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The Effect of Aluminium and Iron-Based Coagulants Used for Lake Recultivation on the Sperm Motility and Fertilisation of the Pike (*Esox lucius* L.)

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ABSTRACT

The objective of the research was to test the effect of iron and aluminium coagulants commonly used for lake re-cultivation, on the pike sperm motility and fertilisation. The coagulants caused changes in the analyzed hydrochemical parameters of the water such as: Fe, Mg^{2+}, TSS, SO_4^{2-} and Cl^-. Selected parameters of sperm motility: MOT, VCL, VSL, VAP, LIN were estimated using CASA. The sperm motility at the moment of activation was the highest in the control sample. With increasing the time of exposure the proportion of sperm motility in the analyzed semen decreased. In the samples with coagulants the values of MOT, VCL and VSL were smaller than in the control sample (statistically significant differences between the control and the samples with PIX and PAX were recorded in the 25^{th}-30^{th} second after activation). The percentage of fertilised eggs was the greatest in the control sample and in the PAX®18 sample (73.11 and 70.42%, respectively), whereas in the PIX®113 it was less than that (54.88%). The shortest larvae were those in the sample with coagulant PIX®113. In the samples with PIX®113 and PAX®18 the survivorship of embryos compared to the control sample was the smallest (43.10 and 51.61%, respectively and control 67.60%) and the proportion of malformed larvae was the highest (34.82 and 24.52%, respectively and control 15.17%).

Keywords: Coagulants, Fertilisation, Lake, Pike, Sperm motility.

INTRODUCTION

Lake eutrophication has a negative effect on water quality which poses a threat to the lake's biodiversity. For this reason various re-cultivation methods are applied in order to improve environmental conditions (Drenner and Hambright, 1999; Grochowska and Brzozowska, 2013; Bidhan *et al.,* 2014). In the last decade chemical compounds have been increasingly used for re-cultivation purposes (Pizarro *et al.,* 1995; Gawrońska *et al.,* 2002; Jančula and Maršálek 2012). In practice the most often used chemicals are iron coagulants of PIX type, for example:

PIX®113 – Water solution of iron(III) sulphate(IV)-$Fe_2(SO_4)_3$,

PIX®111 – Water solution of iron(III) chloride-$FeCl_3$,

PIX®110 – Water solution of iron(III) chlorosulphate-$FeClSO_4$.

The second group of compounds includes aluminium coagulants of PAX type (PAC – polyaluminium Chloride), for example PAX®18 (water solution of polyaluminium chloride), or PAX®25 (water solution of polyaluminium chloride and iron (II) chloride).

The re-cultivation method consists in calculating the doses of coagulants for the reservoir and spreading it on the water

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surface with sprinklers or adding it directly to the bottom deposits (Gawrońska and Brzozowska, 2002; Brzozowska and Gawrońska, 2006; Gawrońska *et al.,* 2002; Tandyrak, 2002; Jančula and Maršálek, 2012). The re-cultivation process using coagulants is practiced in shallow polymictic lakes in spring and early summer (April, May, June), and in autumn in deeper lakes (September, October). It is one of the cheapest re-cultivation methods used in many water bodies worldwide, mainly in small and shallow lakes and, in case of larger water bodies, in a part of the lake as in Lake Annabessacook (USA, 574 ha in area) and Lake Delavan (USA, 720 ha). In Poland inactivation of phosphorus in the water using coagulants was performed in lakes Starodworskie, Długie and Głęboczek (Gawrońska *et al.,* 2002; Tandyrak, 2002; Brzozowska and Gawrońska, 2006).

The coagulants bind phosphates and organic compounds into aggregates which increase their size and settle in the bottom deposits. Chemical precipitation of phosphates in lake water with the use of coagulants decreases the quantity of biogenic compounds, thus limiting the intensity of algal development, and in turn increasing, among others, water transparency, or changing water pH (Ito *et al.,* 2000, Łopata *et al.,* 2007; Piasecki and Zacharzewski, 2010, Jančula and Maršálek, 2012).

As shown by few studies, coagulant-induced changes of lake water parameters may have a negative effect on the plankton, ichthyofauna and benthos (Lelková *et al.*, 2008; Macova *et al*., 2009; Bachand *et al*., 2010; Lewicka-Rataj *et al*., 2014). Studies on the effect of PIX®113 and PAX®18 coagulants added to the water following fertilisation of pike *Esox lucius* (Linnaeus, 1758) eggs and at selected stages of embryogenesis have shown a delay of embryogenesis, decrease in embryo survivorship and increased rate of body deformations in hatching larvae (Bonisławska *et al*., 2012; Tański *et al*., 2013).

Proportion of sperm motility in fish milt determines its quality which is crucial for fertilisation (Billard, 1978; Stoss, 1983). Spermatozoa of most fish species are immobile in the semen plasma. They acquire motility during activation (following contact with water). The sperm motility is triggered by various environmental factors, for example for salmonid fishes it is the difference in concentration of potassium ions between the semen plasma and the water, while for freshwater teleost fishes – a decrease in osmotic pressure (Billard, 1986; Gatti *et al*., 1990). Coagulants used in lake re-cultivation modify the chemical properties of the water; it can be suspected that they may affect the sperm motility and, consequently lead to fertilisation and embryonic development.

Our studies were aimed at determining if and to what extent the coagulants PIX®113 and PAX®18, changing the water properties, could affect fertilisation and embryonic development through their direct effect on the sperm motility parameters.

