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Dual effect of Low- frequency Electromagnetic Field on Muscle Histopathology of Caspian Sea *Cyprinus carpio*

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The effect of electromagnetic field on aquatic organisms has received little attention. In the current study, the effect of 50Hz electromagnetic field on muscle histopathology of Caspian Sea Cyprinus carpio, a species of economic importance, was investigated. A total of 120 healthy fish were used in this study. They were classified randomly in one of two groups as follows: Control or unexposed EMF group and experimental group with 5 different magnetic field intensities (0.1, 1, 3, 5 and 7mT) at 2 different exposure times including 30 and 60 minutes. Fish in the experimental group were exposed only once. Two weeks after exposure, dorsal muscles sectioned transversely, stained and were examined using a light microscope. Histopathologic assessments showed significant difference between control and EMF exposed groups at both 30 min. ($p<0.01$) and 60 min. ($p<0.001$) exposure times. We report for the first time that electromagnetic field in interaction with muscular tissue of *Cyprinus carpio* exhibits a dual effect which depends on the field intensity, and exposure time. At short exposure time (30 min.), EMF stimulates muscle growth process. At longer exposure time (60 min.), EMF can damage muscle tissue and result in muscle necrosis. More research is required to elucidate precise mechanisms involved in muscle hypertrophy and pathologic changes.

Key words: Electromagnetic field (EMF), Muscle hypertrophy,Mmuscle atrophy, Caspian Sea, *Cyprinus carpio*

Nowadays, electromagnetic fields (EMFs) are everywhere around us. In fact, we live in an environment of EMF with naturally origin such as atmospheric and geomagnetic and/or artificial (man-made) origin. Aquatic environment is not an exception and is also influenced by these EMFs.

Available evidence indicates that in aquatic environment these fields originate naturally from physiologic processes within aquatic organisms (bioelectric signals such as muscle and nerve impulses), sea currents and earth electromagnetic field which originates from flow of charged particles in the liquid iron core of our planet [Milton et al., 2012; Krylov et al., 2014]. On the other hand, artificial electromagnetic field is generated around underwater power cables leading electricity to the land and then these electromagnetic waves diffuse in aquatic conductive medium. There is evidence of demonstrated effects of magnetic fields on water quality [Krezemieniewski et al., 2004; Khater and Ibraheim, 2015]. So, aquatic environment and its organisms may also be affected by these fields.

Available evidence have shown that some fish such as elasmobranch fish [Kalmijn, 1982], sharks [Kaijura, 2003; Meyer et al., 2005], rockfish [Nishi and Kawanura, 2005], Japanese eel [Nishi et al., 2004], yellowfin tuna [Walker et al., 1984], trout and Coho salmon [Hellinger and Hoffmann, 2009] are magneto-

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sensitive. This sense helps fish in navigation [Walker, 2003], orientation [Westberg, 1999; Nishi, 2006], migration [Yano, 1997], prey and mate detection, and homing [Milton et al., 2012]. A number of these species such as trout and Coho salmon possess magnetite crystals (Fe_3O_4) which is highly concentrated in snout or anterior head [Wiltschko and Wiltschko, 2005; Hellinger and Hoffmann, 2009]. A line of evidence have shown that artificial EMFs may interact with physiologic behaviors and induce disturbances in fish behaviors including locomotor activity and spatial distributions [Krylov et al., 2014], prey detection and navigation [McMurray, 2007], and swimming behavior [Bevelhimer and Cada, 2013].

The effect of magnetic field on aquatic organisms has received little attention [Bochert and Zettler, 2004], and it is believed that freshwater species may respond differently compared to marine species [Öhman et al., 2007].

Common carp *Cyprinus carpio* is one of the important species of freshwater fish in northern parts of Iran with significant economic impact. Its wild species is a favorite type among residents of northern Iran. Because of overfishing, the population of this species has been reduced over the years. For more production of this rich protein source, it is cultured in the ponds, lagoons and dams. A number of these sites which have been stocked with this popular species are nearby power lines. This raises a question whether or not a non-migratory species such as *Cyprinus carpio* is magnetoreceptive and/or is affected by this artificial EMF.

Compared to other vertebrates, muscle tissues comprise a major percentage of the body weight in fish [Fabbri et al., 1998] which is valuable economically. Thus, its histological study is a useful tool in assessing the effect of environmental parameters such as EMF on meat quality. In the current study, the effect of 50Hz electromagnetic field on muscle histopathology of an important economic species of freshwaters of Caspian Sea, Cyprinus carpio was investigated.

MATERIALS AND METHODS

Electromagnetic field exposure system

Electromagnetic field exposure system consisted of a cylindrical coil, digital temperature controller, digital current intensity control system, oscilloscope, and a platform for placing the specimens inside the coil.

The artificial EMF was generated using a cylindrical coil, 42 cm in length (L), with inner diameter of 9.6 cm and outer diameter 11.5 cm, made of 980 turn of 2.5 mm diameter enameled copper wire (N). Test organisms were positioned inside the coil on an elevated platform to ensure all of them received the same EMF intensity. Values in the center of the coil were calculated according to this formula: $B= \mu^0 NI/L$, where B represents magnetic field intensity measured in Tesla (1 Tesla= 10,000 Gauss), I represents current intensity applied to the coil (Ampere), and $\mu^0=0.256*10$. Magnetic fields were generated by current flow, and field strength was controlled by changing electrical current. Two digital ampere-meters showed the current intensities. Only alternating current (AC) was used in the present study. To create one-way current and to regulate different field intensities, equipment had one key for activating or deactivating processes. Finally, field strengths were checked using a Tesla-meter.

