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RESEARCH ARTICLE

The effect of static magnetic field on Danube huchen, *Hucho hucho* (L.) sperm motility parameters

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Abstract. The distribution range of Danube huchen, *Hucho hucho* (L.) in Polish waters is decreasing, and is currently only 25 to 30% of its original area. Since few data are available concerning Danube huchen, it is necessary to develop a better understanding of its reproduction to improve artificial spawning in hatcheries. Eight sperm motility parameters were assessed using CASA after short-term storage in a static magnetic field. The effect of magnetic field exposure on spermatozoa at fertilization and on sperm morphology (SEM) was also examined. Static magnetic fields had a positive effect on sperm motility parameters, including VCL, which determines fertilization effectiveness; values for this parameter after a 24 h exposure period to fields of different intensity were as follows: 1 mT – 110.09 μm s^{-1}; 5 mT – 111.65 μm s^{-1}; 10 mT – 152.10 μm s^{-1}; control – 102.09 μm s^{-1}. Egg fertilization rates of spermatozoa held for 24 hours in fields of 1mT was 71.32%, 5mT – 58.23%, and 10mT – 59.99%, and in the control – 32.60%. The mean length of spermatozoa was 27.14 ±0.22 μm; the head was elongate; length without the neck was 2.80 ±0.19 μm; the width was 2.0 ±0.08 μm. This study suggests that the method of exposing sperm to magnetic fields might, after more extensive studies, could be used for short-term sperm storage.

Keywords: Huchen, sperm motility (CASA), morphology, fertilization, magnetic field

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Introduction

The Danube huchen, *Hucho hucho* (L.), is the largest representative of the Salmonidae. Until recently, the species inhabited the Danube River and most of its submontane tributaries. Currently, the species occupies only about 25-30% of its original range in Europe, primarily because of river regulation, dam and reservoir construction, major water consumption by industry and agriculture, river pollution, and accelerated eutrophication (Holčík et al. 1988, Holčík 1990). Danube huchen has been categorized recently as an endangered species (Rand 2012). Within its natural distribution range in Poland, single huchen individuals are found only in the Czarna Orawa and its major tributaries. The species has become extinct in Czadeczka Stream (Witkowski et al. 2013). Thanks to introduction, the species is now present in the Dunajec, Poprad, San, and probably the Gwda and Bóbr rivers (Witkowski and Kowalewski 1980, 1988, 1994, Andrzejewski 2000).

Danube huchen has been preserved in Polish waters (*ex situ*) through active protection and artificial breeding (Witkowski 1990, 1996, Witkowski et al. 2013). Wild stocks of this salmon species, which was very popular and caught readily in the past, have become so rare that it has been included in the Polish Red Book of Animals (Głowaciński 2001).

In order to overcome this problem and restore its original population abundance, Danube huchen are bred and reared artificially. Since little data are available concerning the species, a better understanding of Danube huchen reproduction is required to improve hatchery procedures for artificial spawning. Factors that can affect the quality of male gametes have become especially important. Sperm motility is one of the most important criteria in assessing fish sperm quality (Lahnsteiner et al. 1998, Kime et al. 2001, Gage et al. 2004, Zilli et al. 2004). The objective evaluation of sperm motility can be done by computer assisted sperm analysis (CASA). This system reduces differences among technicians and improves the accuracy of final results; additionally it makes the objective assessment of many motility parameters of spermatozoa possible. There is a dependence between sperm motility and fertilization rates (Billard et al. 1986, Gage et al. 2004, Rurangwa et al. 2004).

Static magnetic fields with values higher than those of the Earth's natural background affects many processes during early ontogeny and directional reactions of juvenile and adult fish (Quinn and Groot 1983, Chew and Brown 1989, Tesch et al. 1992, Formicki et al. 2004a, Formicki 2008). Among other processes, magnetic fields affect water uptake by fish eggs (Winnicki et al. 1992, Sadowski et al. 2007), the motility of developing embryos (Winnicki et al. 2004), embryonic respiration (Perkowski and Formicki 1997, Formicki et al. 1998), and the spatial orientation of embryos and larvae of various fish species (Formicki et al. 1997, 2004b, Tański et al. 2005). Our earlier studies on Danube huchen spermatozoa showed that exposure to static magnetic fields prolonged sperm motility (Formicki et al. 1990), but since these experiments were conducted before computer-assisted sperm analysis system (CASA) had became available, sperm motility assessment was done optically under a microscope. The ultrastructure of Danube huchen spermatozoa was also examined using transmission electron microscope (TEM) in the Department of Hydrobiology, Ichthyology and Biotechnology of Reproduction (Radziun and Tomasik 1985).