MATERIAL AND METHODS

Study Area

Sperm activation, fertilisation and egg incubation were conducted in water from Lake Przybiernów (Poland, Zachodniopomorskie voivodeship 53° 45′ 11″ N, 14° 45′ 45″ E). Coagulants PIX®113 and PAX®18 were applied at doses of 50.0 mg dm^{-3} (dose commonly used in lake re-cultivation and in laboratory tests) (Macova *et al*., 2009; Bonisławska *et al*., 2012). Physico-chemical properties of the water were determined prior to and 48 hours after application of the tested coagulants (Table 1).

Hydrochemical Analyses

Physico-chemical parameters of the water were determined with the methods

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Table 1. Mean values and Standard Deviation (SD) hydrochemical parameters of water from Lake Przybiernów (control) and hydrochemical parameters of water 48 h after applying coagulants (PIX®113 and PAX®118) and decrease or increase (in %) compared to values before addition of coagulants.[a]

Sample	Indicator	pH	Alcal	TH	Ca^{2+}	Mg^{2+}	TSS	SSC
			mval dm^{-3}			mg dm^{-3}		cm^3 dm^{-3}
Control		7.5^{b}±0.03	3.6^{b}±0.1	4.6^{b}±0.24	78.2^{a}±3.9	10.2^{a}±0.9	10.0^{a}±1.0	0.0^{a}
PIX®113		7.3^{a}±0.08	3.5^{b}±0.1	4.2^{ab}±0.1	76.2^{a}±1.8	14.6^{b}±1.1	12.0^{a}±1.0	3.0^{b}±0.2
PAX®18		7.4^{ab}±0.10	3.3^{a}±0.05	4.0^{a}±0.2	72.2^{a}±1.0	13.4^{b}±0.5	16.0^{b}±1.0	8.0^{c}±0.2
Reduction before addition %	PIX®113	2.7	2.8	3.4	2.6			
	PAX®18	1.3	8.3	8.0	7.7			
Increase before addition %	PIX®113					42.7	20	100
	PAX®18					30.8	60	100

Sample	Indicator	TRP	TOP	TP	CODCr	Cl-	Fe	SO_4^{2-}
			mgP dm^{-3}			mg dm^{-3}		
Control		0.056^{b}±0.004	0.059^{c}±0.008	0.115^{b}±0.012	27.4^{a}±1.6	24.8^{a}±1.3	0.158^{b}±0.013	48.0^{b}±2.1
PIX®113		0.025^{a}±0.010	0.020^{a}±0.003	0.045^{a}±0.007	24.1^{a}±1.9	26.3^{a}±1.1	0.335^{c}±0.035	53.6^{c}±1.5
PAX®18		0.027^{a}±0.004	0.036^{b}±0.007	0.063^{a}±0.003	25.8^{a}±0.8	39.1^{b}±1.0	0.041^{a}±0.003	40.2^{a}±1.6
Reduction before addition %	PIX®113	55.4	66.1	60.9	12.2			
	PAX®18	51.8	39.0	45.2	6.0		74.1	16.4
Increase before addition %	PIX®113					5.7	112.0	11.7
	PAX®18					57.1		

[a] Alcal: Alcalinity; TH: Total Hardness; TSS: Total Suspended Solids, SSC: Suspended Sediment Concentration. (ANOVA P< 0.05) (Mean values marked with identical superscripts are not significantly different at $P< 0.05$, Duncan's multiple range test).

recommended by APHA (1999). Total alkalinity (titration 0.1N hydrochloric acid against methyl orange), chloride ions (Mohr method: Titration 0.05N $AgNO_3$ against K_2CrO_4 as indicator), total hardness, calcium and magnesium ions (complexometric methods with 0.01N disodium versenate against eriochrome black T and murexide) were determined with titration methods. Chemical Oxygen Demand (COD_{Cr}) was determined with potassium dichromate; the sample was mineralised with sulphuric acid, followed by titration with 0.01N iron-ammonium sulphate against ferroin. Total Suspended Solids (TSSs) were determined by weight: the sample was filtered with glass filter GF/C (produced by Whatman) and air-dried to constant mass at 104±2°C. Easily Settling Suspension (SSC) was determined with volume method using the Imhoff funnel.

Acidity was measured with CP-103 pH-meter produced by Elmetron and conductivity with conductometer CC-101 produced by Elmetron.

The remaining parameters were determined colorimetrically, using spectrophotometer UV-VIS Spectroquant Pharo 300 produced by Merck, measuring absorbance at recommended wave lengths (λ). Total Reactive Phosphorus (TRP) was determined using the method with ammonium molybdate and ascorbic acid as reducer (λ= 882 nm). Total Phosphorus (TP) was determined as phosphorus reacting with potassium persulfate in acid environment following earlier mineralisation. Total Organic Phosphorus (TOP) was calculated from the difference of TP and TRP. Sulphates were determined with barium chloride (λ= 420 nm); the samples were diluted to concentration not exceeding 10 mg dm-3. Total iron was determined with phenanthroline with prior reduction of Fe (III) to Fe (II) using hydroxylamine (λ= 510 nm).

Physico-chemical data of the tested coagulants (according to manufacturer's specification) were: PIX®113 ca. 40-42% water solution with iron (III) sulphate (IV) containing 11.8±0.4% SEM total iron and up to 1% free sulphuric acid; PAX®18 water solution of polyaluminium chloride containing 17.0±0.6% SEM, Al_2O_3 and 20.0±2.0% SEM chloride ions.

