The *in vitro* effect of cypermethrin on quality and oxidative stress indices of rainbow trout oncorhynchus mykiss spermatozoa

Filiz Kutluyer, Fulya Benzer, Mine Erişir, Fatih Öğretmen, Burak Evren İnanan

PII: S0048-3575(15)30041-9
DOI: doi: 10.1016/j.pestbp.2015.10.001
Reference: YPEST 3872

To appear in:

Received date: 8 July 2015
Revised date: 3 October 2015
Accepted date: 5 October 2015

Please cite this article as: Filiz Kutluyer, Fulya Benzer, Mine Erişir, Fatih Öğretmen, Burak Evren İnanan, The *in vitro* effect of cypermethrin on quality and oxidative stress indices of rainbow trout oncorhynchus mykiss spermatozoa, (2015), doi: 10.1016/j.pestbp.2015.10.001

The *in vitro* effect of Cypermethrin on quality and oxidative stress indices of rainbow trout *Oncorhynchus mykiss* spermatozoa

Filiz KUTLUYER[a]†, Fulya BENZER[b,#], Mine ERİŞİR[c,#], Fatih ÖĞRETMEN[d,#], Burak Evren İNANAN[d,#]

[a]Tunceli University, Fisheries Faculty, 62000, Tunceli, Turkey.

[b]Tunceli University, Faculty of Engineering, Department of Food Engineering, 62000, Tunceli, Turkey.

[c]Fırat University, Faculty of Veterinary, Department of Basic Sciences, Elazığ, Turkey.

[d]Muğla Sıtkı Koçman University, Faculty of Science, Department of Bology, 48000, Muğla, Turkey.

Running tittle: The *in vitro* effect of Cypermethrin

†Corresponding author e-mail: filizkutluyer@hotmail.com, Tel: +90 0 554 407 80 65, Fax: +90 0 428 213 18 61.

Abstract

There is limited information on the scientific literature about effect of *in vitro* exposure of fish sperm to pesticides. *In vitro* effect of cypermethrin on sperm quality and oxidative stress has not yet been fully investigated. Therefore, the effects of cypermethrin, a type II pyrethroid insecticide, on quality and oxidative stress of spermatozoa were examined *in vitro*. To explore the potential *in vitro* toxicity of cypermethrin, fish spermatozoa were incubated with different concentrations of cypermethrin (1.025, 2.05 and 4.1 µg/l) for 2h. The motility rate and duration of sperm were determined after exposure to cypermethrin. Reduced glutathione (GSH), glutathione peroxidase (GSH-Px), catalase (CAT) and malondialdehyde (MDA) in spermatozoa were analyzed for determination of oxidant and antioxidant balance. Our results indicated that spermatozoa motility and duration significantly decreased with exposure to cypermethrin. Additionally, activity of GSH-Px ($p<0.05$) and MDA and GSH levels increased in a concentration-dependent manner while CAT activity decreased ($p<0.05$). Consequently, the oxidant and antioxidant status and sperm quality were affected by quantitative changes different concentrations of cypermethrin.

Key words: Cypermethrin, rainbow trout, *Oncorhynchus mykiss*, spermatozoa, oxidative stress indices, sperm quality.

1. Introduction

Synthetic pyrethroid pesticides have received great attention due to usage as widespread and indiscriminate in agriculture, forestry, horticulture and homes in the world [1-3]. Increase in the worldwide annual consumption rate of synthetic pyrethroid pesticides is owing to their low effect of toxicity on mammals and birds and limited soil persistence [4]. Cypermethrin, as a type II pyrethroid insecticide, is a broadly used to control *Lepidoptera* and *Coleoptera* in citrus, moth pests of cotton, fruits, garpes and vegetable crops [3, 5], pest control in soybean culture [6, 7]. It has also been adopted for prevent and treat ticks, lice and scab on sheep and as a treatment against infestation by the parasitic sea louse *Lepeophtheirus salmonis* in intensive salmonid aquaculture [8]. Cypermethrin is very highly toxic to fish and aquatic arthropods (in laboratory tests 96 h LC50 were generally within the range of 0.4–2.8 μg/l and aquatic invertebrates LC50 in the range of 0.01–5 μg/l) [3, 7, 9-11]. Residues of cypermethrin in water and sediment samples from streams and rivers draining major agricultural districts are affected to fish inhabiting these areas due to discharged into waters of the majority of pollutants [12-13].

