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TEMPORAL INFLUENCE OF SPAWNING ON SERUM BIOCHEMICAL PARAMETERS IN BROWN TROUT *SALMO TRUTTA* (TELEOSTEI: SALMONIDAE)

DAMIR SULJEVIĆ*; ANDI ALIJAGIĆ; ERNA ISLAMAGIĆ
University of Sarajevo, Department of Biology, Faculty of Science, Zmaja od Bosne 33-35, Sarajevo, Bosnia and Herzegovina

Abstract

Suljević, D., A. Alijagić and E. Islamagić, 2017. Temporal influence of spawning on serum biochemical parameters in brown trout *Salmo trutta* (Teleostei: Salmonidae). *Bulg. J. Agric. Sci.*, 23 (3): 485–490

In this study were analyzed biochemical parameters in a serum of brown trout (Salmo trutta) during and post spawning. For this purpose 25 fish in the spawning (Group I), 21 fish post-spawning (Group II) and 33 control fish (Group III) were used. Research has shown that during spawning significant reduction in growth occurs, as well as mass loss, particularly in females. Significant positive correlation between the total length and mass has been found. For all biochemical parameters between the three groups significant differences were found ($p<0.05$). We have not found significant differences in the values of serum glucose, cholesterol and chloride between Group I and II; however, protein, triglycerides, AST, sodium, potassium and calcium values were statistically significant ($p<0.05$). For glucose, AST and calcium values no significant differences between Group I and Group II were established. During spawning glucose, AST and calcium values were lower, while other biochemical parameters were increased.

Key words: brown trout; spawning; biochemical parameters; serum electrolytes; glucose
Abbreviations: AST – aspartate aminotransferase; cPG – carp pituitary gland; FOM – final oocyte maturation; FSH – follicle-stimulating hormone; GnRH – gonadotropin-releasing hormone; GPR54 – G protein-coupled receptor-54; GSI – gonadosomatic index; GTH – gonadotrophic hormones; hCG – human chorionic gonadotropin; KiSS – kisspeptin or metastin, protein; LH – luteinizing hormone; VLDL – very low-density lipoproteins; Vtg – vitellogenin

Introduction

Natural environmental stressors, anthropogenic pollution, and hatchery rearing practices may exert considerable impact on developing fish (Von Westernhagen et al., 1988). Spawning of captive broodstock has the negative impact on physiological responses in fish, including metabolism and reproductive development (Cooke, 2002). Also increasing the incidence of embryonic and/or larval malformations is the natural response to environmental stress and in most cases cannot be simply attributed to a single specific factor (Von Westernhagen et al., 1988). Discovery of the KiSS neurones and KiSS1/GPR54 system (de Roux et al., 2003; Seminara et al., 2003) improved understanding link mediating the response to environmental cues and metabolic signals to the reproductive axis (Tena-Sempere, 2006). Two KiSS genes in some fish are encoding two different kisspeptins, KiSS1 and KiSS2 (Kotani et al., 2001), which bind to a receptor.

The most common reproductive dysfunction in fish is the fail of the vitellogenic oocytes undergo to final oocyte maturation (FOM) (Zohar and Mylonas, 2001). Gonadal growth (i.e. sperm count) is reduced in stress especially in male (Takle et al., 2005). Additionally, dysfunctions as delayed ovulation and reduced survival of progeny may occur in female broodstock in fish (Bobe and Labbé, 2010; Mylonas et al., 2010). Migratory fish kept in captivity may exhibit gonads that do not reach the

**Corresponding author*: suljevic.damir@gmail.com

final maturation of oocytes, ovulation and spawning (Mylonas et al., 2010). These final stages can only be produced through hormone treatments with ovaprim, ovaplant, hCG, cPG in the order to increase gonadosomatic index (GSI) (Kucharczyk et al., 1997). Reproductive hormones as GnRHa has proven effective in stimulation of reproductive processes and induces ovulation, spermiation in mullet, Japanese eel, bream (Shehadeh et al., 1973; Miura et al., 1991; Kucharczyk et al., 1997) and in enhancing milt production in fish (Zohar and Mylonas, 2001).

The aim of this study was to analyse impact of spawning on the values of biochemical parameters in brown trout in comparison with values in consuming fish.