Obtaining and Transport of Gametes

Gametes were obtained from adult pike spawners caught in Lake Przybiernów. Eggs were collected from 4 females (mean length 58.5 cm±2.88 SD, mass 1.8 kg±0.41 SD), sperm from 10 males (mean length 56.2 cm ±3.70 SD, mass 1.3 kg±0.28 SD).

Milt collected using syringe with silicone catheters, was placed separately in vials, while eggs (from 4 females) were kept in thermoses of 0.5 dm^3 capacity. The vials and thermoses were then placed in isothermic containers with cooling insets which ensured constant, adequate temperature of 7.0±0.1°C during transport. The duration of transport was 50 minutes.

Sperm Motility

Sperm motility parameters were determined using Computer Assisted Sperm Analysis (CASA) with computer system for sperm motility analysis–SCA (Sperm Class Analyzer ver. 4.0.0, Microptic SL) software. Sperm motility was monitored with a camera (Basler A312fc) coupled with Nikon Eclipse 50i light microscope (10× Negative phase objective).

Mixture of milt and activation liquid (as 1:250) of 5 µl volume was placed in Makler chamber (Sefi – Medical Instruments, Israel). The chamber, made with laser technique, is 10 µm deep, due to which the sperm can move freely during the analysis but are prevented from vertical movements and from disappearing from the field of vision.

The activating liquid was water from the lake without and with addition of coagulants PIX®113 and PAX®18 applied 48 hours earlier. The time between the sperm

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activation and the beginning of the analysis was 3 seconds.

Sperm motility was monitored every 5 seconds (sample 2 after 5 seconds, sample 3 after 10 seconds, etc. until cessation of movement). The analysis of the parameters which are the most important from the point of view of fertilisation: *VCL*– Curvilinear Velocity (μm s^{-1}), *VSL*– Straight-Line Velocity (μm s^{-1}), *VAP*- Average-Path sperm Velocity (μm s^{-1}), *LIN*– Linear motion (%) (VSL/VCL×100), MOT (Motility)– proportion of motile spermatozoa was conducted on each sample (using SCA software) from the moment of activation during 1 second (50 film frames) at 5 seconds intervals, till cessation of movement i.e. 1 second – analysis (50 film frames).

The samples were analysed by the same person and using the same equipment to ensure identical conditions of observation of the spermatozoa from their activation till cessation of movement. Each sample was analysed thrice. Sperm motility assessment was measured at 7.0±0.1°C.

Fertilisation Techniques

Fertilisation was conducted in laboratory with the "dry method", using water from Lake Przybiernów without additions as activation liquid (control) or with addition of coagulants PIX®113 and PAX®18 at the dose of 50.0 mg dm^{-3}. Mixture of eggs from 4 females and sperm from 10 males was used for fertilisation. Eggs were incubated in aquaria of 40 dm^3 capacity. The water in aquaria was aerated, and its temperature was 14.0±0.5°C. Dead eggs were counted and removed daily.

Fertilisation was performed in the laboratory using "dry" method. Mixture of eggs obtained from 4 females (ca. 900 eggs from each) was divided among three dry containers (capacity 1,000 ml) and mixture of milt from 10 males (ca. 100 μl from each) was added to each container, as well as 500 ml of water: from Lake Przybiernów with no additions (control sample) and water with coagulants PIX®113 or PAX®18. After 15 minutes the eggs were rinsed twice with the above-mentioned water. Then the eggs (in equal parts) were delicately placed in 9 aquaria, each of 40 dm3 capacity (three replicates of each experimental variant). The water in the aquaria was aerated, the temperature was 14.0±0.5°C. Whitened eggs were removed and counted daily. The aquaria were kept in the laboratory where the light conditions were similar to those in the spawning ground – no artificial lighting was used. The large volume of water in the aquaria (40 dm^3), considering the small number of eggs, ensured adequate conditions for the development of pike eggs (6 days), without necessity of water exchange.

Eggs and Larvae Morphometrics

Developing embryos were observed in vivo during the experiment. Eggs which had absorbed water were photographed using software NIS Elements Br (20 eggs from each variant), and their diameter was measured. Egg Volume (V) was calculated with the formula:

$V = 4/3\ \pi r^3$ (mm^3)

Yolk spheres inside the eggs were analysed in the same way.

In the last stage of the experiment pike larvae (20 larvae from each variant) were photographed and their total length (*longitudo totalis*– lt) was measured using software MultiScan Base v. 13.01. The volume of yolk sac was calculated using the formula for the Volume of prolate spheroid (V_e) (Blaxter and Hemple 1963):

$V_e = \pi/6\ lh^2$ (mm^3)

Where, *l*: length of yolk sac (mm), *h*: Height of yolk sac (mm).

Analysis of the Course of Embryogenesis

The duration of embryogenesis was expressed in Degree-Day (DD) – (product of

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the number of days of embryogenesis and the mean daily temperature). The fertilisation rate was determined at the stage of blastopore closing in a sample of 100 eggs. Survivorship was assessed after hatching. It was expressed as the percentage of hatched larvae in a group of 100 fertilised eggs (selected at the stage of blastopore closing). The proportion of deformed larvae was the percentage of such larvae among all the hatched ones for each variant.