Sperm quality is the most important due to affect fertilization success, hatching of embryos and survival of embryos, larvae and adults [14-16]. Sperm cells in most fish species release into water and directly exposed to pollutants prior to fertilization [15, 17]. Therefore, determination of the adverse effects of environmental pesticides on the sperm quality is essential in fish with external fertilization [13]. Reactive oxygen species (ROS) generate in consequence of expose of spermatozoa to contaminants due to contain highly polyunsaturated fatty acids (PUFA) in their membranes and a lack of protective cytoplasmic enzymes [15]. Production and increased levels of ROS in fish negatively affect sperm motion parameters and accelerate the process of germ cell apoptosis due to lipid peroxidation [13, 15, 18-21]. Due to these reasons, studies have recently focused on effects of toxicants on sperm quality as bio-

indicator of aquatic pollution in different fish species (*Salmo trutta fario*, *Cyprinus carpio*, *Acipenser ruthenus*, *Heteropneustes fossilis*, *Rutilus frisii kutum*) [4, 13, 15, 21, 22-26]. To best our knowledge, effect of toxicants on rainbow trout (*Oncorhynchus mykiss*) reproduction through ROS induction and sperm quality has not been documented thus far. In this context, the aim of this study was to investigate how spermatozoa physiology is affected by short-term (2 h) *in vitro* exposure of rainbow trout (*O. mykiss*) sperm to cypermethrin, by analyzing oxidative stress indices (lipid peroxidation–MDA), and antioxidant enzyme (Reduced glutathione-GSH, glutathione peroxidase-GSH-Px and catalase-CAT) activity of rainbow trout sperm, as well as spermatozoa motility and viability.

2. Material and Methods

2.1. Broodstock handling and collection of gametes

Rainbow trout sperm was obtained from six males (2-3 years old; 36.6 ± 2.23 cm, 666.42 ± 129.15 g, as mean±SD) reared in the fish farm BUTAŞ Trout Production Facility (Muğla, Turkey) between November and January. The males were anesthetized with 2-phenoxyethanol (0.6 ml L^{-1}) before stripping. The sperm was collected by a gentle abdominal massage, collected into glass vials and stored on ice until use. Caution was exercised to prevent contamination of the semen with urine, feces, blood, mucus or water. Sperm samples with a motility rate ≤90% were excluded from the experiment and the percentage of motile sperm was checked using a light microscope with digital image processing software connected to the computer (Zeiss Axio Scope with AxioVision). Spermatozoa concentration of each of males was estimated microscopically at 200x using a Burker cell hemocytometer.

2.2. Sample preparation

Cypermethrin (technical grade, a-cyano-3 phenoxybenzyl-(IR,IS)cis,trans-3 2, 2-dichlorovinyl)-2,2-dimethylcyclopropanecarcoxylate; empirical formula: $C_{22}H_{19}C_{l2}NO_3$), was obtained from HEKTAŞ Insecticide Limited, Gebze, Kocaeli, Turkey and was dissolved in 96% ethanol and diluted with distilled water to obtain a stock solution of 0.1 g/l. Stock solutions were prepared daily. Analytical grade chemicals were obtained from Sigma Chemicals Co. (USA). Fish sperm from six individual males was pooled. The pooled sample was then diluted with an immobilization medium (NaCl, 103 mmol/l; KCl, 40 mmol/l; $CaCl_2$, 1 mmol/l; $MgSO_4$, 0.8 mmol/l; hepes, 20 mmol/l; pH 7.8) to obtain a sperm density of 6×10^8 cells ml^{-1}. The sperm sub-samples (n = 6) were then exposed for 2 h to final concentrations of 0 (control), 1.025, 2.05 and 4.1 µg/l of cypermethrin dissolved in ethanol. Each experimental condition was triplicated.

2.3. Sperm motility

After the sperm was *in vitro* exposed to the toxicant in the immobilization medium, sperm motility and duration was assessed following a two-step dilution. This was immediately followed by a second five-fold dilution in an activation medium (45 mM NaCl, 5 mM KCl, 30 mM Tris–HCl, pH 8.2) [27]. The percent of motile spermatozoa and motility duration was immediately recorded for 1 min post-activation using a CCD video camera mounted on a phase-contrast microscope (Zeiss Axio Scope with AxioVision) at room temperature (20°C). The percentage of sperm motility was estimated as the cell performing progressive forward movement, while the duration of motility was determined as the time until forward movement stops. Determining the percentage of sperm motility was assessed using an arbitrary scale with 10% interval increments in which non motile represents 0%.

2.4. Oxidative stress and antioxidant indices analyses

The sub-sperm sample was centrifuged at 3000×*g* at 4°C for 10 min and the pellet in an ice bath was homogenized using a Sonopuls HD 2070 ultrasonicator (Bandelin Electronic, Berlin, Germany) with the immobilization buffer. Lipid peroxidation in spermatozoa was measured by the thiobarbituric acid reacting substance (TBARS) method [28], and was expressed in terms of the MDA content, which served as standard of 1,1,3,3-tetraethoxypropane. The TBARS concentration was calculated by the absorption at 535 nm. The content of TBARS was expressed as nanomoles per 10^8 cells. Values were expressed as MDA equivalents in nmol / sperm cells. Glutathione peroxidase (GPx; EC 1.11.1.9) was assayed by the method of Matkovics et al. [29] and expressed as unite per g of protein ($U.g^{-1}$ protein) per 10^8 cells. GSH-Px activity was determined by using cumenehydroperoxide and reduced glutathione (GSH) as co-substrates and the loss of GSH following enzymic reaction at 37°C was measured spectrophotometrically with Ellman's reagent at 412 nm. Spermatozoa CAT activity were determined according to the method of Aebi [30] and expressed as $kat.g^{-1}$ protein per 10^8 cells. The decomposition of H_2O_2 can be directly followed by the decrease of absorbance at 240 nm. The difference in absorbance at 240 nm per time unit allows determining the CAT activity. Glutathione reductase (GR; EC 1.6.4.2) was assayed by the method of Chavan et al. [31] and expressed as $\mu mol.g^{-1}$ protein. The method is based on the capacity of sulfhydryl groups present in whole blood to react with 5, 5'-dithiobis-(2-nitrobenzoic acid) (Ellman's reagent) and form a yellow dye with maximum absorbance at 412 nm. The protein content in spermatozoa was measured by method of Lowry et al. [32].