To the best of our knowledge, there are no data on the motility parameters of Danube huchen spermatozoa with CASA, as there are no scanning electron microscope (SEM) images of spermatozoa.

Thus, the aim of the current study was to asses objectively the effect of static magnetic fields on Danube huchen spermatozoa motility with CASA after short-term static magnetic field exposure during storage and to determine the effect of spermatozoa magnetic field exposure on fertilization and sperm morphology (SEM).

Material and methods

Gamete collection

The research material comprised sperm from five males (aged 7) measuring a total length (TL) of 70-83 ±0.5 cm, and eggs from three females (aged 7) measuring a TL of 69-100 ±0.5 cm that were obtained during the spawning season on 18 April 2012 from spawners at the Fish Breeding Station (Polish Anglers Association) in Łopuszna, in southern Poland (49°48'N, 20°14'E) close to the border with Slovakia. The fish were not stimulated with gonadotropins or other drugs. After anesthetizing the fish in a Propiscin bath at a concentration of 1 ml dm^{-3} of water for 30-60 s (Siwicki et al. 2013), the sperm and eggs were collected by gentle hand-stripping. The sperm and eggs were not contaminated with blood, feces, or urine. The eggs obtained from females that exhibited good morphology and color were used for the fertilization tests. The sperm and eggs were transported to the isothermal laboratory at the Department of Hydrobiology, Ichthyology and Biotechnology of Reproduction by car and airplane with a total transport time of five hours. The material

from each of the individuals was placed in separate test tubes, and kept in thermoses placed in isothermal containers with cooling inserts providing constant, appropriate temperatures that were identical to the water temperature at the spawning ground (4.0±0.1°C). The sperm movement experiment was performed six hours after collection.

Sperm motility characteristics analysis with CASA

Sperm motility parameters were studied immediately upon arrival at the isothermal laboratory (constant temperature 4.0±0.1°C), and again after 24 hours. Computer assisted sperm analysis (CASA) with Sperm Class Analyzer (SCA) v. 4.0.0. (Microptic S.L. Barcelona, Spain) software was used. Sperm motility was monitored with a camera (Basler A312fc, CCD 1/2'' sensor) coupled with a Nikon Eclipse i50 light microscope (10× negative phase objective). Fifty frames s^{-1} were used for determining spermatozoa motility, which was recorded every 5 s for 1 s until no more movement was observed. The spermatozoa were activated in tap water that had been aerated for three days. The sperm was activated at a dilution ratio of 1:250. The spermatozoa were assessed with CASA immediately after activation for 3 s. After rapid mixing, the dilution was placed immediately in a Makler counting chamber, which comprises a base that holds the chamber with a part onto which sperm sub-samples and cover slips are placed. The chamber, made with a laser technique by Sefi Medical Instruments (Israel) is 10 μm deep, making it possible for the spermatozoa to move freely, while remaining on one plane and not disappearing from the field of vision. Spermatozoa movement was assessed by the same technician using the same chamber, which ensured identical observation conditions from activation until the cessation of spermatozoa movement. Three replicate recordings per sample were analyzed. The following sperm motility parameters were determined: VCL – curvilinear velocity (μm s^{-1}), VSL – straight line velocity (μm s^{-1}), VAP – average sperm velocity (μm s^{-1}), LIN – linear motion (%), STR – motion straightness (%), ALH – amplitude of lateral head displacement (μm), WOB – minimum and maximum sperm oscillation index values (%), BCF – beat cross frequency (Hz), MOT – percentage of motile spermatozoa.

Magnetic field

The sperm from each individual was subjected separately to static magnetic field intensities of 1, 5, and 10 mT for a period of 24 hours. Sham-exposed sperm for controls was kept outside of the range of the magnetic fields. All samples were exposed to the Earth's natural magnetic field.

Sperm fertilization

The sperm (100 μl) was mixed with 200 eggs (a mixture from all the females) by gently stirring in 100 ml of water. After incubation for 10 minutes, the eggs were washed three times with fresh water then transferred to 300 ml containers and incubated in one layer at 4.0±0.1°C in the isothermal laboratory. The fertilization rate was evaluated by counting the percentage of gastrula stage embryos in relation to the total number of eggs used.

Scanning electron microscopy

Suspensions of fixed spermatozoa from each male (4% formalin) placed on cover slips were dehydrated and in a critical point drier and then mounted on aluminum stubs and coated with a gold-palladium alloy (Polaron SC7620 Mini Sputter Coater). The samples were examined in a JEOL JSM 6100 scanning electron microscope (Japan).