Statistical Analysis

The results were statistically analysed using Statistica® 9.0 PL, with univariate variance analysis (ANOVA, P< 0.05) and Duncan's test (P< 0.05) for comparisons of the studied hydrochemical parameters, sperm motility parameters (during total time of 45 seconds), egg size, body length and yolk sac volume in pike larvae from eggs incubated in the control sample and in the water with addition of coagulants PIX®113 and PAX®18.

RESULTS

Hydrochemical Conditions

Adding coagulants into the water caused changes in the values of the analysed hydrochemical parameters. ANOVA variance analysis with post-hoc Duncan's test showed that at the significance level of *P*< 0.05, the observed differences between the control sample and the samples with the two coagulants were statistically significant for TRP (Control–PIX®113 and Control–PAX®18 P= 0.002, P= 0.004 respectively), TOP (Control–PIX®113 and Control–PAX®18 P= 0.001,P= 0.008 respectively), TP (Control–PIX®113 and Control–PAX®18 P= 0.000, P= 0.000 respectively), Fe (Control–PIX®113 and Control–PAX®18 P= 0.000, P= 0.001 respectively), SO_4^{2-} (Control–PIX®113 and Control–PAX®18 P= 0.018, P= 0.004 respectively), Mg^{2+} (Control–PIX®113 and Control–PAX®18 P= 0.002, P= 0.010 respectively) and SSC (Control–PIX®113 and Control–PAX®18 P= 0.000, P= 0.000 respectively). For pH the only statistically significant difference was between the control sample and the sample with PIX®113 (P= 0.031), while for alkalinity (P= 0.025), TH (P= 0.016), TSS (P= 0.001) and Cl^- (P= 0.000) significant differences were observed between the control sample and the sample with PAX®18. In the case of COD_{Cr} and Ca^{2+} the observed differences between the control sample and the samples with the two coagulants were statistically insignificant (COD_{Cr}: Control–PIX®113 and Control–PAX®18 P= 0.088, P= 0.439 respectively; Ca^{2+}: Control–PIX®113 and Control–PAX®18 P= 0.652, P= 0.066 respectively) (Table 1).

For pH, alkalinity, total hardness, Ca^{2+} and COD_{Cr} the decrease was small and ranged from 1.3% (pH following application of PAX®18) to 12.2% (for COD_{Cr} following application of PIX®113). The decrease in TRP, TOP and TP was greater and ranged from 39.0 to 66.1%. Application of the tested coagulants caused formation of easily Settling Suspension (SSC) which was not observed in the lake water. The mean SSC volume 48 h after application of PIX ®113 and PAX®18 was 3.0 and 8.0 cm^3 dm^{-3}, respectively (Table 1).

The values of Mg^{2+}, total suspended solids and Cl^- increased after application of the two coagulants. The concentration of chloride ions increased by 5.7% after application of coagulant PIX®113 and by 57.1% after application of PAX®18. In the case of Mg^{2+} application of PIX®113 and PAX®18 caused an increase in the concentration by 42.7 and 30.8%, respectively, and for TSS by 20.0 and 60.0%, respectively (Table 1).

The concentration of Fe and SO_4^{2-} increased or decreased, depending on the tested coagulant. Addition of coagulant PAX®18 decreased the concentration of Fe by 74.1% and SO_4^{2-} by 16.4%. Coagulant PIX®113 caused an increase in Fe

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concentration by 112.0% and SO_4^{2-} concentration by 11.7% (Table 1).

Sperm Motility

The values of selected motility parameters decreased with time, after activation they were smaller in the samples treated with coagulants PIX®113 and PAX®18 than in the control group [Figure 1 (a-e)].

The proportion of Motile spermatozoa (MOT) at the moment of activation was the greatest (52.20 ±15.35 %) in the control; the respective values for the PIX®113 and PAX®18 samples were 36.02±18.38% and 35.16±13.29% (Figure 1-e). Starting with the 30th second after activation the differences in MOT between the control sample and the coagulant samples were statistically significant (Figure 1-a). With the time of exposure the proportion of Motile sperm (MOT) in the 45th second was ten times decreased in the control sample, twenty-five times decreased in the PIX®113 sample and forty times in the PAX®18 sample (Figure 1-a).

In the control sample Curvilinear Velocity (VCL) in the 3rd second of activation was 77.18±16.75 µm s^{-1}, and in the 45th second it decreased to 23.16±5.34 µm s^{-1}. In the coagulant-treated water VCL was smaller immediately after activation: 72.55±18.94 and 71.57±13.37 µm s^{-1} for PIX®113 and PAX®18, respectively (the differences not statistically significant). In the 25th second after activation the differences in *VCL* between the control and the coagulant samples were statistically significant. After 45 seconds *VCL* dropped to 16.09±4.44 and 14.05±5.11 µm s^{-1} for PIX®113 and PAX®18, respectively (Figure 1-b).

The mean Straight-Line Velocity (VSL) in the 3rd second of activation in the control sample was 46.29±10.82 µm s^{-1}, and in the PIX®113 and PAX®18 samples it was 34.53±6.72 and 31.95±8.61 µm s^{-1}, respectively (P> 0.05; not statistically significant). In the 25th second the mean *VSL* in the control sample was 19.32±8.15 µm s^{-1}, in the sample with PIX®113 8.12±3.00 and PAX®18 9.67±3.51 µm s^{-1}. In the 45th second the mean *VSL* was the smallest in the PAX®18 sample: 1.03±0.12 µm s^{-1} (Figure 1-c).