2.5. Statistical assays

All values were expressed as mean±SD and analyzed by SPSS for Win 14.0 software. One-way ANOVA with Duncan test was used to determine whether results of treatments were significantly different from the control group ($p < 0.05$).

3. Results

3.1. Spermatozoa motility

In fresh semen, the percentage and duration of motile spermatozoa was 90±5.0% and 35±7.0 s, respectively. Sperm motility parameters (motility and duration) were determined after 2 h exposure to cypermethrin *in vitro* (Figure 1 and 2). Motility and duration were significantly lower than in the control compared to other concentrations of cypermethrin from 1.025 to 4 µg/l ($P < 0.05$), but did not significantly affect the sperm motility duration ($P > 0.05$).

3.2. Antioxidant responses

Levels of MDA, GSH, GSH-Px and CAT are shown in Figure 1. The results from the present study indicated that an increase in the concentration of cypermethrin caused a slightly increase in Malondialdehyde (MDA) and glutathione (GSH) levels in rainbow trout (*O. mykiss*) but not significant. GSH-Px activity in spermatozoa increased significantly ($P < 0.05$). CAT activity significantly decreased with increasing concentration of cypermethrin ($P < 0.05$).

4. Discussion

Data about the toxicity and effects of pesticides to non-target organisms are important in terms of the assessment of the ecotoxicological risks. Fish is one of non-target aquatic organisms [11]. In this regard, in this study, we examined a toxic effect of cypermethrin on the motility parameters and antioxidant responses in rainbow trout sperm. Overall, we demonstrated that increasing concentrations of cypermethrin decreased motility rate and duration. Additionally, it is suggested that exposure of spermatozoa to increasing cypermethrin concentrations affected the cellular response of rainbow trout (*O. mykiss*) spermatozoa. This may be due to destroy the functional integrity of the axosome and mitochondria of the sperm cells of high doses of cypermethrin or its toxic effect [33, 34].

Over the past 30 years, rainbow trout (Salmonidae) has been used as a alternative model organism in environmental researches due to low rearing costs, an early life-stage ultrasensitive bioassay, a well-described tumor pathology, sensitivity to many classes of carcinogen, responsiveness to tumor promoters and inhibitors, and a mechanistically informative non-mammalian comparative status [35-41]. Due to these reasons, present study was performed and this is the first *in vitro* to examine ROS production in rainbow trout sperm exposed to cypermethrin and relating this to oxidative damage. The results from the present study suggested that spermatozoa quality was impaired by cypermethrin-mediated oxidative stress in rainbow trout sperm. Spermatozoa motility and duration decreased significantly in all treatment groups compared to the control. The present results agree with previous studies about effects of toxicants on sperm quality of different fish species (*Salmo trutta fario*, *Cyprinus carpio*, *Acipenser ruthenus*, *Heteropneustes fossilis*, *Rutilus frisii kutum*) [4, 13, 15, 21, 22-26]. Decrease in motility and viability of spermatozoa may be due to highly permeable to low molecular weight substances [42], increase in the intracellular Ca^{2+} [43] and affect water channels or aquaporins of toxicants [4, 15, 45].

To the best of our knowledge, this is apparently the first report on effect of cypermethrin on rainbow trout (*O. mykiss*) sperm oxidative status, although studies have been conducted about effect of cypermethrin on oxidative status as bio-indicator of aquatic pollution in different tissues and erythrocytes of different species (*Channa punctata*, *Unio elongatulus eucirrus*) [3, 46]. In studies about effect of cypermethrin on oxidative status in tissues and erythrocytes, it was determined that cypermethrin exposure caused oxidative stress. Köprücü et al. [46] demonstrated that lipid oxidation, as measured by mamondialdehyde (MDA), significantly increased in the digestive glands and gills after freshwater mussel (*Unio elongatulus eucirrus*) were exposed to cypermethrin with decreased levels of reduced glutathione (GSH) and catalase (CAT) activity. Ansari et al. [3] reported an increase in GSH content of erythrocytes due to oxidative stress inducing cypermethrin with decrease in SOD activity. Nevertheless, only a few studies have been examined effect of pesticides on oxidative status of spermatozoa in different fish species (*Cyprinus carpio*, *Acipenser ruthenus*) [13, 21, 26]. In these studies, it was determined that ROS production in fish sperm exposed to pesticides caused oxidative damage and reproductive impairment. In a study on oxidative stress through exposure of spermatozoa to duroquinone in common carp (*Cyprinus carpio*), Zhou et al. [21] stated an increase in ROS production as a result of be metabolized by cytochrome P450 reductase, which use a variety of small and large molecules as substrates in enzymatic reactions, or the mitochondrial electron transfer chain in carp sperm. Linhartova et al. [26] determined an increase of lipid peroxidation, content of carbonyl proteins (CP) and SOD activity with increasing concentrations of duroquinone when sterlet (*Acipenser ruthenus*) spermatozoa exposed to duroquinone. Gazo et al. [13] found that TBARS levels, SOD activity and carbonyl proteins (CP) were greater in sterlet (*Acipenser ruthenus*) spermatozoa exposed Vinclozolin. In agreement with the studies noted above our results indicated that Malondialdehyde (MDA) as one of the oxidative damage products of