Statistical analysis

Statistical analysis was performed with Statistica PL v. 9.0 software. The data were subjected to variance analysis (ANOVA). All analyses were performed at significance levels of 0.05. All values were expressed as mean values with confidence intervals or as mean ± SD.

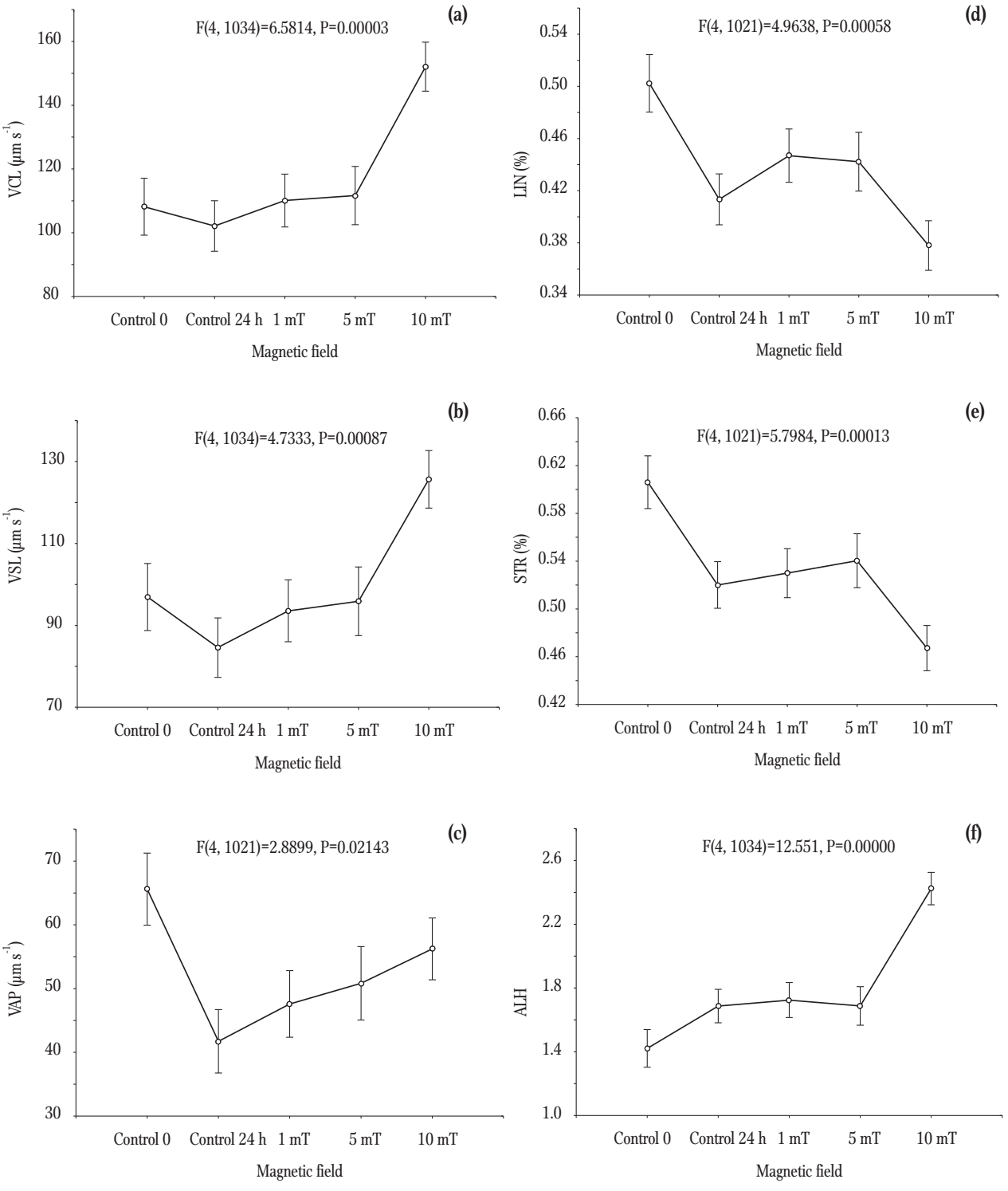

Figure 1. The effect of static magnetic field on: a. curvilinear velocity (VCL); b. average sperm velocity (VAP); c. straight line velocity (VSL); d. linear motion (LIN); e. motion straightness (STR); f. amplitude of lateral head displacement (ALH).