The greatest mean sperm Velocity (VAP) was recorded in the 3rd second of activation in the control sample – 64.84±16.84 µm s^{-1}. At the same time it was smaller for the coagulant-treated samples: PIX®113 60.86±17.79 µm s^{-1} and PAX®18 49.44±16.85 µm s^{-1} (P> 0.05). With time *VAP* decreased in all the samples. The smallest *VAP* values were recorded in the PIX®113 and PAX® samples in the 45th second: 5.97±1.85 and 7.14±2.02 µm s^{-1}, respectively (Figure 1-d)

The mean Linear motion (LIN) was also smaller in the coagulant-treated water compared to the control. Statistical analysis showed significant differences in *LIN* between the control sample and the coagulant samples in the 3rd, 5th and 10th second after activation [Figure 1 (b-d)]. In the 35th second from activation *LIN* dropped very rapidly in the PIX®113 sample to ca. 9.01±3.5% compared to the control where the mean *LIN* was four times greater: 44.55±2.70% (statistically significant difference) (Figure 1-e).

Characteristics of Pike Eggs and Larvae, Embryogenesis

The results of measurements of pike eggs and their yolk spheres showed no significant differences (P> 0.05) between the eggs from the control sample and those from the coagulant-treated samples; it pertained to the diameter of both eggs and yolk spheres and thus to their volume (Table 2).

The proportion of fertilised eggs was the greatest in the control sample and in the PAX®18 sample; it was 73.11 and 70.42%, respectively. In the PIX®13 sample it was 54.88% (Table 2).

Continuous in vivo observation of embryogenesis revealed no differences in the rate of embryonic development among

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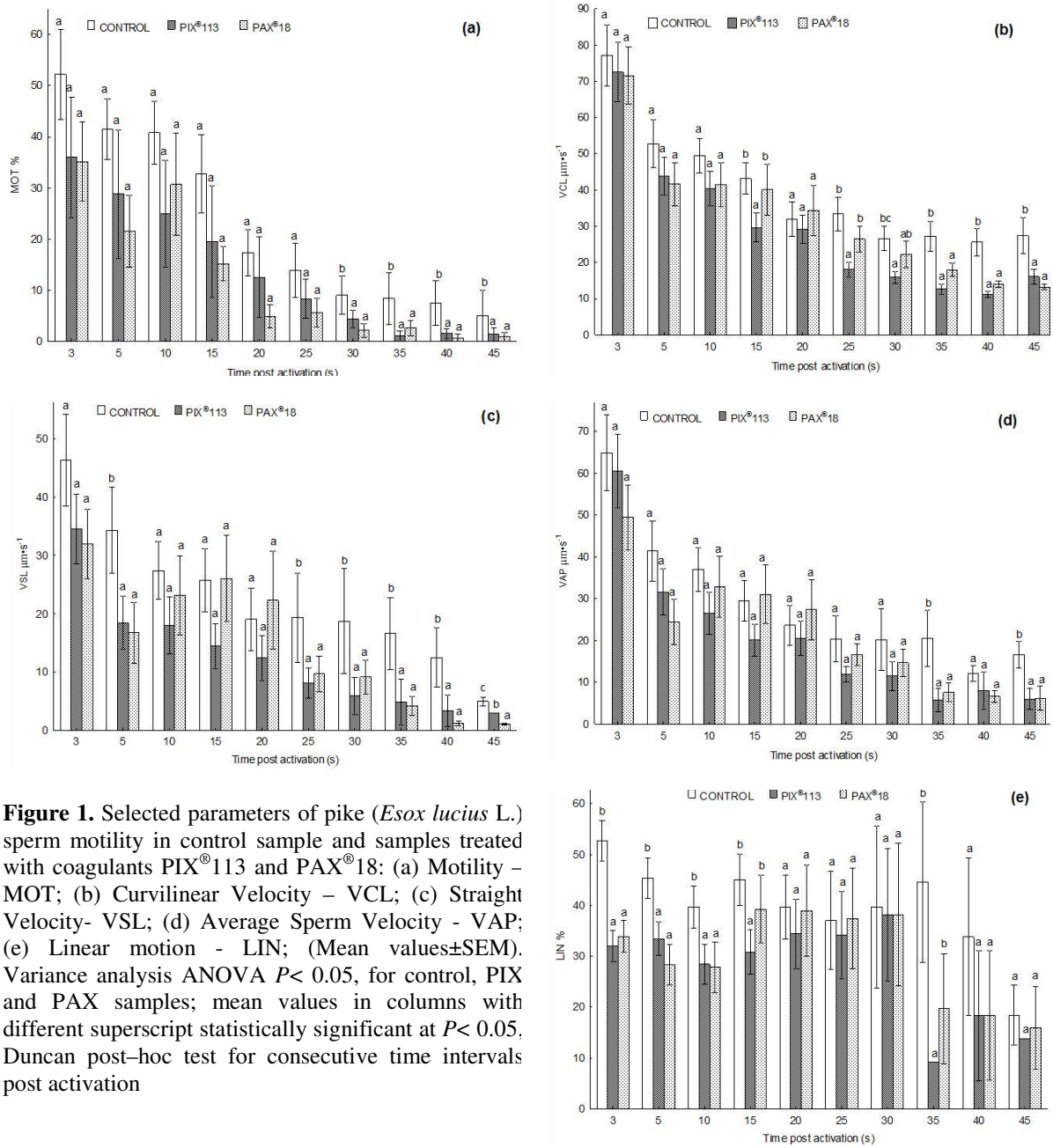

Figure 1. Selected parameters of pike (*Esox lucius* L.) sperm motility in control sample and samples treated with coagulants PIX®113 and PAX®18: (a) Motility – MOT; (b) Curvilinear Velocity – VCL; (c) Straight Velocity- VSL; (d) Average Sperm Velocity - VAP; (e) Linear motion - LIN; (Mean values±SEM). Variance analysis ANOVA $P< 0.05$, for control, PIX and PAX samples; mean values in columns with different superscript statistically significant at $P< 0.05$, Duncan post–hoc test for consecutive time intervals post activation

the studied variants. In the control and coagulant-treated samples the embryos reached consecutive stages of development at the same number of degree-days.