Materials and Methods

Sampling

In this study, 79 specimens of *Salmo trutta* (brown trout) were sampled from the local centre, Centre for Fisheries „Neretva – Konjic" at Boracko Lake (43°33'07"N, 18°01'50"E, 397 m a.s.l.) as it is shown in Figure 1. This Center was established with the aim of protecting, preserving and breeding younger endemic and other salmonid species living in the Neretva river (Eastern Herzegovina, Bosnia and Herzegovina), as well as for its restocking. All fish were divided into three groups. Group I (n = 25) consisted of fish bred for artificial spawning and all data were collected during spawning; Group II (n = 21) also consisted of fish bred for artificial spawning but all data were collected 5 months after spawning period; Group III (or control) (n = 33) consisted of a consumption fish reared for restocking. The analysis was performed in spawning period (January) and post-spawning (June). All analysed specimens were grown in separate pools for spawning. Group I and II were fed with Vitalis Repro and Group III with Ribox 46/22 (Bio-Mar, Denmark).

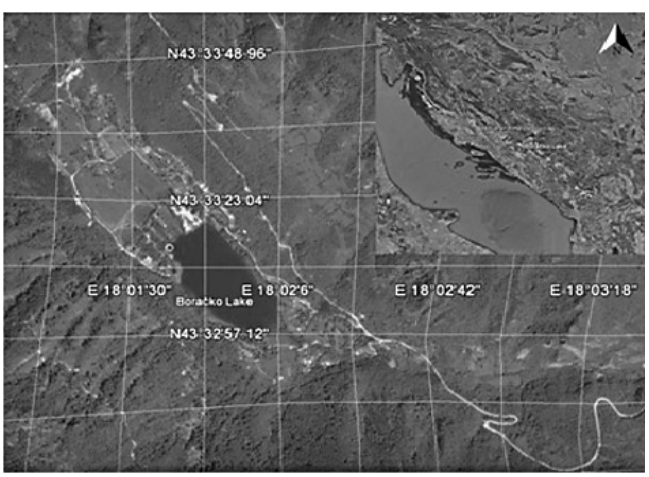

Fig. 1. Centre for Fisheries „Neretva – Konjic" at Boracko Lake

Experimental design

During the experiment, all fish were anaesthetized using Benzoak VET 200 (Vitusapotek, Netherlands) at a concentration of 15-20 ml/10 L for 15 minutes. After treatment, blood samples were collected, and the experimental fish were returned to the pool for recovery. Total length and mass were determined for all sampled fish. Blood samples (2 ml) were taken from the caudal vein without anticoagulant. The blood was centrifuged for 10 min at 2000 rpm in a portable centrifuge (Heraeus Sepatech Biofuge Model 1217 (Heraeus, Germany), serum was separated and stored at the adequate temperature in the portable refrigerator (LABFREEZ, China) and transported to the laboratory for biochemical analysis.

Biochemical analysis

Commercially available diagnostic kits were used for the estimation of biochemical parameters following the instructions of the manufacturer. Biochemical parameters such as concentration of glucose, total proteins, cholesterol, triglycerides and serum mineral (sodium, potassium, chlorides and calcium) and activity of aspartate aminotransferase were analyzed. Standardized methods and modern analyzers have been applied for the testing. Dimension RXL analyzer (Siemens Healthineers, USA) was used for estimation of glucose, cholesterol and triglycerides concentrations and activity of aspartate aminotransferase (AST). Minerals were determined by potentiometry with ion selective electrodes using Vitros DT 60 (Johnson & Johnson, USA), while proteins were analyzed by using a Spectronic 20 Genesys (Thermo Scientific, USA).

Statistical analysis

Data were analyzed with descriptive and analytical statistics using SPSS (Version 20.0, SPSS, Inc., Chicago, IL, USA). Data were presented as means ± 1 SD accompanied by range (minimum and maximum). Significant differences were analyzed using ANOVA, *post hoc* Tukey test, and data distribution by Kruskal-Wallis test. P-values lower than 0.05 ($p<0.05$) were considered as significant and values lower than 0.01 ($p<0.01$) as highly significant.

Results

The results of the morphometric parameters analysis for observed species *Salmo trutta* (Group I – spawning, Group II – post spawning and Group III – control, consumer fish) including mean values, standard deviation and range for 79 specimens are presented in Table 1. Analysis of variance (ANOVA) and *post hoc* analysis (Tukey test) were also performed to establish differences between groups.