Results

Effect of static magnetic field on sperm motility parameters

The analysis of Danube huchen spermatozoon motility parameters showed that after 24 hours VCL in the control sample decreased slightly and was 102.09 μm s^{-1}, while after the material had been transported it was 108.14 μm s^{-1}. In the experimental variants in which the spermatozoa were subjected to static magnetic fields, the values were higher in comparison to the control. The VCL figures for the samples exposed to 1 mT and 5 mT fields were 110.09 μm s^{-1} and 111.65 μm s^{-1}, respectively. The highest VCL was recorded for a sample that was exposed for 24 hours to a magnetic field of 10 mT at 152.10 μm s^{-1} (Fig. 1a). A tendency similar to that described for VCL was observed for VAP. Higher values were recorded for samples exposed for 24 hours to static magnetic fields; the field of 10 mT (125.67 μm s^{-1}) had the most favorable effect, while after 24 hours the VAP of the control sample decreased from 96.94 μm s^{-1} to 84.58 μm s^{-1} (Fig. 1b). VSL in the control sample after transport was 64.51 μm s^{-1}, while after 24 hours the value of this parameter decreased significantly to 41.55 μm s^{-1}. The samples exposed for 24 hours to static magnetic fields showed increasing VSL values with increasing field values, as follows: in a field of 1 mT, VSL was 46.91 μm s^{-1}; in 5 mT – 50.24 μm s^{-1}; in 10 mT – 55.31 μm s^{-1} (Fig. 1c). LIN and STR values in the control samples decreased after 24 hours from 50.23% to 41.33% and 60.60% to 52.00%, respectively (Figs. 1d-e). The values were similar for samples subjected to magnetic fields of both 1 mT and 5 mT at LIN – 44.69% and 44.22%; STR – 52.99% and 54.03%, respectively, but when the magnetic field value was increased to 10 mT these two parameters decreased at LIN – 37.79% and STR – 46.71%. ALH was the smallest in the control immediately after transport, and increases observed after 24 hours were in direct proportion to the value of the magnetic field (Fig. 1f). No statistically significant differences in WOB were found between the control sperm and that subjected to magnetic fields, as follows: after transport – 76%; after 24 h in control – 73%; field 1 mT – 77%; 5 mT – 76%; 10 mT – 75%). The BCF patterns were similar (after transport – 6.23 Hz; after 24 h in control – 6.48 Hz; field 1 mT – 6.03 Hz; 5 mT – 6.32 Hz; 10 mT – 5.62 Hz). MOT in the control was 51.90% after transport, while after 24h it decreased to 32.90%. After 24 h, the highest MOT in samples subjected to magnetic fields was recorded for 1 mT – 37.10%, 10 mT – 35.90%, and 5 mT – 31.20%. The mean spermatozoon concentration was 4.87×10^{9} ml^{-1}.

Fertilization rate

The fertilization rate in the control after transport was 50.20%, and after 24 h it was 32.60%. The values for spermatozoa kept for 24 h in magnetic fields were as follows: 1 mT – 71.32%; 5 mT – 58.23%; 10 mT – 59.99%.

Morphology of spermatozoa

The mean length of Danube huchen spermatozoa was 27.14 μm, including heads, necks, and tails. The shape of the head is typically salmonid with an elongated shape that is oval on one side (Fig. 2). Spermatozoa from a single male exhibit rather wide head size variation: head length ranged from 2.62 to 2.81 μm and head width from 1.70 to 2.20 μm. The neck is distinct with a mean width of 0.28 μm, and its anterior section is embedded deeply in the characteristic implantation hollow of the posterior section of the head. The tail is long, measuring from 20.81 to 26 μm, and is thinner towards the end, but is lacking in additional structures such as a lateral fin; however, some thickening is observed along its length. Table 1 presents the dimensions of various parts of the spermatozoa.

Discussion

The results of the current study comprise the first published data concerning huchen spermatozoa motility that has been exposed to static magnetic fields

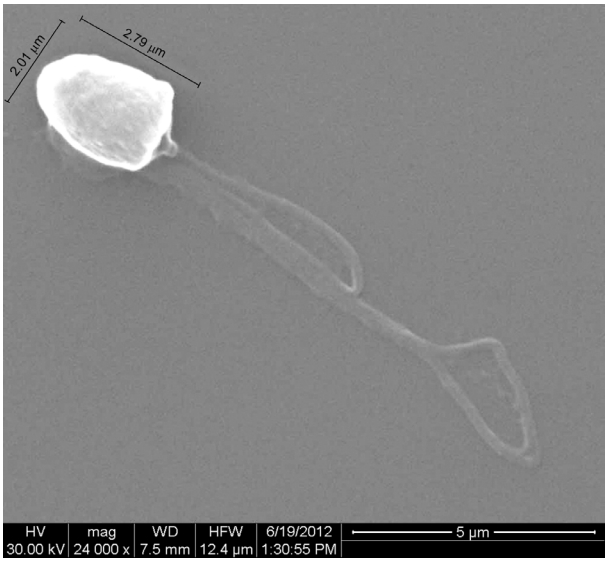

Figure 2. Danube huchen (*Hucho hucho*) spermatozoon, 24,000×.

