall, which is the mating season. This idea was verified by histological examination and hormonal evaluation, alongside physical alterations.

Competing Interests

The authors have no relevant financial or non-financial interests to disclose.

Author Contributions

All authors contributed to the study conception and design. Material preparation, data collection and analysis were performed by Niloufar Fallah and Saber Vatandoust. The first draft of the manuscript was written by Niloufar Fallah, and all authors commented on the manuscript. All authors read and approved the final manuscript.

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Data Availability

The datasets generated during the current study are available from the corresponding author on reasonable request.

Ethical Approval

The project was found to be in accordance to the ethical principles and the national norms and standards for conducting Medical Research in Iran. Additionally, the Research Ethics Committees of Islamic Azad University-Babol Branch approved this study.

REFERENCES

- Arslan, M & Aras, N M 2007, Structure and reproductive characteristics of two brown trout (*Salmo trutta*) populations in the Çoruh River Basin, North-Eastern Anatolia, Turkey. *Turkish Journal of Zoology*, 31: 185-192.
- Billard, R 1983, A quantitative analysis of spermatogenesis in the trout, *Salmo trutta fario*. *Cell and Tissue Research*, 230: 495-502.
- Billard, R 1987, The reproductive cycle of male and female brown trout (*Salmo trutta fario*): A quantitative study. *Reproduction Nutrition Développement*, 27: 29-44.
- Birnie-Gauvin, K, Bordeleau, X, Cooke, S J, Davidsen, J G, Eldøy, S H, Eliason, E J, Moore, A & Aarestrup, K 2021, Life-history strategies in salmonids: The role of physiology and its consequences. *Biological Reviews*, 96: 2304-2320.
- Brown, R S 1994, Spawning and overwintering movements and habitat use by cutthroat trout (*Oncorhynchus clarki*) in the Ram River, Alberta. *Canadian Journal of Fisheries and Aquatic Sciences*, 52(5): 983-992, DOI: 10.1139/f95-097