Table 1
Morphometric parameters of brown trout (*Salmo trutta*)

Groups	Sex	Total lenght (cm)		Mass (g)	
		Mean	Range	Mean	Range
Group I	♀♂ (n=25)	38.92±5.55a	31-47	663.4±290.5a	320-1260
	male (n=12)	41.43±5.47a	35-47	863.3±283.4a	420-1260
	female (n=13)	35.40±3.65a	31-41	478.9±135.4a	320-780
Group II	♀♂ (n=21)	43.17±5.36b	35.5-54.5	885.8±340.2b	438-1743
	male (11)	47.87±3.46b	43-54,5	1160.7±268b	867.2-1743.6
	female (n=10)	38.9±2.15a	35.5-44	635.96±149.8b	438.2-932.2
Group III	♀♂ (n=33)	20.53±0.95c	18-22.5	83.54 ±10.49a	62-108,3
	male (n=14)	20.89±0.82a	20-22	83.96±9.57a	67.5-101
	female (n=19)	20.22±0.96b	18-22.5	83.23±11.65a	62-108.3

Note: a,b,c – different combination of letters shows significant differences ($p<0.05$) between groups

Fish from Group III had the lowest values of total length and mass, with very similar values in males and females. After spawning, we noticed significant increase in total length and body mass and especially for body mass in males, which increased from 863 to 1160 g. Among all three groups, significant differences were obtained for total length and body mass. These differences were mainly due to the presence of very variable morphometric parameters of the fish in post-spawning (for body mass) and in spawning group (for total length) (Table 2).

Table 2
Spearman's rho correlation between total lenght and mass of *Salmo trutta*

Groups	Sex	Spearman's rho correlation	
		R	p-values
Group I	♀♂	.418*	.038
	male	.424.	.170
	female	.615*	.025
Group II	♀♂	.621**	.003
	male	.875**	.001
	female	-.150	.659
Group III	♀♂	.100	.582
	male	-0.18	.952
	female	.130	.596

*The mean diffrerence is significant at the 0.05 level and ** at the 0.01 level

Correlation between total length and the body mass was positive, except for females in post-spawning period (Group II) and the consumer male fish (Group III).

Values of observed biochemical parameters of *Salmo trutta* (spawning, post spawning and consumer groups) including mean values ± 1SD are presented in Table 3.

Values of glucose and potassium concentration as well as activity of aspartate aminotransferase (AST) had an increasing tendency in post-spawning period, while concentrations of proteins, cholesterol, triglycerides, sodium, chloride and calcium had lower values in post-spawning period. Valus of biochemical parameters in Group III (consume fish, control group) were compared with the values of fish after spawning (Group II), assuming that the period of five months will be enough for the establishment of values that preceded spawning period. However, in the post spawn period, the fish had higher concentration of total proteins, sodium, chloride, potassium and calcium, and lower glucose, cholesterol and triglycerides concentrations as well as activity of AST (Table 4).

Table 3
Values of biochemical parameters of brown trout (*Salmo truta*)

	Mean	Glucose (mmol/l)	Proteins (g/l)	Cholesterol (mmol/l)	Triglyceride (mmol/l)	AST (UI)	Na^+ (mmol/l)	Cl^- (mmol/l)	K^+ (mmol/l)	Ca^{++} (mmol/l)
	♀♂	5.99±0.92	49.27±10.85	5.93±2.26	3.00±1.71	203.88±126.55	181.40±17.8	164.72±21.48	1.60±0.89	3.44±0.77
Group I	Male	6.01±1.09	52.59±6.08	5.03±1.44	2.33±1.33	227.17±155.97	185.67±20.54	152.83±23.23	1.90±0.81	3.39±0.95
	Female	5.97±0.77	46.21±13.44	6.76±2.60	3.61±1.84	182.38±93.22	177.46±14.58	175.69±12.51	1.60±0.76	3.49±0.60
	♀♂	6.75±1.91	36.77±7.16	5.57±1.78	1.63±0.65	328.67±144.84	158.71±14.49	161.48±30.84	2.31±0.88	2.92±0.32
Group II	Male	6.72±1.93	36.06±5.99	5.93±1.14	1.68±0.73	353.15±160.76	162.10±12.73	171.60±32.05	2.42±0.79	2.86±0.33
	Female	6.77±1.99	37.42±8.33	5.25±2.22	1.58±0.61	306.09±132.36	155.64±15.88	152.27±27.97	2.21±0.98	2.98±0.31
	♀♂	7.52±1.50	32.57±2.98	7.14±1.12	5.73±2.63	334.27±92.38	150.79±5.76	126.09±5.13	1.44±0.49	2.87±0.17
Group III	Male	7.83±1.61	31.41±2.67	6.60±0.73	5.23±2.60	331.93±113.04	150.36±4.47	125.86±4.05	1.62±0.58	2.84±0.14
	Female	7.29±1.42	33.43±2.97	7.54±1.20	6.09±2.66	336.00±77.04	151.11±6.67	126.26±5.91	1.31±0.36	2.89±0.19