Table 1
Length, width, and diameter of different structures of Danube huchen (*Hucho hucho*) spermatozoa (n = 12) measured in a mix of sperm from five males using a scanning electron microscope (SEM)

Parameter	mean ± SD
Length of head and neck (µm)	3.25±0.20
Length of head without neck (µm)	2.80±0.19
Head width (µm)	2.03±0.08
Neck width (µm)	0.28±0.12
Tail length (µm)	23.89±0.26
Tail width (µm)	0.19±0.11

and controls determined with CASA. Results concerning fertilization rates and spermatozoon morphology determined with scanning electron microscopy (SEM) are also presented. Fertilization rates are determined by many factors, one of the most important being semen quality, including the motility parameters of spermatozoa (Kime et al. 1996, 2001, Lahnsteiner et al. 1998, Chyb et al. 2000, Gage et al. 2004). Using CASA permits conducting objective, standardized analyses and the unification of sperm parameters. This permits researchers to obtain an objective assessment of spermatozoon motility, including detailed information on speed and movement types. The current experiments on the effect of static magnetic fields on the spermatozoon motility parameters indicate that magnetic fields improve some motility parameters, including one of the most important ones – the velocity of curvilinear movement (VCL). Among fish for which fertilization occurs within about ten to fifteen seconds following spawner gamete release, VCL, i.e., total movement – this parameter describes the actual movement and distance covered by the spermatozoa, is of the greatest consequence for fertilization rates (Lanhsteiner et al. 1998, Gage et al. 2004, Tuset et al. 2008a). In comparison to control spermatozoa, those stored for 24 h in a static magnetic field of 10 mT moved faster by 50.01 µm s^{-1}. Similarly, magnetic fields of 1 and 5 mT increased spermatozoon velocity. As compared to other salmonids, Danube huchen spermatozoa moved at a similar speed though just waster (no activation fluid was used). Water with identical parameters was used later for fertilization and egg incubation. The VCL value range for rainbow trout was 110-115 µm s^{-1} (Dietrich et al. 2005), while for brown trout they were 116.3-120.7 µm s^{-1} (Lanhsteiner et al. 2004) and 140.1 µm s^{-1} (Dietrich et al. 2007). In comparison to the spermatozoa of rheophile cyprinids, that of Danube huchen moved faster than those of *Aspius aspius* (L.), *Chondrostoma nasus* (L.), *Leuciscus idus* (L.), and *Leuciscus leuciscus* (L.). The VCL of these species did not exceed 115 µm s^{-1} (Kowalski et al. 2006, Cejko et al. 2011). In the case of *Cyprinus carpio* L. (170.9 µm s^{-1}; Cejko et al. 2011; 97.10 µm s^{-1}; Dietrich et al. 2007) and *Barbus barbus* (L.) (168.0 µm s^{-1}), VCL values were similar to those of the Danube huchen, while the spermatozoa of *Leuciscus cephalus* (L.) showed a much higher VCL at 229.5 µm s^{-1} (Cejko et al. 2011). The LIN of the Danube huchen spermatozoa exceeded 40%, while among *Salmo trutta* L. it was 78% (Dziewulska et al. 2009). The STR values of the huchen (range 46.0–59.60%) was also lower in comparison to other fish species such as *S. trutta trutta* (STR – 82.7%; Dziewulska et al. 2009), *S. trutta fario* (69.3%; Dziewulska et al. 2011). The ALH values for Danube huchen spermatozoa

(1.42–2.42 μm) were comparable to those of *S. trutta trutta* (1.6 μm; Dziewulska et al. 2009).

In earlier studies, the Danube huchen spermatozoa MOT was 69% (Formicki et al. 1990). Depending on the year in which studies were conducted, MOT was 84% in 2000 and 74% in 2003 (Glogowski et al. 2003). In the present study, MOT ranged from 50 to 71%. The relatively small variation in motility could have resulted from the methods applied since in the earlier studies subjective methods were used. It should be borne in mind that the sperm and egg material was transported to the laboratory, and the differences could have resulted from this.