lipid peroxidation significantly increased. This might be associated with increased GSH and decreased CAT activities in spermatozoa after cypermethrin exposure. Decrease in catalase activity could be due to flux of superoxide radicals, which have been reported to inhibit CAT activity [47]. Reduced GSH is one of the most important antioxidant agents, protects cell membranes from lipid peroxidation and the main nonprotein thiol and one of the main reductants found in cells. Increasing of GSH may be due to protective role against oxidative stress-induced toxicity [48]. This oxidative damage in spermatozoa may be due to susceptibility of PUFAs in spermatozoa lipid peroxidation and cell dysfunction as a result of exposure cypermethrin [49].

5. Conclusion

In conclusion, based on the data obtained within the context of this study, rainbow trout spermatozoa are highly sensitive to the presence of lower concentrations of cypermethrin. The antioxidant system possesses a protective response to increased lipid oxidation for prevent cell damage. In addition, sub-lethal doses of cypermethrin significantly affected motility of sperm. According to our results, it is recommended that the use of fish sperm for *in vitro* assays may be practical, quick and cost effective for routine monitoring and toxicological assessment of chemicals compared to another non-invasive method, such as the use of blood cells and tissues. Further studies are necessary to evaluation the mechanisms and relationship between motility and oxidative stress in rainbow trout sperm as well as its consequences on fertilization.

Conflict of interest statement

None.

Acknowledgements

This work was funded by Muğla Sıtkı Koçman University. Authors like to express deep thanks to continuous help of Mehmet KUTLUYER for supply of pesticide standards, and giving valuable scientific background and important consultancies.

References

[1] S.M. Bradberry, S.A. Cage, A.T. Proudfoot, J.A. Vale, Poisoning due to pyrethroids, Toxicol. Rev. 24 (2005) 93–106.

[2] X. Shi, A. Gu, G. Ji, Y. Li, J. Di, J. Jin, F. Hu, Y. Long, Y. Xia, C. Lu, L. Song, S. Wang, X. Wang, Developmental toxicity of cypermethrin in embryo-larval stages of zebrafish, Chemosphere 85 (2011) 1010-1016.

[3] R.A. Ansari, S. Rahman, M. Kaur, S. Anjum, S. Raisuddin, *In vivo* cytogenetic and oxidative stress-inducing effects of cypermethrin in freshwater fish, *Channa punctata* Bloch, Ecotox. Environ. Safe. 74 (2011) 50–156.

[4] P.B. Singh, V. Singh, Bioaccumulation of hexachlorocyclohexane, dichlorodiphenyltrichloroethane, and estradiol-17b in catfish and carp during pre-monsoon season in India, Fish Physiol. Biochem. 34 (2008) 25–36.

[5] M.J. Crawford, A. Croucher, D.H. Hutson, Metabolism of cis- and trans-cypermethrin in rats. Balance and tissue retention study, J. Agric. Food Chem. 29 (1981) 130–135.

[6] P. Carriquiriborde, J. Diaz, H. Mugni, C. Bonetto, A.E. Ronco, Impact of cypermethrin on stream fish populations under field-use in biotech-soybean production, Chemosphere 68 (2007) 613–621.

[7] P. Carriquiriborde, J. Díaz, G.C. López, A.E. Ronco, G.M. Somoza, Effects of cypermethrin chronic exposure and water temperature on survival, growth, sex differentiation, and gonadal developmental stages of *Odontesthes bonariensis* (Teleostei), Chemosphere 76 (2009) 374–380.

[8] A. Moore, C.P. Waring, The effects of a synthetic pyrethroid pesticide on some aspects of reproduction in Atlantic salmon (*Salmo salar* L.), Aquat. Toxicol. 52 (2001) 1–12.

[9] R.R. Sthephanson, Aquatic toxicology of cypermethrin. I. Acute toxicity to some freshwater fish and invertebrates in laboratory tests, Aquat. Toxicol. 2 (1982) 175-185.

[10] B. Sarkar, A. Chatterjee, S. Adhikari, S. Ayyappan, Carbofuran- and cypermethrin-induced histo pathological alterations in the liver of *Labeo rohita* (Hamilton) and its recovery, J. Appl. Ichthyol. 21 (2005) 131-135.