Coil was located in an east-west direction and electromagnetic field was parallel to long axis of fish body. An air conditioning system ventilated the coil chamber (one, 5 cm from the entrance of the coil and the other beneath the coil). Digital temperature controller regulated temperature at 16℃. During exposures, frequency was constant (50Hz) and electromagnetic field intensities varied from 0.1 mT (milli Tesla) to 7mT. There were two exposure times (30 min. and 60 min.) for each EMF intensity. Experimental set up is shown in Fig. 1.

Animals

Test organisms were fresh water common carp

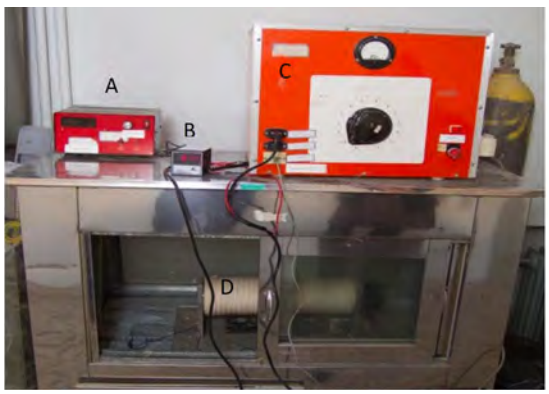

Fig. 1. Experimental set up for EMF exposure to *C. carpio*.(A) Digital temperature controller, (B) Digital ampere-meter, (C) power supply, (D) Electromagnetic field generator.

Cyprinus carpio (length approx. 7-10 cm; mass approx. 15-20 gr) were collected from Shahid Rajaee Fish Farm, in the Sari city near southern coast of Caspian Sea, during April 2015. The experimental protocol was reviewed and approved by the Laboratory Animal Care Committee of Shahid Beheshti University. A total of 120 healthy fish were used in this study. After transport to the laboratory, they were kept in 60 L aquaria (10 fish per tank) at 16℃ temperature and light/dark cycle, 12 hours each, for a period of two months. They were fed once a day with small pieces of fish food and permit to grow and adapt to laboratory conditions. Following acclimation, and at the beginning of experiments, the fish were 25-30 grams in weight and 12-15 cm in length. They were classified randomly in one of two groups as follows with 10 fish per group:

(1) Control group: In this group, fish were placed on the platform inside the coil with no EMF exposure (the system was turned off). There were two control groups for two different exposure time (30 and 60 minutes).

(2) Experimental group: This group was exposed to five different EMF intensities (0.1, 1, 3, 5 and 7mT) at two different exposure times (30 and 60 minutes). Thus, we had 10 experimental or treated groups. Fish were exposed only once to EMF.

For EMF exposures, fish were placed in the acrylic experimental cubes (15 cm× 8 cm× 6 cm) filled with their aquarium water and placed inside the coil. After EMF exposure, the specimens were returned to their aquaria for two weeks.

It should be noted that all of the fish in EMF exposed groups appeared healthy during and at the end of the study and there was no mortality during two weeks after exposure.

Histopathological procedure

Two weeks after EMF exposure, fish were taken from their aquaria, killed and then their dorsal muscles immediately removed, rinsed with saline solution (0.9% w/v concentration), and fixed in formalin solution (10% w/v concentration). They were embedded in paraffin, sectioned transversely (5μ) and stained with hematoxylin and eosin (H &E). Then muscle cross sections were examined and photographed using a light microscope (Optiphot 2; Nikon, Tokyo, Japan). Muscle fibers diameters and the distance between muscle fibers (m) were measured using AxioVision microscope software (viewer 4.8). For each fish 5 microscopic fields were evaluated.

Statistical analysis

Data are presented as mean ± standard error (Mean ± SE) of ten fish. The data were analyzed using analysis of variance (ANOVA) followed by Tukey test. $P<0.05$ was considered statistically significant. All calculations were performed using SPSS/PC software.

RESULTS

The effects of short and long term exposure to EMF on the dorsal muscle measurements are summarized in Tables 1 and 2 respectively. Dorsal muscle photomicrographs of EMF exposed fish in the experimental groups are shown in Fig. 3- 10. Fig. 2 shows dorsal muscle stained section of fish in the con-

Table 1. The effect of EMF short exposure time (30 min.) on dorsal muscle fiber measurements

EMF intensity (mT)	Muscle fiber diameter (μm)		Distance between muscle fibers (μm)	
	Max	Min	Max	Min
0	57.24±3.76	10.02±2.78	15.70±5.38	3.77±1.03
0.1	53.41±6.88	16.71±6.77	16.71±6.77	3.60±0.60
1	62.05±4.79	19.27±5.91	15.77±2.32	3.66±1.35
3	68.00±5.10	17.69±7.11	12.54±4.64	2.04±0.59
5	*72.57±6.03	*30.02±7.63	*11.48±3.38	2.18±1.04
7	**92.54±8.21	**34.36±5.96	**10.10±2.27	*1.61±0.47

Data are presented as mean±S.E.M. for 10 fish. *p<0.05,**p<0.01, *** p<0.001 vs. control (0 mT).

