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Address
ul. Jana Heweliusza 14
10-718 Olsztyn-Kortowo, Poland
tel.: (48) (089) 523-36-61
fax: (48) (089) 523-34-38
e-mail: wydawca@uwm.edu.pl

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THE EFFECTS OF VARIED SOIL AND FOLIAR MINERAL FERTILIZATION LEVELS IN THE PRODUCTION OF HIGH-STARCH POTATOES

***Bożena Bogucka*[1], *Bożena Cwalina-Ambroziak*[2], *Tomasz Zięba*[3]**

[1] Department of Agricultural Technology and Plant Production Management
[2] Department of Phytopathology and Entomology
University of Warmia and Mazury in Olsztyn
[3] Department of Agricultural Technology and Food Storage
Wrocław University of Environmental and Life Sciences

K e y w o r d s: qualitative traits, soil fertilization, foliar fertilization, yield, high-starch potatoes.

A b s t r a c t

The objective of this study was to determine the effect of varied (280 and 420 kg NPK ha^{-1}) soil and foliar application of mineral fertilizers (Basfoliar 12-4-6, ADOB Mn, Solubor DF) on the yield and qualitative traits of late-maturing potato variety Jasia. An exact, two-factorial field experiment was conducted by the randomized split-plot method, in four replications, in the years 2004–2006 in the Masurian Lakeland. The total yield of potato tubers, the content and yield of starch, and the grain size fractions of starch were determined in the study. The applied fertilizers, regardless of their rates, had no significant effect on the total yield of potato tubers and starch yield. The starch content of potatoes was affected by soil fertilization. An increase in the rate of mineral fertilizers from 280 to 420 kg NPK ha^{-1} caused a decrease in starch content. Large starch grains (> 40 μm in diameter), which enable to reduce the overall loss during the removal of starch from the potato pulp, dominated in the experimental material.

EFEKTY ZRÓŻNICOWANEGO NAWOŻENIA MINERALNEGO APLIKOWANEGO DOGLEBOWO I DOLISTNIE W UPRAWIE ZIEMNIAKA SKROBIOWEGO

***Bożena Bogucka*[1], *Bożena Cwalina-Ambroziak*[2], *Tomasz Zięba*[3]**

[1] Katedra Agrotechnologii i Zarządzania Produkcją Roślinną
[2] Katedra Fitopatologii i Entomologii
Uniwersytet Warmińsko-Mazurski w Olsztynie
[3] Katedra Technologii Rolnej i Przechowalnictwa
Uniwersytet Przyrodniczy we Wrocławiu

S ł o w a k l u c z o w e: cechy jakościowe, nawożenie doglebowe, nawożenie dolistne, plon, ziemniak skrobiowy.

Address: Bożena Bogucka, University of Warmia and Mazury in Olsztyn, ul. Oczapowskiego 8, 10-719 Olsztyn, Poland phone: +48 (89) 523 34 65, e-mail: bozena.bogucka@uwm.edu.pl

Abstrakt

Eksperyment, którego celem było przetestowanie wpływu zróżnicowanego nawożenia doglebowego 280 i 420 kg NPK ha^{-1} oraz dolistnego nawozami Basfoliar 12-4-6, ADOB Mn i Solubor DF na plonowanie i cechy jakościowe późnej odmiany Jasia, przeprowadzono w latach 2004–2006 na Pojezierzu Mazurskim. Ścisłe doświadczenie polowe dwuczynnikowe założono metodą losowanych podbloków w 4 powtórzeniach. Zakres badań obejmował ocenę plonu ogólnego bulw ziemniaka, określenie zawartości i plonu skrobi oraz ziarnistości skrobi. Zastosowane dawki, jak i nawozy nie wpłynęły istotnie na plon ogólny bulw ziemniaka i plon skrobi. Zawartość skrobi natomiast była uzależniona od nawożenia doglebowego. Wzrost dawki nawożenia mineralnego z 280 do 420 kg NPK ha^{-1} spowodował obniżenie zawartości skrobi. W przebadanym materiale przeważały duże ziarna skrobi o średnicy >40 μm co powodowało, że podczas wymywania skrobi z miazgi ziemniaczanej straty były mniejsze.

Introduction

Each year 2.5 million tons of starch, i.e. approximately 6% of global starch supplies, are obtained from potatoes (LESZCZYŃSKI 2005). According to SZNAJDER and TARANT (2002), potato starch is characterized by a higher quality than wheat and maize starches. For years Poland has been a leading producer of both potatoes and potato starch (SZCZEPANIAK 2005, REMBEZA 2009).

Potatoes used in the starch industry have to supply high amounts of superior-quality starch. The quality of starch is dependent on the genetic characteristics of potatoes and the applied agricultural practices (LESZCZYŃSKI 2002, STYSZKO 2002, GOPAL, KHURANA 2006). The starch content of potatoes is considerably affected by soil mineral fertilization (LESZCZYŃSKI 1994, GĄSIOR, PAŚKO 1998, CIEĆKO et al. 2004). As demonstrated by JABŁOŃSKI (2005a), potatoes use approximately 50 kg ha^{-1} of 30 t ha^{-1} organic fertilizers applied in the fall, while the other required nutrients are supplied by mineral fertilizers. The N:P:K ratio in fertilizers applied to the soil in which commercial potatoes are grown should be 1:1:1.5 at a nitrogen rate below 100 kg ha^{-1}, and 1:1.2:1.3 at a nitrogen rate above 100 kg ha^{-1}. The Polish potato variety Jasia has higher nutrient and nitrogen requirements (140–160 kg N ha^{-1}) (JABŁOŃSKI 2005a). The foliar application of fertilizers seems promising, due to lower total nutrient loss. Potatoes take up large quantities of micronutrients, including manganese and boron. A boron and manganese deficiency is common in alkaline soils, boron availability is additionally limited in dry periods. Boron has a beneficial influence on root system development and stem growth, and manganese enhances the rate of photosynthesis. Boron has a beneficial influence on root system development, stem growth and starch accumulation, while manganese enhances the rate of photosynthesis (GRZEŚKIEWICZ, TRAWCZYŃSKI 1998, HABERLAND 2000, GOPAL, KHURANA 2006, JABŁOŃSKI 2008a).

The objective of this study was to determine the response of potatoes cv. Jasia to different levels of soil and foliar fertilization. The response was evaluated in view of the interactions between fertilizers and changing temperature and moisture conditions.

Materials and Methods

Field investigations were conducted in the years 2004–2006, at the Production and Experimental Station „Bałcyny" Ltd. in Bałcyny (N = 53°35'49'', E = 19°51'20.3''). A long-term two-factorial experiment was carried out by the randomized split-plot method, in four replications, on grey-brown podsolic soil developed from boulder clay (GRUŻEWSKA, MALICKI 2002). The soil, of quality class IIIa and very good rye complex, was characterized by a high abundance of phosphorus, a high to medium abundance of potassium, a medium abundance of magnesium, and a slightly acidic reaction (Table 1). Potatoes cv. Jasia

Table 1
Selected chemical properties of topsoil before the establishment of the experiment in successive years of the study

Year	Acidity		Available nutrients, mg 100 g^{-1} soil		
	pH in 1N KCL	reaction	P_2O_5	K_2O	Mg
2004	5.62	slightly acidic	16.6	20.0	5.6
2005	6.17	slightly acidic	18.0	23.0	6.7
2006	6.06	slightly acidic	19.5	18.5	6.2

(a Polish, late-maturing, high-starch cultivar) were grown in the experiment. The experimental factors were as follows:

I. Soil NPK fertilization at two rates:

A – 280 kg ha^{-1} (80 N, 80 P, 120 K);

B – 420 kg ha^{-1} (120 N, 144 P, 156 K), 120 kg N ha^{-1} was applied at a divided dose: before planting (80 kg N ha^{-1}) and before the closure of inter-rows (80 kg N ha^{-1}).

II. Foliar application of the following fertilizers:

a – Basfoliar 2-4-6 (8 dm ha^{-1})

b – ADOB Mn (4 dm ha^{-1})

c – Solubor DF (2 dm ha^{-1})

d – ADOB Mn + Solubor DF (2 + 1 dm ha^{-1})
e – ADOB Mn + Basfoliar 12-4-6 (2 + 4 dm ha^{-1})
f – Basfoliar 12-4-6 + Solubor DF(4 + 1 dm ha^{-1})
g – Basfoliar 12-4-6 + ADOB Mn + Solubor DF (2.7 + 1.3 + 0.7 dm ha^{-1})
h – control treatment – no foliar fertilization.

Soil fertilizers applied before planting had the form of potash salt (60%) and granulated triple superphosphate (46%). Nitrogen applied before planting and inter-row closure (variant *B* above) had the form of urea (46%). Foliar fertilizers were applied once, at the stage of complete crop cover (BBCH-scale 309).

Potatoes were grown after cereals, following manure application at a rate of 25 t ha^{-1}. Planting was carried out from the middle of April to the first week of May. Potatoes (certified material, class CA) were planted 40 cm apart, at row spacing of 62.5 cm and the density of 40 000 plants per ha.

Appropriate agricultural management practices were applied, paying particular attention to adequate protection of potatoes against diseases and pests. Cultivation measures included hilling and the application, once during the growing season, of a systemic fungicide (Ridomil Gold MZ 68 WG at a dose of 2 kg ha^{-1}), contact fungicides (Penncozeb 80 WP at a dose of 2 kg ha^{-1}, Gwarant 500 SC at a dose of 2 dm^{-3} ha^{-1}), a translaminar fungicide (Pyton 60 WG at a dose of 1.5 kg ha^{-1}) and insecticides (Mospilan 20 SP 80 g ha^{-1} and Actara 25 WG at a dose of 80g ha^{-1}). Dicotyledonous and selected monocotyledonous weeds were controlled with the herbicide Aphalon 450 SC, applied at a dose of 2 dm^{-3} ha^{-1}.

The growing season lasted from 146 days in 2006 to 161 days in 2005. The duration of flowering was similar in all three experimental years: from 17 days in 2006 to 23 days in 2004. Potatoes were harvested in the last week of September or in the first week of October.

The Sielianinow coefficient was determined based on average temperatures and precipitation totals, indicating the dry spells that affected potato yields (Bac et al. 1998). The growing seasons of 2004 and 2006 were humid, with no extreme air temperatures. The year 2005 was characterized by a too low moisture content of soil.

The total yield of potato tubers, the content and yield of starch were determined in the study. Starch content was determined by the gravimetric method proposed by Reimann and Parow. This value, together with the potato tuber yield, provided a basis for calculating starch yield (Czerko et al. 1999). The grain size fractions of starch were determined in 2006, with the use of a laser particle size analyzer, in cooperation with the Department of Agricultural Technology and Food Storage, Wrocław University of Environmental and Life Sciences (Lin et al. 2005, Chang et al. 2006, Lu et al. 2008).

The obtained results were verified statistically by an analysis of variance. The significance of the effect of the experimental factors on potato yield, the content and yield of starch was estimated by an analysis of variance for long-term two-factorial experiments, and their effect on the grain size fractions of starch was evaluated by an analysis of variance for short-time two-factorial experiments (limited to the year 2006). Differences between mean values in treatments were determined by Duncan's T test, at a significance level of $p = 0.05$. The partial correlation between starch yield and potato yield and the starch content of potato tubers was calculated using linear regression equations, according to the formula:

$$y = a + b \cdot x,$$

where:

x – independent variable (explanatory variable: potato yield and starch content, respectively),

y – dependent variable (explained variable) corresponding to value x (starch yield),

a – regression constant (free term) – indicating the intercept point of the regression line and the y axis,

b – slope of the regression line indicating the change in dependent variable y for each unit change in independent variable x.

The degree of determination (explanation) of variable y by variables x was expressed as the coefficient of determination (R^2) (FILIPIAK, WILKOS 1998).

The effect of weather factors on potato yield has been widely discussed in literature. The yield of potato tubers is significantly affected by temperature and precipitation, in both quantitative and qualitative terms (LESZCZYŃSKI 1994, BOMBIK et al. 1999, KALBARCZYK 1999). In wet years potato tubers have a lower starch content, whereas in dry years they accumulate more starch and are less susceptible to flesh darkening (ZGÓRSKA, FRYDECKA-MAZURCZYK 1981, BOLIGŁOWA, TRĘTOWSKI 1986, ROZTROPOWCZ, WIERZEJSKA 1986, GĄSIOR, PAŚKO 1998, GRZEŚKIEWICZ, TRAWCZYŃSKI 2002).

The growing seasons of 2004 and 2006 were humid, with no extreme air temperatures (Table 2, Table 3). The year 2004 offered optimal conditions for potato growth and development, except in September – which was too dry, and in May – when precipitation was by 51% higher than the long-term average. This resulted in the highest potato yield over the entire experimental period. In 2006, the only extremely dry month was July, while precipitation in May, August and September exceeded the long-term average by 51%, 88% and 79% respectively. Late potato cultivars show the highest water demand in July, August and September, when the yield levels increase. The year 2005 year was

least favorable to potato growing due to soil moisture deficiency in April, June, August (dry spell) and September (extreme dry spell). Rainfall total during the growing season of 2005 reached 267 mm, and it was by 29% lower than the long-term average.

Table 2
Meteorological data for the growing seasons 2004–2006 and means of the years 1961–2000

Specification	Year	Month					
		Apr	May	Jun	Jul	Aug	Sept
Air temperature [°C]	2004	8.9	11.8	15.3	17.0	19.2	14.2
	2005	8.2	11.6	14.2	19.7	16.9	18.1
	2006	7.6	14.0	14.4	22.5	18.9	16.4
1961–2000	–	7.0	12.5	15.8	17.2	16.8	12.6
Rainfall total [mm]	2004	51.5	87.1	90.6	78.8	89.3	41.9
	2005	22.0	68.2	35.4	83.9	39.6	17.9
	2006	24.2	87.2	83.5	27.1	141.7	105.6
1961–2000	–	35.4	57.6	69.5	81.6	75.2	59.0

Table 3
Values of the Sielianinow coefficient (*K*)

Year	Month						Entire growing season
	Apr	May	Jun	Jul	Aug	Sept	
2004	1.93	2.38	1.97	1.50	1.50	0.98	1.71
2005	0.89	1.90	0.83	1.37	0.76	0.33	1.01
2006	1.06	2.00	1.93	0.39	2.50	2.14	1.67

K: 0–0.5 – extreme dry spell
0.6–1.0 – dry spell
1.0–2.0 – humid spell
>2.1 – wet spell

Results and Discussion

The highest potato yield, at 58.12 t ha^{-1} (Table 3), was noted in the year 2004 which was found to be optimal for potato growing due to, among others, the most favorable weather conditions (Table 4). The year 2005 was extremely unfavorable, which resulted in a substantially lower potato yield (by 25%, i.e. 14.66 t ha^{-1}) compared with 2004. In 2006 potato yield reached 48.50 t ha^{-1}.

The present experiment showed no correlation between the total yield of potato tubers and the varied levels of soil and foliar fertilization. An increase in the rates of soil fertilizers, from 280 kg to 420 kg NPK ha^{-1}, resulted

Table 4
Effect of varied soil and foliar mineral fertilization levels on potato yield in the experimental period

Experimental factors		Total yield of potato tubers [ha^{-1}]	Starch content [%]	Starch yield [t ha^{-1}]
Soil fertilization	*A*	49.66	18.78	9.26
	B	50.40	18.50	9.29
Foliar fertilization	*A*	49.68	18.63	9.20
	B	49.73	18.44	9.12
	C	49.70	18.76	9.24
	D	49.77	18.65	9.23
	E	49.68	18.57	9.16
	f	50.42	18.93	9.52
	g	50.12	18.62	9.27
	h (control)	51.14	18.53	9.43
Mean		50.03	18.64	9.27
Year of study	2004	58.12	18.23	10.61
	2005	43.46	21.07	9.15
	2006	48.50	16.61	8.06
LSD (α = 0.05) for years		1.37	0.19	0.29
soil fertilization		n.s.	0.15	n.s.
foliar fertilization		n.s.	n.s.	n.s.

I. Soil mineral fertilization NPK: *A* – 280 kg ha^{-1}; *B* – 420 kg ha^{-1};
II. Foliar mineral fertilization: *a* – Basfoliar 2-4-6, *b* – ADOB Mn, *c* – Solubor DF, *d* – ADOB Mn + Solubor DF, *e* – ADOB Mn + Basfoliar 12-4-6, *f* – Basfoliar 12-4-6 + Solubor DF, *g* – Basfoliar 12-4-6 + ADOB Mn + Solubor DF, *h* – control treatment – no foliar fertilization
n.s. – non-significant difference

in an insignificant yield increment of 0.74 t ha^{-1}, i.e. 1.5% (Table 4). The highest potato tuber yield (51.14 t ha^{-1}) was attained in the control treatment, where no foliar fertilizers were applied. In a study by JABŁOŃSKI (2009 b), total potato yield increased by 3.6 t ha^{-1} as a result of an increase in soil fertilization levels by 179 kg NPK ha^{-1}, to 385 kg NPK ha^{-1}. GĄSIOR, PAŚKO (1998), BERNAT (2002), and JABŁOŃSKI (2005b) reported a significant increase in total potato yield as the rates of soil-applied nitrogen fertilizers were increased from 50 to 150–200 kg N ha^{-1}, at constant rates of phosphorus and potassium. In a series of experiments, CIEĆKO et al. (2004) demonstrated a correlation between higher NPK rates and an increase in total potato yield. On the other hand, SŁOWIŃSKI et al. (1995) reported that potassium and phosphorus fertilizers had no effect on the total yield of potato tubers.

The combination of two foliar fertilizers, Basfoliar 12-4-6 and Solubor DF (*f*), was found to be most effective, and the potato yield of 50.42 t ha^{-1} recorded in this treatment was higher than the yields noted for the remaining variants of foliar fertilization. However, the noted effect was statistically non-significant. An increment in total potato tuber yield of 5.7% to 19%, in comparison

with treatments with no foliar fertilization, have been reported by many authors (BOLIGŁOWA 1995, GRZEŚKIEWICZ, TRAWCZYŃSKI 1998, JABŁOŃSKI, DRYJAŃSKA 1998, JABŁOŃSKI 1999, 2003, 2006a,b, 2008b, 2009b, HABERLAND 2000, JABŁOŃSKI, BERNAT 2001, SAWICKA 2003, TRAWCZYŃSKI, KOPENEC 2007, GĄSIOROWSKA 2010).

During the three-year experimental period, potatoes cv. Jasia accumulated 18.64% starch on average. The growing season of 2005 did not support the development of potato tubers, but it contributed to starch accumulation. The average starch content of potatoes cv. Jasia was 21.07%. The lowest starch content, at 16.61%, was recorded in the growing season of 2006; it was by 4.5% lower than in 2005. The starch content of potatoes in 2004 was higher than in 2006, but significantly lower than in 2005 (characterized by insufficient soil moisture content).

Different soil fertilization levels had a statistically significant effect on the starch content of potato tubers. An increase in mineral fertilizer rates, from 280 to 420 kg NPK ha^{-1}, caused an average decrease in starch content of 0.28%. However, the negative impact of NPK soil fertilization was noted only in 2005 and 2006, and it was caused by weather conditions (Table 5). The decrease in starch accumulation resulting from the increase in NPK rates reached 0.61% in 2005 and 0.37% in 2006. The above is consistent with the findings of BERNAT (2002) and JABŁOŃSKI (2005b), who also observed a decrease in the starch content of potatoes following the soil application of increasing nitrogen rates.

Table 5

Effect of the interaction between the year of study and mineral soil and foliar fertilization on the starch content (%) of potato tubers

Experimental factors	Year of study			Mean
	2004	2005	2006	
Soil fertilization				
*A	18.16	21.38	16.80	18.78
B	18.31	20.77	16.43	18.50
LSD (α = 0.05) = 0.26				
Foliar fertilization				
*a	18.69	20.80	16.39	16.63
B	18.38	20.81	16.13	18.44
C	18.06	21.69	16.54	18.76
D	18.19	21.08	16.68	18.65
E	17.50	21.16	17.05	18.57
F	19.13	20.86	16.79	18.93
G	18.06	21.33	16.48	18.62
h (control)	17.88	20.84	16.86	18.53
LSD (α = 0.05) = 0.52				

* – legend as in Table 4

Similar observations were made by CIEĆKO et al. (2004) for increasing NPK rates. In a series of experiments conducted in 2003–2006, JABŁOŃSKI (2009b) noted no effect of increased rates of soil mineral fertilizers (from 206 to 385 kg NPK ha^{-1}) on starch accumulation.

Foliar fertilization had no significant effect on starch accumulation. The combination of Basfoliar 12-4-6 and Solubor DF (*f*) contributed to an insignificant increase in the starch content of potatoes, compared with the control treatment (Table 4). However, there was an interaction between the years of study and foliar fertilization (Table 5). In 2004, the highest starch content (by 1.25% higher than in the control treatment) was determined in potatoes fertilized with Basfoliar 12-4-6 + Solubor DF (*f*). In 2005, the highest starch accumulation (by 0.85% higher than in the control treatment) was observed in the treatment with Solubor DF (*c*). In 2006, the highest starch content was noted in potatoes fertilized with ADOB Mn+ Basfoliar 12-4-6 (*e*), but the difference between this treatment and control was non-significant. A decrease in starch content was reported for treatment (*b*), with ADOB Mn.

The results of numerous research studies indicated a beneficial influence of selected foliar fertilizers on starch accumulation in potatoes. GRZEŚKIEWICZ and TRAWCZYŃSKI (1998), BOLIGŁOWA (1995, 2003), and JABŁOŃSKI (2006a,b; 2008a,b) found that the starch content of potato tubers increased significantly (by 0.7% to 2.5%) in comparison with the control treatment as a result of the application of various foliar fertilizers. JACOBSEN et al. (1998), HABERLAND (2000), JABŁOŃSKI and BERNAT (2001), JABŁOŃSKI (2003), TRAWCZYŃSKI and KOPENEC (2007) reported that foliar fertilizers had no effect on the starch content of potatoes. In experiments by JABŁOŃSKI and DRYJAŃSKA (1998), and JABŁOŃSKI (1999, 2009b), foliar fertilization contributed to a significant decrease in the starch content of potatoes.

During the entire experimental period, weather conditions had the greatest influence on the total yield of potato tubers and starch yield. Starch yield is the outcome of total potato yield and starch content. The highest starch yield (10.61 t ha^{-1}) was attained in 2004, while lowest (8.06 t ha^{-1}) – in 2006 (Table 4). An insignificant increase in starch yield was noted in the treatment with Basfoliar 12-4-6 + Solubor DF (f), compared with the control treatment with no foliar fertilization.

JABŁOŃSKI (2006 a, b; 2008 b) performed a series of experiment with a new generation of fertilizers (ENTEC perfekt, Basfoliar 36 E, Nitrophoska 12 specjal, Nitrophoska 15 perfekt) and reported an increase in starch yield as high as 35%, compared with treatments without foliar fertilization. In a study by Jabłoński and Bernat (2001), the application of the foliar fertilizer ADOB Mn allowed to increase starch yield by 9.6%. Gąsiorowska (2010) observed an increase in starch yield by 1 t ha^{-1} in treatments fertilized with Ekosol K.

Jabłoński (2009 a) demonstrated that starch yield increased by 0.5 t ha^{-1} as a result of the application of Nutrifol, in comparison with the control treatment with no foliar fertilization.

Starch yield was significantly correlated with the total yield of potato tubers (Figure 1). The value of the determination coefficient (100 R^2) shows that total potato yield explained the variation in starch yield in 59%. The correlation between starch yield and the percentage content of starch in tubers was weaker, but also significant (Figure 2). This trait determined starch yield in 9%.