[11] J. Velisek, T. Wlasow, P. Gomulka, Z. Svobodova, R. Dobsikova, L. Novotny, M. Dudzik, Effects of cyperhethrin on rainbow trout (*Oncorhynchus mykiss*), Vet. Med. 51(10) (2006) 469-476.

[12] D. Marino, A. Ronco, Cypermethrin and chlorpyrifos concentration levels in surface water bodies of the Pampa Ondulada, Argentina, Bull. Environ. Contam. Toxicol. 75 (2005) 820–826.

[13] I. Gazo, P. Linhartova, A. Shaliutina, M. Hulak, Influence of environmentally relevant concentrations of vinclozolin on quality, DNA integrity, and antioxidant responses of sterlet *Acipenser ruthenus* spermatozoa, Chem. Biol. Interact. 203 (2013) 377–385.

[14] D.W.T. Au, C.Y. Lee, K.L. Chan, R.S.S. Wu, Reproductive impairment of sea urchins upon chronic exposure to cadmium. Part I. Effects on gamete quality, Environ. Pollut. 111 (2001) 1–9.

[15] Z. Li, P. Li, B. Dzyuba, T. Randak, Influence of environmental related concentrations of heavy metals on motility parameters and antioxidant responses in sturgeon sperm, Chem. Biol. Interact. 188 (2010a) 473–477.

[16] Z.H. Li, P. Li, M. Rodina, T. Randak, Effect of human pharmaceutical Carbamazepine on the quality parameters and oxidative stress in common carp (*Cyprinus carpio* L.) spermatozoa, Chemosphere 80 (2010b) 530–534.

[17] M.J.G. Gage, C.P. Macfarlane, S. Yeates, R.G. Ward, J.B. Searle, G.A. Parker, Spermatozoal traits and sperm competition in Atlantic salmon: relative sperm velocity is the primary determinant of fertilization success, Curr. Biol. 14 (2004) 44–47.

[18] C.N. Ong, H.M. Shen, S.E. Chia, Biomarkers for male reproductive health hazards: are they available? Toxicol. Letters 134 (2002) 17–30.

[19] A. Agarwal, R.A. Saleh, M.A. Bedaiwy, Role of reactive oxygen species in the pathophysiology of human reproduction, Fertil. Steril. 79 (2003) 829–843.

[20] M.A. Baker, R.J. Aitken, The importance of redox regulated pathways in sperm cell biology, Mol. Cell. Endocrinol. 216 (2004) 47–54.

[21] B.S. Zhou, W.H. Liu, W.H.L. Siu, D. O'Toole, P.K.S. Lam, R.S.S. Wu, Exposure of spermatozoa to duroquinone may impair reproduction of the common carp (*Cyprinus carpio)* through oxidative stress, Aquat. Toxicol. 77 (2006) 136-142.

[22] E. Rurangwa, A. Biegniewsk, E. Slominska, E.F. Skorkowski, F. Ollevier, Effect of tributyltin on adenylate content and enzyme activities of teleost sperm: a biochemical approach to study the mechanisms of toxicant reduced spermatozoa motility, Comp. Biochem. Physiol. Part C 131 (2002) 335–344.

[23] G.J. Dietrich, M. Dietrich, R.K. Kowalski, S. Dobosz, H. Karol, W. Demianowicz, J. Glogowski, Exposure of rainbow trout milt to mercury and cadmium alters sperm motility parameters and reproductive success, Aquat. Toxicol. 97 (2007) 277–284.

[24] P.B. Singh, V. Sahu, V. Singh, S.K. Nigam, K. Hement, Sperm motility in the fishes of pesticide exposed and from polluted rivers of Gomti and Ganga of north India, Food Chem. Toxicol. 46 (2008) 3764–3769.

[25] F. Fadakar Masouleh, B. Mojazi Amiri, A.R. Mirvaghefi, M.A. Nemtollahi, *In vitro* Effects of diazinon on male reproductive tissue and sperm motility of Caspian Kutum (*Rutilus frisii kutum*), Res. J. Environ. Toxicol. 5 (2011) 108-116.

[26] P. Linhartova, I. Gazo, A. Shaliutina, M. Hulak, The *in vitro* effect of duroquinone on functional competence, genomic integrity, and oxidative stress indices of sterlet (*Acipenser ruthenus*) spermatozoa, Toxicol. Vitro 27 (2013) 1612–1619.

[27] F. Ögretmen, B.E. Inanan, M. Ozturk, Protective effects of propolis on cryopreservation of common carp (*Cyprinus carpio*) sperm, Cryobiology 68 (2014) 107-112.

[28] Z.A. Placer, L.L. Cushman, B.C. Johson, Estimation of product of lipid peroxidation (malonyldialdehyde) in biochemical systems, Anal. Biochem.16 (1966) 359–364.

[29] B. Matkovics, I. Szabo, I.S. Varga, Determination of enzyme activities in lipid peroxidation and glutathione pathways, Laboratoriumi Diagnosztika 15 (1988) 248–249.