Table 2. The effect of EMF long exposure time (60 min.) on dorsal muscle fiber measurements

EMF intensity (mT)	Muscle fiber diameter (μm)		Distance between muscle fibers (μm)	
	Max	Min	Max	Min
0	54.12±4.36	14.32±2.80	14.23±4.27	2.63±0.69
0.1	53.96±5.20	12.16±5.40	16.71±6.77	3.86±0.94
1	51.83±3.76	13.25±3.91	19.30±2.32	4.64±1.80
3	54.90±4.28	11.36±4.11	*21.63±4.64	4.58±1.80
5	55.28±7.08	*10.22±4.23	**27.48±5.38	*5.95±1.25
7	59.32±3.92	**8.99±5.71	***35.05±4.17	**6.64±0.77

Data are presented as mean±S.E.M. for 10 fish. *p<0.05,**p<0.01, *** p<0.001 vs. control (0 mT).

trol group. It should be mentioned that for brevity limited stained sections are included in the article.

Muscle fiber measurements showed significant difference between control and EMF exposed groups at both 30 min. (p<0.01) and 60 min. (p<0.001) exposure times. In addition, there was a significant difference between EMF exposed groups in two exposure times

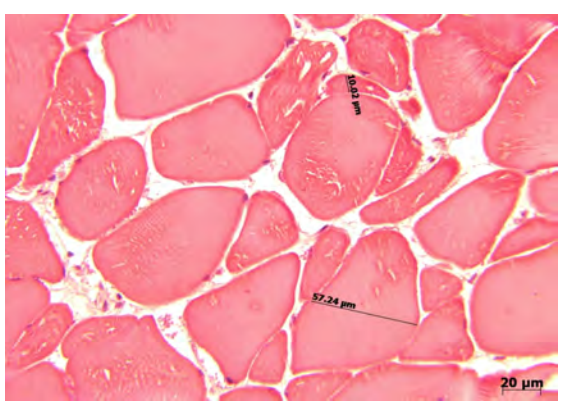

Fig. 2. Light micrograph after Hematoxylin-eosin staining of fish dorsal muscle of control group. Polygonal shaped muscle fibers with their nuclei in the periphery or at the edges of the fibers are seen in transverse section. Muscle fibers, endomysium and muscle tissue structure are normal. The diameter of muscle fibers is between 10-57 μm in this microscopic field.

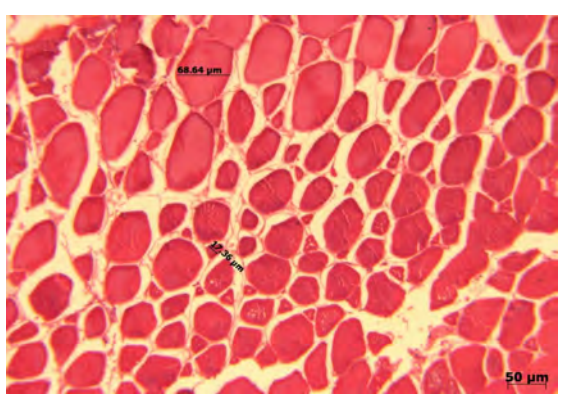

Fig. 3. Light micrograph after Hematoxylin-eosin staining of dorsal muscle section of a fish in treated group at field strength of 3mT (30 min.) 2 weeks after EMF exposure. The diameter of muscle fibers is between 17-68 μm in this section.

($p<0.01$).

As shown in Fig. (3- 5), at shorter exposure time (30 min.) no pathologic lesion was seen in muscles of exposed group at different field intensities as compared to the control. Furthermore, the diameter of muscle fibers increased and the distance between fibers decreased statistically in an intensity dependent manner (Table1). Statistical analysis showed that this increase in diameter of muscle fibers was significant at field intensities 5mT ($p<0.05$) and 7mT ($p<0.01$) and reached its peak value at field intensity of 7mT (92.54 ± 8.21µm). Indeed, post hoc analysis showed significant difference between EMF exposed groups at different filed intensities ($p<0.05$). There was significant difference in diameter of muscle fibers at 0.1, 1 and 3mT field intensities with those of 5 and 7mT. The distance between muscle fibers decreased sig-

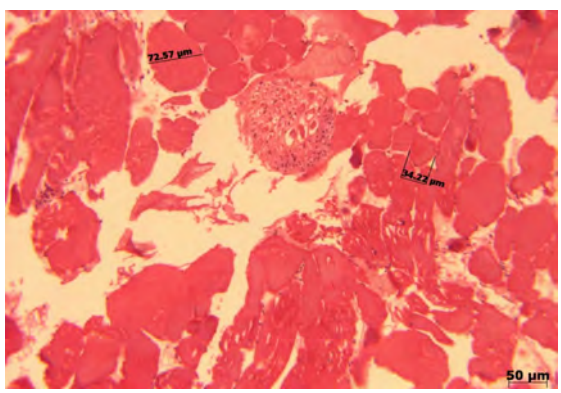

Fig. 4. Light micrograph after Hematoxylin-eosin staining of dorsal muscle section of a fish in treated group at field strength of 5mT (30 min.) 2 weeks after EMF exposure. The diameter of muscle fibers is between 34-72 µm in this field. A nerve is seen in the figure.

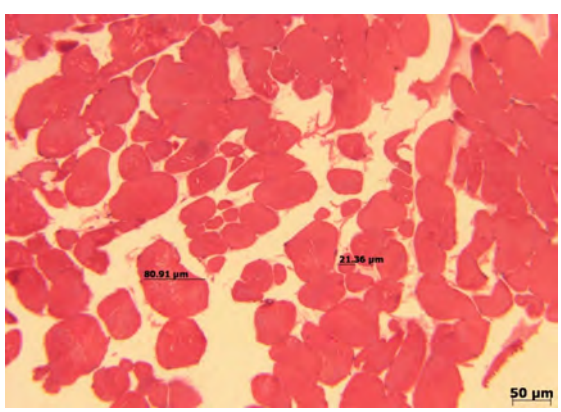

Fig. 5. Light micrograph after Hematoxylin-eosin staining of dorsal muscle section of a fish in treated group at field strength of 7mT (30 min.) 2 weeks after EMF exposure. The diameter of muscle fibers is between 21-80 µm in this microscopic field.