Spermatozoa concentration, which determines sperm quality, depends on fish species, age, condition, and reproductive behavior. Normal gonadal functioning can be assessed with this parameter (Rurangwa et al. 2004, Dietrich et al. 2008), and after transport it was 4.87×10^9 ml^{-1} in the Danube huchen In the studies by Glogowski et al. (2003) from 2000 and 2003, the concentration was 6.81 $\times10^9$ ml^{-1} (2000) and 5.19 $\times10^9$ ml^{-1} (2003), while in *S. trutta trutta* the mean concentration was $19.5\pm3.9\times10^9$ ml^{-1} (range 10.9-26.6 $\times10^9$ ml^{-1}) (Dziewulska et al. 2009).

Morphological analysis of the huchen spermatozoa shows that the head shape is elongated and oval, which is characteristic of salmonids such as *S. trutta* (Tuset et al. 2008b) and is determined by external factors such as very fast water flow. The large, oval heads of salmonid spermatozoa, in contrast to the round heads of cyprinid or pike, *Esox lucius* L. spermatozoa, pose relatively lesser resistance in the water that decreases the rate of sperm cloud dispersion thus increasing the probability of fertilization. The total spermatozoa size recorded in our experiments is somewhat smaller than that determined with the transmission electron microscope (TEM) (Radziun and Tomasik 1985), which can be explained by individual characters since the size of spermatozoa varies among males, and can also vary among ejaculations. Tuset et al. (2008b) associated spermatozoon length with its motility and the time it took to reach the egg surface. Longer spermatozoa could reach the micropyle before shorter spermatozoa did, but this is only true for sperm expelled close to the micropyle. If expelled at a distance, then shorter sperm executing more curvilinear movements find it easier to circulate an egg and fertilize it. The absence of the lateral fin at the end of the tail, which, for example, is present in pike (Alavi et al. 2009), is associated with different environmental conditions of reproduction, and in rapidly flowing montane streams this produces additional resistance, thus contributing to sperm cloud dispersal before reaching the egg surface.

An explanation for the changes in the motility parameters of the Danube huchen spermatozoa effected by static magnetic fields can be found in changes of spermatozoon membrane permeability. Chains of single-domain magnetite particles are capable of perceiving the direction and intensity of magnetic fields. Magnetite particles are connected by microtubule-like strands with many ion channels in the cell membrane, which, by shifting position, can open and close through the mediation of microtubule-like strand ion channels, which causes changes in the cell membrane potential and membrane permeability (Kirschvink et al. 2001, Walker et al. 2002).

Based on our current results, it can be concluded that short-term semen storage in low intensity static magnetic fields has a positive effect on selected motility parameters, which, in turn, improves fertilization rates. After more extensive studies, this method could prove useful for short-term storage of Danube huchen sperm.

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References

Alavi S.M., Rodina. M, Viveiros A.T., Cosson J., Gela D., Boryshpolets S., Linhart O. 2009 – Effects of osmolality on sperm morphology, motility and flagellar wave parameters in Northern pike (*Esox lucius* L.) – Theriogenology 72: 32-43.

Andrzejewski W. 2000 – Results of an attempt to introduce of the Danube salmon, *Hucho hucho* (L.) into the River Gwda and its tributaries (northwestern Poland) – Acta Hydrobiol. 42: 85-93.

Billard R., Christen R., Cosson M.P., Gatty J.L., Letellier L., Renard P., Saad A. 1986 – Biology of the gametes of some teleost species – Fish Physiol. Biochem. 2: 115-120.

Cejko B.I., Kowalski R.K., Sarosiek B., Judycka S., Głogowski J. 2011 – Parameters of spermatozoon motility of cyprinids – the significance of sperm quality in wild fish artificial reproduction – In: New species in aquaculture - reproduction, fry, prevention (Eds) Z. Zakęś, K. Demska-Zakęś, A. Kowalska, Wyd. IRS, Olsztyn: 211-222 (in Polish).

Chew G.L., Brown G.E. 1989 – Orientation of rainbow trout (*Salmo gairdneri*) in normal and null magnetic fields – Can. J. Zool. 67(3): 641-643.

Chyb J., Kime D.E., Mikołajczyk T., Szczerbik P., Epler P. 2000 – The influence of zinc on sperm motility of common carp - a computer assisted studies – Arch. Pol. Fish. 8: 5-14.

Dietrich G.J., Kowalski R., Wojtczak M., Dobosz K., Goryczko K., Ciereszko A. 2005 – Motility parameters of rainbow trout (*Oncorhynchus mykiss*) spermatozoa in relation to sequential collection of milt, time of *post-mortem* storage and anesthesia – Fish Physiol. Biochem. 31: 1-9.

Dietrich G.J., Żabowska M., Wojtczak M., Słowińska M., Kucharczyk D., Ciereszko A. 2007 – Effects of different surfactants on motility and DNA integrity of brown trout (*Salmo trutta fario*) and common carp (*Cyprinus carpio*) spermatozoa – Reprod. Biol. 7: 127-142.