[30] H. Aebi, Catalase, in: H.U. Bergmeyer (Eds.), Methods of Enzymatic Analysis. Academic press, London, 1984, pp. 671-684.

[31] S. Chavan, L. Sava, V. Saxena, S. Pillai, A. Sontakke, D. Ingole, Reduced Glutathione: Importance of specimen collection, J. Clin. Biochem. 20(1) (2005) 150-152.

[32] O.H. Lowry, N.J. Farr, A.L. Rosenbrough, R.J. Randall, Protein measurements with the folin phenol reagent, J. Biol. Chem. 193 (1951) 265-275.

[33] A. Atessahin, M.N. Bucak, P.B. Tuncer, M. Kizil, Effect of antioxidant additives on microscopic and oxidative parameters of Angora goat semen following the freeze-thawing process, Small Ruminant Res. 77 (2008) 38-44.

[34] K. Kledmanee, S. Taweedet, P. Thaijongruk, P. Chanapiwat, K. Kaeoket, Effect of L-cysteine on chilled carp (*Cyprinus carpio*) semen qualities, Thai J. Vet. Med. 43(1) (2013) 91-97.

[35] J.D. Hendricks, T.R. Meyers, D.W. Shelton, J.L. Casteel, G.S. Bailey, Hepatocarcinogenicity of benzo[a]pyrene to rainbow trout by dietary exposure and intraperitoneal injection, J. Natl. Cancer Inst. 74 (1985) 839–851.

[36] J.D. Hendricks, D.W. Shelton, P.M. Loveland, C.B. Pereira, G.S. Bailey, Carcinogenicity of dietary dimethylnitrosomorpholine, N-methyl-N′-nitro-Nnitrosoguanidine, and dibromoethane in rainbow trout, Toxicol. Pathol. 23 (1995) 447–457.

[37] J.D. Kelly, G.A. Orner, J.D. Hendricks, D.E. Williams, Dietary hydrogen peroxide enhances hepatocarcinogenesis in trout: correlation with 8-hydroxy-2′-deoxyguanosine levels in liver DNA, Carcinogenesis 13 (1992) 1639–1642.

[38] G.S. Bailey, D.E. Williams, J.D. Hendricks, Fish models for environmental carcinogenesis: the rainbow trout, Environ. Health Perspect. 104 (1996) 5–21.

[39] R.B. Walter, G.S. Timmins, S.C. Tilton, G.A. Orner, A.D. Benninghoff, G.S. Bailey, D.E. Williams, Carcinogenesis models: focus on Xiphophorous and rainbow trout, in: P.J. Walsh (Eds.), Oceans and Human Health: Risks and Remedies from the Seas. Elsevier, 2008, pp. 586–611.

[40] D.E. Williams, The rainbow trout liver cancer model: Response to environmental chemicals and studies on promotion and chemoprevention, Comp. Biochem. Physiol. C 155 (2012) 121-127.

[41] F. Kutluyer, E. Aksakal, Aquatic Model Organisms and Their Use in Biotechnology, Anadolu J. Agricult. Sci. 28(2) (2013) 101-107.

[42] F. Lahnsteiner, B. Berger, T. Weismann, Sperm metabolism of the teleost fishes *Chalcalburnus chalcoides* and *Oncorhynchus mykiss* and its relation to motility and viability, J. Exp. Zool. 284 (1999) 454–465.

[43] M.V. Rao, B. Gangadharan, Antioxidative potential of melatonin against mercury induced intoxication in spermatozoa *in vitro*, Toxicol. Vitro 22 (2008) 935–942.

[45] M. Kuwahara, Y. Gu, K. Ishibashi, F. Marumo, S. Sasaki, Mercury-sensitive residues and pore site in AQP3 water channel, Biochemistry 36 (1997) 13973–13978.

[46] K. Köprücü, S. Mişe Yonar, E. Şeker, Effects of cypermethrin on antioxidant status, oxidative stress biomarkers, behavior, and mortality in the freshwater mussel *Unio elongatulus eucirrus*, Fish. Sci. 76 (2010) 1007–1013.

[47] S. Pandey, I. Ahmad, S. Parvez, B. Bin-Hafeez, R. Haque, S. Raisuddin, Effect of endosulfan on antioxidants of freshwater fish *Channa punctatus* Block. I. Protection against lipid peroxidation in liver by copper pre-exposure, Arch. Environ. Contam. Toxicol. 41 (2001) 345–352.

[48] S. Parvez, S. Raisuddin, Pre-exposure to copper modulates non-enzymatic antioxidants in liver of *Channa punctata* (Bloch) exposed to the herbicide paraquat, Bull. Environ. Contam. Toxicol. 76 (2006) 545–551.

[49] D. Sanocka, M. Kurpisz, Reactive oxygen species and sperm cells, Reprod. Biol. Endocrinol. 2 (2004) 12.

Legends

Fig. 1. *In vitro* effects of cypermethrin on spermatozoa motility rate (%) in *Oncorhynchus mykiss*. Data are presented as means±SD. Superscript letters indicate significant differences among samples at the same time post-activation (ANOVA, $P < 0.05$).