The grain size fractions of starch were determined in potato tubers harvested in 2006. Starch grains with a diameter > 40 μm are considered most desirable, since too small granules (< 20 μm) cannot be used for industrial

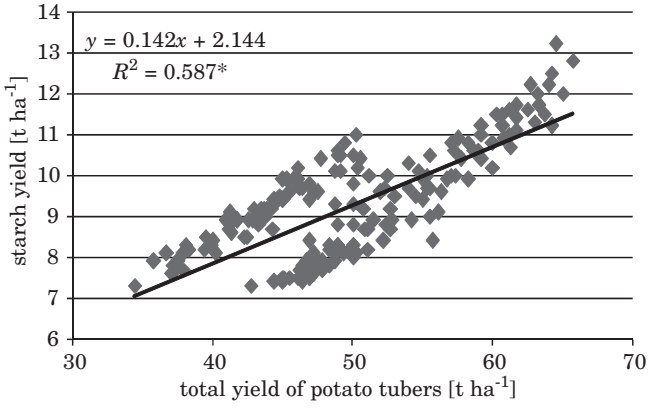

Fig. 1. Partial correlation between starch yield [t ha^{-1}] (y) and the total yield of potato tubers [t ha^{-1}] (x) (R^2 – coefficient of determination, * significant at $p = 0.05$)

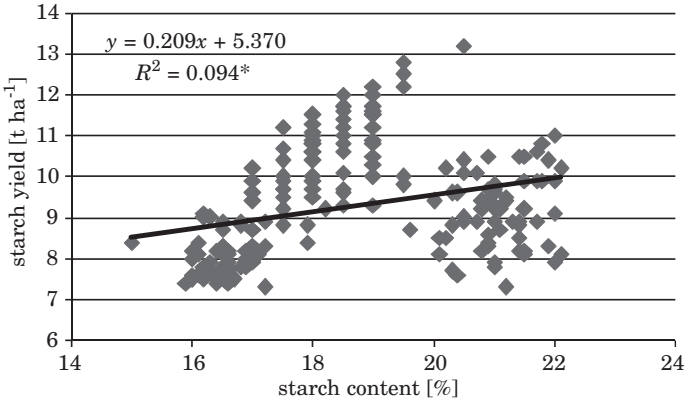

Fig. 2. Partial correlation between starch yield [t ha^{-1}] (y) and the starch content (%) (x) of potato tubers (R^2 – coefficient of determination, * significant at $p = 0.05$)

purposes. Soil mineral fertilization had a significant effect on starch grain size. Large starch grains (> 40 μm) dominated in the treatment fertilized with 280 kg NKP ha^{-1}, accounting for 58.25% of all grains, while small starch globules (<20 μm in diameter) accounted for only 9.91% (Table 6). Starch in the treatment fertilized with 420 kg NPK ha^{-1} was characterized by a higher share of 20–40 μm and <20 μm fractions. The applied foliar fertilizers had no effect on the size of starch granules. According to HASSE and PLATE (1996), and HAASE (2000), the content of large starch grains is a varietal property.

Table 6

Effect of varied soil and foliar fertilization levels on the grain size fractions of starch

Experimental factors		Grain size fractions [%]		
		<20 μm	20–40 μm	>40 μm
*A	*a*	9.80	31.04	59.15
	B	10.21	31.25	58.54
	C	10.14	32.08	57.78
	D	10.66	33.28	56.06
	e	11.02	33.39	55.60
	f	10.18	32.89	56.93
	g	11.02	32.72	56.26
	h (control)	9.91	31.84	58.25
Mean		10.37	32.31	57.32
*B	*a*	12.67	33.84	53.49
	b	11.50	34.67	53.83
	c	10.83	34.33	54.83
	d	11.22	34.62	54.16
	e	10.83	33.20	55.97
	f	11.53	34.12	54.35
	g	10.75	33.83	55.42
	h (control)	12.86	34.57	52.57
Mean		11.52	34.15	54.33
LSD (α = 0.05) for:				
soil fertilization		0.72	0.78	1.28
Foliar fertilization		n.s.	n.s.	n.s.

* – legend as in Table 4

Conclusions

1. An increase in NPK rates from 280 to 420 kg ha^{-1} not only did not improve the total yield of potatoes cv. Jasia, but it decreased their starch content and deteriorated starch grain size distribution.

2. The foliar fertilizers applied in the study had no effect on the yield and qualitative traits of potatoes.

3. Supplemental fertilization with NPK rates higher than 280 kg ha^{-1} seems unnecessary if potatoes are grown in compact and nutrient-abundant soil fertilized with manure at a dose of 25 t ha^{-1}.

4. Weather conditions had a more significant effect on the total yield of potato tubers, the starch content of potatoes and starch yield than mineral fertilization.

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THE EFFECT OF EXPLANT TYPE ON SOMATIC EMBRYOGENESIS INDUCTION IN *PISUM SATIVUM* L.

***Kamilla Górska-Koplińska*[1], *Anna Źróbek-Sokolnik*[2], *Ryszard J. Górecki*[1], *Dariusz J. Michalczyk*[1]**

[1] Department of Plant Physiology and Biotechnology
[2] Department of Botany and Nature Protection
University of Warmia and Mazury in Olsztyn

K e y w o r d s: pea (*Pisum sativum* L.), somatic embryogenesis, somatic embryos.

Abbreviation: DAF – Days after flowering.

A b s t r a c t

The effect of explant type on somatic embryogenesis induction in *Pisum sativum* (cv. Oskar and an unregistered line HM-6) was studied. Shoot apices, leaf primordia, and epicotyl fragments of axenically grown, etiolated seedlings, as well as embryonic axes and cotyledon fragments isolated from zygotic embryos at different stages of development, were used as explants. Somatic embryogenesis was induced essentially as described by Griga in 1998 – MS salts and sucrose, B5 Gamborg vitamins, picloram (2.5 μM). After induction period (14 days) all cultures were transferred to the differentiation medium (basal medium as above, auxin omitted). Both in Oskar and HM-6, only shoot apices developed somatic embryos and (with significantly lower frequency) adventitious shoots.

WPŁYW RODZAJU EKSPLANTATU NA INDUKCJĘ SOMATYCZNEJ EMBRIOGENEZY U *PISUM SATIVUM* L.

***Kamilla Górska-Koplińska*[1], *Anna Źróbek-Sokolnik*[2], *Ryszard J. Górecki*[1], *Dariusz J. Michalczyk*[1]**

[1] Katedra Fizjologii i Biotechnologii Roślin
[2] Katedra Botaniki i Ochrony Przyrody
Uniwersytet Warmińsko-Mazurski w Olsztynie

S ł o w a k l u c z o w e: groch, somatyczna embriogeneza, somatyczne zarodki.

Skróty: DPK – dzień po kwitnieniu.

Address: Anna Źróbek-Sokolnik, University of Warmia and Mazury, pl. Łódzki 1, 10-719 Olsztyn, Poland, phone: +48 (89) 523 49 27, e-mail: a.zrobeksokolnik@uwm.edu.pl

Abstrakt

W pracy zbadano zdolność wybranych rodzajów eksplantatów grochu odmiany Oskar oraz linii HM-6 do indukowania somatycznej embriogenezy. Jako eksplantaty zastosowano wierzchołki pędu, zawiązki liści i fragmenty epikotyli izolowane z aksenicznie hodowanych, etiolowanych siewek, jak również osie zarodkowe i fragmenty liścieni pobierane z zarodków zygotycznych na różnych etapach ich rozwoju. Somatyczną embriogenezę pobudzano w zasadzie wg procedury opisanej w 1998 r. przez Grigę, tj. na pożywce zawierającej sole i sacharozę jak w zestawie MS, witaminy zestawu B5 Gamborga oraz pikloram (2.5 μM). Po okresie indukcji (14 dni) wszystkie hodowle przenoszono na pożywkę różnicującą (podłoże podstawowe jak wyżej, ale bez auksyny). Zarówno u odmiany Oskar, jak i linii HM-6 zarodki somatyczne oraz (z istotnie niższą wydajnością) pędy przybyszowe uzyskiwano jedynie z wierzchołków pędów młodych siewek.

Introduction

Somatic embryos have been obtained from *in vitro* cultures of virtually all economically important plant species. However, various species, as well as cultivars (PODWYSZYŃSKA et al. 1997) and lines (GAIN et al. 1998) differ widely in embryogenic potential and requirements. The choice of explant (and nutrient medium, as well) is a critical factor determining the success of SE induction. Fragments of young seedlings and immature zygotic embryos are generally considered the most suitable explant sources (LAKSHMANAN and TAJI 2000, HITA et al. 2003, BELMONTE et al. 2007). Fragments of flowers can also be very embryogenic (PINTO-SINTRA 2007, SINGH et al. 2007, STEINMACHERA et al. 2007) and in bulbiferous plants somatic embryogenesis can be induced on fragments of bulb scales (eg. DUQUENNE et al. 2006).

Somatic embryogenesis in *Pisum sativum* L., unlike other legumes, is difficult to induce (LAKSHMANAN and TAJI 2000). With this in mind, we tested the effect of explant type on somatic embryogenesis induction in pea. Explants successfully applied for SE induction in other legumes were considered.

Materials And Methods

Plant Material

To obtain axenically growing seedlings, seeds were surface sterilized in 5% water solution of Chloramine B for 15 minutes followed by three washes with sterile distilled water. Disinfected seeds were placed in sterile tubes (25 dm^{-3} capacity) containing moist cotton wool. After germination (in darkness at 25–26°C for 4 days): shoot apices, leaf primordia and epicotyl fragments (0.1 cm long) were excised from seedlings, using a dissecting microscope, and placed on induction medium.

To obtain zygotic embryos, seeds were placed in pots filled with compost soil and sand (4:1, v/v). Seeds were regularly watered with tap water. The humidity of substrate was kept at 60–70%. Florovit fertilizer was applied three times: at the stage of five leaves, at the beginning of flowering and fruiting. Pods were collected 14, 18 or 22 days after flowering. Unopened pods were surface sterilized in 5% water solution of Chloramine B for 10–12 minutes followed by several washes with sterile distilled water. Embryonic axes and fragments of cotyledons (0.1 cm long) were isolated from zygotic embryos and placed on induction medium.

Seeds of *P. sativum* cv. Oskar and line HM-6 were kindly provided by AGRITECH Ltd. Czech Republic.

In vitro cultures

The explants were subjected to 14-days induction on basal medium described by GRIGA (1998), which contained MS salts (MURASHIGE and SKOOG 1962), Gamborg B5 vitamins (GAMBORG et al. 1968), 3% sucrose and 2.5 μM picloram. During the induction period cultures were kept in growth room under 16/8 hours (light/darkness) photoperiod at temperatures of 23–24°C at day and 19–20°C at night.

After induction all cultures were transferred to the differentiation medium (basal medium without phytohormones) and incubated in growth room at light/temperature conditions as given above. Three weeks later the physiological state of explants, morphogenetic responses of cultures and the efficiency of somatic embryogenesis were evaluated. Observations were repeated every 7–10 days. The efficiency of somatic embryogenesis was defined as number of somatic embryos per explant (expressed as per cent).

Statistical Analysis

One-way analysis of variance (ANOVA) was applied. The values analyzed were means of five series with four replicates within each series. Standard errors (± SE) were also determined. The obtained average values were separated using Duncan's multiple range test $P \leq 0.05$. The Microsoft Excel 2007 and STATISTICA 8.0 computer programs were used.

Results And Discussion

Among nine types/developmental stages of explants only one kind of explant, that is shoot apices, formed somatic embryos during differentiation culture. This is valid for both Oskar and HM-6, although these genotypes differed quite distinctly in the efficiency of somatic embryogenesis induction (Table 1) and the rate of embryo formation. In Oskar cultivar most embryoids appeared on explants after 25 days of culture, whereas in cultures of HM-6 line, somatic embryos appeared later, 35–45 days after explant isolation. Rather unexpectedly, no structures resembling somatic embryos developed on embryonic axes, although elongation and rooting of these explants could easily be observed (Table 2).

Table 1
The frequency of somatic embryogenesis and organogenesis on explants isolated from pea zygotic embryos or seedlings (mean ± S.E)

Cultivar	Explant	Somatic embryogenesis and regeneration efficiency [%]		
		somatic embryos	shoots	roots
Oskar	shoot apex	53.3 ±12.0^{a}	6.7 ± 3.3^{e}	20.0 ± 5.8^{d}
	leaf	0.0	0.0	36.7 ± 18.6^{e}
	epicotyle	0.0	0.0	16.7 ± 3.3^{df}
	embryonic axis 14 DAF	0.0	0.0	10.0 ± 5.8^{f}
	embryonic axis 18 DAF	0.0	0.0	0.0
	embryonic axis 22 DAF	0.0	0.0	26.7 ± 3.3^{d}
	cotyledon 14 DAF	0.0	0.0	0.0
	cotyledon 18 DAF	0.0	0.0	0.0
	cotyledon 22 DAF	0.0	0.0	0.0
HM-6	shoot apex	36.7 ± 6.7^{b}	6.7 ± 3.3^{e}	16.7 ± 6.7^{df}
	leaf	0.0	0.0	6.7 ± 3.3
	epicotyle	0.0	0.0	20.0 ± 5.8^{d}
	embryonic axis 14 DAF	0.0	0.0	20.0 ± 15.3^{d}
	embryonic axis 18 DAF	0.0	0.0	43.3± 12.0^{e}
	embryonic axis 22 DAF	0.0	0.0	36.7 ± 6.7^{e}
	cotyledon 14 DAF	0.0	0.0	23.3 ± 3.3^{d}
	cotyledon 18 DAF	0.0	0.0	0.0
	cotyledon 22 DAF	0.0	0.0	0.0

Values followed by the same superscript are not significantly different at 5% level (data in columns were compared).

Callus tissue developed on all explants, most rapidly during the first 30 days of culture, and covered about 10 to 100% of explants surface. The size and compactness of callus depended on explant type (Table 2). Embryonic axes formed soft callus, while on cotyledons it was compact with ragged surface and it was rigid and nodular on epicotyls. When embryonic axes of 18 or 22-days-

Table 2

Morphogenetic responses of explants after 60 days of *in vitro* culture

Explant		Responses	
		OSKAR	HM-6
Seedling	shoot apical	somatic embryogenesis, shoots, roots	somatic embryogenesis, shoots, roots
	leaf	roots	roots
	epicotyle	explant elongation, roots, green-white callus	explant elongation, roots, green callus
Zygotic embryo	embryo axis 14 DAF	bright-green callus, roots	bright-green or cream--yellow callus on hypocotyl and root, roots
	embryo axis 18 DAF	green callus	axis germination, root covered by callus
	embryo axis 22 DAF	roots	roots, necrosis
	cotyledon 14 DAF	cream-green callus on whole explant	roots
	cotyledon 18 DAF	cream-green callus covering whole explant	explants decay
	cotyledon 22 DAF	green callus	cream-green callus

-old seedling were used as explants, callus growth was very intense and it resulted in a four-fold increase of explant size. In case of embryonic axes isolated from 14-days-old seedlings callus proliferation increased approximately twice the explant size. The weakest morphogenetic reactions were observed on cotyledons isolated from 18-days-old zygotic embryos. Most of these explants formed only minute callus on the edges, and at 35 day of culture callus tissue started decaying. Necrosis was observed also on other explants, but less it covered less than 15% of their surface.

Rhizogenesis was observed on all types of explants, except cotyledons of immature zygotic embryos (18 and 22 DAF) – Table 2. In cultures of embryonic axes, roots developed from preexisting shoot apices, from the cells surrounding vascular bundles. Some adventitious roots were also formed on apical parts of explants. They reached 30–35 mm length and were covered by soft cream-bronze callus. In cultures of shoot apices roots developed in basal part of explants or in apical part of explant closely to developing shoot primordium. Adventitious roots were also formed by epidermal cells of epicotyles and cotyledons (14 DAF), as well as cells of leaf vascular bundles.

Various species, cultivars and genetic lines of legumes may differ widely in efficiency of somatic embryogenesis induction. Cultivar and genotype of explants cells affect both embryo formation but also its potential for vigorous growth (NADOLSKA-ORCZYK 1992, BRODA and TORZ 1997, GRIGA 1998, WALTER

and PARROT 2001, TOMLIN et al. 2002). The choice of explant, type of tissue and age of seedling used as explant source are considered critical factors that determine the success of somatic embryogenesis in legumes (GRIGA 1998, NADOLSKA-ORCZYK et al. 1994).

In our experiments with pea only shoot apices proved embryogenic. The suitability of this type of explant for somatic embryogenesis induction in pea was previously reported by LOISEAU et al. (1995) and GRIGA (1998). Leaves have not been used very often as explants in legumes, however they were suitable for *Cicer arietinum* (DINESHKUMAR et al. 1995), *Medicago arborea* (HITA et al. 2003) and *Medicago sativa* (MCKERSIE and BROWN 1996, RUDUŚ et al. 2000). Zygotic embryos or their fragments (especially cotyledons) have been successfully used as explant source by several authors studying legumes (HITA et al. 2003, BRODA and TORZ 1997, NADOLSKA-ORCZYK 2000, TOMLIN et al. 2002, GOGATE and NADGAUDA 2003). The stage of embryo development is considered an important factor for efficient SE induction (LOISEAU et al. 1996, RASHID 2001).

Conclusions

In our experiments, somatic embryogenesis could not be obtained either from axes or cotyledons of zygotic embryos at three stages of development/maturity (14, 18 and 22 DAF – Table 1). However, this does not seem to be a limitation typical for all *Pisum sativum* cultivars. Somatic embryogenesis induction on embryonic axes and cotyledons of *Pisum sativum* was achieved by NADOLSKA-ORCZYK et al. (1994) and LOISEAU et al. (1996).

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ENERGY EXPENDITURE IN TRITICALE CULTIVATION WITH DIFFERENT MICROELEMENTS FERLILIZATION TECHNIQUE

Marek Raczkowski

Departament of Technical Science
Chair of Foundation of Safety
University of Warmia and Mazury in Olsztyn

K e y w o r d s: triticale, ferlilization, microelements, energy expenditure.

A b s t r a c t

The purpose of study was determination the energetic expenditure of triticale cultivation at different fertilization technique. There were calculated expenditure of treatments, natural resources, materials, durable means and equivalent of real work. It was showed, that the sum of the total energy expenditure in triticale cultivation was from 16723.2 to 21864.8 MJ ha^{-1}. The lower expenditures were with soil fertilization technique without objects with higher manganese dose (10.0 kg ha^{-1}) and with copper, zinc and manganese applied together at nitrogen dose 80 and 120 kg ha^{-1} independent with microelement dose. The higher sum of total energy expenditure was with all microelements applied together before triticale sawing (to soil) depend on ferlilization before sawing with copper, zinc or manganese. There was not significant different in the same case with spray application of studied microelements. In the energy expenditure structure of triticale cultivation the higher participation (64.4–71.7%) was shoved with raw and materials, the lower (17.2–21.5% and 8.9–11.4%) with immediate energy carriers and exploitation expenditure and, the lowest (2.2–2.7%) with human work.

ENERGOCHŁONNOŚĆ PRODUKCJI PSZENŻYTA W WARUNKACH ZRÓŻNICOWANEJ TECHNIKI NAWOŻENIA MIKROELEMENTAMI

Marek Raczkowski

Wydział Nauk Technicznych
Katedra Podstaw Bezpieczeństwa
Uniwersytet Warmińsko-Mazurski w Olsztynie

S ł o w a k l u c z o w e: pszenżyto, nawożenie, mikroelementy, energochłonność.

Address: Marek Raczkowski, University of Warmia and Mazury, ul. Okrzei 1a, 10-266 Olsztyn, Poland, phone: +48 (89) 526 63 80, e-mail: marek.raczkowski@uwm.edu.pl

A b s t r a k t

Celem badań było określenie nakładów energetycznych w uprawie pszenżyta w warunkach zróżnicowanej techniki nawożenia. Obliczono energochłonność zabiegów, surowców, materiałów, środków trwałych i ekwiwalentu pracy ludzkiej. Stwierdzono, że suma skumulowanych nakładów energetycznych w uprawie pszenżyta wynosiła od 16 723,2 do 21 864,8 MJ ha^{-1}. Niższe nakłady występowały w doglebowej technice nawożenia, z wyjątkiem obiektów z większą dawką manganu (10,0 kg ha^{-1}) oraz z równoczesnym zastosowaniem miedzi z cynkiem i manganem na tle 80 i 120 kg ha^{-1} azotu, niezależnie od dawki mikroelementów. Do zwiększenia sumy skumulowanych nakładów energetycznych przyczyniło się zastosowanie łączne mikroelementów przed siewem pszenżyta (doglebowo) w porównaniu z nawożeniem przedsiewnym wyłącznie miedzią, cynkiem lub manganem. Nie stwierdzono natomiast istotnej różnicy w takim samym przypadku po dolistnej aplikacji badanych mikroelementów. W strukturze energochłonności uprawy pszenżyta największy udział miały surowce i materiały (64,4–71,7%), mniejszy bezpośrednie nośniki energii (17,2–21,5%) i nakłady eksploatacyjne (8,9–11,4%), a najmniejszy praca ludzka (2,2–2,7%).

Introduction

In the plant production process, a lot importance is the estimation of the energetic expenditure (WIELICKI, BŁAŻEJ 1988, PLOUFFE et al. 1995, WÓJCICKI 2000). The obtained results of the energetic materials of some agriculture yields or enterprises are different with the study methods, so there are not the clear arrangements (WIELICKI, BŁAŻEK 1988).

The study of the estimation of the material and energetic expenditures effectiveness in the agricultural production was conducted in two levels: the macroeconomic with energetic expenditure of the all agriculture as the energetic balances (PAWLAK 1981) and the microeconomic with the analyses of the differential levels energetic expenditures of some farms (SOKOLIK 1986). In the economic practice was not possibility to the estimation of the agricultural production effectiveness energetic expenditure without uniform method and with applied of the different technologies. This deficit in the previous studies with the estimation of the effectiveness energetic expenditures in the agricultural production was supplement by ANUSZEWSKI (1987) in the basis the estimation technological method in the wide meaning the energetic expenditures of the basic agricultural products.

In the paper were showed the results of six years field experiments with influence of different microelements applying techniques on the expenditure production of Maja spring triticale.

Research conditions

The field experiments were carried out in 1994–2000 years on soil with clay granulometric composition, 3 complex agricultural usefulness (unsuitable for

wheat), low copper and medium phosphorus, potassium, zinc and manganese wealthy and light acidity reaction.

The experiments were found the random blocks method in 4 repeats. The harvesting area was 10 m^2.

Spring triticale variety Maja was cultivated after winter wheat with seeds sawing in dose of 250 kg ha^{-1} and with 15 cm rows distance.

In the all study triticale complex were fertilized before sawing with superphosphate in 70 kg ha^{-1} dose (P_2O_5) and 56% potassium salt in 100 kg ha^{-1} dose (K_2O). The fertilize objects were different with the kind and the apply way of nitrogen and microelements (Table 1).

Table 1

Fertilization techniques

Object	Fertilization	Fertilization							
		A – to soil				*B* – to soil and foliar application			
		N [kg ha^{-1}]	Cu	Zn	Mn	N [kg ha^{-1}]	Cu	Zn	Mn
			[kg ha^{-1}]				[kg ha^{-1}]		
1	N_1	$80(40_k+40_s)$	–	–	–	$80(40_k+40_{sd})$	–	–	–
2	N_1+Cu_1		5	–	–		0.2	–	–
3	N_1+Zn_1		–	5	–		–	0.2	–
4	N_1+Mn_1		–	–	5		–	–	0.2
5	N_1+Cu_1, Zn_1, Mn_1		5	5	5		0.2	0.2	0.2
6	N_1+Florovit$_1^e$	–	–	–	–		0.2+0.4+0.4		
7	N_1+Cu_2	$80(40_k+40_s)$	10	–	–		0.4	–	–
8	N_1+Zn_2		–	10	–		–	0.4	–
9	N_1+Mn_2		–	–	10		–	–	0.4
10	N_1+Cu_2, Zn_2, Mn_2		10	10	10		0.4	0.4	0.4
11	N_2	$120(40_p+40_k+40_s)$	–	–	–	$120(40_p+40_k+40_{sd})$	–	–	–
12	N_2+Cu_1		5	–	–		0.2	–	–
13	N_2+Zn_1		–	5	–		–	0.2	–
14	N_2+Mn_1		–	–	5		–	–	0.2
15	N_2+Cu_1, Zn_1, Mn_1		5	5	5		0.2	0.2	0.2
16	N_2+Cu_2		10	–	–		0.4	–	–
17	N_2+Zn_2		–	10	–		–	0.4	–
18	N_2+Mn_2		–	–	10		–	–	0.4
19	N_2+Cu_2, Zn_2, Mn_2		10	10	10		0.4	0.4	0.4
20	N_2+Florovit$_2^e$	–	–	–	–	–	0.4+0.8+0.8		

p – before sowing; *k* – in tillering time; *s* – in shooting time; *d* – foliar application; *e* – doses of Cu+Zn+Mn.

The triticale cultivation and harvest methods were conducted with according to the agro technological requirement typical for this plants species.