Table 4
Analysis of variance (ANOVA) and normal distribution (Kruskal-Wallis test)

Parameters	ANOVA			Kruskal-Wallis test	
	Group I i II	Group II i III	Group I,II,III	Chi-Square	Asymp. Sig.
Glucose	.112	.091	.001*	13.99	.001*
Proteins	.001*	.000*	.000*	31.67	.000*
Cholesterol	.574	.000*	.003*	11.61	.003*
Triglycerides	.000*	.000*	.000*	40.59	.000*
AST	.000*	.762	.000*	24.72	.000*
Na+	.000*	.000*	.000*	42.63	.000*
Cl-	.572	.000*	.001*	39.12	.000*
K+	.004*	.000*	.000*	16.11	.000*
Ca++	.000*	.381	.000*	20.71	.000*

After spawning, significant differences were established for the most of the observed biochemical parameters, except for concentration of glucose, cholesterol and chloride. Comparison of biochemical parameters between Group II and Group III (post-spawn and control group) have shown significant differences for all parameters, which was also noticed when we combined all three groups together. Kruskal-Wallis test showed statistically significant deviation from the normal distribution when we compared all three groups ($p<0.01$).

Discussion

Hematological and biochemical parameters reflect the physiological condition of the fish and are important for the adequate monitoring, particularly during artificial fish breeding. Various environmental negative factors have impact on reduced fish reproduction in natural habitats, so artificial breeding became common. The amount of dissolved oxygen, water temperature, osmotic imbalance cause stress reactions which prevent maturation in ovaries (Rottman et al., 1991). Many studies focused on the analysis of stress reactions during spawning period by monitoring biochemical parameters like glucose and cortisol (Demas et al., 1978). Analysis of the fish morphometric parameters during and after spawning showed increasement in their size (total length and weight). During spawning almost all energy is used to the development of secondary sex characteristics, development of gametes and special sexual behaviour patterns (Brett, 1995; Pinson, 2005; Wiegand et al., 2007). This is the reason why the breeding flocks of fish are much bigger during post-spawning period. Males were generally larger than females due to demanding reproductive process in females during spawning, and this fact was confirmed by the negative Pearson correlation between the total length and body weight in females.

The values of cholesterol and triglyceride concentrations were high during the spawning and reduced later, while in the consume fish values of cholesterol and triglyceride concentrations were very high. Lipid concentration values were high during spawning, because of several reasons. Lipids are the major source of metabolic energy in the reproductive cycle and oocyte development. They originate from tissue deposits, from the diet intake or lipids can be synthesized *de novo* in the ovarian follicles (Wiegand, 1996). A large percentage of total lipids is catabolized in the energy necessary for the reproductive cycle, while the other energy molecules such as a protein vitellogenin (Vtg) or very low-density lipoproteins (VLDL) are stored as yolk reserve (Sargent, 1995). Many lipid metabolic pathways are important during spawning and their lack can significantly change fish production rate (Norton and Macfarlane, 1999). Otherwise, changes of various lipids in gonads, muscles and liver of adult fish are directly related to a process of sexual maturation and spawning (Huynh et al., 2007). Thus, during the ovarian development in fish, components essential for the viability of eggs and survival of the larvae are incorporated in oocytes. Large amount of lipid is stored in the muscles prior to spawning, however during spawning more lipids are mobilised to the liver and at the end of spawning to the ovaries (Rainuzzo et al., 1997).