Fig. 2. *In vitro* effects of cypermethrin on spermatozoa motility duration (s) in *Oncorhynchus mykiss*. Data are presented as means±SD. Superscript letters indicate significant differences among samples at the same time post-activation (ANOVA, $P < 0.05$).

Fig. 3. Effect of cypermethrin on (a) MDA, (b) GSH, (c) GSH-Px and (d) CAT of spermatozoa in *Oncorhynchus mykiss*. Data are presented as means±SD. Superscript letters indicate significant differences among samples at the same time post-activation (ANOVA, $P < 0.05$).

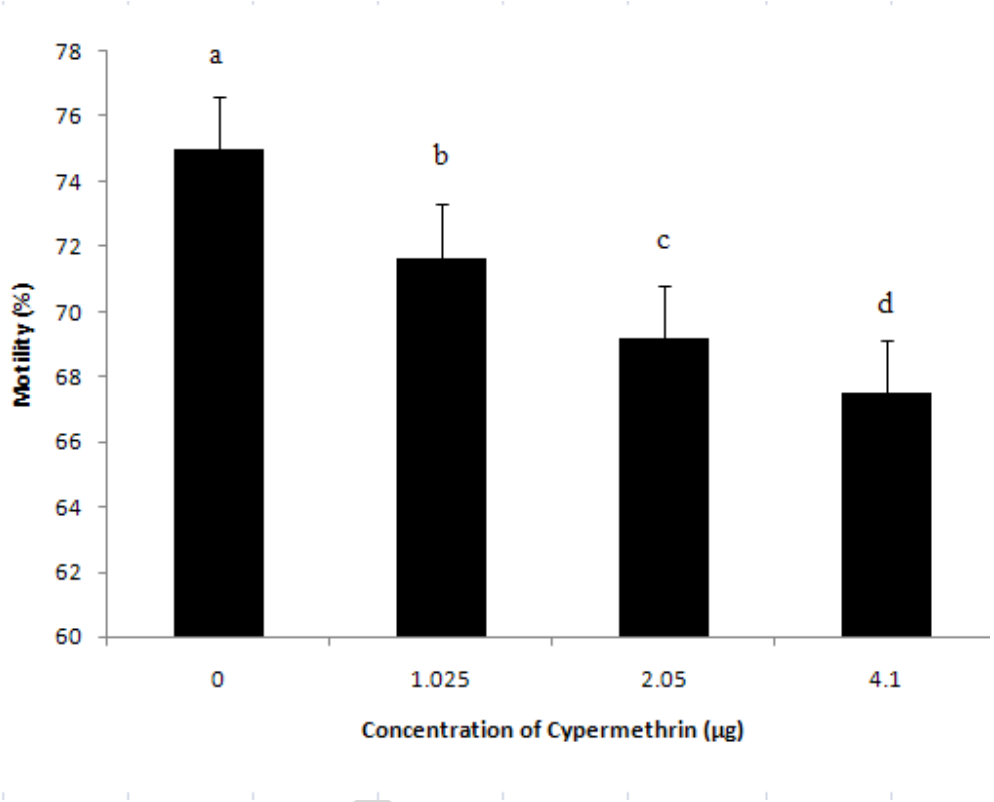

Fig. 1. *In vitro* effects of cypermethrin on spermatozoa motility rate (%) in *Oncorhynchus mykiss*. Data are presented as means±SD. Superscript letters indicate significant differences among samples at the same time post-activation (ANOVA, $P < 0.05$).

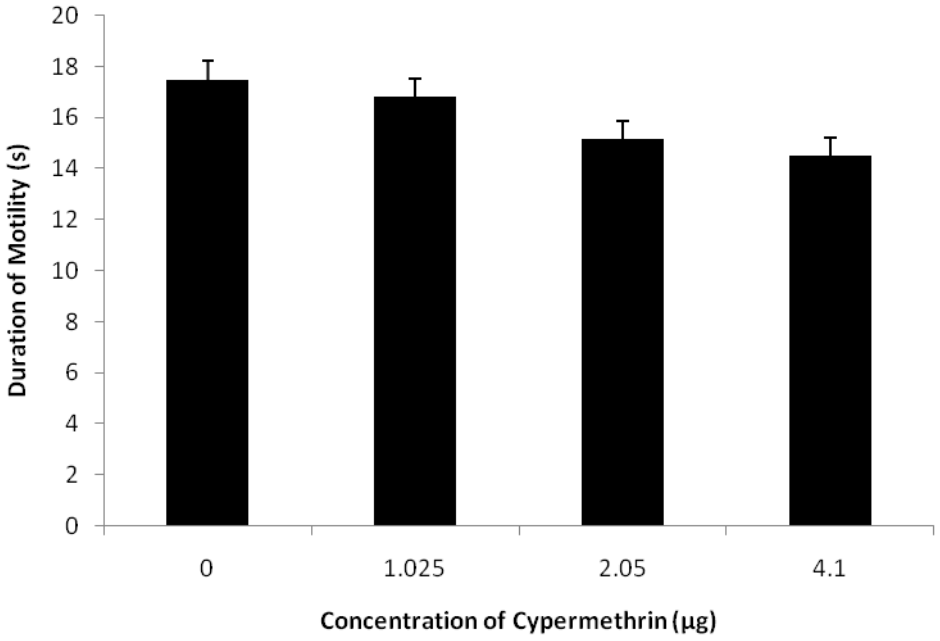

Fig. 2. *In vitro* effects of cypermethrin on spermatozoa motility duration (s) in *Oncorhynchus mykiss*. Data are presented as means±SD. Superscript letters indicate significant differences among samples at the same time post-activation (ANOVA, $P < 0.05$).