The operation production expenditures of spring triticale variety Maja cultivation were calculated by MET method (ANUSZEWSKI 1987) in MJ ha^{-1} for the consume some energy carries (fuel), natural resources, materials, durable means (tractors and accompany machines) and human work equivalent.

The results were statistically work out and significance of variations has been calculated using the Tukey's test, at the level obtained different gravity of the trust range $\alpha = 0.05$.

Results and Discussion

It has been found out that in North-South Poland condition the total sum of the energy expenditure in spring triticale Maja cultivation was from 16 723.2 to 21 864.8 MJ ha^{-1} (Table 2). The lower expenditures were with soil technique fertilization, exception to objects of higher manganese dose (10.0 kg ha^{-1}) and of copper, zinc and manganese in total dose with 80 and 120 kg ha^{-1} nitrogen dose independently to microelements dose. The factors deciding about high of expenditure, like to JANKOWSKI et al. (1998) or KISIEL and DOMSKA (1994) were machines and fertilizers kind. The higher sum the energy expenditure was with total microelements dose fertilization before triticale sowing (to soil) depends on this fertilization only with copper, zinc or manganese. There was not important different at the similar techniques with study microelements foliar application.

In the structure of the energy expenditure of triticale cultivation with microelements soil fertilization (Figure 1) the most participation (65.3–71.7%) were row and materials (Em_i). Considerably lower (appropriately 17.2–20.9% and 8.9–10.8%) were participations of immediate energy carries (Enp_i) and exploitation expenditure (Eim_i). The relatively low and not very different values (2.2–2.6%) were showed in human work participation (Ezl_i). The most effectiveness cultivation objects in accident of carried energy expenditure were with soil fertilization 80 or 120 kg ha^{-1} nitrogen dose and 5 kg ha^{-1}copper or zinc dose (objects 2, 3, 12 and 13).

In the triticale technique foliar application with nitrogen and microelements, the energy expenditure participation mainly was more similar (Figure 2). There was lower row and materials participation (EM_i) from 64.4 to 68.6% depend on soil fertilization technique and, similar with immediate energy carries (Enp_i), exploitation expenditure (Eim_i) and human work (Ezl_i), appropriately in size 18.8–21.5%, 10.2–11.4% and 2.4–2,7%. The lower row and material participation were with lower nitrogen dose and some microelements

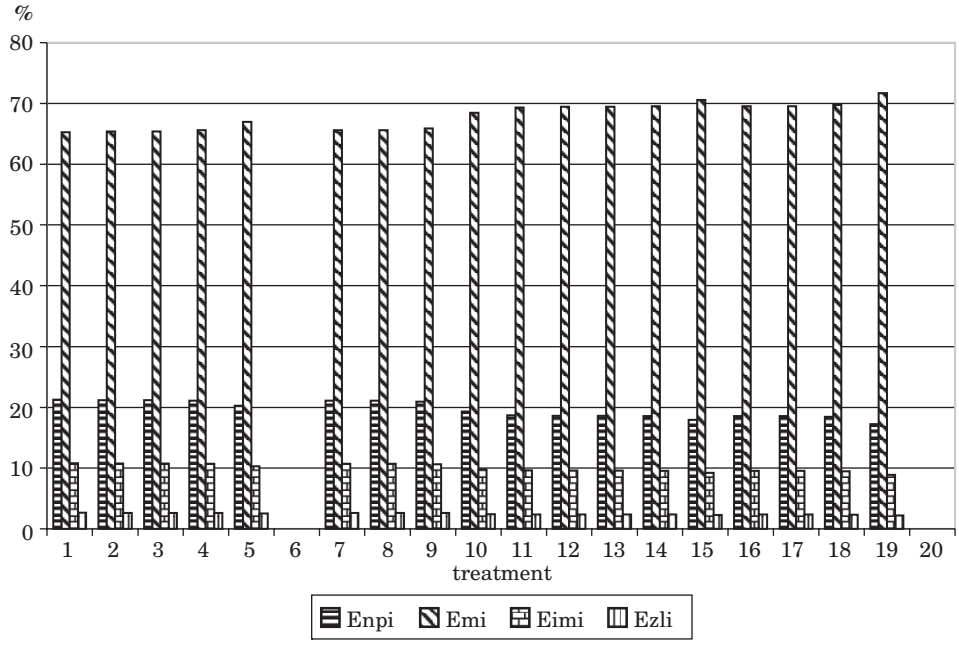
Table 2
Sum of total energetic expenditure in cultivation of spring triticale Maja cv. [MJ ha^{-1}]

Treatment	Fertilization	Fertilization	
		A – to soil	*B* – to soil and spray application
1	N_1	16 723.2^a	16 954.4^a
2	N_1+Cu_1	16 793.2^a	16 957.2^a
3	N_1+Zn_1	16 793.2^a	16 957.2^a
4	N_1+Mn_1	16 863.2^a	16 960.0^a
5	N_1+Cu_1, Zn_1, Mn_1	17 563.2^b	16 988.0^a
6	N_1+Florovit$_1$		16 987.4^a
7	N_1+Cu_2	16 863.2^a	16 960.0^a
8	N_1+Zn_2	16 863.2^a	16 960.0^a
9	N_1+Mn_2	17 003.2^a	16 965.6^a
10	N_1+Cu_2, Zn_2, Mn_2	18 403.2^b	17 021.6^a
11	N_2	20 184.8^c	20 441.3^b
12	N_2+Cu_1	20 254.8^c	20 444.1^b
13	N_2+Zn_1	20 254.8^c	20 444.1^b
14	N_2+Mn_1	20 324.8^c	20 446.9^b
15	N_2+Cu_1, Zn_1, Mn_1	21 024.8^d	20 474.9^b
16	N_2+Cu_2	20 324.8^c	20 446.9^b
17	N_2+Zn_2	20 324.8^c	20 446.9^b
18	N_2+Mn_2	20 464.8^c	20 452.5^b
19	N_2+Cu_2, Zn_2, Mn_2	21 864.8^d	20 508.5^c
20	N_2+Florovit$_2$		20 474.3^b

Ferlilizer doses (kg ha^{-1}): N_1 – 80 (40+40), N_2 – 120 (40+40+40), Cu_1, Zn_1, Mn_1 – 0.2 (*A*), 5.0 (*B*), Cu_2, Zn_2, Mn_2 – 0.4 (*A*), 10.0 (*B*), Florovit$_1$ – 0.2+0.4+0.4 (Cu+Zn+Mn), Florovit$_2$ – 0.4+0.8+0.8 (Cu+Zn+Mn), *a, b, c, d* – differences of value in each column marked with the same letters are issignificant at $\alpha = 0.05$.

fertilization (objects 1–8) except higher dose (0.4 kg ha^{-1}) manganese without and together with copper and zinc (objects 9 and 10). However, the fertilization technique with 120 kg ha^{-1} nitrogen dose without and together with microelements (objects 11–30) were decreased the participation of the immediate energy carries (fuel) and the conitant measures (durable means – tractors and machines). KAMIŃSKI and ROSZKOWSKI (2001) were showed that one of the way to become cheaper of the plant production is the technique of foliar nitrogen fertilization applied together with plant protection. In this case, nitrogen is about 40% effectively used from urea solution applied to leaves depend on soil fertilization.

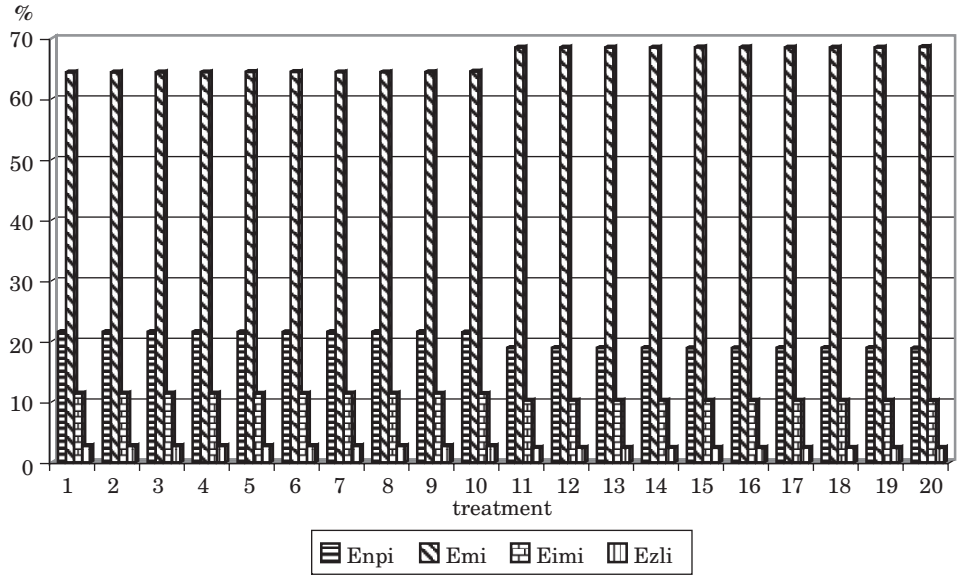
The structure kind of the carried energy expenditure in the experiment was different, and like as in studies of KISIEL and DOMSKA (1994), JANKOWSKI et al.



Explanation of treatment – see Table 2, Enp_i – immediate energy carries, Em_i – row and materialss, Eim_i – exploitation expenditure, Ezl_i – human work.

Fig. 1. Structure of energetic expenditure in cultivation of spring triticale with soil microelements ferlilization

(1998) and STARCZEWSKI et al. (2008), there was high the row and materials participation (64.4–71.7%). The obtained results are showing on the important significance of the natural sources and materials, which determined about 50% of the total expenditure. STARCZEWSKI et al. (2008) were showed that most energy (56.4%) is carried with fertilizers. Moreover, authors were found that all cultivation operations carried mean 29.4% energy and, on the differential of this value influenced another work time and fuel consumption. While, ANUSZEWSKI and SOKOLIK (1985) were showed that in the study agricultural farms were more than 60% the raw and materials in total expenditure and, immediate energy carries from 20 to 40%. Moreover, there was more different human work participation about 10 to 15%. The lowest expenditures (3–10%) were in the exploitation of the durable means. ANUSZEWSKI and GROTKOWSKA (1986) were analyzed the production effectiveness of some agricultural yields and showed the most participation of the immediate energy carries (fuel and electric energy) in the expenditure and, also were showed that with expenditure energetic value increase was not be the same with proportional increase of the agricultural production. After all, the expenditure estimation and



Explanation of treatment – see Table 2 and Figure 1.

Fig. 2. Structure of energic expenditure in cultivation of spring triticale with microelements spray application

comparison should be the base to choice of the technology variant with the lowest expenditure.

The researches with the expenditure plant production also were showed on the quality of the plough work connected with size, shape, place of work main surface and ploughing speed (PLOUFFE et al. 1995, YAVUZCAN et al. 1998).

Conclusion

1. Based on the obtained date it has been found that the total energy expenditure of spring triticale Maja cultivated in North-South Poland condition was from 16 723.2 to 21 864.8 MJ ha^{-1}. The lower expenditure was with the soil fertilization technique, except objects with higher manganese dose (10 kg ha^{-1}) and with applying together of copper, zinc and manganese in comparison with 80 and 120 kg ha^{-1} nitrogen dose, independent to microelement dose.

2. The microelements applied together before triticale sowing (to soil) were increased the total sum of the energy expenditure depend on fertilization before sowing only copper, zinc or manganese. Whereas, there was not different with the same technique but microelements foliar application.

3. In the expenditure structure of the triticale cultivation was the most participation of row and materials (64.4–71.7%), the lower of immediate energy carries (17.2–21.5%) and exploitation expenditure (8.9-11.4%) and, the least of human work (2.2–2.7%).

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CHLORINATED HYDROCARBONS IN FEED AND TISSUES OF TURKEY HENS FROM A BREEDING FLOCK AND THEIR CONTENT IN EGG YOLKS AND BLOOD OF POULTS

***Andrzej Faruga*[1], *Renata Pietrzak-Fiećko*[2], *Stefan S. Smoczyński*[1]**

[1] Department of Poultry Breeding
[2] Department of Commodities and Food Analysis
University of Warmia and Mazury in Olsztyn

K e y w o r d s: turkeys, DDT, blood, egg yolk, yolk sack, abdominal fat.

A b s t r a c t

The aim of the study was to determine the content of chlorinated hydrocarbons (DDT, DDE, DDD and γ-HCH) in different tissues of turkeys from a reproductive flock. Linear correlation coefficients between the contents of chlorinated hydrocarbons in blood of the turkey hens and their concentrations in egg yolks; in egg yolk and their concentrations in blood of poults; in egg yolk and their concentrations in yolk sack of poults, in blood of the turkey hens and their concentrations in abdominal fat of the hens were determinated. Significant correlations were determined only between the contents of chlorinated carbohydrates in blood and their concentration in egg yolks of the layers. The contents of chlorinated hydrocarbons in the research material did not arouse serious hygienic and toxicological concerns.

CHLOROWANE WĘGLOWODORY W PASZY I TKANKACH INDYCZEK STADA REPRODUKCYJNEGO ORAZ ICH ZAWARTOŚĆ W ŻÓŁTKACH JAJ I KRWI PISKLĄT

***Andrzej Faruga*[1], *Renata Pietrzak-Fiećko*[2], *Stefan S. Smoczyński*[1]**

[1] Katedra Drobiarstwa
[2] Katedra Towaroznawstwa i Badań Żywności
Uniwersytet Warmińsko-Mazurski w Olsztynie

S ł o w a k l u c z o w e: indyki, DDT, krew, żółtka jaj, woreczek żółtkowy, tłuszcz sadełkowy.

Address: Stefan S. Smoczyński, University of Warmia and Mazury, pl. Cieszyński 1, 10-719 Olsztyn, Poland, phone, +48 (89) 523 39 74, e-mail: stesmo@uwm.edu.pl

Abstract

Celem badań było określenie zawartości chlorowanych węglowodorów (DDT, DDE, DDD i γ-HCH) w różnych tkankach indyczych stada reprodukcyjnego. Określono liniową korelację między zawartością chlorowanych węglowodorów w krwi indyczek a ich zawartością w żółtkach jaj; między zawartością chlorowanych węglowodorów w żółtkach jaj a ich zawartością w krwi piskląt; między zawartością chlorowanych węglowodorów w żółtkach jaj a ich zawartością woreczkach żółtkowych piskląt; między zawartością w krwi a zawartością w tłuszczu sadełkowym indyczek. Stwierdzono istotne korelacje tylko między zawartością chlorowanych węglowodorów w krwi indyczek a ich zawartością w żółtkach jaj. Zawartości chlorowanych węglowodorów w badanym materiale nie budzą zastrzeżeń higienicznych i toksykologicznych.

Introduction

Recent studies have found that chlorinated carbohydrates and their derivatives still occur in the environment and in food. Circulating in the environment, they are subject to bioaccumulation in particular links of the food chain. In Poland, preparations containing DDT have been withdrawn from use since 1976, yet they are still used in some countries of Asia and Africa for fighting insects that transfer malaria as well as in crops of tobacco and cotton, DDT was used in the East Germany for forest spraying until the 1980s. By penetrating into atmosphere, this compound is adsorbed onto molecules of liquids suspended in it and in that form may be transferred for long distances (STRUCIŃSKI et al. 2000).

Organic chlorine hydrocarbons are a group of xenobiotics with special toxicological significance which, due to considerable stability in the environment and lipophilic character, pose a high risk to the health of humans and animals. This compounds affects the marrow and morphological elements of blood as well as the immunological and nervous systems. Methyl sulfone metabolites of DDT exert a toxic effect on adrenal glands bringing about death of the cells. The DDT influences also the hormonal system causing spontaneous miscarriages, anomalies in the reproductive systems, and shortening of lactation period in mammals (STRUCIŃSKI et al. 2000. VIDAEFF and SEVER 2005).

Presence of organochlorine hydrocarbons has been reported, among others, in animal milk (JUSZKIEWICZ and NIEWIADOWSKA 1984, PIETRZAK-FIEĆKO et al. 2000), tissues of slaughter animals (FARUGA et al. 2008b, NIEWIADOWSKA et al. 1995, 2008. ULRICH and RASZYK 2002) and game (JANICKI et al. 2007, RODZIEWICZ and HAJDUK 1995) as well as hen eggs (AURIGI et al. 2000. NIEWIADOWSKA et al. 1996, SMOCZYŃSKI et al. 1979). Blood as a liquid tissue of a live organism serves an important physiological function. Its multi-oriented role may be fulfilled only without the occurrence of extrinsic factors that make it impossible. Extrinsic substances often determined in human

blood include chlorinated hydrocarbons (SYROWATKA et al. 1981). Many studies have also addressed the content of chlorinated hydrocarbons in the blood of slaughter animals (AMAROWICZ et al. 1987. SMOCZYŃSKI et al. 1984). Their concentration in blood may indicate contamination of other body tissues. Studies carried out on birds (GLICK 1974) demonstrated that DDT affected a decrease in blood level of immunoglobulins G and M. Results of experiments carried out on hens (ADAMCZYK 1971) also point to translocation of DDT through blood from adipose tissue to muscles. Data on the content of chlorinated hydrocarbons in blood and eggs of domestic fowls may also be useful in breeding, especially in its intensive form.

The aim of the study was to determine the content of chlorinated hydrocarbons (DDT, DDE, DDD and γ-HCH) in different tissues of turkeys from a reproductive flock and linear correlation coefficients between the contents of these compounds in blood of the turkey hens and their concentrations in egg yolks; in egg yolk and their concentrations in blood of poults; in egg yolk and their concentrations in yolk sack of poults, in blood of the turkey hens and their concentrations in abdominal fat.

Material and Methods

Experiments were carried out on 50 turkey hens (BUT-5) kept in individual cages. All layers were provided equal and optimal environmental conditions. Over the entire laying period, the turkey hens were fed *ad libitum* a complete mixture. They were inseminated every 7th day with semen diluted at a ratio of 1:1. Eggs for hatching were collected 6 times a day, stored for a period of 7 days and incubated in Petersime incubators. During incubation, the hatching of poults was monitored. In addition, in the 3–4, 11–12 and 19–20 week of the laying season, blood and 2 eggs were collected from each layer, and the contents of γ-HCH, DDE, DDD and DDT were assayed in whole blood and egg yolk lipids. The content of chlorinated carbohydrates was also determined in the complete mixture, abdominal fat of turkey hens after the reproductive period as well as in blood and yolk sacks of one-day-old poults. Chlorinated hydrocarbons from the complete mixture, abdominal fat of hens, egg yolks and yolk sacks were isolated according to the method described by AMAROWICZ et al. (1986), and those from blood of turkey hens and poults – according to SYROWATKA et al. (1979). Separation and quantitative determination of chlorinated hydrocarbons were conducted by means of gas chromatography using a PU 4600 chromatograph by UNICAM with an electron capture detector and a glass column (2.1 x 4 mm) filled with Supercoport 100/120 covered with a liquid phase of 1.5% SP-2250 + 1.95%

SP-2401. The temperature of the detector was 250°C, the injector was 225°C and the column was 195°C. Argon was used as a carrier gas at a flow rate of 60 cm^3/min.

Identification was carried out by comparing retention times of peaks in a standard mixture and experimental sample. Quantitative calculations were performed by means of Unicam 4880 software. To provide the quality of results and control of the methods applied, samples of reference material were also analyzed. In addition, linear correlations were computed between the contents of chlorinated hydrocarbons in the samples examined.

Results and Discussion

During 20 weeks of the laying season, all birds displayed good welfare and high reproductivity. The mean number of eggs obtained from one layer was 114, the weight of eggs was 84.6 g and the brooding of poults from fertilized eggs was 90.05%.

Table 1 presents the mean values and variability coefficients of the contents of chlorinated carbohydrates in the complete mixture, blood of turkey hens and poults, egg yolk, yolk sack and the abdominal fat of the hens. In the feed mixture, the content of γ-HCH (0.0128 ng g^{-1}) was higher than that of DDT (0.0001 ng g^{-1}). Analyses also demonstrated a higher concentration of DDT metabolites (DDE-0.0117 ng g^{-1}. DDD-0.0155 ng g^{-1}) than DDT itself. whose content reached as little as 0.0001 ng g^{-1}. The total content of DDT and its metabolites in the feed mixture was low and accounted for 0.0273 ng g^{-1}. The content of HCH in blood of turkey hens (0.0002 ng g^{-1}) was lower than that in blood of one-day-old poults (0.0013 ng g^{-1}). Contrary results were reported for the content of DDT, a higher concentration of which was noted in blood of turkey hens (0.0021 ng g^{-1}) than in the blood of the poults (0.0001 ng g^{-1}). In addition, the blood of the hens was found to contain a higher concentration of DDT than of its metabolites: DDE 2-fold and DDD 5-fold. An opposite tendency was demonstrated in the blood of the poults, in which the concentration of DDT (0.0001 ng g^{-1}) was lower than that of its metabolites (DDE-0.0028 ng g^{-1}. DDD-0.0003 ng g^{-1}).

Low percentage contribution of DDT in Σ DDT and high contribution of metabolites of DDT: DDE (to 42.8%) and DDD (to 56.8%) in complete mixture testifies that they are not introduced to environment at present. High percentage contribution of DDT in blood of turkey hens (55.3%) and abdominal fat of the hens (84.7%) can showed that DDT is able to accumulate in tissues of organism. Very low (amount subliminal) levels of DDT don't activate of defensive mechanism and facilitate accumulation in organism.

Table 1

Contents of chlorinated carbohydrates in the research material (ng g^{-1})

Chlorinated carbohydrates	Statistical measures	Complete mixture $n=30$	Blood of turkey hens $n=50$	Egg yolk $n=100$	Abdominal fat of the hens $n=50$	Blood of poults $n=50$	Yolk sack of poults $n=50$
γ-HCH	x	0.0128	0.0002	0.0149	0.0012	0.0013	0.0354
	SD	0.003	0.0001	0.009	0.0003	0.0007	0.021
	range	0.010– –0.015	0.000– –0.0003	0.0056– –0.024	0.0008– –0.0015	0.0006– –0.0019	0.014– –0.059
	v	19.98	80.47	62.40	28.32	50.43	60.56
DDE	x	0.0117	0.0013	0.0068	0.0076	0.0028	0.0174
	SD	0.005	0.0015	0.0029	0.0018	0.0024	0.0114
	range	0.007– –0.0167	0.000– –0.0028	0.0039– –0.0097	0.0058– –0.0094	0.0004– –0.0052	0.006– –0.0288
	v	42.31	115.09	43.16	23.41	86.86	65.38
	%	42.8	34.2	41.5	13.4	87.5	32.4
DDD	x	0.0155	0.0004	0.0063	0.0011	0.0003	0.0321
	SD	0.0129	0.0013	0.0029	0.0005	0.0007	0.0767
	range	0.0025– –0.0285	0.000– –0.0017	0.0034– –0.0093	0.0006– –0.0016	0.000– –0.0009	0.000– –0.1087
	v	83.75	325.47	47.04	42.17	219.45	238.87
	%	56.8	10.5	37.5	1.9	9.4	59.6
DDT	x	0.0001	0.0021	0.0037	0.0481	0.0001	0.0043
	SD	0.0001	0.0136	0.0056	0.0157	0.0006	0.0089
	range	0.000– –0.0002	0.000– –0.0156	0.000– –0.0094	0.0324– –0.0638	0.000– –0.0007	0.000– –0.0132
	v	141.42	646.06	152.8	32.74	587.31	206.12
	%	0.4	55.3	21.0	84.7	3.1	8.0
Σ DDT	x	0.0273	0.0038	0.0168	0.0568	0.0032	0.0536
	SD	0.0079	0.0156	0.0081	0.0162	0.0027	0.0773
	range	0.0194– –0.0353	0.000– –0.0194	0.0086– –0.0249	0.0406– –0.0731	0.0005– –0.0059	0.000– –0.1309
	v	29.12	410.00	48.46	28.60	84.81	144.24

x – mean values. v – variability coefficients (%), % – percentage contribution of DDT metabolites in Σ DDT

Linear correlation coefficients between the contents of chlorinated hydrocarbons in blood of the turkey hens and their concentrations in egg yolks (Table 2), ranged from –0.036 to +0.654 and in the case of DDD (+0.534). DDT (+0.549) and sum of DDT (+0.654) were statistically significant ($p < 0.05$, 0.01), which may suggest that the transport of these compounds proceeds via the circulatory system to other organs. The coefficients of correlation obtained between contents of chlorinated hydrocarbons in the egg yolk of turkey hens and blood of poults (Table 2) were statistically insignificant (ranged from –0.216 to +0.020). However, the coefficient of correlation computed for DDE of –0.400 was close to being significant. In turn, no correlation was calculated for the content of DDT since it occurred only in a few blood samples.