Major variations in blood glucose concentrations during and after spawning were not noticed. Consuming fish had evident highest glucose concentration due to larger energy requirements, overcrowding of pools and body size. Normal glucose concentrations indicate that stress reaction is absent and that glucose (stored in form of glycogen) is not a significant source of energy during spawning. Glucose concentration depends on carbohydrates in diet and lipid concentrations which are primarily used as the energy source during spawning. Interestingly, in Teleostean serum glucose is not associated with the concentration of glycogen in the liver (Love, 1980) and is not associated with reproduction (Hutton, 1968). Plasma glucose concentration in female tench is significantly higher during spawning if it is compared to the concentration two months before spawning (Svoboda et al., 2000). These high plasma glucose concentrations may be a consequence of the gonade development (Robertson et al., 1961; UIM et al., 1988). Low carbohydrate levels of in the organs and during different seasons are the reason why carbohydrates are not main energy resource in the fish body (Mathana et al., 2012). However, obtained glucose values in tench are higher in comparison to the other fish species and are very similar to glucose values during induced stress period (Suljević et al., 2015).

We noticed that protein concentration was higher during spawning and decreased after spawning, while the lowest values were observed in the control group. High protein level during spawning could be the result of high-protein diet (Bhuyan et al., 2003). Luskova (Luskova, 1997; Luskova, 1998) studied total protein level throughout the year and found intersex differences but only in grayling, while in brown trout and chub there were no evident intersex concentration differences. Protein levels in muscle are higher during the spawning season, and there is no difference in prespawning period and postspawning period, while protein concentrations in the liver and the kidney are equal (Mathana et al., 2012). Studies also showed a seasonal variation of protein level, but only in the muscles of the species *Lethrinus lentjan*. Low protein levels after spawning may be the result of seasonal and physiological needs such as maturation cycle and the depletion of the gonads (Azam, 2004; Boran and Karaçam, 2011).

In our study values of AST activity were low during spawning and increase after spawning period. Changes in enzyme activity during artificial insemination of common carp were not evident (Svoboda et al., 2000). But even so, very small number of references is available regarding this topic.

The values of sodium and chloride concentrations remain similar during spawning and after spawning period. Potassium concentrations were low and, otherwise, calcium concentrations were higher during spawning. Values of the electrolyte concentration in brown trout were much higher in comparison to tench (Suljević et al., 2015). During spawning increasement of calcium concentration can be considered as the adaptation at the cellular level: ions of calcium as a secondary messenger activates metabolically complex processes in the cell, while potassium ions trigger the parasympathetic system, which has an impact on cardiac activity. In *Salmo salar* (Stoss et al., 1983) sperm motility is regulated by the external concentration of potassium ions, which is usually lower than potassium concentration in the seminal plasma, as previously described in rainbow trout. Average electrolyte concentration in common carp is very stable during and after spawning (Svoboda et al., 2000).

Conclusion

Biochemical properties of blood and spawning can give very precise and significant results about the health of fish during reproduction and serve as base for future research and improvement of certain living conditions for more productive spawning and rearing. Spawning fish consume less food, and an additional loss of metabolic energy may have negative impact on production of gamets, which potentially could result in reduced reproductive fitness. Therefore, biochemical analyses of blood give important information regarding the health of fish in hatcheries. Our research has shown a strong correlation between metabolism and reproductive strategies of brown trout. Furthermore, this research points out a very important aspect of diet in fish when preparing for spawning. Fish could use carbohydrates for metabolic needs, however lipids are the most important source of energy for the different stages of maturation, and finally, proteins are active participants in the maturation phase.

References

Aroua, S., F. A. Weltzien, N. Le Belle and S. Dufour, 2007. Development of real-time RTPCR assays for eel gonadotropins and their application to the comparison of in vivo and in vitro effects of sex steroids. *General and Comparative Endocrinology,* **153**: 333-343.

Azam, K., M. Y. Ali, M. Asaduzzaman, M. Z. Basher and M. M. Hossain, 2004. Biochemical assessment of selected fresh fish. *Journal of Biological Sciences*, **4** (1): 9-10.

Bhuyan, H. R., M. B. Chowdhury, K. K. Nath, P. Seal and M. A. Hag, 2003. Studies on the biochemical parameters of Cynoglossids in the Kutuboha Channel, Bangladesh. *Bangladesh Journal of Science and Industrial Research,* **38**: 91-96.

Bobe, J. and C. Labbé, 2010. Egg and sperm quality in fish. *General and Comparative Endocrinology,* **165**: 535-548.

Boran, G. and H. Karaçam, 2011. Seasonal changes in proximate composition of some fish species from the Black Sea. *Turkish Journal of Fisheries and Aquatic Sciences*, **11**: 1-5.