Dziewulska K., Rzemieniecki A., Domagała J. 2009 – Characteristics of sea trout (*Salmo trutta* m. *trutta*) spermatozoon motility – In: Reproduction, fry, and prevention in salmonids and other species (Eds) Z. Zakęś, K. Demska-Zakęś, A. Kowalska, D. Ulikowski, Wyd. IRS Olsztyn: 93-96 (in Polish).

Dziewulska K., Czerniawski R., Gancarczyk J., Domagała J. 2011 – Biological characteristics of brown trout (*Salmo trutta* m. *fario*) sperm from a local population in the Słupnica River (the buffer zone of the Drawno National Park) – In: New species in aquaculture - reproduction, fry, prevention (Eds) Z. Zakęś, K. Demska-Zakęś, A. Kowalska, Wyd. IRS, Olsztyn: 133-139 (in Polish).

Formicki K. 2008 – Magnetoreception – In: Fish Larval Physiology (Eds.) R.N. Finn, B.G. Kapoor, Science Publisher, USA: 461-491.

Formicki K., Bonisławska M., Jasiński M. 1997 – Spatial orientation of trout (*Salmo trutta* L.) and rainbow trout (*Oncorhynchus mykiss* Walb.) embryos in natural and artificial magnetic field – Acta Ichthyol. Piscat. 27: 29-40.

Formicki K., Perkowski T. 1998 – The effect of a magnetic field on the gas exchange in rainbow trout *Oncorhynchus mykiss* embryos (Salmonidae) – Ital. J. Zool. 65, Suppl.: 475-477.

Formicki K., Sobociński A., Winnicki A., 1990 – Motility of spermatozoa of Danube salmon (*Hucho hucho* L.) exposed to magnetic field prior to activation – Pol. Arch. Hydrobiol. 37(3): 435-443 (in Polish).

Formicki K., Tański A., Sadowski M., Winnicki A. 2004a – Effects of magnetic fields on fyke net performance – J. Appl. Ichthyol. 20: 402-406.

Formicki K., Sadowski M., Tański A., Korzelecka-Orkisz A., Winnicki A. 2004b – Behaviour of trout (*Salmo trutta* L.) larvae and fry in constant magnetic field – J. Appl. Ichthyol. 20: 290-294.

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

Glogowski J., Kowalewski M., Demianowicz W., Dietrich G., Wojtczak M., Kowalski R., Sarosiek B., Ciereszko A. 2003 – Cryoconservation of Danube salmon (*Hucho hucho*) sperm – In: Trout farming – production, environment, prevention – Wyd. IRS Olsztyn: 84-95 (in Polish).

Głowaciński Z. 2001 – Polish Red Book of Animals. Vertebrates – PWRiL, Warszawa, 452 p. (in Polish).

Holčík J. 1990 – Conservation of the huchen, *Hucho hucho* (L.), (Salmonidae) with special reference to Slovakian rivers – J. Fish Biol. 37(Suppl. A): 113-121.

Holčík J., Hensel K., Nieslanik J. Skácel L. 1988 – The Eurasian huchen, *Hucho hucho*, largest salmon of the Word – Dr W. Junk Publisher., Dordrecht – Boston – Lancaster, 239 p.

Kime D.E., Ebrahimi M., Nysten K., Roelants I., Rurangwa E., Moore H.D.M., Ollevier F. 1996 – Use of computer assisted sperm analysis (CASA) for monitoring the effects of pollution on sperm quality of fish; application to the effects of heavy metals – Aquat. Toxicol. 36: 223-237.

Kime D.E., Van Look K.J.W., McAllister B.G., Huyskens G., Rurangwa E., Ollevier F. 2001 – Computer-assisted sperm analysis (CASA) as a tool for monitoring sperm quality in fish. – Comp. Biochem. Physiol. 130: 425-433.

Kirschvink J.L., Walker M.M., Diebel C.E. 2001 – Magnetite-based magnetoreception – Curr. Opin. Neurobiol. 11: 462-467.

Kowalski R., Hliwa P., Andronowska A., Król J., Dietrich G.J., Wojtczak M., Stabiński R., Ciereszko A. 2006 – Semen biology and stimulation of milt production in the European smelt (*Osmerus eperlanus* L.) – Aquaculture 261: 760-770.

Lahnsteiner F., Berger B., Weismann T., Patzner R.A. 1998 – Determination of semen quality of the rainbow trout, *Oncorhynchus mykiss*, by sperm motility, seminal plasma parameters, and spermatozoa metabolism – Aquaculture 163: 163-181.