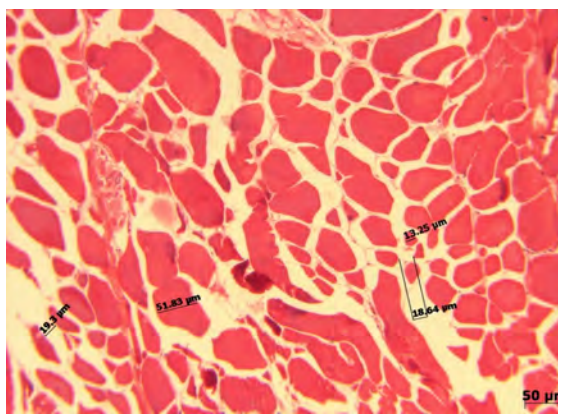

Fig. 6. Light micrograph after Hematoxylin-eosin staining of dorsal muscle section of a fish in treated group at field strength of 1mT (60 min.) 2 weeks after EMF exposure. The diameter of muscle fibers is between 13-51 µm and the distance between muscle fibers is 18-19 µm in this microscopic field.

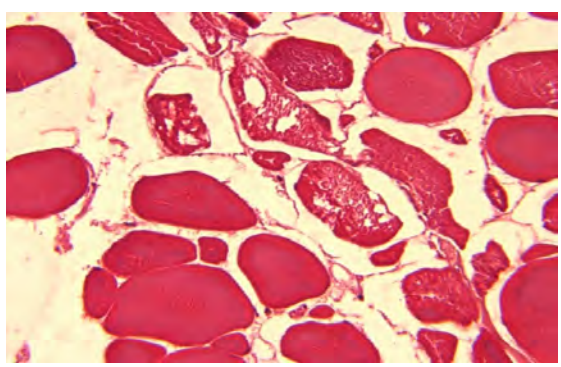

Fig. 7. Light micrograph after Hematoxylin-eosin staining of dorsal muscle section of a fish in treated group at field strength of 5mT (60 min.) 2 weeks after EMF exposure. Disorganization of sarcoplasmic contractile elements of a number of muscle fibers is shown here (X250).

nificantly at field intensities 5mT ($p<0.05$) and 7mT ($p<0.01$) compared to control group.

At longer exposure time (60 min.), as shown in Table 2, the distance between muscle fibers increased in a field intensity dependent manner. Statistical analysis showed that the distance between muscle fibers increased significantly at field intensities 3mT ($p<0.05$), 5mT ($p<0.01$) and 7mT ($p<0.001$) as compared control group and reached to its maximum value at field intensity of 7mT (Table 2 & Fig. 8). Furthermore, the diameter of muscle fibers decreased significantly compared to control groups in an intensity-dependent manner ($p<0.01$). Adverse effects of EMF appeared gradually in muscular tissue. These include contractile elements disorganization (Fig. 7), thrombosis (Fig. 9), atrophy and necrosis (Fig. 8 and 10).

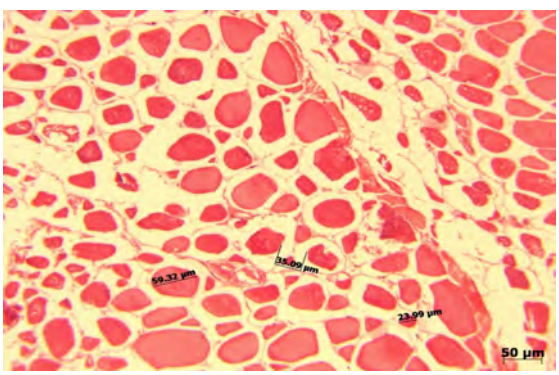

Fig. 8. Light micrograph after Hematoxylin-eosin staining of dorsal muscle section of a fish in treated group at field strength of 7mT (60 min.) 2 weeks after EMF exposure. The distance between muscle fibers was more than control (mean ± standard error in control group = 14.23 ± 4.27 µm) and was 35 µm in this microscopic field.

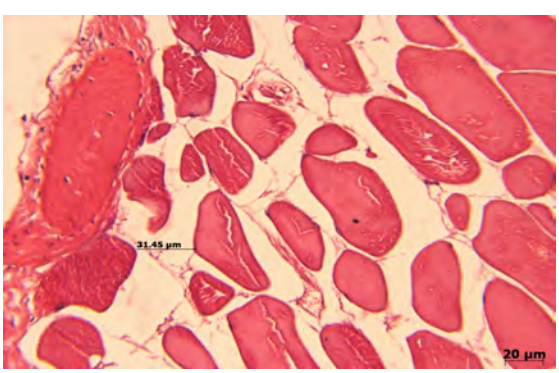

Fig. 9. Light micrograph after Hematoxylin-eosin staining of dorsal muscle section of a fish in treated group at field strength of 7mT (60 min.) 2 weeks after EMF exposure. The distance between fibers is more than control and was 31 µm. An arteriole thrombus (in left side) is seen in this section.