The coefficient of correlation computed between the contents of chlorinated carbohydrates in egg yolk and yolk sacks (Table 2) fluctuated between –0.042 and 0.442 and in all cases was statistically insignificant, which is difficult to explain and requires further investigations. It should be emphasized that the correlation coefficient calculated for γ-HCH was close to being significant. No statistically significant coefficients of correlation were determined between the contents of chlorinated hydrocarbons in the blood and abdominal fat of turkey hens, in contrast to the correlation between their concentrations in blood and egg yolks of the hens (Table 2).

Table 2

Linear correlation coefficients between the contents of chlorinated hydrocarbons in selected materials

Specification		Blood of turkey hens				
		γ-HCH	DDE	DDD	DDT	Σ DDT
Egg yolk	γ-HCH	0.193				
	DDE		–0.036			
	DDD			0.534*		
	DDT				0.549*	
	Σ DDT					0.654**
		egg yolk				
		γ-HCH	DDE	DDD	DDT	Σ DDT
Blood of poults	γ-HCH	–0.216				
	DDE		–0.400			
	DDD			0.020		
	DDT				X	
	Σ DDT					–0.038
		egg yolk				
		γ-HCH	DDE	DDD	DDT	Σ DDT
Yolk sack of poults	γ-HCH	0.442				
	DDE		0.324			
	DDD			0.144		
	DDT				–0.042	
	Σ DDT					0.247
		blood of the turkey hens				
		γ-HCH	DDE	DDD	DDT	Σ DDT
Abdominal fat of the hens	γ-HCH	0.213				
	DDE		–0.039			
	DDD			–0.115		
	DDT				0.048	
	Σ DDT					0.004

Explanation: *$p < 0.05$; **$p < 0.01$; X – inanalysable value

No statistically significant coefficients of correlation between the contents of chlorinated hydrocarbons in the turkey blood and egg yolk lipids and number of laid eggs, weight of eggs and hatchability from fertile eggs were

determined in our earlier research (FARUGA et al. 2008a). Content of chlorinated hydrocarbons (DDT) in blood of turkey was ranged 0.0009–0.0081 ng g^{-1} however in turkey egg lipids was higher 0.0122–0.0210 ng g^{-1} (FARUGA et al. 2008a).

Aulakh et al. determined 0.91 mg kg^{-1} Σ DDT in feed while in chicken muscle – 0.24 mg kg^{-1} and that higher residues were in eggs (0.51 mg kg^{-1}) compared to muscle (AULAKH et al. 2006).

Furusawa research indicate that in fats from tissues of laying hens and eggs yolk DDT and DDE were transferred throughout the tissues and egg yolk while DDD was detected only in the liver after oral administration single dose of p.p'-(DDT) – 1 mg kg^{-1} body weight (FURUSAWA 2002).

Conclusions

The contents of chlorinated hydrocarbons in the samples examined were small and did not arouse serious hygienic or toxicological concerns. Significant correlations were determined only between the contents of chlorinated carbohydrates in blood and egg yolks of the layers. The low coefficients of correlation computed between contents of chlorinated hydrocarbons in egg yolks and their concentrations in the blood and yolk sacks of the poults as well as between their contents in blood and abdominal fat of the hens. The explanation the way of transportation of the chlorinated hydrocarbons from the blood of turkey hens to other tissues and from egg yolks to tissues of the poults requires further investigations.

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INFLUENCE OF POLLUTION SOURCES OF POINTS AND AREALS DIFFERENTIATION ON THE CONCENTRATION OF PHOSPHORUS AND NITROGEN COMPOUNDS IN WATER OF MAŁY JEZIORAK LAKE

Danuta Domska, Katarzyna Wojtkowiak

Chair of Foundation of Safety
Warmia and Mazury University in Olsztyn

K e y w o r d s: Mały Jeziorak Lake, water, nitrogen and phosphorus compounds.

A b s t r a c t

There were showed influence of the points (local and city reception basin outflow) and the areas (recreation area and wasteland) differentiation of pollution sources on the concentration of nitrogen and phosphorus compounds in water of Mały Jeziorak Lake. There were analyzed NO_3^-, NO_2^-, NH_4^+, total P and PO_4^{-3} content. Results of the studies were indicated that the most components concentration was low in I class of water cleanliness. There was more ammonium and nitrate nitrogen content near the recreation area and more nitrate nitrogen content in spring and summer time near the dairy outflow. Moreover, higher total phosphorus content but not more than in I class of water cleanliness norm were in the lake water with village rain outflow and near the recreation area and, phosphates content in II class water cleanliness in spring time near the recreation area.

ODDZIAŁYWANIE ZRÓŻNICOWANIA PUNKTOWYCH I OBSZAROWYCH ŹRÓDEŁ ZANIECZYSZCZEŃ NA ZAWARTOŚĆ ZWIĄZKÓW FOSFORU I AZOTU W WODZIE JEZIORA MAŁY JEZIORAK

Danuta Domska, Katarzyna Wojtkowiak

Katedra Podstaw Bezpieczeństwa
Uniwersytet Warmińsko-Mazurski w Olsztynie

S ł o w a k l u c z o w e: jezioro Mały Jeziorak, woda, związki azotu i fosforu.

Address: Danuta Domska, University of Warmia and Mazury, ul. Okrzei 1a, 10-266 Olsztyn, Poland, phone: +48 (89) 526 63 80, e-mail: danuta.domska@uwm.edu.pl

Abstrakt

W pracy określono wpływ zróżnicowania źródeł zanieczyszczeń punktowych (odpływ wód burzowych i ścieków z mleczarni) oraz obszarowych (teren rekreacyjny i nieużytek) na zawartość związków azotu i fosforu w wodzie części misy jeziora Mały Jeziorak. W metodyce badań uwzględniono analizę zawartości NO_3^-, NO_2^-, NH_4^+, P ogółem i PO_4^{-3}. Na podstawie uzyskanych wyników wykazano bezpośredni, a także zróżnicowany, wpływ punktowych źródeł zanieczyszczeń oraz obszaru przylegającego do linii brzegowej jeziora Jeziorak Mały oraz okresu wegetacyjnego na zawartość mineralnych związków azotu i fosforu, a tym samym na jakość wody badanej tafli jeziora. Stwierdzono, że zawartość większości badanych związków była przeważnie niska i mieściła się w klasie I czystości wód. W wodzie jeziora przylegającego do terenu rekreacyjnego było więcej azotu amonowego i azotanowego, a w miejscu odpływu ścieków z mleczarni w okresie wiosenno-letnim – więcej azotu azotanowego. Większą zawartość fosforu ogółem, nieprzekraczającą jednak norm I klasy czystości wód, zaobserwowano ponadto w wodzie jeziora, do której spływały osiedlowe wody burzowe i przy granicy z terenem rekreacyjnym, a fosforanów (w granicach normy II klasy czystości wód) – wiosną w okolicy terenu rekreacyjnego.

Introduction

The degradation of the environment is one from the most problem. It is connected with quality worsening of surface waters and considerable depend on phosphorus and nitrogen compounds content. These compounds have a important part in the eutrophication process of lakes when the phosphorus content is in norm of II class water cleanness and there is more nitrate nitrogen content (SZOSZKIEWICZ and SZOSZKIEWICZ 1997). The water properties depend on climate, basin kind and location and also human activity (SZCZYGIELSKI 1996, RACZKOWSKI and WARECHOWSKA 2002, DOMSKA et al. 2005, PULIKOWSKI et al. 2005, DOMSKA et al. 2006, KOC, SIDORUK 2006). The excessive phosphorus and nitrogen compounds concentration can be caused not only with point pollutions but also way of spatial economy connected with urbanization, industrialization, agricultural production on the basin area and tourism development without effective methods of the waters protection (RACZKOWSKI and WARECHOWSKA 2002, BECHMANN et al. 2005, DOMSKA et al. 2005, 2006, KOC and SIDORUK 2006, KOC at al. 2009).

The purpose of this paper was the estimation of the different points (outflow of storm waters and dairy sewerages) and area (recreation area and waste land) pollutions on nitrogen (NO_3^-, NO_2^- and NH_4^+) and phosphorus (total P and PO_4^{-3}) content in the some part of the lake basin of Mały Jeziorak.

Material and Metodhs

Mały Jeziorak Lake is situation in the centre of Iława town of Warmia and Mazury province. The lake surface is 26.0 ha, maximum depth – 6.4 m and

water volume – 890.9 thousand m^3. Out of natural storm waters flow, there are conducted back to lake the storm waters with town interceptors. Those waters are partially cleaned from derivative petroleum substances and organic pollution in the separator.

In the purpose of the points and area influence on water quality there were collected of water samples on April, June and September 2009 year according to standard PN-88/C-04663/04. The sites of water samples were located in the deepest place of lake (6.4 m) and in littoral zone near outflow place of town and storm waters and dairy sewerages, recreation area (town park) and waste land with shore covered with reed and bulrush (Figure 1).

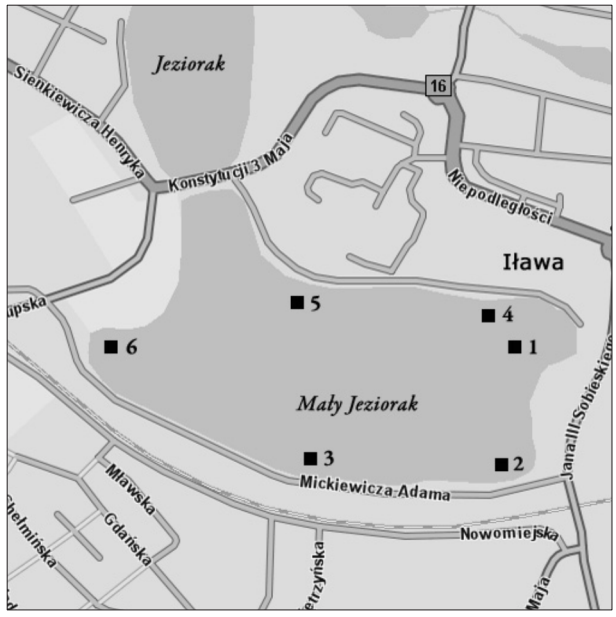

Fig. 1. Location of research stands: 1 – deepest place, 2 – local reception basin, 3 – city reception basin, 4 – dairy sewages, 5 – recreation area, 6 – waste land

In the water samples was immediately determinated nitrogen (NO_3^-, NO_2^- and NH_4^+) and phosphorus (total P and PO_4^{-3}) compounds content. In the analytical analysis was regarded the methods universally accepted in the agricultural and chemical stations.

The obtained results was statistically calculated by Tukey test of the variant analyse at the considerable differences of the confidence interval $p = 0.05$.

Results and Discusion

The analyzed nitrogen compounds in water was low and it was in standard of I class water cleanness (Rozporządzenie MOŚ... 2004). There was ammonium nitrogen content from 0.143 to 0.180 mg dm^{-3}, nitrate nitrogen from 0.764 to 1.30 mg dm^{-3} and nitrite nitrogen – from 0.002 to 0.070 mg dm^{-3} (Table 1). A little more ammonium nitrogen content in the lake surface was only in spring time and nitrate nitrogen in all study time and in the both cases

Table 1

Nitrogen concentration in water of Mały Jeziorak Lake (mg dm^{-3})

Research position	$N\text{-}NH_4^+$			$N\text{-}NO_3^-$			$N\text{-}NO_2^-$		
	sampling time								
	A	*B*	*C*	*A*	*B*	*C*	*A*	*B*	*C*
1	0.158	0.170	0.160	0.764	0.820	0.795	0.003	0.002	0.004
2	0.156	0.168	0.155	0.900	0.880	0.863	0.060	0.050	0.023
3	0.145	0.155	0.150	0.780	0.760	0.910	0.070	0.057	0.014
4	0.143	0.155	0.157	0.890	0.925	0.850	0.018	0.014	0.018
5	0.180	0.170	0.170	1.130	0.980	1.005	0.018	0.022	0.016
6	0.150	0.143	0.150	0.830	0.930	0.768	0.010	0.009	0.005
$LSD_{0.05}$	0.015			0.060			0.003		

A – spring, *B* – summer, *C* – autumn

near the recreation area. PULIKOWSKI et al. (2005) considered that different changes of nitrogen compounds concentration in water can be caused with big content balancing of its in all year. WOJTAS and DĄBEK (2006) were showed that nitrite and ammonium nitrogen concentration in water of Olecko Wielkie Lake was decreased in spring and summer time and nitrate nitrogen concentration was higher in summer time. In turn of SZYMCZYK and GLIŃSKA-LEWCZUK (2007) study was showed relatively high concentration $N\text{-}NO_3^-$ (average 1.25 mg dm^{-3}) in winter-spring time and low (average 0.18 mg dm^{-3}) – in spring-summer time. The nitrite nitrogen concentration in water of Mały Jeziorak was important different between deepest lake place and another studied sites, at the same time, it was highest near storm outflows on spring-summer time. The different in this case were from 0.038 to 0.68 mg dm^{-3} and, it was higher than DOMSKA et. al. (2006) showed in water of Narie Lake as effect of agriculture area (arable soils). There was also higher nitrite nitrogen content (from 0.007 to 0.020 mg dm^{-3}) in water with outflow of dairy sewerages, near recreation area and on spring-summer time near waste

land. According to KRUK (1996), the lakes showed big resistance to degradation only in case the forest nearness, while a big risk are intensive tourism and area adaptation for the recreation necessity. Other authors (KOC et al. 1966, DOMSKA et al, 2005) are point out to direct influence of agricultural areas, particularly arable soils and also intensive tourism on some form of mineral nitrogen content in waters.

Table 2

Phosforus concentration in water of Mały Jeziorak Lake (mg dm^{-3})

Research position	Total P			PO_4^{-3}		
	Sampling time*					
	A	*B*	*C*	*A*	*B*	*C*
1	0.217	0.271	0.234	0.158	0.163	0.149
2	0.310	0.288	0.330	0.210	0.270	0.232
3	0.239	0.231	0.286	0.180	0.213	0.220
4	0.246	0.286	0.278	0.225	0.275	0.276
5	0.263	0.215	0.235	0.467	0.278	0.320
6	0.162	0.256	0.287	0.200	0.245	0.250
$LSD_{0.05}$	0.032			0.038		

* see Table 1.

There was a little difference of phosphorus content in the surface water of lake Mały Jeziorak in the study time (Table 2). Total phosphorus concentration was from 0.162 to 0.330 mg dm^{-3}, so it was not higher then standard of I class water cleanness (Rozporządzenie MOŚ... 2004). The most phosphorus content was in water near the community storm outflow (site 2) in all study time, particularly in spring and autumn time. Moreover, higher total phosphorus content was near town storm outflow (site 3) in autumn time and in surface water lake near town park (site 5) in spring time depend on the content of this in deepest lake place (site 1). DOMSKA et al. (2006) was showed higher total phosphorus content in water Narie Lake in standard of III class water cleanness near arable soils in spring and summer time. In turn of GLIŃSKA-LEWCZUK (2005) was showed that P-PO_4^{-3} in water of Łyna river was increased average from 0.26 mg dm^{-3} in spring time to average 0.70 mg dm^{-3} in summer time. But first of all, some authors (SZOSZKIEWICZ, SZOSZKIEWICZ 1997, BECHMANN et al. 2005, DOMSKA et al. 2005) are point out on a big influence of agricultural areas nearness on total phosphorus content in the surface waters. In the conducted studies was showed clear influence of fertilized in autumn time, the town park recreation area nearness (site 5) on phosphates content in water lake Jeziorak Mały. In this case, there was 0.467 mg dm^{-3} of these

compounds in spring time, so it was changed water cleanness from first to second class. The similar results were showed in studies of BARTOSZEWICZ (2005) and DOMSKA et al. (2006) with the influence of the neighbours area on phosphates content on land of Kościańsk Plain and in water of Narie Lake. SZYMCZYK and GLIŃSKA-LEWCZUK (2007) were point out on the variability of $P\text{-}PO_4^{-3}$ concentration in Jagiełek Lake near Olsztyn, where it was tendency to increase from average 0.26 mg dm^{-3} in spring time to average 0.79 mg dm^{-3} in summer time. The outflows from dairy sewerage and storm were increased phosphates content in water lake Mały Jeziorak but without change of water cleanness class. According to BARTOSZEWICZ (2005), first at all, phosphates concentration increased in surface waters in condition of intensive rainfalls.

Conclusion

1. The mineral nitrogen compounds concentration in water lake Mały Jeziorak was mainly low in standard of I class water cleanness.

2. In water of lake near the recreation area was more ammonium and nitrate nitrogen content and in place of dairy sewerages inflow was more nitrate content in spring-summer time.

3. There was showed higher total phosphorus content, but not more than standard of I class water cleanness, in lake water with inflow of community storm waters and near the recreation area and, higher phosphates content in standard of II class water cleanness was in lake water near the recreation area in spring time.

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EVALUATION OF THE ECOLOGICAL STATUS AND DIVERSITY OF MACROPHYTES OF DRAINAGE DITCHES THREATENED BY A PESTICIDE TOMB*

***Mirosław Grzybowski*[1], *Józef Szarek*[2], *Krystyna A. Skibniewska*[3], *Janusz Guziur*[4], *Joanna Duriasz*[1]**

[1] Chair of Applied Ecology
[2] Chair of Pathophysiology, Forensic Veterinary Medicine and Administration
[3] Chair of Foundations of Safety
[4] Department of Fish Breeding and Rearing
University of Warmia and Mazury in Olsztyn

K e y w o r d s: species diversity, macrophyte, pesticide tomb, drainage ditch.

A b s t r a c t

The research was aimed at determining whether a pesticide tomb affects the ecological status of drainage ditches located in its vicinity as well as how it affects the diversity of the flora of the ditches and in which direction its effect on the surrounding environment is. In addition, the results of the study are a contribution to a comparative analysis of methods for macrophyte evaluation of watercourses used in Poland and to identify the possibilities of their application in investigations of artificial watercourses under the pressure of pesticides. Values of indices of the hydromorphological evaluation (HQA, HMS) were similar for all drainage ditches examined. This points to a negligible effect of the pesticide tomb on the hydromorphological characteristics of the analyzed watercourses. The pesticide tomb was found to affect the ecological status and floral diversity of the drainage ditches. The lowest values of macrophyte and diversity indices were recorded in the first and the second watercourse, whereas higher ones were in the third watercourse. Changes in the indices of ecological status and those of floral diversity observed in the watercourses examined show that the effect of the pesticide tomb on the surrounding ecosystem is consistent with topographic features and proceeds in a north-easterly direction. Both methods of the macrophyte evaluation of the ecological status of watercourses (MTR, MIR) yield different numeric values, yet their results are comparable in terms of the tendency of changes in the ecological status of the analyzed watercourses. In both methods, the highest rank was reported for the third watercourse. The MTR index diversifies the examined watercourses to a smaller extent than the MIR index. The MIR method is better under conditions of lowland Poland for the evaluation of the ecological status of artificial watercourses, as it enables stronger diversification of the ecological status of the watercourses examined due to a higher number of indicatory species.

Address: Mirosław Grzybowski, University of Warmia and Mazury, ul. Oczapowskiego 5, 10-719 Olsztyn, phone: +48 (89) 523 37 42, e-mail: grzybomi@uwm.edu.pl
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OCENA STANU EKOLOGICZNEGO ORAZ RÓŻNORODNOŚCI GATUNKOWEJ ROŚLINNOŚCI ROWÓW MELIORACYJNYCH POŁOŻONYCH W SĄSIEDZTWIE MOGILNIKA PESTYCYDOWEGO

***Mirosław Grzybowski*[1], *Józef Szarek*[2], *Krystyna A. Skibniewska*[3], *Janusz Guziur*[4], *Joanna Duriasz*[1]**

[1] Katedra Ekologii Stosowanej
[2] Katedra Patofizjologii, Weterynarii Sądowej i Administracji
[3] Katedra Podstaw Bezpieczeństwa
[4] Katedra Biologii i Hodowli Ryb
Uniwersytet Warmińsko-Mazurski w Olsztynie

S ł o w a k l u c z o w e: różnorodność gatunkowa, makrofity, rowy melioracyjne, mogilnik pestycydowy.

A b s t r a k t

Przeprowadzone badania mają na celu stwierdzenie, czy mogilnik pestycydowy wpływa na stan ekologiczny rowów melioracyjnych znajdujących się w sąsiedztwie, jak również, w jaki sposób wpływa na różnorodność ich flory oraz jaki jest kierunek tego oddziaływania. Wyniki badań są wkładem do analizy porównawczej metod oceny makrofitów cieków Polski. Istnieje możliwość ich zastosowania w badaniach sztucznych cieków wodnych będących pod wpływem pestycydów. Wartości wskaźników hydromorfologicznej oceny dla rowów melioracyjnych (HQA, HMS) były zbliżone. Wskazuje to na nieznaczny wpływ mogilnika pestycydowego na hydromorfologiczne cechy badanych cieków. Stwierdzono wpływ mogilnika pestycydowego na stan ekologiczny oraz różnorodność roślinności rowów melioracyjnych. Niższą wartość wskaźników makrofitowych oraz wskaźników różnorodności zanotowano w cieku pierwszym i drugim, wyższą w cieku trzecim. Na podstawie zmian wskaźników stanu ekologicznego oraz wskaźników różnorodności roślinności w badanych ciekach stwierdzono, że wpływ mogilnika pestycydowego na otaczające ekosystemy jest zgodny z ukształtowaniem terenu i przebiega w kierunku północno-wschodnim. Obie metody makrofitowej oceny stanu ekologicznego cieków (MTR, MIR) dają liczbowo odmienne wartości. Wyniki są jednak porównywalne w zakresie tendencji zmian stanu ekologicznego w badanych ciekach. W obu metodach najwyższą wartość uzyskał ciek trzeci. Wskaźnik MTR w mniejszym stopniu różnicuje badane cieki niż wskaźnik MIR. Metoda MIR jest lepsza w warunkach Polski niżowej do oceny stanu ekologicznego cieków sztucznych, ponieważ pozwala silniej różnicować stan ekologiczny badanych cieków dzięki większej obecności gatunków wskaźnikowych.

Introduction

Watercourses are an inseparable element of environment, they create living space for a number of flora and fauna species and constitute a significant element of balanced landscape persistence. Apart from tree-covered areas, permanent grasslands and natural watercourses, drainage ditches are a part of a network of biocenters, bioways and elements working together to play a protective role for soil and a gene pool of plants and animals, as well as of elevating the stability of the entire landscape system.

Drainage ditches in Warlity Wielkie (a village at the edge of which a pesticide tomb is located) play an important functional role by draining excessive water from areas around fish ponds. The drainage ditches pass through an area located near a closed pesticide tomb (< 1 km) – Figure 1.