Lahnsteiner F., Mansour N., Berger B. 2004 – The effect of inorganic and organic pollutants on sperm motility of some freshwater teleost – J. Fish Biol. 65: 1283-1297.

Perkowski T., Formicki K. 1997 – Effects of constant magnetic fields on respiration of rainbow trout (*Oncorhynchus mykiss* Walb.) embryos – Acta Ichthyol. Piscat. 27: 41-56.

Quinn T.P., Groot C. 1983 – Orientation of chum salmon (*Oncorhynchus keta*) after internal and external magnetic field alteration – Can. J. Fish. Aquat. Sci. 40: 1598-1606.

Radziun K., Tomasik L. 1985 – Ultrastructure of *Hucho hucho* (L.) spermatozoa – Acta Ichthyol. Piscat. 15: 129-140.

Rand P. 2012 – Historic Meeting held on the Danube salmon (*Hucho hucho*): The largest salmon on the World – Newsletter of the Freshwater Fish Specialist Group, 2: 19-22.

Rurangwa E., Kime D.E., Ollevier F., Nash J.P. 2004 – The measurement of sperm motility and factors affecting sperm quality in cultured fish – Aquaculture 234: 1-28.

Sadowski M., Winnicki A., Formicki K., Sobociński A., Tański A. 2007 – The effect of magnetic field on permeability of egg shells of salmonid fishes – Acta Ichthyol. Piscat. 37: 129-135.

Siwicki A. K., Kowalewski M., Kazuń K. 2013 – Application of Propiscin - a safe anesthetic for huchen (*Hucho hucho*) culture – Arch. Pol. Fish. 21: 229-231.

Tański A., Formicki K., Korzelecka-Orkisz A., Winnicki A. 2005 – Spatial orientation of fish embryos in magnetic field – Electron. J. Ichthyol. 1: 21-34.

Tesch F.W., Wendt T., Karlsson L. 1992 – Influence of geomagnetism on the activity and orientation of the eel *Anguilla anguilla* L., as evident from laboratory experiments – Ecol. Freshwat. Fish. 1: 52-60.

Tuset V.M., Dietrich G.J, Wojtczak M, Słowińska M., de Monserrat J., Ciereszko A. 2008a –Comparison of three staining techniques for the morphometric study of rainbow trout (*Oncorhynchus mykiss*) spermatozoa – Theriogenology 69: 1033-1038.

Tuset VM., Dietrich G.J., Wojtczak M., Słowińska M., de Monserrat J., Ciereszko A. 2008b – Relationships between morphology, motility and fertilization capacity in rainbow trout (*Oncorhynchus mykiss*) spermatozoa – J. Appl. Ichthyol. 24: 393-397.

Walker M.M., Dennis T.E., Kirschvink J.L. 2002 – The magnetic sense and its use in long-distance navigation by animals – Curr. Opin. Neurobiol. 12: 735-744.

Winnicki A., Formicki K., Sobociński A. 1992 – Water uptake by trout (*Salmo trutta* L.) eggs exposed after activation to magnetic field – Acta Ichthyol. Piscat. 22: 155-161.

Winnicki A., Korzelecka-Orkisz A., Sobociński A., Tański A., Formicki K. 2004 – Effects of the magnetic field on different forms of embryonic locomotor activity of northern pike, *Esox lucius* L. – Acta Ichthyol. Piscat. 34: 193-203.

Witkowski A. 1990 – Threats to the huchen, *Hucho hucho* (L.), in Europe – Chrońmy Przyr. Ojcz. 46: 47-53 (in Polish).

Witkowski A. 1996 – The Huchen, *Hucho hucho* (L.) (Salmonidae) - a species saved for the Polish waters – Zool. Pol. 41 (Suppl.): 131-136.

Witkowski A., Kowalewski M. 1980 – Acclimatization and distribution of huchen in Poland – Gosp. Ryb. 1: 6-9 (in Polish).

Witkowski A., Kowalewski M. 1988 – Danube salmon in Poland - current state and prospects – Gosp. Ryb. 11: 9-11 (in Polish).

Witkowski A., Kowalewski M. 1994 – The Huchen *Hucho hucho* (L.) in Poland – Lindberger Hefte 4: 129-138.

Witkowski A., Goryczko K., Kowalewski M. 2013 – The history of huchen, *Hucho hucho* (L.), in Poland – distribution, restoration and conservation – Arch. Pol. Fish. 21: 161-168.

Zilli L., Schiavone R., Zonno V., Storelli C., Vilella S. 2004 – Adenosine triphosphate concentration and beta-D-glucuronidase activity as indicators of sea bass semen quality – Biol. Reprod. 70: 1679-1684.