Advanced cleavage –small-cell morula– was observed on 10,0 DD of embryogenesis in all the samples. Yolk sphere epiboly (blastopore closure) was completed on 30 DD, and gastrulation started. On 47 DD delicate pigments appeared in the embryos' eyes, and on 62 DD slow heartbeats started. Hatching in all the samples started simultaneously after 6 days (90 DD) and lasted 24 hours.

The larvae hatched in the control sample and in the PAX®18 samples they were the

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longest (Table 3). Their mean total length was 9.20 mm and 9.17 mm, respectively. The larvae from the PIX®113 samples were shorter (8.91 mm), and their yolk sac had the greatest volume (6.25 mm^3) ($P< 0.05$) (Table 3).

The most numerous deformations were observed in the larvae from the samples treated with coagulant PIX®113 (34.82%). They varied, and included mainly body deformations: C-shaped larvae and axial and lateral spine curvature. In that sample survivorship of the embryos was the smallest and amounted to 43.10% (Table 3).

DISCUSSION

Hydrochemical analyses of the water from Lake Przybiernów are in agreement with the studies by Tański *et al.* (2012) and WIOŚ in 2013. Hydrochemical parameters of the water used in the experiments classify it as acidification-resistant (alkalinity of 3.8 mval dm^{-3}), while nitrogen and phosphorus concentrations are characteristics of water bodies from the boundary of eutrophic and hypertrophic, which was also shown for the lake's water in earlier studies (Bonisławska *et al*., 2012; Tański *et al.,* 2013). However, because of the poor condition of aquatic vegetation (macrophytes), the absence of underwater meadows and the algal blooms (especially intensive in August) Lake Przybiernowskie was classified as ecological class V (WIOŚ, 2014).

The observed qualitative changes in the water following addition of the tested coagulants were typical, dependent on the precipitation processes under the effect of component compounds of the coagulants. The solutions of the tested coagulants have low pH, and thus decrease the water's pH. However, because of the high alkalinity of the water, the pH decrease under the effect of coagulants was relatively small. The tested coagulants acidify the aquatic environment to a lesser extent compared to other alternative precipitating substances (Ito *et al*., 2000; Konieczny *et al*., 2007;

Table 2. Characteristics of pike (*Esox lucius* L.) eggs from control and coagulant-treated samples.[a]

Dimension	Treatment		
	Control	PIX®113	PAX®18
Egg diameter (mm)	2.74^{a}±0.06	2.70^{a}±0.07	2.74^{a}±0.05
Yolk sphere diameter (mm)	2.28^{a}±0.08	2.31^{a}±0.08	2.30^{a}±0.10
Egg volume (mm^3)	10.75^{a}±0.70	10.32^{a}±0.78	10.85^{a}±0,59
Yolk sphere volume (mm^3)	6.24^{a}±0.69	6.47^{a}±0.64	6.46^{a}±0.98
Fertilisation success (%)	73.11^{b}±0.44	54.88^{a}±0.46	70.42^{b}±0.50
Number of incubated eggs	1086	1123	1488

[a] Mean values and Standard Deviation (SD) are given in the table. Variance analysis ANOVA $P< 0.05$; for each sample mean values in rows marked with different superscript are statistically significantly different at $P< 0.05$; Duncan post–hoc test.

Table 3. Characteristics of pike (*Esox lucius* L.) larvae from control sample, PIX®113 and PAX®18 samples.[a]

	Control	PIX®113	PAX®18
Total length (mm)	9.20^{b} ±0.32	8.91^{a} ±0.42	9.17^{b}±0.37
Yolk sac volume (mm^3)	5.64^{a} ±0.47	6.25^{b} ±0.68	5.53^{a} ±0.50
Survivorship %	67.60^{b}±0.47	43.10^{a}±0.49	51.61^{a}±0.50
Malformed larvae %	15.17^{a}±0.19	34.82^{b}±0.44	24.52^{b}±0.40

[a] Mean values and Standard Deviation (SD) are given in the table. Variance analysis ANOVA $P< 0.05$; for each sample mean values in rows marked with different superscript are statistically significantly different at $P< 0.05$; Duncan post–hoc test.

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Łopata *et al*., 2007; Piasecki and Zacharzewski, 2010). The use of coagulants affects the precipitation of the total, easily precipitating suspension, and reduces concentration of various forms of phosphorus, which is advisable in lake re-cultivation. High reduction rates apply mainly to phosphorus compounds (analogously to our studies), for example Gawrońska *et al*. (2002), besides decrease in organic matter content, showed a more than twofold decrease in phosphorus concentration under the effect of coagulants; this was mainly associated with reduction of reactive phosphorus. Application of coagulants may also increase concentration of their component salts. In the case of PIX®113 increase in concentration of iron and sulphate ions was observed, and for PAX®18 – increase in concentration of chloride ions; similar changes in those concentrations were recorded by Tański *et al*. (2013).