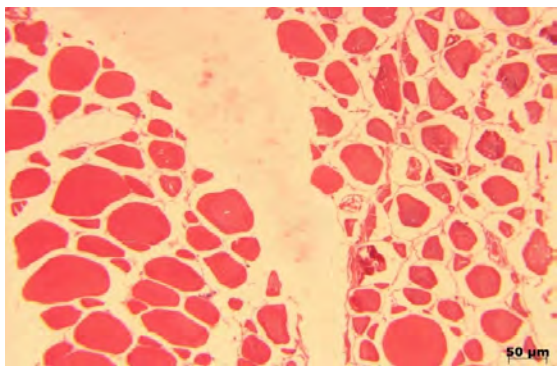

Fig. 10. Light micrograph after Hematoxylin-eosin staining of dorsal muscle section of a fish in treated group at field strength of 7mT (60 min.) 2 weeks after EMF exposure shows muscle fiber atrophy and disorganization of myofibrils (in right side) and necrosis (in left side). Eosinophilic cytoplasm and pyknotic nuclei of necrotic cells are shown in this slide (X100).

DISCUSSION

Previous researches have shown that some aquatic species can detect and respond to EMF [Nishi et al., 2006; Hellinger and Hoffmann, 2009; Milton et al., 2012]. The literature on magnetoreception in common carp Cyprinus carpio, one of the most widely cultured species all over the world, is quite scarce.

In the current study, the effect of 50Hz electromagnetic field on muscle histopathology of Caspian Sea, Cyprinus carpio was investigated. Results showed EMF induced changes in the dorsal muscle histology of *Cyprinus carpio*. This study provides more evidence that this non-migratory species is EMF sensitive and can respond to changes in artificial EMF.

There is limited evidence in the literature that shows this species is sensitive to EMF. It has been previously reported that magnetic exposure altered the circulation in embryos and larvae of carp *(Cyprinus carpio)* during development and causes an increase in heart rhythm especially in early stage of development [Formiciki and Winnicki, 1998]. Another study by Hart et al. [2012] has shown that carps orient themselves in the geomagnetic field (along the North-South axis) at traditional Christmas sale in plastic circular tubs. It is believed that an animal with the ability to orient its movements according to the geomagnetic field has a magnetic compass sense [Kennth et al., 2000].

In our study, all EMF-exposed animals appeared healthy and there was no mortality during and at the end of study. It is possible that immune system stimulation may be the mechanism of no mortality in EMF exposed groups in the present study. Consistent with the present observation, Cuppen et al. [2007] observed EMF treatment reduced mortality rate in fantail goldfish infected with ectoparasites (such as trichodina). They reported that low frequency electromagnetic field exposure induced mild stress to cells and cytokines, alarms molecules, are produced which increase immune system activity.

Bochert and Zettler [2004] who studied exposure of several marine benthic animals such as North Sea prawn and round crab to static magnetic fields showed no difference in survival rate between control and experimental groups. Krzemieniewski et al. [2004] reported that biomass decreased and mortality increased in European catfish (*Silurus glanis*) after exposure to a constant magnetic field. These differences in mortality rate may be due to difference in magnetic field intensity, exposure time and/or difference between species.

Short Term Exposure

In our study, histological observations showed that in EMF-exposed groups with shorter exposure time (30 min.) no muscle pathologic changes occurred. Also the diameter of muscle fibers increased and the distance between fibers decreased in an intensity-dependent manner, i.e. hypertrophy increased with increase in field intensity. This indicates mild to moderate muscle fiber hypertrophy as shown in Fig. 4, 5. This observation can be interpreted as positive effect of EMF on muscle growth process. There is a possibility that this effect continues in longer duration (greater than 2 weeks) and may result in more muscle mass production with a positive economic impact.

Long Term Exposure

For the experimental groups exposed for longer time (60 min.), negative effect of EMF was observed. A decrease in muscle fiber diameter and increase in distance between fibers was observed. This shows mild to severe muscle atrophy of majority of muscle fibers as shown in Fig. 6 and 8. At maximum field strength of 7mT, besides atrophy, thrombus was also observed which is a pathologic indicator, and can cause tissue damage due to ischemia. This condition may be irreversible and result in muscle necrosis.

Overall based on the results of this research, one can say that artificial EMFs exert dual effect on fish muscular tissue; in short term exposure (30 min.) EMF stimulates muscle growth (positive effect), whereas in long term exposure (60 min.), it can result in muscle necrosis (negative effect). The exact mechanism of dual effects of EMF on fish muscle tissue modifications is not clear, but on the basis of available evidence in literature cellular stress response and oxidative stress are the most likely.

The precise mechanism of EMF on muscle hypertrophy is not clear. But there are evidence that have shown the effect of EMF on synthesis of stress proteins such as hsp70, heat shock protein, and contractile proteins such as actin and myosin [Blank, 2012; Cheon et al., 2012; Rodrı´guez-De la Fuente et al., 2012]. These proteins synthetized during cellular stress response which is a protective mechanism

of cells against environmental harmful stimuli that cause damage to macromolecules and increase cellular resistance to adverse effects of EMF [Kultz, 2005; Blank, 2012]. There is evidence that fish are sensitive to both chronic and acute environmental changes and exhibit a classical stress response [Gurcu et al., 2010].

We propose that existence of electromagnetic waves can be considered as environmental stress in our study which has hypertrophic effect due to production of stress proteins such as contractile proteins and hsp70. In agreement to this hypothesis, Kee et al. [2008] reported hsp70 triggers cardiac hypertrophy in vitro and in vivo conditions. On the basis of available evidence, hsp70 induces in response to damaging stress in skeletal muscle and has a key role in muscle plasticity [Miyabara et al., 2012; Senf, 2013]. Indeed, hsp70 was identified in red skeletal muscle of stressed common carp [Poltronieri et al., 2008]. Other evidences confirm stress response following electromagnetic field exposure [Tokalov and Gutzeit, 2004; Blank, 2009].