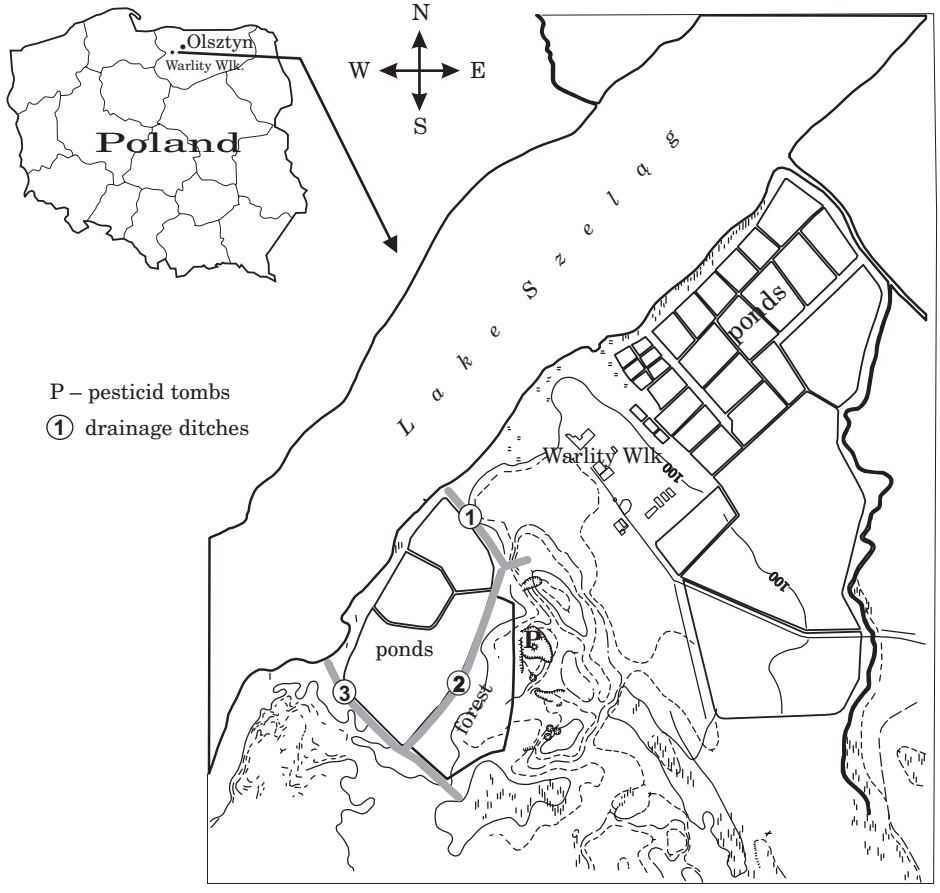

Fig. 1. Location of the study area

Pesticide tombs are one of the most severe threats to the natural environment in Poland. In Warlity Wielkie, near Ostróda, in the Iławski Lake District, a pesticide tomb was in operation until November 3, 2004. It was one of the 16 landfill sites of non-utilized pesticides in the province Warmia and Mazury in the years 1960–1970. The tomb was used as a landfill of 54 tones of toxic substances disposed of in 36 silos and 2 unprotected cavities. Since 2003, complex analyses of the edaphic and aquatic environment have been carried

out in this area (SKIBNIEWSKA et al. 2003, GRZYBOWSKI et al. 2003, 2004, 2005, 2005a,b, 2006, 2010, SAWICKA-KAPUSTA et al. 2005, SZAREK et al. 2004, 2006, 2007a, 2007b, 2007c, ZAKRZEWSKA et al. 2005, ZMYSŁOWSKA et al. 2005).

The research was aimed at determining whether a pesticide tomb affects the ecological status of drainage ditches located in its vicinity as well as how it affects the diversity of the flora of the ditches and in which direction its effect on the surrounding environment is. In addition, the results of the study are a contribution to a comparative analysis of methods for macrophyte evaluation of watercourses used in Poland and to identify the possibilities of their application in investigations of artificial watercourses under the pressure of pesticides.

Characteristics of the study area

A site survey was carried out in the summer of 2007 on drainage ditches (Figure 1). The examined drainage ditches surrounding three ground fish ponds, they are typical artificial watercourses, draining the area around the ponds (Figure 1). They are located in the Iławski Lake District, in the province of Warmia and Mazury, in Ostróda Commune. The first and the third watercourse discharge waters to Lake Szeląg Wielki, whereas the second stream links the second and the third watercourse together. Lake Szeląg Wielki is a typical ribbon-like narrow and very elongated lake. Runoff from the aquifer proceeds south-west from Lake Pauzeńskie and Lake Drwęckie. Its size accounts for 599 ha, its maximum depth for 35.5 m, and mean depth for 13.5 m. Until 2004 a pesticide tomb was located ca. 50 m south of the drainage ditches. It was separated from the second watercourse by a thin belt of anthropogenic forest (*Sambucus nigra-Picea abies* and that of *Sambuco racemosi-Pieceetum*).

Materials and Methods

The experimental material for analyses was collected during field work in the period of April–July 2007. The nomenclature of vascular plants was adopted after RUTKOWSKI (2006). The study also involved bryological analyses that were carried out on moss collected from stones and wood in watercourse channels. In the case of mosses determined by macroscopic characteristics, only a floristic census was made. The collected materials were assayed in a laboratory according to the following keys (SMITH 1996, NYHOLM 1960–1969, 1986, 1993, 2001). The nomenclature of mosses was adopted after OCHYRA et al. (2003).

The hydromorphological evaluation of the watercourses was conducted based on the British method: River Habitat Survey (RHS) (RAVEN et al.1989), considering modifications introduced for Poland (SZOSZKIEWICZ et al. 2007). Analyses were carried out by trained staff (GRZYBOWSKI 2007). The RHS was performed on three representative stretches (500-metres long) of the examined watercourses. The following indices were calculated: Habitat Quality Assessment Score (HQA) and Habitat Modification Score (HMS).

The HQA evaluates the presence and diversity of natural elements of river bed and valley, including: physical characteristics of the bed and banks, structure of flora on the slope, type of flora in the bed, land utilization at a distance of 50 m from the bed, stand density, and other characteristics indicating the natural character.

The HMS determines the extent of modifications in the morphology of a watercourse by means of the number and type of water structures, fortifications, and profile changes.

For the macrophyte evaluation, two methods were selected, namely: the British method – the Mean Trophic Ranking (MTR) (HOLMES et al. 1999) and the Polish method – the River Macrophyte Index (RMI) (SZOSZKIEWICZ et al. 2006a). Both the methods assume conducting analyses on 100-metre stretches of a watercourse. Analyses were carried out on the three watercourses examined, consideration was given to all species growing permanently in the water.

The MTR is based on the quantitative and qualitative assessment of the species composition of plants, out of which a list of 128 species indicative of macrophytes was selected: 8 algae, 23 mosses, 7 liverworts, 3 pteridophytes, 47 monocotyledonous, and 40 dicotyledonous. The MTR index is calculated based on a nine-degree Species Cover Value (SCV) and Species Trophic Rank (STR):

$$MTR = \frac{\sum (STR \cdot SVC)}{\sum SVC} \cdot 10$$

Three degrees of watercourses degradation are distinguished in the interpretation of the MTR index (HOLMES et al. 1999) (Table 1).

The River Macrophyte Index for Rivers – MIR (SZOSZKIEWICZ et al. 2006b) is computed based on the ratio of the quotient of Species Trophic Rank for a given species (L_i), weight coefficient for a given species (W_i), and Species Cover Value for a given species according to a 9-degree scale (P_i) (the same as for MTR; P_i = SVC) to the quotient of weigh coefficient for a given species (W_i) and Species Cover Value for a given species (P_i). The MIR may attain values

Table 1
Boundary values of MTR index [25] and MIR index [26] and their interpretation for watercourses

MTR		MIR	
degree of watercourse degradation		ecological status class	type of watercourse: sandy and organic
I	> 65 non-eutrophicated watercourses	very good	≥ 44.5
II	25–65 watercourses degraded as a result of eutrophication or severely threatened by proceeding eutrophication	good	44.5–35.0
IIa	45–65 at risk of degradation	moderate	35.0–25.4
IIb	25–45 degradation as a result of eutrophication is evident	poor	25.4–15.8
III	< 25 considerable degradation	bad	< 15.8

from 10 (the most degraded) to 100 (the best). In the case of lowland rivers, the highest values of MIR do not exceed 60 (SZOSZKIEWICZ et al. 2006a).

$$MIR = \frac{\sum (L_i\ W_i \cdot P_i)}{\sum W_i \cdot P_i} \cdot 10$$

The MIR considers 146 species indicative of macrophytes: 9 algae, 22 mosses, 10 liverworts, 3 pteridophytes, 53 monocotyledonous and 49 dicotyledonous. According to the Framework Water Directive, five classes of the ecological status of watercourses are distinguished in the interpretation of Macrophyte Index for Rivers (SZOSZKIEWICZ at al. 2006a) – Table 1.

The diversity evaluation was carried based on the Shannon-Wiener index (SHANNON, WEAVER 1949). The distribution of species in a phytocenosis was described by means of the Pielou Evenness index [*J*] (MAGURRAN 1988).

In order to depict floral diversity in the examined drainage ditches, use was made of an ordination technique – Principal Component Analysis [PCA]. The PCA is a method elaborated by PEARSON (1901), and adapted for plant analysis by GOODAL (1954) and especially by ORLOCI (1966). It enables determining the main directions of changes in flora along theoretical environmental gradients.

Diversity indices and PCA were computed by means of Multi Variate Statistical Package (MVSP) ver. 3.1.

Results

Diversity indices of aquatic and rush flora calculated for the examined drainage ditches were the highest for the third watercourse and the lowest for the first watercourse (Table 2).

Table 2
Values of indices of diversity, hydromorphology and macrophyte evaluation for the drainage ditches in Warlity Wielkie

				Watercourse 1	Watercourse 2	Watercourse 3
Number of species				20	22	27
Shannon-Wiener index				3.877	4.127	4.519
Pielou Evenness index				0.897	0.925	0.950
RHS	HQA			27	28	29
	HMS			53	61	53
MTR/MIR indices	STR	L_i	W_i	SVC=P_i	SVC=P_i	SVC=P_i
1	2	3	4	5	6	7
Algae						
Cladophora sp.	1	1	2	5	5	1
Oedogonium sp.	1	1	1	–	1	1
Stigeoclonium sp.	1	1	1	–	1	–
*Rhizoclonium** sp.	–	1	1	–	–	1
Mosses:						
Brachythecium rutabulum	3	3	2	4	3	1
Pteridophytes and horsetails						
Equisetum fluviatile	5	6	2	–	4	3
Equisetum palustre	5	5	2	–	–	1
*Thelypteris palustris**	5	5	2	–	1	1
Dicotyledonous:						
Nasturcium officinalis	5	5	2	1	–	–
Veronica anagalis-aquatica	3	4	2	1	1	–
*Veronica beccabunga**	–	4	1	–	1	1
*Mentha aquatica**	–	5	1	–	–	1
*Myosotis palustris**	–	4	1	–	–	1
Monocotyledonous:						
Carex acutiformis	3	4	1	3	–	–
Carex riparia	4	4	2	–	–	1
Carex vesicaria	6	6	2	–	–	1
Glyceria maxima	–	3	1	–	1	2
Iris pseudoacorus	5	6	2	1	–	–
Lemna minor	4	2	2	9	6	1
Lemna gibba	2	1	3	–	1	–
*Phragmites australis***	4	–	–	2	4	3
*Carex paniculata**	–	5	1	–	–	3
*Scirpus sylvaticus**	–	5	2	1	1	5

cont. table 2

1	2	3	4	5	6	7
Accompanying species (not being indicators):						
Plagiomnium undulatum	–	–	–	2	2	1
Leptodictyum riparium	–	–	–	2	1	–
Amblystegium serpent	–	–	–	1	1	1
Dicranum scoparium	–	–	–	1	1	
Orthotrichum affinae	–	–	–	–	–	1
Galium palustre	–	–	–	1	–	1
Salix fragilis	–	–	–	3	–	2
Juncus effuses	–	–	–	1	–	1
Cirsium oleraceum	–	–	–	1	–	–
Filipendula ulmaria	–	–	–	1	1	–
Poa annua	–	–	–	1	–	–
Urtica urens	–	–	–	1	–	–
Lythrum salicaria	–	–	–	–	–	1
Alnus glutinosa	–	–	–	–	1	1
Poa trivialis	–	–	–	–	–	1
Carex pseudocyperus	–	–	–	–	–	1
Epilobium hirsutum	–	–	–	–	2	–
Galium aparine	–	–	–	–	2	–
Urtica dioica	–	–	–	–	2	–
MTR:	31.9	31.9	37.3			
MIR:	26.0	28.6	43.5			

* – indicatory species only in the MIR method
** – indicatory species only in the MTR method

The computed MTR indices (Table 2) were within the range of 25–45 and indicated evident eutrophication. Of all the watercourses examined, the highest value of this index was recorded for the third watercourse. In turn, the first and the second watercourses obtained the similar value. The MIR index diversifies the ecological status of the watercourses under scrutiny. The ecological status of the third watercourse was good, whereas that of watercourses 1 and 2 was moderate. The first watercourse obtained the lowest value of the RMI index out of all streams examined.

Indices of diversity were the lowest for the first watercourse and the highest for the third watercourse (Table 2).

Results of hydromorphological evaluation of the watercourses based on the RHS method were as follows:

– the index (HQA), amounted to based on the presence and diversity of natural elements of a watercourse and river bed values 27 for the first watercourse, 28 for the second watercourse and 29 for the third watercourse, which indicated their low naturalness (Table 2).

– the index (HMS) that determining the range of modifications in watercourse morphology was higher than the HQA index in all watercourses

examined (Table 2). The highest value of HMS was recorded in the second watercourse, which is due to two spillways noticed. The second watercourse is a connecting link between the first and the third watercourse, hence, the higher number of hydrotechnical structures. All the other parameters of the Habitat Modification Score (HMS) of a watercourse evaluated in ten profiles and in the entire 500-m stretch in the synthetic perspective, such as: modification of bed and banks, were the same.

Discussion

The diversity indices calculated for the examined drainage ditches demonstrated appeared to be higher in the third watercourse located east of the pesticide tomb, thus indicating higher biological diversity. This points to an oriented action of the pressure of the pesticide tomb on the surrounding ecosystems, which is consistent with the results obtained in analyses of macrophytes of Szeląg Wielki Lake (GRZYBOWSKI et al. 2005a, 2007b) and flora of the anthropogenic forest adjoining the pesticide tomb (GRZYBOWSKI et al. 2006, 2010b).

So far, drainage ditches have been a sparse subject of RHS evaluation (SZOSZKIEWICZ et al. 2006b). The obtained results of the hydromorphological assessment of the watercourses examined were typical of the artificial watercourses.

In Poland, the MTR method has been used for years in scientific research (SZOSZKIEWICZ et al. 2002, ZBIERSKA at al. 2002a,b, 2004, GRZYBOWSKI et al. 2010a). It has proved to be useful under conditions of Poland and its draft adaptation has been postulated by SZOSZKIEWICZ et al. (2002, 2005). So far, in Poland this method has been applied in analyses of over 300 uniform water streams, mainly on the lowlands (SZOSZKIEWICZ et al. 2006a).

The MIR method is novel in Poland; to date it has been applied in investigations of 110 river stretches located on 76 rivers (SZOSZKIEWICZ et al. 2006a), artificial watercourses have not been studied.

The analysis of watercourses assayed so far with the MTR method demonstrated that this method needs to be adjusted to Polish conditions (SZOSZKIEWICZ et al. 2006a, GRZYBOWSKI et al. 2010a), which has also been confirmed in the reported study. The number of indicatory species used in the macrophyte evaluation of watercourses in the MTR method accounted for 16, and that used in the MIR method for 22. Owing to the higher number of indicatory macrophytes, the MIR method enabled better demonstration of diversification between the watercourses examined as compared to the MTR method.

PCA ordination provides a picture of basic directions of diversification in the occurrence of species in the watercourses under scrutiny. It is a multi-species extension of a multiple regression, and the environmental variable is a theoretical variable. The length of a vector reflects diversity in species contribution in the ordination space and is proportional to its share in watercourses. Species observed in the first and the second watercourse are bound more strongly with environmental gradient represented by the first ordination axis (Figure 2), assuming that the I ordination axis of the PCA ordination reflects the environmental factor generated by the pesticide tomb, which has been confirmed by results of hydromorphological and macrophyte evaluation and those of macrophyte diversity of the watercourses examined, then the greatest effect was observed for the first watercourse, slightly smaller one for the second and the smallest one for the third watercourse analyzed in the study. This points to a greater pressure of the pesticide tomb exerted on the first and second watercourse than on the third one. It confirms the assumed direction of pesticide distribution in the environment around the pesticide tomb (GRZYBOWSKI et al. 2010a,b).

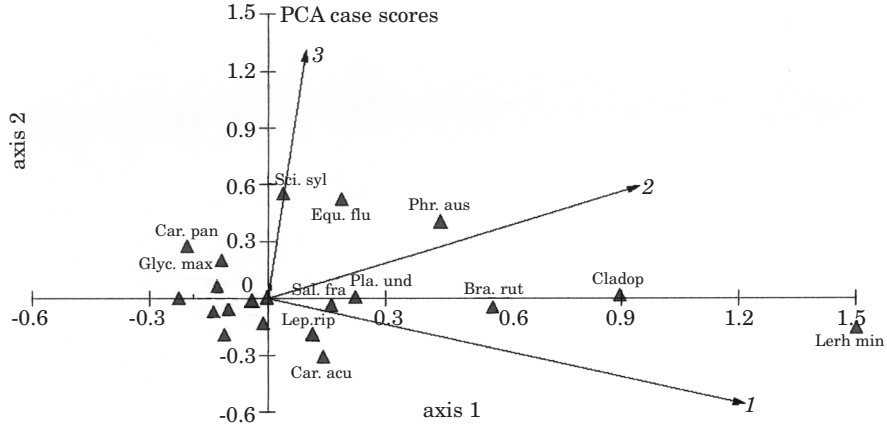

Fig. 2. Principle Component Analysis [PCA] of aquatic and rush flora of the drainage ditches in Warlity Wielkie

Conclusions

1. Values of indices of the hydromorphological evaluation (HQA, HMS) were similar for all drainage ditches examined. This pointed to a negligible effect of the pesticide tomb on the hydromorphological characteristics of the analyzed watercourses.

2. The pesticide tomb was found to affect the ecological status and floral diversity of the drainage ditches. The lowest values of macrophyte and diversity indices were recorded in the first and the second watercourse, whereas higher ones were in the third watercourse.

3. Changes in the indices of ecological status and those of floral diversity observed in the watercourses examined showed that the effect of the pesticide tomb on the surrounding ecosystem was consistent with topographic features and proceeds in a north-easterly direction.

4. Both methods of the macrophyte evaluation of the ecological status of watercourses (MTR, MIR) yield different numeric values, yet their results are comparable in terms of the tendency of changes in the ecological status of the analyzed watercourses. In both methods, the highest rank is reported for the third watercourse.

5. The MTR index diversifies the examined watercourses to a smaller extent than the MIR index. The MIR method is better under conditions of lowland Poland for the evaluation of the ecological status of artificial watercourses, as it enables stronger diversification of the ecological status of the watercourses examined due to a higher number of indicatory species.

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A COMPARISON OF THE ECONOMIC EFFECTIVENESS OF VARIOUS SPAWNING AGENTS FOR STIMULATING THE REPRODUCTION OF THE CULTURED AND WILD FORMS OF THE COMMON BARBEL *BARBUS BARBUS* (L.)

Anna Hakuć-Błażowska, Krzysztof Kupren, Konrad Turkowski, Katarzyna Targońska, Daniel Żarski, Dariusz Kucharczyk

Department of Lake and River Fisheries
University of Warmia and Mazury in Olsztyn

K e y w o r d s: hormonal stimulation, economic effectiveness.

A b s t r a c t

The objective of this study was to evaluate the economic effectiveness of various hormonal products for stimulating the reproduction of the cultured and wild forms of the common barbel *Barbus barbus* (L.). Three hormonal products were analyzed: Ovopel, Ovaprim (cultured and wild fish) and hCG (cultured fish). The economic effectiveness of hormonal stimulants was analyzed in view of ovulation rates, the price of hormonal products and spawner mortality rates. The cost of hormonal injection per 1000 eggs and 1000 hatchlings was adopted as an indicator of economic effectiveness. The best results were reported for hormonal stimulation using synthetic GnRH analogues during induced spawning of the barbel in a group of cultured fish. The above product's effectiveness in the controlled spawning of cyprinids is demonstrated by high ovulation rates and high embryo survival rates.

PORÓWNANIE OPŁACALNOŚCI ZASTOSOWANIA RÓŻNYCH PREPARATÓW HORMONALNYCH DO STYMULACJI ROZRODU BRZANY *BARBUS BARBUS* (L.) – FORMY HODOWLANEJ I DZIKIEJ

Anna Hakuć-Błażowska, Krzysztof Kupren, Konrad Turkowski, Katarzyna Targońska, Daniel Żarski, Dariusz Kucharczyk

Katedra Rybactwa Jeziorowego i Rzecznego
Uniwersytet Warmińsko-Mazurski w Olsztynie

S ł o w a k l u c z o w e: stymulacja hormonalna, opłacalność.

Address: Anna Hakuć-Błażowska, University of Warmia and Mazury, ul. Michała Oczapowskiego 5, 10-719 Olsztyn, Poland, phone: +48 (89) 523 44 36, e-mail: hakuc.blazowska@uwm.edu.pl

Abstrakt

Celem badań przedstawionych w pracy była ocena opłacalności zastosowania różnych preparatów hormonalnych do stymulacji rozrodu brzany *Barbus barbus* (L.) zarówno jej formy hodowlanej, jak i dzikiej. Do badań przyjęto trzy środki hormonalne: Ovopel, Ovaprim (forma hodowlana i dzika) i hCG (forma hodowlana). Oceniono opłacalność zastosowania tych preparatów hormonalnych do stymulacji rozrodu brzany, biorąc pod uwagę płodność ryb, cenę preparatów hormonalnych i śmiertelność tarlaków. Jako miarę opłacalności przyjęto koszt iniekcji hormonalnej w przeliczeniu na uzyskane 1000 ziaren ikry i 1000 sztuk wylęgu. Najbardziej opłacalna okazała się stymulacja hormonalna za pomocą syntetycznych analogów GnRH w trakcie kontrolowanego rozrodu brzany w grupie ryb hodowlanych. Wynika to ze skuteczności tych środków hormonalnych w kontrolowanym rozrodzie ryb karpiowatych, o której świadczy wysoki odsetek owulacji, jak i wysoki odsetek przeżywalności zarodków.

Introduction

The progressing loss of habitats and the extinction of rheophilic fish of the family Cyprinidae in recent years calls for the urgent implementation of protection programs. The is an immense demand for adequately selected stocking material of those valuable fish species to support stock management in open waters (MEJZA et al. 1996, AUGUSTYN 2002, KUJAWA 2004, PENCZAK et al. 2004, WOJDA 2004, WOLNICKI 2005, BOLLAND et al. 2008). Two main methods are applied to produce spawners of rheophilic cyprinid fish. The first is artificial spawning in a controlled environment (KUCHARCZYK 2002, JAMRÓZ et al. 2008a,b, KREJSZEFF et al. 2009, 2010, ŻARSKI et al. 2008b, 2009, WOLNICKI, MYSZKOWSKI 1998, CIEŚLA et al. 2000b, MYSZKOWSKI et al. 2000), and the second involves artificial reproduction of caught wild spawners in a hatchery both with and without hormonal stimulation (KOURIL et al. 1988, CIEŚLA et al. 2000a, AUGUSTYN 2002). The production of stocking material based on the artificial spawning of rheophiles is gaining new significance as advances are made in the reproduction biotechnology, thus contributing to higher production in commercial aquaculture (KUPREN et al. 2008b, TURKOWSKI et al. 2008). The success of induced spawning is determined by the choice of the right hormonal preparation and its dose. Hormonal stimulation takes place at the level of the hypothalamus, the pituitary gland and the gonads (BIENIARZ and EPLER 1991, PETER and YU 1997, ZOHAR and MYLONAS 2001, PODHOREC and KOURIL 2009, YARON et al. 2009). Hormonal products which are most frequently used in the induced spawning of cyprinids include the homogenate of carp and bream pituitary glands (CIEŚLA 1998, KUCHARCZYK et al. 2008), human chorionic gonadotropin (hCG) (KUCHARCZYK et al. 1997a,b,c, 2005), gonadotropin-releasing hormone (GnRH) and its analogues, often in combination with a dopamine antagonist (KREJSZEFF et al. 2008, 2009, 2010, ŻARSKI et al. 2008b, 2009).

Artificial spawning and breeding has to deliver economic advantages. There is a scarcity of publications discussing the economic aspects of rearing the larvae of rheophilic cyprinid fish under controlled conditions (KUPREN et al. 2008b, TURKOWSKI et al. 2008). The cost of performing hormonal stimulations in the reproduction of rheophilic fish also receives weak coverage in the related literature (KŁODZIŃSKA and OKONIEWSKI 1998, HAKUĆ-BŁAŻOWSKA et al. 2009). The objective of this study was to evaluate the economic efficiency of various hormonal products for stimulating the reproduction of wild and cultured forms of the common barbel *Barbus barbus* (L.).