Spermatozoa of teleost fishes are released directly into the water and thus exposed to dangers which are associated with changes in the water's chemical composition. Our studies showed that the use of coagulants in order to reduce the content of biogenic substances in the water had an unfavourable effect on the studied parameters of pike sperm motility and, consequently, on the fertilisation and embryogenesis. The end result was a decrease in successful hatching and an increase in the proportion of deformed larvae. Fish sperm motility is known to be affected by such environmental factors as temperature, concentration of univalent and bivalent ions, osmolality, pH, or magnetic field (Cosson *et al*., 1999; Alavi and Cosson 2005; 2006; Dietrich *et al*., 2007, Alavi *et al*., 2009; Ciereszko *et al*., 2010; Formicki *et al*., 2013; Dziewulska *et al*., 2013). It has been shown that also pollution and toxic substances (including heavy metals and pesticides) in the water affect sperm motility parameters (Abascal *et al*., 2007; Singh *et al*., 2008; Dietrich *et al*., 2010; Zhi-Hua *et al*., 2010; Kalbassi *et al*., 2014).

In our studies the water treated with PIX®113 showed a very large increase in the content of Fe – 112.0% and a smaller increase in Mg^{2+}– 42.7% and TSS– 20%, SO_4^{2-}– 11.7% and Cl^-- 5.7%. Only three concentrations increased in the water treated with PAX®18: TSS – 60.0%, Mg^{2+}– 30.8 and Cl^-–57.1% (Table 1). The increase in the values of these parameters might be the reason for the deterioration of the sperm motility parameters in the case of both coagulants, but mainly PIX®113.

The increased values of the studied parameters probably had a negative effect on the proportion of fertilised eggs and survivorships of the larvae among which the percentage of malformations was the highest.

The effect may be explained by the fact that the viability and motility of fish sperm depend on many factors, among others on the adequate concentration of univalent and bivalent ions. Increased concentration of such can by ions as K^+, Na^+, Ca^{+2}, Mg^{+2} decreases the percentage of motile spermatozoa (Cosson, 2004; Alavi and Cosson, 2006; Dietrich *et al.*, 2010; Dziewulska and Domagała, 2013). Na^+, K^+, Cl^- oraz Ca^{+2}, Mg^{+2} ions prevail in fish seminal plasma (Alavi and Cosson 2006). Some of them are responsible for initiation of sperm motility (e.g. K^+ in rainbow trout), which proceeds normally when the ion concentration in the water is adequate compared to that concentration in the semen plasma (Billard, 1978; Bondarenko *et al*., 2014). The increased values of Mg^{+2} and Cl^- (in case of PIX®113 and PAX®18) and SO_4^{2-} ions (in case of PIX®113) in the water with coagulants may have caused disturbances during fertilisation and embryonic development of the pike. Earlier research by Eddy and Talbot (1983) showed that the increase in the concentration of bivalent ions Ca^{+2}, Mg^{2+}, SO_4^{2-} in the water may also negatively affect the process of formation of PeriVitelline Space (PVS) in fish ovaries, resulting in disturbances of embryonic development.

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The 112% Fe content in the water with PIX®113 recorded in our studies may also have caused deleterious changes in the sperm motility parameters and disturbances in the embryonic development. Increased iron content in the water can increase oxygen consumption and thus affect biological life. In oxygen conditions bivalent iron easily oxidises to trivalent iron which precipitates as iron hydroxide (III) or iron oxides (III). Iron concentration exceeding 0.35 mg dm^{-3} can be dangerous to fishes since the hydroxides, forming colloidal suspensions, settle on the eggs and decrease oxygen supply to the developing embryos (Szczerbowski, 2008).

Also, the increased TSS concentration in the water with coagulants, through settling on the egg surface, may cause deterioration in oxygen conditions which in turn has a negative effect on the embryonic development (Schubel *et al.*, 1974; Soulsby *et al*., 2001; Bonisławska *et al*., 2011).

Our results are among the few confirmations of the significant effect of application of the tested chemicals on aquatic organisms. Earlier studies on the effect of coagulants PIX®113 and PAX®18 on the growth and mortality of the copepod *Daphnia magna* Straus., have shown that both substances at doses which are commonly used for lake re-cultivation cause a significant decrease in the copepod biomass, and PIX®113 causes an increase in the copepod mortality of up to 24% (Piasecki and Zacharzewski, 2010).

The use of PAX-18 for re-cultivation of natural waters may pose potential threat to organisms because of the presence and accumulation of aluminium. Depending on the water pH, temperature and organic matter content, aluminium may occur in toxic form (Freeman and Everhart 1971; Baker and Schofield 1982; Howells *et al*., 1990). Decrease in water pH, i.e. acidification, increases solubility of aluminium, iron, copper, zinc, nickel, lead and cadmium. The negative effect of acidification on organisms consists in the fact that increased solubility of aluminium is accompanied by production of toxic Al^{3+} ions (Lampert and Sommer, 1996). Negative environmental effects of the use of aluminium coagulants associated with aluminium toxicity may occur at pH below 6.0 (Exley *et al.,* 1996). In our studies the dose of coagulants was 50 mg·dm^{-3} and thus no great decrease in the water pH was observed. It should also be remembered that the lake's water is acidification-resistant and thus probably the toxic form of aluminium which could affect the sperm motility and embryogenesis did not appear in the water. Macova *et al*. (2009) estimated PAX®18 toxicity at various ontogenetic stages of carp *Cyprinus carpio* (Linnaeus, 1758) (developing embryos, larvae) and found no significant effect of PAX®18 at the dose of 50 mg·dm^{-3} on the course of embryonic development.