At longer exposure time (60 min.), it appears cellular stress response attenuates with EMF intensity increase. Thus, cells are not able to produce enough stress proteins (such as contractile proteins and hsp70) against EMF effects to protect themselves. Under these conditions EMF tolerance develops. Our hypothesis confirms findings of DiCarlo et al. [2002] who reported when EMF tolerance develops in the chick embryos, cytoprotection decrease. On the other hand, oxidative stress overcomes EMF- stress response. In another words, oxidative stress does not appear to be a strong inducer of stress proteins production under these conditions. There are some evidences which confirm this hypothesis [Ozdemirler et al., 2005; Adachi et al., 2009]. We propose both of these mechanisms including oxidative stress and stress response decrease cause disruption of cellular resistance and result in EMF induced adverse effects such as myofibrillar disorganization, muscle atrophy, thrombus formation and muscle necrosis.

Oxidative stress appears to be an important mechanism for biological response to EMF in different cellular systems [Kovacic and Somanathan, 2010; Consales et al., 2012]. Increased EMF exposure can change cellular balance by generating reactive oxygen species (ROS) [SimkÓ, 2007; Canseven et al., 2008; Akdag et al., 2010]. Disruption of balance between ROS production and antioxidant defense may activate a cascade of events, leading to structural changes in muscle. There are evidences confirming the effect of oxidative stress on muscle waste [Buck and Chojkier, 1996; Moylan and Reid, 2007], muscle atrophy [Powers et al., 2007; Aucello et al., 2009; Musarò et al., 2010], muscle pathogenesis [Musarò and Fulle, 2009 ; Musarò et al., 2010; Betancor et al., 2013], and myofibrillar force decrease [Hardin et al., 2008]. Other studies showed that substances that interfere with oxidative stress prevent muscle atrophy and improve its contractile characteristics [Arbogast et al., 2007; Aleem et al., 2013]. Steinberg [2013] proposed oxidative stress and ROS production can lead to direct oxidation of many contractile proteins, resulting in sarcomere structural changes in muscle.

In this study, exposure to EMF at higher intensity in longer time exposure (60 min.) as shown in Fig. 7 resulted in disorganization of contractile elements of a number of muscle fibers. This confirms findings by Ciejka et al. [2010] who showed that protein concentration decreased in the rat muscles homogenates after exposure to low frequency electromagnetic field. This change in contractile proteins and organelles in muscular tissue can result in muscle atrophy where proteolytic enzymes are activated and result in the muscle fiber shrinkage [Bonaldo and Sandri, 2013].

A study by Raeker et al. [2014] on myofibrillogenesis in zebrafish embryos showed that optimal striated muscle function depends on this high level myofibrillar organization. Disruption of the muscle structural integrity, in turn, induces changes in its physiology.

In conclusion, we report for the first time that EMF interaction with muscular tissue of common carp *C. carpio* exhibits a dual effect depending on the field intensity, and exposure time. At shorter exposure time (30 min.), EMF stimulates muscle growth process. If confirmed by further research on the evaluation of muscle mass, application of this finding in aquaculture may result in more muscle mass production of this popular species, with positive economic impact. On the other hand, at longer exposure time (60 min.), EMF can damage muscle tissue and result in muscle necrosis. More research is required to elucidate precise mechanisms involved in muscle hypertrophy and pathologic changes.

REFERENCES

Adachi M, Liu Y, Fujii K, Calderwood SK, Nakai A, Imai K, Shinomura Y. (2009). Oxidative Stress Impairs the Heat Stress Response and Delays Unfolded Protein Recovery. PLOS ONE 4(11): e7719. doi:10.1371/journal.pone.0007719.

Akdag MZ, Dasdag S, Ulukaya E, Uzunlar AK, Kurt MA, Taşkin A.(2010). Effects of extremely low-frequency magnetic field on caspase activities and oxidative stress values in rat brain. Biol Trace Elem Res 138(1-3): 238-249.

Aleem SB, Hussain MM, Faroog Y. (2013). Levo-Carnitine reduces oxidative stress and improves contractile functions of fast muscles in type 2 diabetic rats. Iranian biomedical journal 17(1): 29-35.

Arbogast S1, Smith J, Matuszczak Y, Hardin BJ, Moylan JS, Smith JD, Ware J, Kennedy AR, Reid MB. (2007). Browman-Birk inhibitor concentrate prevents atrophy, weakness, and oxidative stress in soleus muscle of hindlimb-unloaded mice. J Appl Physiol; 102: 956-964.

Aucello M, Dobrowolny G, Musaro A. (2009). Localized accumulation of oxidative stress causes muscle atrophy through activation of an autophagic pathway. Autophagy 5(4): 527-9.

Betancor MB, Caballero MJ, Benitez-Santana T, Saleh R, Roo J, Atalah E, Izquierdo M. (2013). Oxidative status and histological changes in sea bass larvae muscle in response to high dietary content of docosahexaenoic acid DHA. J Fish Dis 36(5): 453-65.

Bevelhimer MS, Cada GF. (2013). Behavioral responses of representative freshwater fish species to electromagnetic fields. Transactions of the American Fisheries Society, 142: 3, 802-813.