Materials and Methods

Spawners and fish manipulation

Barbel spawners were obtained from two sources. Cultured fish (F_3 generation) were acquired from the Czarci Jar Fish Farm near Olsztynek (NE Poland). The stock originated from fish caught in the Vistula Basin. It was kept in tanks with a capacity of 1000 dm^3 and an environment control option (KUJAWA et al. 1999). The first group of wild fish (WF-1) was caught in the Narew River in Dębe in the fall, and it was transported to the Czarci Jar Fish Farm where it was kept in a flow-through earthen pond. Starting in the fall, both groups were kept in natural temperatures. The photoperiod of cultured fish was similar to the natural regime and it was controlled by a light clock. Both groups were fed the same diet comprising trout pellet and frozen chironomids in a weight ratio of 1:1 (1.5% fish biomass). In late spring when water temperature reached 15°C, both groups were kept under identical conditions in fiberglass tanks with a capacity of 1 m^3. Fish density did not exceed 30 kg m^{-3}. The third experimental group (WF-2) comprised fish caught in the Vistula Basin (dam on the Narew River in the village of Dębe) in the spring, immediately before the spawning season, when water temperature in the river reached 15°C. The fish were transported to the Czarci Jar Fish Farm where they were placed in tanks and kept under the same conditions as the remaining two groups.

Prior to any manipulation, fish were anesthetized in a bath of 0.5 cm^3 dm^{-3} 2-phenoxyethanol solution (Sigma-Aldrich, Germany). Temperature in the tanks was 15°C on the day of spawner introduction. Hormonal injections were performed after a four-day adaptive period during which water temperature was gradually increased to 17°C. Fish from both hatchery groups were exposed to a constant photoperiod of 14 h (14 L : 10 D).

Experiments

The experiments were carried out within a single breeding season. The first experiment was performed on the group of cultured fish, and it verified the efficacy and economic effectiveness of selected hormonal products. Prior to the first injection, fish were marked and randomly divided into groups subject to the applied hormonal product. Hormonal stimulation was performed using three products: Ovopel ([D-Ala^6, Pro^9-Net]-mGnRH) (Unic-trade, Hungary) (homogenized in 0.9% NaCl solution and administered according to the method described by Horvhth et al. 1997), Ovaprim ([D-Arg^6, Pro^9-Net]-sGnRH) (Syndel, Canada) (PETER et al. 1993) and hCG – human chorionic gonadotropin (Argent, USA). Saline solution injections (0.9% NaCl) were used in the control group. Hormonal product doses are presented in Table 1.

Table 1
Hormonal doses applied to stimulate the reproduction of the common barbel (cultured form) during the natural spawning season

Group	Males	Females	
	hormonal dose	preliminary dose	release dose
1 control	+	+	+
2	0.25 ml Ovaprim	0.1 ml Ovaprim	0.5 ml Ovaprim
3	1000 IU hCG	500 IU hCG	2000 IU hCG
4	0.5 Ovopel granules	0.1 Ovopel granules	1 Ovopel granule

+ 0.9% NaCl injections

Table 2
Hormonal doses (in terms of kg BW) applied to stimulate the reproduction of the barbel (wild and cultured forms) during the natural spawning season

	Cultured fish		Wild fish	
	1st injection	2nd injection	1st injection	2nd injection
Control	+	+	+	+
Ovopel [granule]	0.1	1.0	0.1	1.0
Ovaprim [ml]	0.1	0.5	0.1	0.5

+ 0.9% NaCl injections

The second experiment compared the efficacy and economic effectiveness of two hormonal stimulants in the induced spawning of wild (groups WF-1 and WF-2) and cultured fish (females). Similarly to the first experiment, fish were marked and divided into groups (subject to the applied hormonal stimulant) before the first injection. The stimulation was performed using two products:

Ovopel and Ovaprim. Saline solution injections (0.9% NaCl) were used in the control group. Hormonal product doses for cultured fish and wild fish are presented in Table 2.

hCG injections were administered intramuscularly under the dorsal fin. Ovaprim and Ovopel was injected intraperitoneally under the ventral fin. After the first injection, the temperature of water was increased over a period of 17 h to reach 18°C. The second hormonal injection was administered 12 h after the first injection. Males received a single injection at the time of the second manipulation in females. Next, temperature was raised to 19–20°C after the second treatment.

Gamete acquisition

Recognition of ovulation began 15 h after the second injection. Barbel females in every group were monitored every three hours in the course of successive 15 hours. Gametes were acquired from spawners by massaging and applying gentle pressure to the abdomen. Eggs were collected from each female into separate plastic bowls. To determine the effect of hormonal stimulants on the biological quality of gametes, the eggs from each female (three samples of 100–200 eggs each) were fertilized with mixed semen collected from five males. The samples were incubated separately in water at 19–20°C. Embryo survival rates were determined upon hatching. After the experiment, spawners were kept in the pond for another 14 days to determine whether experimental manipulation increased fish mortality rates.

The body weight of fish in each group and the reported results (including fertility and embryo survival rates) were evaluated by an analysis of variance (ANOVA) and Tukey's post-hoc test at a significance level of $\alpha = 0.05$. The values expressed in terms of percent were subjected to arcsine transformation before statistical analyses.

Economic effectiveness of hormonal stimulation

The economic effectiveness of hormonal products stimulating reproduction was analyzed in view of ovulation rates, the price of hormonal products and spawner mortality rates. The cost of hormonal injection per 1000 eggs and 1000 hatchlings was adopted as an indicator of economic effectiveness. Calculations were performed in view of the purchasing price of selected hormonal products, converted into Polish zloty in accordance with the exchange rate quoted on the day of purchasing Ovaprim and hCG stimulants. The cost of

purchasing 10000 IU hCG was USD 33, 10 ml Ovaprim – USD 25.50, 1 Ovopel granule – EUR 0.4. The cost of a single Ovopel, Ovaprim and hCG dose per kg BW of fish was calculated. At the next stage, the total cost of every hormonal product was computed based on the ovulation rates and spawner mortality rates in every group. Fixed cost components that were not affected by the type of the applied hormonal product (e.g. equipment depreciation, lighting, labor) were not accounted for in the calculations (HAKUĆ-BŁAŻOWSKA et al. 2009).

Results

Efficacy and economic effectiveness of hormonal stimulation in fish cultures

Hormonal stimulation clearly affected barbel spawning (Table 3). Ovulation rates in experimental fish groups were significantly higher (53–77%) than in the control group (30%). The highest percentage of ovulating females was noted following the administration of Ovaprim and Ovopel. The highest percentage of hatched embryos was reported in the group stimulated with Ovaprim. Embryo viability was higher in all experimental groups than in the control group. Hormonal stimulation and fish manipulation did not increase spawner mortality rates.

Table 3
The results of stimulating the reproduction of the common barbel (cultured form) with various hormonal products

Fish group	Control	Ovaprim	Ovopel	HCG
Number of males	12	11	12	12
Average body weight of males	108 + 12	110 + 14	114 + 21	109 + 18
Male mortality rate [%]	0	0	0	0
Number of females	10	13	15	15
Average body weight of females	128 + 22	133 + 25	127 + 32	129 + 30
Percentage of ovulating females	30	77	73	53
Fecundity [eggs/kg]	$1078 + 221^{b}$	$1547 + 325^{a}$	$1654 + 322^{a}$	$1089 + 159^{b}$
Hatching rate [%] (± SD)	$66.3 + 3.4^{c}$	$83.2 + 4.1^{a}$	$77.2 + 3.0^{b}$	$75.0 + 2.9^{b}$
Female mortality rate [%]	0	0	0	0

Data marked by the same letters in rows do not differ statistically

The costs of applying different hormonal products to stimulate barbel reproduction in a commercial farm varied significantly. The cost of one dose per kg BW of male fish reached: Ovaprim – PLN 1.66, Ovopel – PLN 0.72 and

hCG – PLN 8.25. As regards females, the cost of a combined product dose (preliminary dose and release dose) was: Ovaprim – PLN 3.82, Ovopel – PLN 1.58 and hCG – PLN 20.63. Total Ovaprim costs and total Ovopel costs differed more than two-fold, reaching PLN 6.60 and PLN 3.01, respectively, while total hCG costs were significantly higher at PLN 50.71.

The costs of hormonal stimulation of the barbel in terms of 1000 eggs and 1000 hatchlings are presented in Figure 1. The cost of hormonal stimulation in terms of 1000 eggs was the lowest for Ovopel and Ovaprim at PLN 1.74 and PLN 4.15, respectively. hCG (PLN 45.41 per 1000 eggs) proved to be the least cost-effective hormonal product. Similar differences in the profitability of different stimulants were produced by calculating hormonal injection costs per 1000 hatchlings.

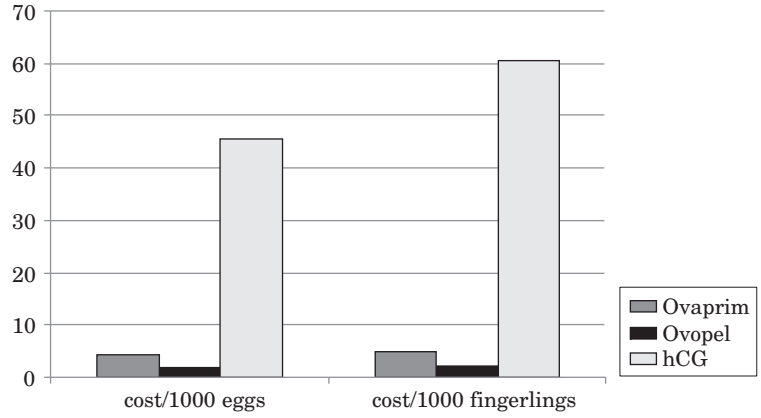

Fig. 1. The profitability of various hormonal products in the artificial spawning of cultured barbel

Efficacy and economic effectiveness of hormonal stimulation in cultured and wild fish

Hormonal stimulants had a considerable effect on the investigated reproduction parameters of the barbel, in particular its cultured form (Tables 4–6). In the control group, none of the wild-type females matured sexually, and mortality rates reached 50% in groups WF-1 and WF-2. The ovulation rate in the cultured group was 30%. A comparison of the reproductive performance of cultured fish and wild fish administered Ovopel showed much higher ovulation rates in the group of cultured fish (80%) than wild fish (0–13%) (Table 5). Significant differences in embryo hatching rates were not determined. The mortality of wild spawners was as high as 50% of the stock.

Table 4

The results of artificial spawning in the control group of wild and cultured fish

Fish group	Cultured	WF-1	WF-2
Number of females	10	6	6
Average body weight of females	0.324	3.874	3.587
Percentage of ovulating females	30%	0	0
Fecundity [eggs/kg]	1054 + 124	0	0
Hatching rate [%] (± SD)	64.2 + 3.8	–	–
Female mortality rate [%]	0	50	50

Table 5

The results of stimulating the reproduction of the common barbel (cultured and wild forms) with Ovopel

Fish group	Cultured	WF-1	WF-2
Number of females	10	8	8
Average body weight of females	0.254	3.245	3.687
Percentage of ovulating females	80	13	0
Fecundity [eggs/kg]	1654	1020	0
Hatching rate [%] (± SD)	79.3 + 63.6^a	80.2 + 3.0^a	0
Female mortality rate [%]	0	13	50

Data marked by the same letters in rows do not differ statistically

Table 6

The results of stimulating the reproduction of the common barbel (cultured and wild forms) with Ovaprim

Fish group	Cultured	WF-1	WF-2
Number of females	10	8	8
Average body weight of females	0.287	3.875	3.364
Percentage of ovulating females	90	25	13
Fecundity [eggs/kg]	1875	1645	1587
Hatching rate [%] (± SD)	82.2 + 3.3^b	81.1 + 3.7^b	89.2 + 1.2^a
Female mortality rate [%]	0	38	50

Data marked by the same letters in rows do not differ statistically

Similar results were noted following the administration of Ovaprim (Table 6). Ovulation rates were higher and embryo hatching rates were insignificantly higher than in the groups stimulated with Ovopel. Mortality rates of wild-type fish also reached 50%. In general, Ovaprim produced more satisfactory results than Ovopel.

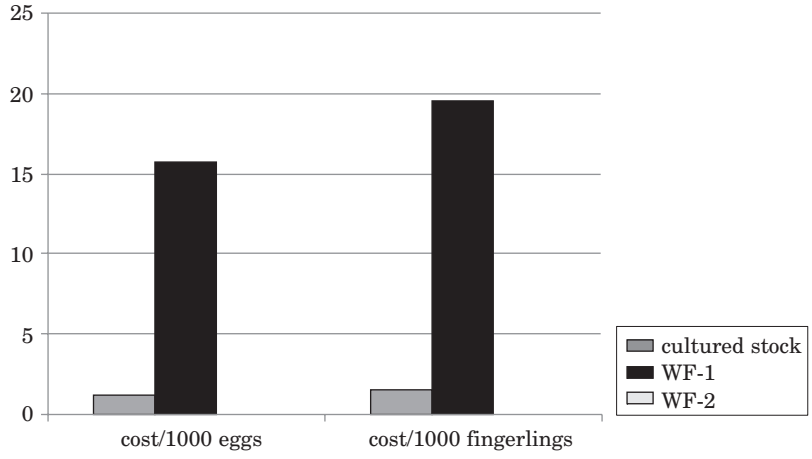

Fig. 2. The profitability of Ovopel in the artificial spawning of the common barbel: wild and cultured forms

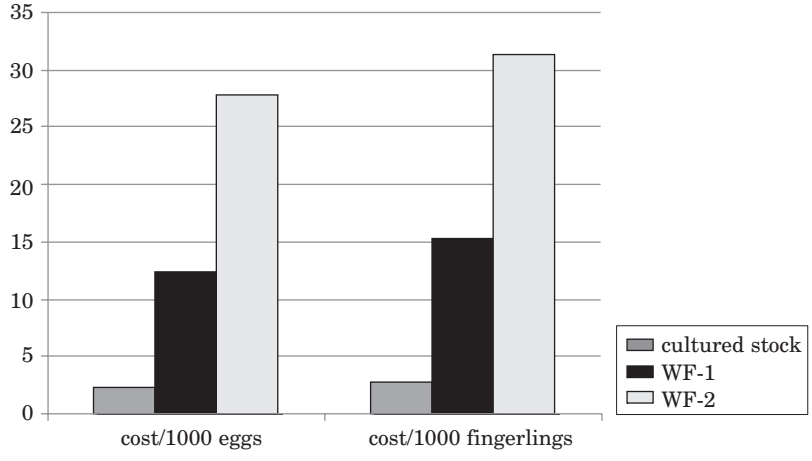

Fig. 3. The profitability of Ovaprim in the artificial spawning of the common barbel: wild and cultured forms

The costs of stimulating the reproduction of cultured and wild barbel with Ovopel varied significantly. The cost of a single Ovopel dose per kg BW of female fish reached an average of PLN 1.58 for both wild and cultured forms. The total cost of administering Ovopel was significantly higher in both wild fish groups (WF-1 – PLN 41.02 and WF-2 – PLN 46.60) than in cultured fish (PLN 4.01) which resulted from the fact that the body weight of cultured females was several times lower. When spawner mortality rates were taken into account, the cost of hormonal manipulation increased by PLN 13 (WF-1) and PLN 52 (WF-2).

The costs of Ovopel stimulation in cultured and wild fish (WF-1) per 1000 eggs and 1000 hatchlings are presented in Figure 2. Group WF-2 was not taken into account due to an absence of ovulating females. The cost of hormonal stimulation per 1000 eggs was significantly lower in cultured fish (PLN 1.20) than in wild fish where it reached PLN 15.69. Ovopel injections proved to be less profitable in wild fish than in cultured fish also in terms of 1000 hatchlings.

Discussion

The production of stocking under controlled conditions, including of rheophilic cyprinid fish, is becoming the predominant source of income for many fish farms in Poland (WOJDA 2004, TURKOWSKI et al. 2008). Stocking material is produced by artificial spawning. Under controlled conditions, selected species, such as the burbot, *Lota lota* L., or the perch, *Perca fluviatilis* (L.), may reproduce with or without hormonal stimulation (KUCHARCZYK et al. 1996, 1998c, SZCZERBOWSKI et al. 2009, ŻARSKI et al. 2010). Hormonal stimulation is not required in aquarium fish and domesticated fish species (KREJSZEFF et al. 2009, KUCHARCZYK et al. 2010). Yet in most cases, the production of high quality gametes and abundant stocking material may require hormonal stimulation (BRZUSKA et al. 2000, 2005, KUCHARCZYK et al. 2005, 2008, CEJKO et al. 2008, 2010, ŻARSKI et al. 2009).

In induced spawning, the selection of the appropriate hormonal stimulant determines ovulation success. There are scant data on the controlled reproduction of the common barbel (WOLNICKI and MYSZKOWSKI 1998, CEJKO et al. 2009, KAMIŃSKI et al. 2009). The effect of hCG on the artificial spawning of selected fish species has been described by KUCHARCZYK et al. (1998b). Except for a small number of species, hCG proved to be ineffective in most rheophilic cyprinid fish (KUCHARCZYK et al. 1997a,b). Satisfactory results were reported as regards the induced spawning of the rudd and the chub stimulated with hCG (KUCHARCZYK et al. 1997c, KREJSZEFF et al. 2010). Recent years have witnessed the growing popularity of synthetic GnRH analogues with the addition of dopamine inhibitors. Many Polish researchers reported highly satisfactory results in stimulating the reproduction of most rheophilic cyprinid species with the use of Ovopel (CIEŚLA 1998, KŁODZIŃSKA and OKONIEWSKI 1998, KUCHARCZYK et al. 1998, ŚLIWIŃSKI 1998, KUCHARCZYK et al. 1999, TARGOŃSKA-DIETRICH et al. 2004, JAMRÓZ et al. 2008b, KREJSZEFF et al. 2008, ŻARSKI et al. 2008b, HAKUĆ-BŁAŻOWSKA et al. 2009) as well as Ovaprim (KUCHARCZYK et al. 2007, JAMRÓZ et al. 2008a,b, ŻARSKI et al. 2008a,b, HAKUĆ-BŁAŻOWSKA et al. 2009).

The results of the present experiment point to the high effectiveness of Ovopel and Ovaprim in the induced spawning of farmed barbel fish. hCG was marked by lower efficacy in comparison with GnRH analogues. Hormonal stimulation with hCG produced ovulation rates of 53%, while the administration of Ovaprim and Ovopel induced ovulation in 77% and 73% of females, respectively. Other authors also noted the high effectiveness of Ovopel and Ovaprim in the artificial spawning of other rheophilic cyprinids (KUCHARCZYK 2002, TARGOŃSKA-DIETRICH et al. 2004, JAMRÓZ et al. 2008b, KREJSZEFF et al. 2008, 2009, ŻARSKI et al. 2008a. 2009, TARGOŃSKA et al. 2010). In comparison with the control group, the results of hormonal injections in wild barbel also validate the efficacy of Ovopel and Ovaprim in artificial spawning. GnRH analogues stimulated ovulation in a small percentage of females, but the absence of hormonal stimulation resulted in zero ovulation. The above findings suggest that GnRH analogues are highly effective in the induced spawning of the common barbel, and they point to the economic effectiveness of spawner breeding in fish farms. As suggested by a limited number of comparative studies, hormonal stimulation delivers more satisfactory results in cultured rather than in wild fish (TARGOŃSKA-DIETRICH et al. 2004, KREJSZEFF et al. 2009, 2010). Hormonal stimulation involving any of the analyzed spawning agents also contributed to higher embryo survival rates (in both cultured and wild fish groups) in comparison with the control group.

The dynamic growth of commercial fish stock production in pond and lake farms prompts research into the economic profitability of the process. To date, few studies investigating the problem have been published (KŁODZIŃSKA and OKONIEWSKI 1998, TLUSTY 2002, TURKOWSKI 2002, HAKUĆ-BŁAŻOWSKA et al. 2008, 2009, KUPREN et al. 2008b, TURKOWSKI et al. 2008). In cultured fish production was significantly more profitable with the involvement of GnRH synthetic analogues than hCG. The cost of a single dose of human chorionic gonadotropin was more than five-fold higher than the cost of a single Ovaprim dose and 13-fold higher than an Ovopel dose. The results of studies evaluating the economic effectiveness of hormonal stimulation in the ide *Leuciscus idus* (L.), the asp *Aspius aspius* (L.) (HAKUĆ-BŁAŻOWSKA et al. 2009) and the pikeperch *Stizostedion lucioperca* (L.) (HAKUĆ-BŁAŻOWSKA et al. 2008, KUPREN et al. 2008a) also pointed to the higher profitability of GnRH synthetic analogues in artificial spawning. However, smaller fertility of common barbell causes larger costs of hormonal stimulation in comparison to results of artificial reproduction of ide, or asp.

When the economic effectiveness of Ovopel and Ovaprim was compared in the artificial reproduction of cultured and wild barbel fish, more satisfactory results were reported for Ovopel. Yet the most significant differences in the overall cost of hormonal stimulation were noted when the same product was

used in cultured fish and wild fish. The cost of hormonal stimulation with Ovopel was ten-fold higher in wild-type female fish than in cultured fish. Taking into account spawner mortality rates, hormonal simulation costs increased in the group of wild fish, compared with cultured fish. The above was attributed mainly to the average body weight of females (wild females were more than ten-times larger than cultured females) and spawner mortality rates (noted only in the group of wild fish). The differences in economic profitability should be leveled out when production costs are calculated in terms of 1000 eggs and 1000 hatchlings, yet the eggs yield of both wild fish groups did not reduce the difference in Ovopel's profitability. There were no sexually mature females in group WF-2, and the ovulation rate in group WF-1 reached 13%. Due to an absence of ovulating females, group WF-2 was not taken into account in cost calculations. The stimulation of cultured fish with Ovopel proved to be more profitable even in view of embryo survival rates in the studied groups.

Similar differences in economic effectiveness were reported in respect of Ovaprim. In view of the body weight of fish and spawner mortality rates, the overall cost of hormonal stimulation in wild fish groups exceeded simulation costs in cultured fish 15-fold. Ovaprim was also a less cost-effective stimulant in wild fish due to their significantly lower ovulation rates (in particular in group WF-2). The low profitability of hormonal treatments in wild-type fish resulted from the low effectiveness of induced spawning in this group of fish. As regards economic profitability, the most satisfactory results were reported in respect of GnRH synthetic analogues administered during the induced spawning of cultured fish.

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OSMOLALITY OF SEMINAL PLASMA AS AN INDICATOR OF MILT CONTAMINATION WITH URINE BASED ON THE EXAMPLE OF THE TENCH *Tinca tinca* (L.)

***Beata Irena Cejko*[1], *Daniel Żarski*[2], *Katarzyna Targońska*[2], *Sławomir Krejszeff*[2], *Dariusz Kucharczyk*[2], *Jan Glogowski*[1,3]**

[1] Department of Gamete and Embryo Biology, Institute of Animal Reproduction and Food Research, Polish Academy of Sciences in Olsztyn
[2] Department of Lake and River Fisheries
[3] Department of Ichthyology
University Warmia and Mazury in Olsztyn

K e y w o r d s: *Tinca tinca* (L.), CPH, Ovopel, Ovaprim, milt, spermatozoa, seminal plasma osmotic pressure, urine.

A b s t r a c t

Milt was collected from the tench *Tinca tinca* (L.) following hormonal stimulation with carp pituitary homogenate (CPH, group I, $n = 9$), Ovopel (group II, $n = 8$) and Ovaprim (group III, $n = 9$). Males non-stimulated fish were used as a control (group IV, $n = 6$). The parameters determined included the total volume of milt (TVM, ml) and the volume per kg of the males' body weight (VOM, ml kg^{-1} b.w.), total number of spermatozoa produced by the males (TSP, $\times 10^9$) and the number of spermatozoa per kg of their body weight (TNS, $\times 10^9$ kg^{-1} b.w.). Moreover, attempts were made to show the effect of the hormone preparations on spermatozoa motility (%), their concentration in milt ($\times 10^9$ ml^{-1}) and the total protein content in seminal plasma (mg ml^{-1}). Osmotic pressure of the seminal plasma (mOsm kg^{-1}) was determined to check if the milt samples were contaminated with urine. Pearson's linear correlation was also determined between the osmolality, on the one hand, and the spermatozoa motility and concentration of spermatozoa in milt of the groups examined in the study, on the other. The significance of differences between the analysed parameters was checked with Tukey's test (One-way ANOVA, $\alpha = 0.05$). Motility and concentration of spermatozoa in the remained relatively low, not exceeding 22% and $5.0 \cdot 10^9$ ml^{-1} in each of the groups. Using CPH, Ovopel or Ovaprim did not result in any significant increase ($P > 0.05$) in the amount of milt obtained (TVM, VOM) or the total amount of spermatozoa produced as compared to the control group. Significant differences ($P < 0.05$) were found only between the TNS values for group I (CPH), and group IV (control). Osmolality of the seminal plasma did not exceed 120 mOsm kg^{-1} in any of the groups under examination. Its low values as well as low motility and low concentration of spermatozoa in milt indicate that milt was contaminated with urine, which is also corroborated by a significant correlation between osmolality and motility of spermatozoa in group III ($R^2 = 0.828$; $P < 0.001$) and IV ($R^2 = 0.983$; $P < 0.001$) and between osmolality and concentration of spermatozoa in each of the groups ($R^2 = 0.447$; $P < 0.05$, group I; $R^2 = 0.964$; $P < 0.001$, group II; $R^2 = 0.768$; $P < 0.001$, group III and $R^2 = 0.924$; $P < 0.001$; group IV).