- Caputo, V, Giovannotti, M & Splendiani, A 2010, Pattern of gonad maturation in a highly stocked population of brown trout (*Salmo trutta* L., 1758) from Central Italy. *Italian Journal of Zoology*, 77: 14-22.
- Chaves-Pozo, E, Mulero, V, Meseguer, J & García Ayala, A 2005, An overview of cell renewal in the testis throughout the reproductive cycle of a seasonal breeding teleost, the gilthead seabream (*Sparus aurata* L). *Biology of Reproduction*, 72: 593-601.
- Dodson, J J, Aubin-Horth, N, Thériault, V & Páez, D J 2013 The evolutionary ecology of alternative migratory tactics in salmonid fishes. *Biological Reviews*, 88: 602-625.
- Dziewulska, K & Domagala, J 2003 Histology of salmonid testes during maturation. *Reproduction Biology*, 3: 47-61.
- Er, A & Dik, B 2014 The effects of florfenicol on the values of serum tumor necrosis factor-and other biochemical markers in lipopolysaccharide-induced endotoxemia in brown trout. *Mediators of Inflammation*, 2014.
- Fatima, S, Adams, M & Wilkinson, R 2018, Seasonal variation in the profile of sex steroids and histological testicular development of brook trout (*Salvelinus fontinalis* Mitchell) during the annual reproductive cycle in Tasmania. *Australian Journal of Zoology*, 65: 313-318.
- Fleming, I A & Reynolds, J D 2004, Salmonid breeding systems. *Evolution illuminated: salmon and their relatives*. pp. 264-294.
- Fregeneda-Grandes, J M, Hernández-Navarro, S, Fernandez-Coppel, I A, Correa-Guimaraes, A, Ruíz-Potosme, N, Navas-Gracia, L M, Aller-Gancedo, J M, Martín-Gil, F J & Martín-Gil, J 2013 Seasonal and sex-related variations in serum steroid hormone levels in wild and farmed brown trout *Salmo trutta* L. in the north-west of Spain. *Journal of Water and Health*, 11: 720-728.
- GROSS, M R & SARGENT, R C 1985, The evolution of male and female parental care in fishes. *American Zoologist*, 25: 807-822.
- Hossain, M. & Yoshimatsu, T 2014, Dietary calcium requirement in fishes. *Aquaculture nutrition*, 20, 1-11.
- Liu, C, Yue, S, Solarz, J, Lee, J & Li, L 2021 Improving the sexual activity and reproduction of female zebrafish with high testosterone levels. *Scientific Reports*, 11, 3822.
- Lobón-Cervía, J & Sanz, N 2017, Brown trout: Biology, ecology and management, Wiley Online Library.
- Mlaćo, N, Katica, A & Pilić, S 2014, Comparative histology of testes of brown (*Salmo trutta* m. *fario*) and California (*Oncorhynchus mykiss*) trout during the spawning period. *Biotechnology in Animal Husbandry*, 30: 489-497.
- Muhlfeld, C C, Dauwalter, D C, D'angelo, V S, Ferguson, A, Giersch, J J, Impson, D, Koizumi, I, Kovach, R, McGinnity, P & Schöffmann, J 2019 Global status of trout and char: Conservation challenges in the twenty-first century. *Trout and char of the world*, pp. 717-760.
- Mukhopadhyay, S & Sinha, G M 1986 Annual cyclical changes in the testicular activity of an Indian freshwater major carp, *Labeo rohita* (Hamilton). *Gegenbaurs Morphologisches Jahrbuch*, 132: 303-321.
- Mylonas, C C, Sullivan, C V & Hinshaw, J M 1994, Thyroid hormones in brown trout (*Salmo trutta*) reproduction and early development. *Fish Physiology and Biochemistry*, 13: 485-493.
- Pavlov, E, Bush, A, Kostin, V & Pavlov, D 2020, Growth and early maturation of brown trout *Salmo trutta* in the Alatsoya River (Republic of Karelia). *Inland Water Biology*, 13: 640-647.
- Penney, Z L & Moffitt, C M 2014, Histological assessment of organs in sexually mature and post-spawning steelhead trout and insights into iteroparity. *Reviews in Fish Biology and Fisheries*, 24: 781-801.
- Rippere-Lampe, K E, Lang, M, Ceri, H, Olson, M, Lockman, H A & O'brien, A 2001 Cytotoxic necrotizing factor type 1-positive *Escherichia coli* causes increased inflammation and tissue damage to the prostate in a rat prostatitis model. *Infection and Immunity*, 69: 6515-6519.
- Salehi, A, Faraji, S, Shafaei, A, Kangarlou, K & Behzadi, A 2023, Potential of natural antioxidants for upregulating apoptosis in cervical cancer cells: A Cellular and Molecular Study. *Journal of Chemistry*, 2023.
- Salehi, A, Hosseini, S M & Kazemi, S 2022a, Antioxidant and anticarcinogenic potentials of propolis for dimethylhydrazine-induced colorectal cancer in Wistar rats. *BioMed Research International*, 2022.
- Salehi, A, Hosseini, S M & Kazemi, S 2025 Propolis ameliorates renal, liver, and pancreatic lesions in Wistar rats. *Biotechnology and Applied Biochemistry*. 72(2): 437-448. DOI: 10.1002/bab.2674.

- Salehi, A, Razavi, M & Vahedi Nouri, N 2022b, Seasonal prevalence of helminthic infections in the gastrointestinal tract of sheep in Mazandaran Province, Northern Iran. *Journal of Parasitology Research*, 2022.
- Santos, D, Rocha, E, Malhão, F, Lopes, C, Gonçalves, J F & Madureira, T V 2021, Multi-parametric portfolio to assess the fitness and gonadal maturation in four key reproductive phases of brown trout. *Animals*, 11: 1290.
- Saraniemi, M, Huusko, A & Tahkola, H 2008, Spawning migration and habitat, use of adfluvial brown trout, *Salmo trutta*, in a strongly seasonal Boreal River. *Boreal Environment Research*, 13: 121-132.
- Smialek, N, Pander, J & Geist, J 2021, Environmental threats and conservation implications for Atlantic salmon and brown trout during their critical freshwater phases of spawning, egg development and juvenile emergence. *Fisheries Management and Ecology*, 28: 437-467.
- Valaei, A, Azadeh, F, Mostafavi Niaki, S T, Salehi, A, Shakib Khoob, M, Mirebrahimi, S H O, Kazemi, S & Hosseini, S M 2022, Antioxidant and anticancer potentials of the olive and sesame mixture against dimethylhydrazine-induced colorectal cancer in Wistar rats. *BioMed Research International*, 2022: 5440773.