Brett, J. R., 1995. Energetics. In: C. Groot, C. Margolis and W. C. Clarke (Eds.). Physiological Ecology of Pacific Salmon. *University of British Columbia Press*, Vancouver, pp. 1-66.

Cooke, S. J., J. F. Schreer, D. H. Wahl and D. P. Philipp, 2002. Physiological impacts of catch-and-release angling practices on largemouth bass and smallmouth bass. In: D. P. Philipp and M. S. Ridgway (Eds), Black Bass: Ecology, Conservation, and Management. *American Fisheries Society,* Bethesda, MD, USA, pp. 489-512.

Demael, A. and D. Garin, 1978. Effects of thermal stress on some metabolic parameters of tench living in different oxygenated waters. *Cah. Lab. Hydrobiol*., **7**: 15-26.

de Roux, N., E. Genin, J. C. Carel, F. Matsuda, J. L. Chaussain and E. Milgrom, 2003. Hypogonadotropic hypogonadism due to loss of function of the KiSS1-derived peptide receptor GPR54. *Proceedings of the National Academy of Sciences,* **100:** 10972-10976.

Hutton, K. E., 1968. Characteristics of the blood of adult pink salmon at three stages of maturity. *Fishery Bulletin of the Fish and Wildlife Service*, **66**: 195-202.

Huynh, M. D., D. D. Kitts, C. Hu and A. W. Trites, 2007. Comparison of fatty acid profiles of spawning and non-spawning Pacific herring, *Clupea Harengus Pallasi*. *Comparative Bio-*

chemistry and Physiology – Part B: Biochemistry & Molecular Biology, **146**: 504-511.

Kotani, M., M. Detheux, A. Vandenbogaerde, D. Communi, J. M. Vanderwinden, E. Le Poul, S. Brezillon, R. Tyldesley, N. Suarez-Huerta, F. Vandeput, C. Blanpain, S. N. Schiffmann, G. Vassart and M. Parmentier, 2001. The metastasis suppressor gene KiSS-1 encodes kisspeptins, the natural ligands of the orphan G proteincoupled receptor GPR54. *The Journal of Biological Chemistry*, **276**: 34631-34636.

Kucharczyk, D., R. Kuzawa, M. Luczynski, J. Glogawski, I. Babiak and E. Wyszomirska, 1997. Induced spawning in bream, Abramis brama (L), using carp and bream pituitary extract and hCG. *Aquaculture Research,* **28**: 139-144.

Love, R. M., 1981. The Chemical Biology of Fishes. *Academic Press,* London, 943 pp.

Lusková, V., 1997. Influence of spawning on enzyme activity in the blood plasma of fish. *Polskie Archiwum Hydrobiologii,* **44**: 57-66.

Lusková, V., 1998. Factors affecting haematological indices in free-living fish populations. *Acta Veterinaria Brno*, **67:** 249-255.

Mathana, P., S. T. Raj, C. R. K. Nair and T. Selvamohan, 2012. Seasonal changes in the biochemical composition of four different tissues of red spotted emperor *Lethrinus lentjan* (Family: Lethrinidae). *Annals of Biological Research,* **3** (11): 5078-5082.

Miura, T., K. Yamauchi, Y. Nagahama and H. Takahashi, 1991. Induction of spermatogenesis in male Japanese eel, *Anguilla japonica*, by a single injection of human chorionic gonadotropin. *Zoological Science*, **8**: 63-73.

Mylonas, C. C., A. Fostier and S. Zanuy, 2010. Broodstock management and hormonal manipulations of fish reproduction. *General and Comparative Endocrinology*, **165**: 516-534.

Norton, E. C. and R. B. MacFarlane, 1999. Lipid class composition of the viviparous yellowtail rockfish over a reproductive cycle. *Journal of Fish Biology,* **54:** 1287-1299.

Pinson, A. M., 2005. Energy use, migration time and spawning success of adult Chinook salmon returning to the South Fork of the Salmon River in Central Idaho. Master's Thesis. *University of Idaho:* Moscow, Idaho, pp. 25-27.

Rainuzzo, J. R., K. I. Reitan and Y. Olsen, 1997. The significance of lipids at early stages of marine fish: a review. *Aquaculture,* **155:** 103-115.