Blank M. (2012). The Cellular Stress Response: EMF-DNA Interaction. Prepared for the BioInitiative Working Group. Section7.

Blank M, Goodman R. (2009). Electromagnetic fields stress living cells. Pathophysiology 16 (2-3):71-8.

Bochert R, Zettler ML. (2004). Long- term exposure of several marine benthic animals to static fields. Bio electromagnetics 25: 498-502.

Bonaldo P, Sandri M. (2013). Cellular and molecular mechanisms of muscle atrophy. Disease models & Mechanisms 6: 25-39.

Buck M, Chojkier M. (1996). Muscle wasting and dedifferentiation induced by oxidative stress in a murine model of cachexia is prevented by inhibitors of nitric oxide synthesis and antioxidants. EMBO J. 15: 1753-1765.

Canseven AG, Coskun S, Seyhan N. (2008). Effects of various extremely low frequency magnetic fields on the free radical processes, natural antioxidant activities in the heart and liver tissues. Indian Journal of Biochemistry & Biophssics 45: 326-331.

Cheon S, Park I, Kim M. (2012). Pulsed electromagnetic field elicits muscle recovery via increase of HSP 70 expression after crush injury of rat skeletal muscle .J.Phys.Ther.Sci 24: 589-592.

Ciejka E, Skibska B, Kleniewska P, Goraca A. (2010). Influence of low frequency magnetic field on chosen parameters of oxidative stress in rat's muscles. Pol Merkur Lekarski. 29(174):361-4.

Consales C, Merla C, Marino C, Benassi B. (2012). Electromagnetic fields, oxidative stress, and neurodegeneration. International journal of cell biology. Volume 2012, Article ID 683897, 16 pages.

Cuppen JJM, Wiegertjes GF, Lobee HWJ, Savelkoul HFJ, Elmusharaf MA, Beynen AC, Grooten HNA, Smink W.(2007). Immune stimulation in fish and chicken through weak low frequency electromagnetic fields. Environmentalist 27, Issue 4, 577-583.

Di Carlo A, White N, Guo F, Garrett P, Litovitz T. (2002). Chronic electromagnetic field exposure decreases HSP70 levels and lowers cytoprotection. J Cell Biochem. 84(3):447-54.

Fabbri E, Capuzzo A, Moon TW. (1998). The role of circulating catecholamines in the regulation of fish metabolism: An overview. Comp. Biochem. Physiol.

Pharmacol. Toxicol. Endocrinol. 120: 177-192.

Formicki K, Winnicki A. (1998). Reactions of fish embryos and larvae to of constant magnetic fields. Italian J. Zool. 65, 479-482.

Gurcu B, Yildiz S, Koca YB, Koca S. (2010). Investigation of histopathological and cytogenetic effects of heavy metals pollution on *Cyprinus carpio* (Linneaus, 1758) in the GÖlmarmara Lake, Turkey. Journal of Animal and Veterinary Advances 9(4): 798-808.

Hardin BJ, Campbell KS, Smith JD. (2008). TNF-alpha acts via TNFR1 and muscle- derived oxidants to depress myofibrillar force in murine skeletal muscle. J Appl Physiol 104: 694-699.

Hart V, KustaT, Nemec P, Blahova V, Jezek M, Novakova P, Begall S, Cerveny J, Hanzal V, Malkemper EP, Stipek K , Vole C, Burda H. (2012). Magnetic Alignment in Carps: Evidence from the Czech Christmas Fish Market. PLOS One 7(12): e51100 doi: 10.1371/journal. Pone. 0051100. Epub 2012 Dec 5.

Hellinger J, Hoffmann KP. (2009). Magnetic field perception in the Rainbow Trout, Oncorhynchus mykiss. Journal of Comparative Physiology A- Neuroethology, Sensory, Neural, and Behavioral.

Kaijura SM. (2003). Electroreception in neonatal bonnet head sharks, Sphyrna tiburo. Marine biology 143: 603-611.

Kalmijn AJ. (1982). Electric and magnetic field detection in elasmobranch fishes. Science 218, no.4575: 916-918.

Kee HJ, Eom GH, Joung H, Shin S, Kim JR, ChoYK, Choe N, Sim BW, Jo D, Jeong MH, Kim KK, Seo JS, Kook H. (2008). Activation of Histone Deacetylase 2 by Inducible Heat Shock Protein 70 in Cardiac Hypertrophy. Circulation Research 103: 1259-1269.

Kenneth J, Lohmann KJ and Sönke J. (2000). The neurobiology of magnetoreception in vertebrate animals. Trends Neurosci 23(4): 153-9.

Khater ZZK, Ibraheim MH. (2015). Ecological studies on the effect of magnetic field on water. Int. J. Curr. Res. Aca. Rev 3 (8), pp. 262-279.

Kovacic P, Somanathan R. (2010). Electromagnetic fields: mechanism, cell signaling, other bioprocess, toxicity, radicals, antioxidants and beneficial effects. Journal of Receptors and Signal Transduction. Volume 30, Issue 4.

Krylov VV, Izyumov G, Izvekov EI, Nepomnyasshchikh VA. (2014). Magnetic fields and fish behavior. Biology Bulletin Reviews l4(3): 222-231.

Krzemieniewski M, Teodorowicz M, Debowski M, Pesta J. (2004). Effects of a constant magnetic field on water quality and rearing of European sheatfish *Silurus glanis L*. larvae. Aquacult. Res 35: 568-573.

Kultz D. (2005). Molecular and evolutionary basis of the cellular stress response. Annual Reviews of Physiology 67: 225-257.