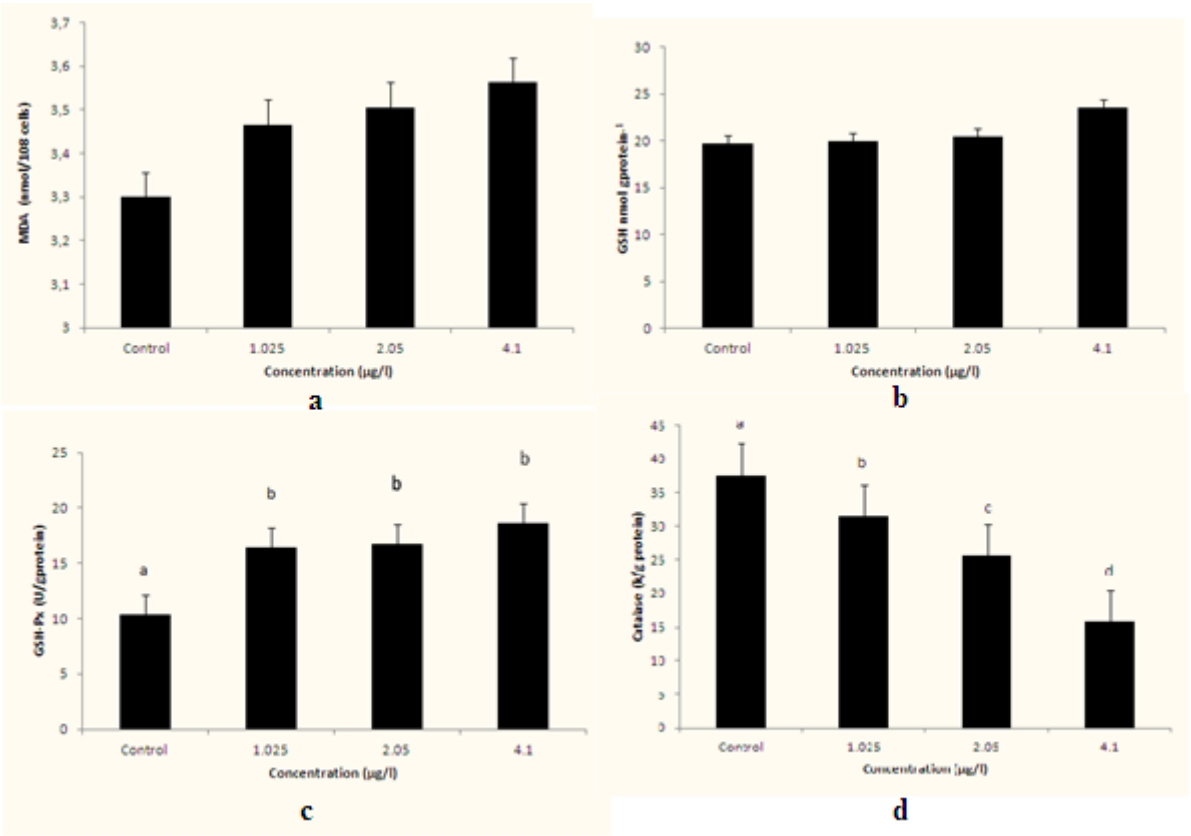

Fig. 3. Effect of cypermethrin on (a) MDA, (b) GSH, (c) GSH-Px and (d) CAT of spermatozoa in *Oncorhynchus mykiss*. Data are presented as means±SD. Superscript letters indicate significant differences among samples at the same time post-activation (ANOVA, $P < 0.05$).

Highlights

- Motility rate and duration of spermatozoa decreased with exposure to cypermethrin.
- GSH-Px activity of spermatozoa increased significantly.
- MDA and GSH levels slightly increased while CAT activity decreased significantly.

Graphical abstract

Fish are affected by residues of cypermethrin in water and sediment samples due to discharge into waters of the majority of pollutants. Sperm cells in most fish species release into water and directly expose to pollutants prior to fertilization. Reactive oxygen species (ROS) generate in spermatozoa due to contain highly polyunsaturated fatty acids (PUFA) in their membranes and a largely lack of protective cytoplasmic enzymes. Increased lipid peroxidation of polyunsaturated fatty acids in the sperm plasma membrane can reduce the fluidity of the membrane and lead to loss of cell function, thereby reducing sperm motility and duration when spermatozoa exposed to cypermethrin.