Robertson, O. H., N. A. Krupp, C. B. Favour, S. Hane and S. F. Thomas, 1961. Physiological changes occurring in the blood of the pacific salmon (*Oncorhynchus tshawytscha*) accompanying sexual maturation and spawning. *Endocrinology*, **68**: 325-337.

Rottmann, R. W., J. V. Shireman and F. A. Chapman, 1991. Introduction to hormone-induced spawning of fish. *SRAC Publication*, 421 pp.

Sargent, J. R., 1995. Origins and functions of lipids in fish eggs: nutritional implications. In: N. R Bromage and R. R Roberts (Eds), Broodstock Management and Egg and Larval Quality. *Blackwell Science*, Oxford, pp. 353-372.

Seminara, S. B., S. Messager, E. E. Chatzidaki, R. R. Thresher, J. S. Acierno, J. K. Shagoury, Y. Bo-Abbas, W. Kuohung, K. M. Schwinof, A. G. Hendrick, D. Zahn, J. Dixon, U. B. Kaiser, S. A. Slaugenhaupt, J. F. Gusella, S. O'Rahilly, M. B. Carlton, W. F Crowley, S. A. Aparicio and W. H. Colledge, 2003. The GPR54 gene as a regulator of puberty. *New England Journal of Medicine,* **349:** 1614-1627.

Shehadeh, Z. H., W. D. Madden and T. P. Dohl, 1973. The effects of exogenous hormone treatment on spermiation and vitellogenesis in the grey mullet, *Mulgil cephalus*. *Journal of Fish Biology,* **5:** 479-487.

Stoss, J. and T. Reftsie, 1983. Short term storage and cryopreservation of milt from Atlantic salmon and sea trout. *Aquaculture,* **30:** 229-236.

Suljević, D., E. Islamagić and M. Fočak, 2015. The effects of high temperature level on electrolytes and glucose concentration in tench (*Tinca tinca* Linnaeus, 1758) serum. *Veterinaria,* **64** (2): 60-64.

Suzuki, K., Y. Nagahama and H. Kawauchi, 1988. Steroidogenic activities of two distinct salmon gonadotropins. *General and Comparative Endocrinology,* **71:** 452-458.

Svoboda, M., J. Kouril, J. Hamačkova, P. Kalab, L. Savina, Z. Svobodova and B. Vyukusova, 2000. Biochemical profile of blood plasma of tench (*Tinca tinca* L.) during pre- and post-spawning period. *Acta Veterinaria Brno,* **70:** 259-268.

Swanson, P., 1991. Salmon gonadotropins: reconciling old and new ideas. In: A. P. Scott, J. P. Sumpter, D. E. Kime and M. S. Rolfe (Eds). Reproductive Physiology of Fish. FishSymp, Sheffield, pp. 2-7.

Takle, H., G. Baeverfjord, M. Lunde, K. Kolstad and Ø. Andersen, 2005. The effect of heat and cold exposure on HSP70 expression and development of deformities during embryogenesis of Atlantic salmon (*Salmo salar*). *Aquaculture,* **249:** 515-524.

Tena-Sempere, M., 2006. KiSS-1 and reproduction: focus on its role in the metabolic regulation of fertility. *Neuroendocrinology,* **83**: 275-281.

Von Westernhagen, H., W. S. Hoar and D. J. Randall, 1988. Sublethal effects of pollutants on fish eggs and larvae. *Fish Physiology,* **11:** 253-346.

Wiegand, M. D., 1996. Composition, accumulation and utilization of yolk lipids inteleost fish. *Reviews in Fish Biology and Fisheries*, **6**: 259-286.

Wiegand, M. D., T. A. Johnston, W. C. Leggett, K. E. Watchorn, A. J. Ballevona, L. R. Porteous and J. M. Casselman, 2007. Contrasting strategies of ova lipid provisioning in relation to maternal characteristics in three walleye (*Sander vitreus*) populations. *Canadian Journal of Fisheries and Aquatic Sciences,* **64:** 700-712.

Zohar, Y. and C. C. Mylonas, 2001. Endocrine manipulations of spawning in cultured fish: from hormones to genes. *Aquaculture*, **197**: 99-136.

Zuim, S. M. F., A. A. M. Rosa and N. Castagnolli, 1988. Sex and sexual cycle influences over metabolic parameters in pacu *Piaractus mesopotamicus* (Holmberg, 1887). In: Proceeding of Aquaculture International Congress, Vancouver, 74 pp.

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