Address: Beata Irena Cejko, Institute of Animal Reproduction and Food Research, Polish Academy of Sciences, Olsztyn, tel: +48 (089) 539 31 33, e-mail: b.cejko@pan.olsztyn.pl

OSMOLALNOŚĆ PLAZMY NASIENIA JAKO WSKAŹNIK ZANIECZYSZCZENIA MLECZA MOCZEM NA PRZYKŁADZIE LINA *Tinca tinca* (L.)

***Beata Irena Cejko*[1], *Daniel Żarski*[2], *Katarzyna Targońska*[2], *Sławomir Krejszeff*[2], *Dariusz Kucharczyk*[2], *Jan Glogowski*[1,3]**

[1] Zakład Biologii Gamet i Zarodka
PAN, Olsztyn
[2] Katedra Rybactwa Jeziorowego i Rzecznego
[3] Katedra Ichtiologii
Uniwersytet Warmińsko-Mazurski w Olsztynie

S ł o w a k l u c z o w e: *Tinca tinca* (L.), CPH, Ovopel, Ovaprim, mlecz, plemniki, ciśnienie osmotyczne plazmy nasienia, mocz.

A b s t r a k t

Mlecz pozyskano od lina *Tinca tinca* (L.) po stymulacji hormonalnej za pomocą homogenatu przysadki mózgowej karpia (CPH, grupa I, $n = 9$), Ovopelu (grupa II, $n = 8$) oraz Ovaprimu (grupa III, $n = 9$). Grupę kontrolną (IV, $n = 6$) stanowiły samce, których nie stymulowano. Określono całkowitą objętość pozyskanego mlecza (TVM, ml) oraz objętość przypadającą na kg masy ciała samców (VOM, ml kg^{-1} m.c.), całkowitą liczbę wyprodukowanych przez samce plemników w miliardach (TSP, $\times 10^9$) oraz liczbę przypadającą na kg masy ich ciała (TNS, $\times 10^9$ kg^{-1} m.c.). Dodatkowo podjęto próbę wykazania wpływu zastosowanych preparatów hormonalnych na ruchliwość plemników (%) ich koncentrację w mleczu ($\times 10^9$ ml^{-1}) oraz stężenie białka ogólnego (mg ml^{-1}) w plazmie nasienia. W celu sprawdzenia czy pobrane próby mlecza nie zostały zanieczyszczone moczem, oznaczono ciśnienie osmotyczne plazmy nasienia (mOsm kg^{-1}) oraz przeprowadzono prostoliniową korelację Pearsona między osmolalnością a ruchliwością i koncentracją plemników w mleczu badanych grup. Istotność różnic w wartościach analizowanych parametrów weryfikowano testem Tukey'a (One-way ANOVA, $\alpha = 0.05$). Wartości ruchliwości i koncentracji plemników w mleczu utrzymywały się na stosunkowo niskim poziomie nieprzekraczającym 22% oraz $5.0 \cdot 10^9$ ml^{-1} w każdej z badanych grup. Zastosowanie CPH, Ovopelu oraz Ovaprimu nie doprowadziło do istotnego wzrostu ($P > 0.05$) objętości pozyskanego mlecza (TVM, VOM) oraz całkowitej ilości pozyskanych plemników (TSP) w porównaniu z grupą kontrolną. Istotne różnice ($P < 0.05$) stwierdzono jedynie w wartościach TNS między grupą I (CPH) a grupą IV (kontrolną). Osmolalność plazmy nasienia nie przekraczała 120 mOsm kg^{-1} w każdej z badanych grup. Niskie jej wartości jak również wartości ruchliwości oraz koncentracji plemników w mleczu wskazują na zanieczyszczenie mlecza moczem. Stwierdzona istotna zależność między osmolalnością a ruchliwością plemników w grupie III ($R^2 = 0.828$; $P < 0.001$) i IV ($R^2 = 0.983$; $P < 0.001$) oraz między osmolalnością a koncentracją plemników w każdej z badanych grup (odpowiednio: $R^2 = 0.447$; $P < 0.05$, grupa I; $R^2 = 0.964$; $P < 0.001$, grupa II; $R^2 = 0.768$; $P < 0.001$, grupa III oraz $R^2 = 0.924$; $P < 0.001$; grupa IV) potwierdza ten fakt.

Introduction

The tench *Tinca tinca* (L.) is a stenothermial species which lives in inland waters on all continents. In natural conditions it is frequently bred in carp ponds where it spawns, which in the Polish geographical zone lasts from June to August (BRYLIŃSKA, BRYLIŃSKI 2000). Due to its high taste quality and an

important role played by it in leisure activities (angling) and pond management (polyculture) (MAMCARZ, SKRZYPCZAK 2006, SKRZYPCZAK, MAMCARZ 2006), the tench is a valuable breeding fish, which is also confirmed by an increase in its production in aquaculture (FAO).

Tench reproduction under controlled conditions is relatively difficult to carry out, especially in fish from wild populations (MAMCARZ et al. 2006, KUCHARCZYK et al. 2007, KUJAWA et al. 2010). Another serious problem is the small amount of milt produced in the process, and its frequent contamination with urine disqualifies such samples for fertilisation, short-term storage or cryopreservation (LINHART et al. 2003). In order to improve the milt quality, immobilising solutions are used while it is being extracted or soon afterwards (RODINA et al. 2004). Their function has been found not only to block the spermatozoa motion apparatus but-as is the case with the tench – to restore energy supplies. Moreover, when stored in immobilising solutions (10 hours, 0–4°C), milt can be successfully used to fertilization of gametes fish of the same species (RODINA et al. 2004).

Reproduction of cyprinids under controlled conditions is based on available hormonal preparations (KUCHARCZYK et al. 1997, 2008, BRZUSKA, BIAŁOWĄS 2002, KREJSZEFF et al. 2008, 2010, ŻARSKI et al. 2009, TARGOŃSKA et al. 2010). When used, they affect not only gamete maturation, but also their biological value (YARON 1995, ZOHAR, MYLONAS 2001). Milt quality is sometimes regarded as being of marginal importance in fish reproduction, although a significant effect of its parameters (motility and concentration of spermatozoa in milt) on the fertilisation process has been shown (GLOGOWSKI et al. 1999, KUCHARCZYK et al. 2005, TARGOŃSKA et al. 2008). Hormonal stimulation in the tench is carried out mainly with carp pituitary homogenate (CPH) and gonadoliberine analogues. They have an impact on the volume of milt and the amount of spermatozoa produced by males fish (LINHART et al. 1995b, GELA et al. 2006, CAILLE et al. 2006).

As many hormonal preparations are commercially available which are used in cyprinid reproduction, the aim of this study was to compare the effectiveness of CPH and two synthetic, complex preparations, i.e. Ovopel [(D-Ala^6, Pro^9 NEt)-mGnRH]+metoklopramide and Ovaprim [(D-Arg^6, Pro^9 NEt)-sGnRH]+domperidon. Their effectiveness was evaluated by taking the total volume of milt (TVM, ml) and the volume per kg of the males' body weight (VOM, ml kg^{-1} b.w.), total number of spermatozoa in billions (TSP, $\times 10^9$) and their number per kg of males; b.w. (TNS, $\times 10^9$ kg^{-1} b.w.). Moreover, an attempt was made to show the effect of hormonal preparations on motility of spermatozoa (%) and their concentration in milt ($\times 10^9$ ml^{-1}) as well as total protein content in seminal plasma (mg ml^{-1}). In order to check whether the milt samples were contaminated with urine, seminal plasma osmotic pressure (mOsm kg^{-1}) was also determined.

Materials and Methods

Origin and transport of fish

Wild brood male tench with body weights of 0.28–0.98 kg were caught with an electric generator in Lake Sasek Wielki during a spawning period, i.e. in June, 2008. The fish were transported on the same day in bags with oxygen to the aquarium hall of the Department of Lake and River Fisheries of the University of Warmia and Mazury in Olsztyn. After being brought to the hatchery, they were put into 1.000 dm^3 tanks in water at a temperature of 17°C (KUJAWA et al. 1999).

Hormonal stimulation and manipulations with spawners

Following three-day adaptation, the fish were divided into four experimental groups and intraperitoneal hormonal stimulation was carried out with the three hormonal preparations. Group I ($n=9$) was given CPH at 2.0 mg kg^{-1} of b.w. (Argent, USA), group II ($n=8$) Ovopel at 1 pellet kg^{-1} of b.w. (Unic-trade, Hungary, HORVÁTH et al. 1997), and group III ($n=9$) Ovaprim at 0.5 ml kg^{-1} of b.w. (Syndel, Canada). Group IV, control ($n=6$), consisted of fish which were not stimulated hormonally but were given physiological saline instead. Following the stimulation, the fish were put into the tanks and the water temperature was raised to 19°C. Since female tench usually lay spawn 16 hours after being stimulated, the male fish were caught after that time, their body weight was determined and milt was collected by delicately massaging the abdominal parts. All the brood fish procedures were carried out after they were anaesthetised with 2-phenoxyethanol at 0.5 ml l^{-1} (Sigma-Aldrich, St. Louis, MO).

Determination of the volume of milt collected and number of spermatozoa

The total volume of milt produced (TVM) was measured while it was being collected, using sterile syringes, calibrated every 0.01 ml. The volume of milt (VOM) was calculated using the males' body weights and TVM. The values of TVM and concentration of spermatozoa in milt ($\times 10^9$ ml^{-1}) were used to calculate the total number of spermatozoa (TSP). The latter TSP and the males' b.w. were used to calculate the number of spermatozoa per kg of the brood fish b.w. (TNS).

Determination of motility and concentration of spermatozoa in milt, total protein content in seminal plasma and the seminal plasma osmotic pressure

Motility of spermatozoa in milt (%) was determined by a subjective method, using a light microscope and 400x magnification. Spermatozoa were activated with hatchery water, and the value was given with an accuracy of ±10% (GLOGOWSKI, CEJKO 2008). Concentration of spermatozoa in milt was determined using the spectrophotometric method (CIERESZKO, DĄBROWSKI 1993), after the milt was diluted 500x with 0.7% NaCl (Sigma-Aldrich, St. Louis, MO). Absorbance of the samples was measured on a Specol 11 spectrophotmeter (Carl Zeiss Jena, USA) at $\alpha = 530$ nm. The concentration values were substituted in the equation of the standard curve, prepared earlier for the tench (the cytometric method, BIELAŃSKI 1979). Seminal plasma was obtained by centrifugation milt samples and the supernatatn was decanted to test tubes; its osmolality (mOsm kg^{-1}) was measured using a Vapor Pressure Osmometer 5520 (WESCOR, Logan, UT, USA). The total protein content in seminal plasma (mg ml^{-1}) was determined by the method presented by LOWRY et al. (1951), after diluting it 40× with 0.85% NaCl (Sigma-Aldrich, St. Louis, MO).

Statistical analysis

The results were characterised using the arithmetic average ($\bar{x}$) and standard deviation (±SD); the significance of the differences in the milt parameters analysed was checked with the Tukey's test (One-way ANOVA, $\alpha = 0.05$). In order to show the relationship between osmolality of seminal plasma and motility and concentration of spermatozoa in milt, Pearson's linear correlation was determined. All the analyses were performed using the GraphPad Prism 4 software (GraphPad Software Inc., USA).

Results

The smallest amount of milt (TVM) was extracted from the fish which were not stimulated hormonally (0.26 ml), whereas much larger amounts of milt were collected from the males which were given hormonal preparation, although the TVM values for the groups under examination did not differ significantly ($P > 0.05$; Table 1). The volume of milt per kg of brood fish b.w. (VOM) was similar in groups I and III (1.42 and 1.48 ml kg^{-1} b.w. respectively). The VOM values in the other groups did not exceed 1 ml kg^{-1} b. w., but no

significant differences were found between the parameter values ($P > 0.05$; Table 1). The total number of spermatozoa collected from the fish (TSP) was similar in the milt from fish which had been given CPH and Ovaprim ($3.82 \cdot 10^9$ and $3.36 \cdot 10^9$, respectively). The values in the other groups were much lower, but they did not differ significantly from those found in the other two groups ($P > 0.05$; Table 1). The most similar values were found in the groups which had been given CPH and Ovaprim (group I: $6.68 \cdot 10^9$ kg^{-1} b.w. and group III: $6.28 \cdot 10^9$ kg^{-1} b.w.), whereas the TNS values were much lower in the control group and in the one which had been given Ovopel (Table 1). Significant differences between TNS values were found between group I and group IV ($P < 0.05$).

Table 1

Arithmetic average and standard deviation (±SD) of the total volume of milt (TVM, ml) and the volume per kg of body weight (VOM, ml kg^{-1} b.w.), total number of spermatozoa (TSP, $\times 10^9$) and the number of spermatozoa per kg of body weight (TNS, $\times 10^9$ kg^{-1} b.w.) of the tench *Tinca linca* (L.) obtained after stimulation of spermation with CPH (group I), Ovopel (group II) and Ovaprim (group III); non-stimulated male fish (group IV)

Fish group	TVM [ml]	VOM [ml kg^{-1} b.w.]	TSP [$\times 10^9$]	TNS [$\times 10^9$ kg^{-1} b.w.]
I (n = 9)	0.84 ± 0.97^a	1.42 ± 1.03^a	3.82 ± 3.74^a	6.68 ± 3.98^a
II (n = 8)	0.46 ± 0.24^a	0.91 ± 0.43^a	1.37 ± 1.23^a	2.53 ± 1.74^{ab}
III (n = 9)	0.83 ± 0.50^a	1.48 ± 0.63^a	3.36 ± 2.45^a	6.28 ± 4.07^{ab}
IV (n = 6)	0.26 ± 0.14^a	0.53 ± 0.42^a	0.82 ± 0.90^a	1.80 ± 2.42^b

Mean values with the some letters do not differ significantly ($P > 0.05$)

The percentage of motility spermatozoa in each of the groups was low and it did not exceed 22%, with no significant differences between average values of motility ($P > 0.05$; Table 2). No significant differences ($P > 0.05$) were also found between concentrations of spermatozoa in milt, although the highest values were found in milt samples from fish in group I, i.e. from the male fish stimulated with CPH ($5.0 \cdot 10^9$ ml^{-1}), whereas the lowest ones were found in milt samples from group II, i.e. from male fish stimulated by Ovopel ($2.9 \cdot 10^9$ ml^{-1}). The total protein content in seminal plasma from the fish which had been given CPH, Ovopel or Ovaprim was similar (0.72; 0.58; 0.62 mg ml^{-1}), whereas it was nearly twice as high (1.2 mg ml^{-1}) in group IV (control), but the differences were not significant as compared with the others ($P > 0.05$; Table 2).

Table 2

Arithmetic average and standard deviation (±SD) of spermatozoa motility (%), seminal plasma osmotic pressure (mOsm kg^{-1}), concentration of spermatozoa in milt ($\times 10^9$ ml^{-1}) and total protein content in seminal plasma (mg ml^{-1}) of the tench *Tinca linca* (L.) obtained after stimulation of spermation with CPH (group I), Ovopel (group II) and Ovaprim (group III); non-stimulated male fish (group IV)

Fish group	Motility of spermatozoa [%]	Seminal plasma osmotic pressure [mOsm kg^{-1}]	Concentration of spermatozoa [$\times 10^9$ ml^{-1}]	Total protein content [mg ml^{-1}]
I (n = 9)	20.56 ± 17.93^a	119 ± 28.90^a	5.05 ± 1.40^a	0.72 ± 0.30^a
II (n = 8)	21.25 ± 18.07^a	100 ± 48.34^a	2.88 ± 1.74^a	0.58 ± 0.21^a
III (n = 9)	17.22 ± 21.38^a	98 ± 36.85^a	4.03 ± 2.18^a	0.62 ± 0.31^a
IV (n = 6)	9.17 ± 12.81^a	79 ± 38.55^a	3.26 ± 2.33^a	1.2 ± 0.64^a

Mean values with the same letters do not differ significantly ($P > 0.05$).

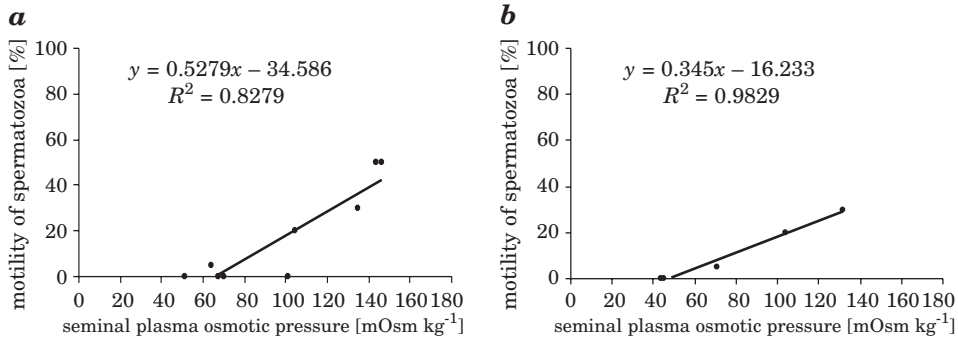

Fig. 1. Relationship between motility of spermatozoa (%) and seminal plasma osmotic pressure (mOsm kg^{-1}) in tench *Tinca tinca* (L.) stimulated hormonally with Ovaprim (*a*) and in the control group (*b*)

Osmotic pressure in seminal plasma did not exceed 120 mOsm kg^{-1} in any of the groups under study, with the highest values determined for plasma samples from fish in the control group (79 mOsm kg^{-1}). The values of osmolality determined for various groups of fish did not differ significantly ($P > 0.05$; Table 2). A significant correlation was found between motility of spermatozoa in milt, and osmolality of seminal plasma in group III ($R^2 = 0.828$; $P < 0.001$; Figure 1a) and group IV ($R^2 = 0.983$; $P < 0.001$; Figure 1b). The correlation for the other two groups was not significant, with the R^2 coefficients adopting the value of: $R^2 = 0.280$ in group I and $R^2 = 0.066$ in group II. A significant relationship was found between concentration of spermatozoa in milt and osmolality of seminal

plasma in each of the groups under study, with Pearson's coefficients equal to: $R^2 = 0.447$ ($P < 0.05$; group I; Figure 2a), $R^2 = 0.964$ ($P < 0.001$; group II; Figure 2b), $R^2 = 0.768$ ($P < 0.001$; group III; Figure 2c) and $R^2 = 0.924$ ($P < 0.001$; group IV; Figure 2d).

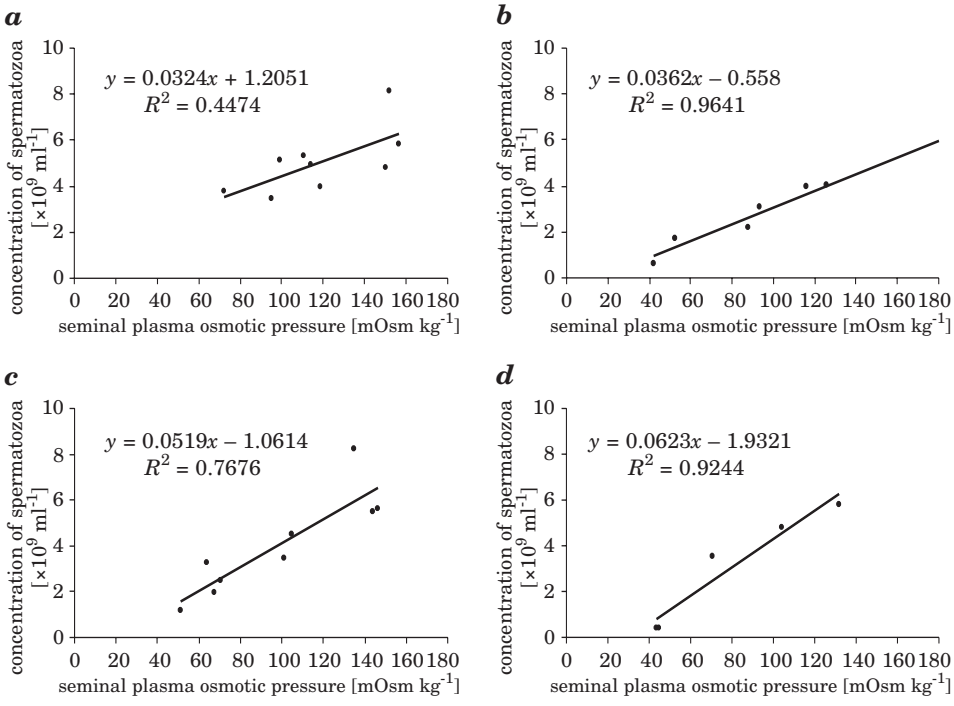

Fig. 2. Relationship between concentration of spermatozoa in milt ($\times 10^9$ ml^{-1}) and seminal plasma osmotic pressure (mOsm kg^{-1}) in tench *Tinca tinca* (L.) stimulated hormonally with CPH (*a*), Ovopel (*b*), Ovaprim (*c*) and in the control group (*d*)

Discussion

The effectiveness of spawning is impacted by many factors on which reproduction success depends; hence it is very important to optimise the procedures used in reproduction biotechniques. These include: the way brood fish are obtained and their origin (KUCHARCZYK et al. 2007, KREJSZEFF et al. 2008, 2009, 2010), thermal conditions and the possibility of controlling them (TARGOŃSKA et al. 2010, ŻARSKI et al. 2010, CEJKO et al. unpublished; TARGOŃSKA et al. unpublished), as well as the types and doses of hormonal agents (YARON 1995, KUCHARCZYK et al. 2005). The economic profitability of an undertaking is also very important (TURKOWSKI et al. 2008, HAKUĆ-BŁAŻOWSKA et al. 2009, 2010).

A small number of hatched larvae is a bottleneck for controlled tench reproduction and it largely determines the profitability of production and rearing of the fish. One of the reasons for this is poor quality of the milt used for spawn fertilisation, frequently contaminated with urine (LINHART et al. 2003). Spermatozoa motility in salmonids and acipenserids is controlled mainly by the concentration of K^+, ions, whereas the proper osmotic pressure of seminal plasma plays a decisive role in cyprinids and in marine fish (ALAVI, COSSON 2006). Due to its low osmolality, below 100 mOsm kg^{-1}, urine which gets into milt is the major factor which prematurely activates spermatozoa motility in tench (LINHART et al. 2003). The average values of osmotic pressure, which were determined in each of the groups, correspond to low values of spermatozoa motility, which indicates that milt is contaminated with urine when collected by the traditional method. This is corroborated by a significant correlation, which has been found between osmolality of seminal plasma and spermatozoa motility in groups III and group IV as well as high values of Pearson's coefficients. The highest values motility of spermatozoa and seminal plasma osmolality in group I and II and the absence of significant correlations between those values probably indicates that contamination with urine might be very small in those groups. The tench milt is especially susceptible to contact with urine due to the vicinity of spermatic ducts and urine bladder (LINHART et al. 2003), but contamination with urine has also been observed in milt of other species, e.g. in carp *Cyprinus carpio* L. (PERCHEC et al. 1995), pikeperch *Sander lucioperca* (L.) (CEJKO et al. 2008) and rainbow trout *Oncorrhynchus mykiss* (Walbaum) (GLOGOWSKI et al. 2000).

The results presented in this paper do not point to any hormonal preparation which, when used, would be more or less effective in inducing tench spermation. This is corroborated by the absence of any significant differences between the parameters analysed in this study, both in terms of the volume of milt collected from a male fish (TVM, VOM), the number of spermatozoa (TSP, TNS), concentration and motility of spermatozoa in the fish groups under examination. Concentration of spermatozoa in milt ranges from 1 to $20 \cdot 10^9$ ml^{-1} (ŻUROMSKA 1981, MOCZARSKI, KOŁDRAS 1982, LINHART, BILLARD 1995a), and although the values of the parameters do not usually exceed $10 \cdot 10^9$ ml^{-1}, their low values in the tench are not a coincidence (LINHART et al. 1986). The low concentration of spermatozoa in milt collected from the male fish under examination may have been caused by poor quality of the brood fish or lack of a positive effect of the selected hormonal preparations on the last stages of spermatozoa maturation in spermatic ducts. However, it is more probable that the urine which reached the milt caused not only premature activation of spermatozoa motility, but it also reduced its concentration by dilution. These assumptions may be confirmed by the low concentration values and by the

results of Pearson's correlations (high values of R^2) between the concentration and osmolality of seminal plasma in each of the groups under examination. A similar situation of a decrease concentration of spermatozoa in the tench milt, which results from its contamination, was described by ŻUROMSKA (1981) and CAILLE et al. (2006).