Other studies, involving juvenile stages (2-3 months old) of *Danio rerio* (Hamilton, 1822), showed that the dose of LC 50 PAX®18 during 96 h was within 737.3 – 783.2 mg dm^{-3}. The value of LC_{50} for the species' embryos expressed as 120 hours LC_{50} was within 645.0-889.1 mg dm^{-3}. The mortality increased with the coagulant concentration and for 1,400 mg dm^{-3} it was ca. 90% (Macova *et al*., 2010). Similarly, Lopus *et al*. (2009) in their studies on the effect of coagulant PAX®XL9 on the fecundity, hatching and mortality of *Oryzias latipes* (Temminck and Schlegel, 1846) observed its negative effect when it was applied in high doses.

In 2011 Bonisławska *et al.* started research on the effect of PIX®113 and PAX®18 on the pike embryogenesis. They added PIX®113 and PAX®18, at doses of 6.25 and 50.0 mg dm^{-3}, to the lake water in which the embryos developed. In the case of PAX®18 at 50.0 mg dm^{-3}, the pike embryogenesis was delayed and the hatching larvae were significantly shorter by more than 0.5 mm than those from the remaining variants (Bonisławska *et al*., 2012). The reason was the quantity of total suspension which, in the variant with PAX®18 at 50.0 mg dm^{-3}, was more than three times higher (32.0 mg dm^{-3})

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than the natural value in the water used in the studies (control variant– 10.0 mg dm^{-3}). They also showed that the hatching success in the water treated with coagulants at various concentrations and in coagulant-free water (control) decreased distinctly with increasing coagulant concentration – 71% in the control sample, 20% in the PAX®18 (50.0 mg dm^{-3}) sample (Bonisławska *et al*., 2012). Subsequent studies, with the use of coagulants only at the dose of 50.0 mg dm^{-3}, indicate their different effects on the developing pike embryos at consecutive development stages. It was observed that with progressing embryogenesis and embryo growth the precipitated aggregates formed under the effect of PIX®113 and PAX®18 caused disturbances to an increasingly lesser extent and thus successful hatching increased (Tański *et al*., 2013). In the samples treated with coagulants at first (gastrulation or formation of germ layers) and second ("eyeing" stage– the pigment appears in the embryo's eyes) stages of embryogenesis, the embryonic development slowed down as a result of impeded gas exchange (Tański *et al.,* 2013).

CONCLUSIONS

Our results indicate that application of coagulants during pike spawning, when sperm and eggs are released into the water, causes a decrease in sperm motility parameters. The effects include decrease in the number of fertilised eggs, in embryos' survivorship, and thus decrease in the number of hatching larvae – but mainly in the variant with PIX®113.

In order to avoid increase in losses during embryonic development of various fish species of spring and summer spawning, the dates of application of coagulants should be delayed in relation to the spawning dates which additionally depend on thermal conditions. Introducing a different form of adding coagulants to the water instead of sprinkling them on the water surface, for example adding them directly to the bottom deposits, may make it possible to avoid the harmful effects on the fish sperm and developing embryos.

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اثر منعقد کنند های پایه آهن استفاده شده در بازکشت (lake recultivation) بر تحرک اسپرم و لقاح(*Esox lucius* L.)pike

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چکیده

هدف از این پژوهش، بررسی اثر منعقدکننده های آهن و آلومینیومی معمول استفاده شده در بازکشت بر تحرک اسپرم و لقاح pike است. منعقد کننده ها منجر به تغییرات در پارامترهای هیدروشیمی تجزیه و تحلیل شده آب مانند: Cl^- ،Fe, Mg^{2+}, TSS, SO_4^{2-} می شود. پارامترهای انتخاب شده تحرک اسپرم: MOT, VCL, VSL, VAP, LIN توسط CASA بررسی شدند. تحرک اسپرم در نمونه کنترل و در لحظه فعال سازی بالاتر بود. با افزایش قرار گرفتن، نسبت تحرک اسپرم در مایع منی تجزیه و تحلیل شده، کاهش یافت. در نمونه با منعقد، مقدار MOT, VCL و VSL کمتر از نمونه شاهد بود (تفاوت معنادار بین شاهد و نمونه های با PIX و PAX در ثانیه های ۲۵ و ۳۰ ثانیه پس از فعال سازی ظبط شد). درصد تخم بارور در نمونه شاهد بیشتر بود و به ترتیب در شاهد و در نمونه $PAX^{®}18$ ۷۳.۱۱ درصد و ۷۰.۴۲ ٪ بود در حالیکه در $PIX^{®}113$، ۵۴.۸درصد بود. کوتاهترین لارو در نمونه های با انعقاد کننده های $PIX^{®}113$ بودند. در نمونه های با $PIX^{®}113$ و $PAX^{®}18$ بقای جنین در مقایسه با شاهد کمتر بود(به ترتیب ۴۳.۱۰ و ۵۱.۶۱درصد و در شاهد ۶۷٪) و درصد لارو ناقص بیشتر بود (به ترتیبب ۳۴.۸۲ ٪ ، ۲۴.۵۲٪ و در شاهد ۱۵.۱۷ ٪).

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