McMurray G. (2007).Wave energy ecological effects workshop ecological assessment briefing paper. Hatfield marine science center, Oregan State University. October 11-12.

Meyer CG, Holland KN, Papastamatiou YP. (2005). Sharks can detect changes in the geomagnetic field. Journal of the Royal Society Interface 2: 129-130.

Milton L, Bull A, Schroeder D. (2012). EMF and marine organisms, 28-29.

Miyabara, EH, Nascimento,TL, Rodrigues,DC, Moriscot, AS, Davila,WF, Aitmou,Y., Detombe PP, Mestril R. (2012). Overexpression of inducible70-kDa heat shock protein in mouse improves structural and functional recovery of skeletal muscles from atrophy. Pflugers Arch. 463: 733-741.

Moylan JS, Reid MB. (2007). Oxidative stress, chronic disease, and muscle wasting. Muscle Nerve 35: 411-429.

Musarò A1, Fulle S, Fanò G. (2010). Oxidative stress and muscle homeostasis. Curr Opin Clin Nutr Metab Care 13(3): 236-42.

Musarò A1, Fulle S. (2009). The role of oxidative stress in the physiopathology of skeletal muscle. In: Balsano C, editor. Oxidative stress, dysregulation of cell homeostasis and induction of cell transformation. Kerala, India: research Signopost 1-18.

Nishi T, Kawanura G. (2005). Magnetosensitivity in the darkbanded rockfish Sebastes inermis. Nippon Suisan Gakkaishi 72(1): 27-33.

Nishi T. (2006). Studies on the magnetosensitivity and magnetocompass orientation in the Japanese eel Anguilla japonica. Fish 55(2): 107-159.

Nishi T, Kawamura G., Matsumoto K. (2004). Magnetic sense in the Japanese eel, *Anguilla japonica*, as determined by conditioning and electrocardiography. The Journal of Experimental Biology 207: 2965-2970.

Öhman MC, Sigray P, Westerberg H. (2007).Offshore windmills and the effects of electromagnetic fields on fish. Ambio 36(8): 630-633.

Ozdemirler Erata G, Kanbağli O, Durlanik O, Bulut T,

Toker G, Uysal M. (2005). Induced oxidative stress and decreased expression of inducible heat shock protein 70 (ihsp70) in patients with colorectal adenocarcinomas. Jpn J Clin Oncol 35(2): 74-8.

Poltronieri C, Negrato E., Bertotto D., Majolini D., Simontacchi C., Radaelli G. (2008). Immunohistochemical localization of constitutive and inducible Heat Shock Protein 70 in carp (Cyprinus carpio) and trout (Oncorhynchus mykiss) exposed to transport stress. European Journal of Histochemistry. vol. 52 issue 3 (July-September): 191-198.

Powers SK, Kavazis AN, McClung JM. (2007). Oxidative stress and disuse muscle atrophy. J Appl Physiol 102: 2389-2397.

Raeker MO, Shavit JA, Dowling JJ, Michele DE, and Russel W. (2014). Membrane-myofibril cross-talk in myofibrillogenesis and in muscular dystrophy pathogenesis: Lessons from the Zebrafish. Frontiers in Physiology Volume 5:14. Published online 2014 January 28. doi: 10.3389/fphys.2014.00014.

Rodrı´guez-De la Fuente AQ, Alcocer-Gonza´ lez JM, Antonio Heredia-Rojas J, Rodrı´guez-Padilla C, Rodrı´guez-Flores LE, Santoyo- Stephano MA, Castan˜eda- Garza E, Tame´z-Guerra RS. (2012). Effect of 60 Hz electromagnetic fields on the activity of hsp70 promoter: an in vivo study. www.cellbiolintrep.org & volume19 (1) art: e00014.

Senf SM. Skeletal muscle heat shock protein70: diverse functions and therapeutic potential for wasting disorders. (2013). Perspective article Volume 4, Article 330. www.frontiersin.org.

SimkÓ M. (2007). Cell type specific redox status is responsible for diverse electromagnetic field effects. Current Medicinal Chemistry 14(10): 1141-1152.

Steinberg SF. (2013). Oxidative stress and sarcoplasmic proteins. Circ Res.18; 112(2): 393-405.

1. Tokalov SV, Gutzeit HO. (2004). Weak electromagnetic fields (50 Hz) elicit a stress response in human cells. Environ Res 94(2): 145-51.

Walker MM. (2003). Sensory system, perception, and learning, magnetic sense in fishes. Current Opinion in Neurobiology 12: 735-744.

Walker MM, Kirschvink JL, Chang SBR, Dizon AE. (1984). A candidate magnetic sense organ in the yellowfin tuna, *Thunnus albacares*. Science 224: 751-753.

Westerberg H. (1999). Effect of HVDC cables on eel orientation. Technische Eingriffe in Marine Lebensraume, pp 1-6. Insel Vlim, Sweden. As cited in Gill (2005) Offshore Renewable Energy: Ecological Implications of Generating Electricity in the Coastal Zone. Journal of Applied Ecology 42: 605-615.

Wiltschko W, Wiltschko R. (2005). Magnetic orientation and magnetoreception in birds and other animals. Journal of Comparative Physiology A- Neuroethology, Sensory, Neural, and Behavioral Physiology 191(8): 675-693.

Yano A, Ogura M, Sato A, Sakak Y, Shimizu Y, Baba N, Nagasawa K. (1997). Effect of modified magnetic field on the ocean migration of maturing chum salmon, Oncorhynchus keta. Marine Biology 129(3): 523-530.

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