The intensity of spermatozoa maturation in spermatic ducts in natural conditions is determined largely by environmental conditions (temperature, light); however, hormonal stimulation is also important under controlled conditions. Although stimulation by properly selected hormonal preparations positively affects the volume of milt collected from male fish (TVM, VOM) as well as the amount of spermatozoa (TSP, TNS), (CAILLE et al. 2006, CEJKO et al. 2010), the spermatozoa quality, expressed as the percentage of their motility, is also affected by the procedure of milt collected (GLOGOWSKI et al. 2000, LINHART et al. 2003). This has been corroborated by literature reports, but also by the results of experiments presented in this paper, where the spawning of brood fish by the traditional method resulted in contamination of milt samples with urine, causing the percentage of motility spermatozoa to decrease.

Seminal plasma osmotic pressure is one of important indicators of the tench milt quality. Despite proper handling of brood fish and properly conducted hormonal stimulation, it is often difficult to obtain good quality milt. Although contamination of milt with blood does not disqualify it for being used for spawn fertilisation (WŁASOW et al. 1999, CIERESZKO et al. 2004), contamination with urine makes the use of such samples for fertilisation impossible, and all the more so for short-term storage or cryopreservation of milt. Catheterisation of brood fish may be an alternative to the traditional method of tench milt colection. The method has been successfully applied by GLOGOWSKI et al. (2000) in rainbow trout, thereby minimising the risk of contamination. This method can be used with success in bigger male fish, so it is difficult to draw conclusions about its effectiveness in small brood fish. Testing immobilising solutions (RODINA et al. 2004) in order to improve the milt quality is also recommended.

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EFFECTS OF TEMPERATURE ON SURVIVAL, DEFORMATIONS RATE AND SELECTED PARAMETERS OF NEWLY HATCHED LARVAE OF THREE RHEOPHILIC CYPRINIDS (GENUS *LEUCISCUS*)*

Krzysztof Kupren, Andrzej Mamcarz, Dariusz Kucharczyk

Department of Lake & River Fisheries
University of Warmia and Mazury in Olsztyn

K e y w o r d s: incubation temperature, hatching success, abnormalities, *Leuciscus leuciscus*, *Leuciscus idus, Leuciscus cephalus*.

A b s t r a c t

Experiments were conducted to determine full range of tolerated and optimal water condition for eggs incubation of three species from genus *Leuciscus* i.e: dace *Leuciscus leuciscus* (L.), ide *Leuciscus idus* (L.), and chub *Leuciscus cephalus* (L.). Spawners were caught from rivers of the northern (Pasleka River drainage) and central (Pilica River drainage) parts of Poland. Fertilized eggs were incubated under controlled conditions at ten different constant temperatures ranging from 4.5 to 29.0°C. The optimal temperature ranges for the incubation of dace, ide and chub eggs were 7.5 to 12.3°C; 15.7°C and 19.0 to 23.0°C, respectively (considering hatching percentage, incidence of abnormalities and size of hatched larvae), which are close to the water temperature during spawning season. This study also reveals that embryos of studied species can adapt to increasing water temperature due to global warming up to 23.0°C (dace and ide) and 27.5°C (chub). Besides, some inter-population differences in the response to temperature were observed.

WPŁYW TEMPERATURY WODY NA PRZEŻYWALNOŚĆ, ODSETEK DEFORMACJI ROZWOJOWYCH ORAZ WYBRANE PARAMETRY LARW TRZECH GATUNKÓW KARPIOWATYCH RYB REOFILNYCH Z RODZAJU *LEUCISCUS*

Krzysztof Kupren, Andrzej Mamcarz, Dariusz Kucharczyk

Katedra Rybactwa Jeziorowego i Rzecznego
Uniwersytet Warmińsko-Mazurski w Olsztynie

S ł o w a k l u c z o w e: temperatura inkubacji, procent wyklucia, deformacje rozwojowe, *Leuciscus leuciscus*, *Leuciscus idus, Leuciscus cephalus*.

Address: Krzysztof Kupren, University of Warmia and Mazury, ul. Oczapowskiego 5, 10-957 Olsztyn, Poland, phone: +48 89 523 44 36, e-mail: krzysztof.kupren@uwm.edu.pl
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Abstrakt

Przeprowadzono badania określające zakres temperatur tolerowanych i optymalnych dla inkubacji ikry trzech gatunków należących do rodzaju *Leuciscus*: jelca *Leuciscus idus* (L.), jazia *Leuciscus idus* (L.) i klenia *Leuciscus cephalus* (L.). Tarlaki pozyskano z Polski północnej (rzek dorzecza Pasłęki) oraz centralnej (rzek dorzecza Pilicy). Zapłodnioną ikrę inkubowano w warunkach kontrolowanych, w szerokim zakresie stałych temperatur wynoszącym od 4,5 do 29.0°C. Biorąc pod uwagę procent wyklucia, odsetek zdeformowanych larw oraz ich rozmiar optymalnymi warunkami inkubacji dla ikry jelca i klenia okazały się zakresy odpowiednio 7.5–12.3°C i 19.0–23.0°C. Dla jazia była to temperatura 15.7°C. Uzyskane wartości temperatur optymalnych są zbliżone do temperatur występujących podczas tarła. Przeprowadzone badania ujawniają również, że rozwijające się embriony badanych gatunków są w stanie tolerować temperaturę wody sięgającą 23.0°C (jelec, jaź) oraz 27.5°C (kleń). W trakcie doświadczenia zaobserwowano również pewne międzypopulacyjne różnice w badanych parametrach.

Introduction

Among the inland freshwater fish species of Europe, rheophilic cyprinids are one of the most sensitive to changes in the environment that stem primarily from constructions on rivers and pollution (SAUNDERS et al. 2002). Systematic studies of the rivers of Poland indicate there are permanent access limitations for many rheophilic fish including ide, *Leuciscus idus* (L.), dace, *Leuciscus leuciscus* (L.) and chub, *Leuciscus cephalus* (L.) (WITKOWSKI et al. 2004, PENCZAK et al. 2004). This is why interest is growing in the restoration of this species to European waters, and, consequently, efforts in the field of aquaculture are intensifying. Most of research aimed at developing reproductive biotechnologies (e.g.: KUCHARCZYK 2002, KREJSZEFF et al. 2008, 2009, ŻARSKI et al. 2009, CEJKO et al. 2010, HAKUĆ-BŁAŻOWSKA et al. 2010, TARGOŃSKA et al. 2010), and nursery techniques for dace, ide and chub (i.e. SHIRI HARZEVILI et al. 2003, 2004, KUJAWA 2004, KUPREN et al. 2008a, ŻARSKI et al. 2008). One of the main aspects of these researches are determination of the thermal conditions of incubation and rearing of fish embryos and larvae. Data concerning the thermal preferences of the different fish species is also very important in the context of overall warming of the water due to climatic changes (DAUFRESNE and BOËT 2007).

Temperature directly influences the developmental rate and development is faster at increasing temperature (KOKUREWICZ 1969, HERZIG and WINKLER 1986, KAMLER 1992, KUCHARCZYK et al. 1997, DAS et al. 2008, KORWIN-KOSSAKOWSKI 2008). However increase in temperature beyond tolerates thermal limits may also elevate the mortality and the percentage of abnormal embryos. In general, thermal limits are narrower for early stages of development (embryonic and larval). Later stages (juvenile and adults) are clearly less stenothermal (ELLIOTT 1981, COSSINS and BOWLER 1987, KUCHARCZYK et al.

1998, BERMUDES and RITAR 1999, ŻARSKI et al. 2010). Thermal history or acclimation temperature may also affect the temperature tolerance of embryos and larvae (KOKUREWICZ 1971, KUJAWA et al. 1997).

The influence of water temperature on chosen aspects of egg incubation for dace or ide has been studied by KENNEDY (1969), FLOREZ (1972), MILLS (1980), RECHULICZ et al. (2002), KUPREN et al. (2008a). Data on chub are presented by PENAZ (1968), PENAZ and STERBA (1969), ECONOMOU et al. (1991), CALTA (2000), ELLIOTT (1981) and KUPREN et al. (2008a). Most of these reports are incomplete because in most cases these data do not show full range of tolerated temperatures, in which normal development of embryos is possible, or they are limited to information concerning fish from given specific populations. However many fish species have a wide geographical range of occurrence, and the thermal conditions to which the particular populations are adapted may differ considerably (KOKUREWICZ 1971, MANN 1996, KUCHARCZYK et al. 1997). Besides data concerning thermal tolerance of developing eggs together with information concerning hatched individuals (i.e. abnormalities, size of body) would allow determine range of optimal temperatures for incubation (KOKUREWICZ 1969, KAMLER 1992). These information are very important not only for practical purposes but also from the physiological and ecological point of view.

This study investigated the effect of incubation temperature conditions on mortality, abnormalities of newly hatched larvae and body size parameters of newly hatched larvae of three rheophilic cyprinids from the genus *Leuciscus*, important species for European conservational aquaculture.

Materials and Methods

Spawners of dace *Leuciscus leuciscus* (L.), ide *Leuciscus idus* (L.) and chub *Leuciscus cephalus* (L.) were caught at different times before natural spawning (between February and June) from two parts of Poland: rivers of central Poland (Pisa River drainage) (dace: 10 females with body weight of 250–345 g and 12 males with body weight of 70–300 g; ide: 25 females of 820–1200 g and 14 males of 320–760 g; chub: 20 females of 290–365 g and 16 males of 136–350 g) and northern part of Poland (Pasleka River drainage) (dace: 13 females with body weight of 240–295 g and 10 males 80–280 g; ide: 12 females of 780–1150 g and 14 males of 300–650 g; chub: 14 females with body weight of 300–390 g and 12 males with body weight of 160–350 g). After collection the fishes were transported to the hatchery of the Department of Lake and River Fishery of the University of Warmia and Mazury and placed in 1000 dm^3 separate tanks with the possibility of thermal regulation, aeration and controlled

photoperiod (KUJAWA et al., 1999). For the purpose of spawning synchronization all obtained females and males of each species were subjected to hormonal stimulation. In the case of all the species Ovopel (Unic – Trade, Hungary) (HORVATH et al. 1997), in two doses of 0.2 and 1.0 granules per kg was used as the preparation stimulating maturation. All handling procedure was made using the methods described by KUCHARCZYK et al. (2005) for common bream (*A. brama* L.). The interval between the injections was 24 h for dace and ide and 12 h for chub. Following the second hormonal injection the water temperature in the tanks with spawners was increased to 12.0°C for dace, 14.5°C for ide and 18°C for chub (KREJSZEFF et al. 2008, 2009, KUCHARCZYK et al. 2008, ŻARSKI et al. 2009). That temperatures occur during spawning (MANN 1996) and are recommended for reproduction conducted under controlled conditions (KUPREN et al. 2008a, KUCHARCZYK et al. 2008, TARGOŃSKA et al. 2008). Before manipulations, spawners were anaesthetized in a solution of 2-phenoxyethanol (0.5 mg dm^{-3}) (Sigma-Aldrich, Germany). Milt was collected with plastic syringes and kept at 4°C before further treatment. Females were checked every three hours between 20 and 48th hours after resolving injections. Eggs were collected to plastic vessel end were next fertilized using dry method with pooled sperm collected from at least a few males.

Conditions of incubation

Fertilized eggs of the three studied species were incubated next at ten constant temperatures (4.5; 7.5; 9.5; 12.3; 15.7; 19.0; 23.0; 25.0; 27.5 and 29.0°C). The time of thermal adaptation to the given constant temperature was 1.5°C h^{-1}. Each experimental variant consisted of two lighted and aerated 40 dm^3 aquaria submerged in the 1000 dm^3 tank with water. The bath (1000 dm^3 tank) was equipped with controllable heater adjusting water temperature with an accuracy up to 0.1°C. In each of the aquaria the eggs were incubated on two Petri dishes (150–180 eggs/dish). The dishes were additionally placed in baskets of fine mesh. Water temperature during incubation was measured with the accuracy of 0.1°C four times a day. For secure stable and good conditions of incubation (oxygen saturation > 80%, ammonia and nitrite < 0.1 and 0.05, respectively) the water in the aquaria were changed daily (min 50% of volume). A fixed photoperiod of 12L:12D (Light: Dark) was maintained with light exposure from 7.00–19.00 h.

Hatching success in individual experimental groups was expressed as the ratio of hatched, normal developed embryos to the number of incubated eggs. The percentage of hatched, deformed embryos (abnormalities) (i.e.: a curvature of the spine, shortened body, shortened yolk sac, deformed skull, deformed eyes and cardiac edema) was also recorded.

Morphological measurements after hatching

At the moment of mass hatching (about 50% embryos hatched) 30 individuals were sampled from each replicate for morphological measurements. Larvae were scanned using DP-Soft software from SZ CPV Olympus stereoscopic microscope mounted with Olympus DP 12 digital camera connected to a computer. The total length, height and length of the yolk sac were measured with the accuracy of 0.01 mm. The measurements of the yolk sac were used for determination of its volume (BLAXTER and HEMPEL 1963).

Statistical analysis

Differences between groups regarding mean diameter of hydrated eggs (50 eggs from each group were tested), survival, embryonic total length and volume of yolk sac were analyzed with analysis of variance (ANOVA) and Tuckey's *post hoc* test ($\alpha = 0.05$). Survival and abnormalities percentages were normalised using arcsine transformation (SOKAL and ROHLF 1969). The differences were regarded as significant at $p < 0.05$.

Results

Mean diameter of hydrated eggs of dace, ide and chub were clearly diferentiated. Mean diameter of eggs of each species which were originated from two populations located in different parts of Poland did not differ significantly (Table 1).

Table 1
Mean diameter of hydrated eggs of dace, ide and chub originated from northern (N) and central (C) Poland. Means (± SD) with different letters are significantly different (Tukey test P < 0.05)

Species	Eggs diameter [mm]
Dace (N)	2.23 + 0.12^a
Dace (C)	2.19 + 0.06^a
Ide (N)	2.28 + 0.12^b
Ide (C)	2.26 + 0.12^b
Chub(N)	1.89 + 0.06^c
Chub(C)	1.89 + 0.07^c

Hatching success and abnormalities

The water temperature range the incubated eggs were exposed to was found to affect the hatching success and incidence of abnormalities (Figure 1 and Figure 2, Table 2). Dace embryos from northern part of Poland hatched at temperatures from 7.5 to 23.0°C. Incubation temperature of 23.0°C was lethal for embryos from central part of Poland. Generally the best results of dace incubation (to 61.3% of survival) were observed in temperatures from 9.5 to 15.7°C (Figure 2, Table 2). Survival rate in others treatments was significantly lower. Significant highest incidences of abnormalities (ranging 11.4%) were at sublethal temperatures (19.0 and 23.0°C for fish from central and northern Poland, respectively) – Table 2.

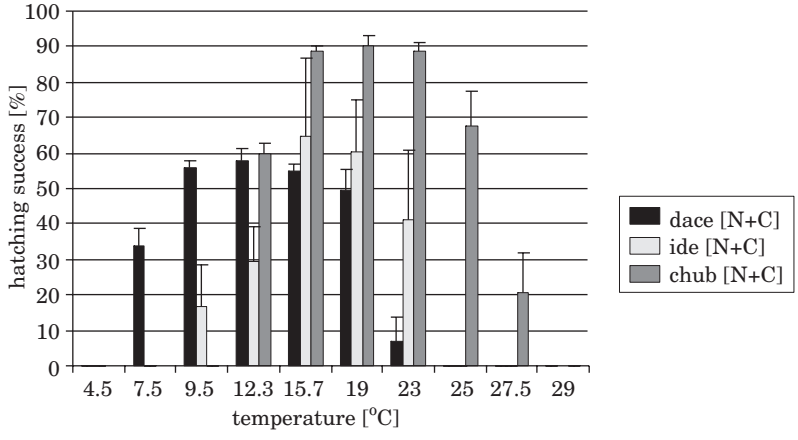

Fig. 1. The hatching success of dace, ide and chub larvae. Means (± SD) from two studied populations (N+C)

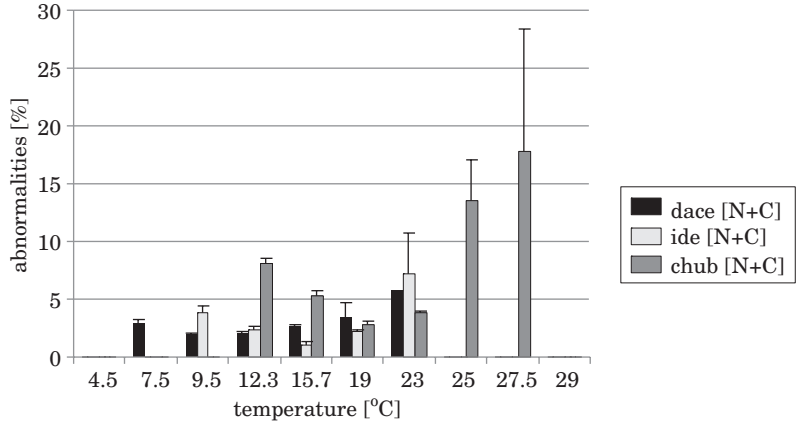

Fig. 2. Abnormalities of newly hatched dace, ide and chub larvae originated from hatching in different temperatures. Means (± SD) from two studied populations (N+C)

Table 2

The hatching success and abnormalities of newly hatched dace, ide and chub larvae originated from northern (N) and central (C) hatching in different temperatures (treatments with viable embryos). Means (± SD) in the same column with different letters are significantly different ($P < 0.05$)

Species	Temperature [°C]	Hatching success [%]	Abnormalities [%]
Dace (N)	7.5	29.0 ± 2.2^{c}	$1.2+ \ 0.3^{c}$
	9.5	54.4 ± 10.7^{a}	2.0 ± 0.13^{d}
	12.3	54.5 ± 2.1^{a}	2.1 ± 0.2^{cd}
	15.7	52.8 ± 7.5^{ab}	2.4 ± 0.7^{cd}
	19.0	55.5 ± 5.8^{a}	2.0 ± 0.1^{d}
	23.0	13.8 ± 3.2^{d}	11.4 ± 1.1^{a}
Dace (C)	7.5	38.7 ± 7.9^{b}	2.7 ± 0.2^{cd}
	9.5	57.6 ± 3.7^{a}	1.9 ± 0.3^{d}
	12.3	61.3 ± 5.5^{a}	2.0 ± 0.3^{d}
	15.7	57.0 ± 4.4^{a}	2.8 ± 0.3^{cd}
	19.0	43.9 ± 5.5^{b}	4.7 ± 0.5^{b}
Ide (N)	9.5	28.5 ± 3.4^{e}	4.4 ± 0.6^{b}
	12.3	39.2 ± 1.7^{d}	2.0 ± 0.3^{bcd}
	15.7	86.8 ± 5.0^{a}	1.3 ± 0.2^{cd}
	19.0	75.1 ± 4.2^{b}	1.9 ± 0.3^{bcd}
	23.0	60.7 ± 7.3^{c}	10.7 ± 1.6^{a}
Ide (C)	9.5	4.5 ± 2.7^{f}	3.4 ± 0.3^{b}
	12.3	20.0 ± 1.5^{e}	2.7 ± 0.2^{bc}
	15.7	42.4 ± 1.9^{d}	0.8 ± 0.3^{d}
	19.0	45.3 ± 2.9^{d}	2.4 ± 0.1^{bcd}
	23.0	22.0 ± 2.6^{e}	3.8 ± 0.6^{b}
Chub (N)	12.3	57.4 ± 2.4^{c}	6.8 ± 0.5^{c}
	15.7	90.0 ± 1.4^{a}	3.2 ± 0.8^{d}
	19.0	93.3 ± 0.7^{a}	2.9 ± 0.2^{d}
	23.0	90.9 ± 0.9^{a}	3.5 ± 0.4^{d}
	25.0	58.1 ± 2.9^{c}	20.0 ± 2.1^{b}
	27.5	31.6 ± 3.8^{d}	28.4 ± 2.7^{a}
Chub (C)	12.3	62.6 ± 3.3^{c}	9.3 ± 0.5^{c}
	15.7	87.9 ± 3.2^{a}	7.4 ± 0.8^{c}
	19.0	87.3 ± 2.0^{a}	2.7 ± 0.2^{d}
	23.0	$86.3 \ +1.7^{a}$	4.0 ± 0.12^{d}
	25.0	77.4 ± 2.2^{b}	7.1 ± 0.4^{c}
	27.5	9.8 ± 4.5^{de}	7.4 ± 0.9^{c}

Hatching of ide originating from two parts of Poland was observed in almost identical temperatures, as in dace (9.5–23.0°C). The highest mean eggs survival was observed at 15.7 and 19.0°C (75.1–86.8% for fish from north part of Poland and 42.4%–45.3% for fish from central part), lowest was at 9.5°C (28.5% and 4.5% for fish from north and central Poland) – Table 2. Level of morphological abnormalities was highest at 23.0°C. For ide originating from north part of Poland mean percent of abnormalities reached 10.7% and was almost three times higher than registered in fish from central Poland at the

same temperature. This rate was also significantly different from that observed in other treatments (Table 2).

Chub embryos from two studied part of Poland had the same range of tolerated temperatures ranging from 12.3 to 27.5°C. The highest survivals (86.3 to 93.3%) were at temperatures from 15.7 to 23.0°C (Table 2), significantly different from others treatments. The favourable incubation temperatures (survival > 50%) ranged from 12.3 to 25.0°C (Table 2). The highest incidence of abnormalities reaching 28.4% (27.5°C, northern population) was noted at two (highest and lowest) limits temperature. Differences in percent of abnormal embryos were clearer among treatments of northern population (Table 2).

Total length and yolk sac volume of hatched embryos

Larval total length and yolk volume were related to temperature at hatch (Figure 3, Figure 4, Table 3). Total length of dace at hatching time ranged in different temperatures from 5.78 to 8.30 mm in northern population, and from 6.37 to 7.42 mm in central population (Table 3). Their yolk sac volumes reached 0.33–0.92 mm^3 and 0.26–0.49 mm^3, respectively). The highest mean total length was recorded in the larvae hatched from eggs incubated at 9.5 and 12.3°C. Embryos incubated in these regimes showed also the lowest yolk sac volume (Table 3).

The total length of ide larvae from northern population ranged from 6.01 to 7.23 mm, and their yolk sac volumes varied from 0.66 to 1.13 mm^3. The total length of ide from central Poland ranged from 5.89 to 7.52 mm, and their yolk sac volumes varied from 0.63 to 0.97 mm^3. The highest mean total length was

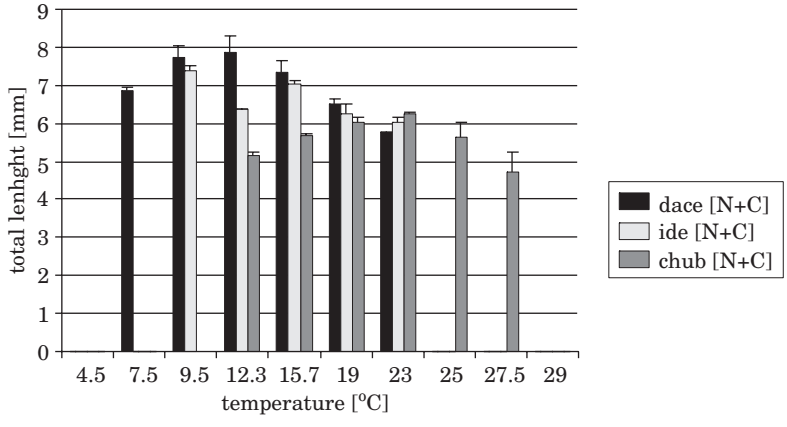

Fig. 3. Mean total length of dace, ide and chub larvae at the moment of hatching (50% individuals hatched). Means (± SD) from two studied populations (N+C)

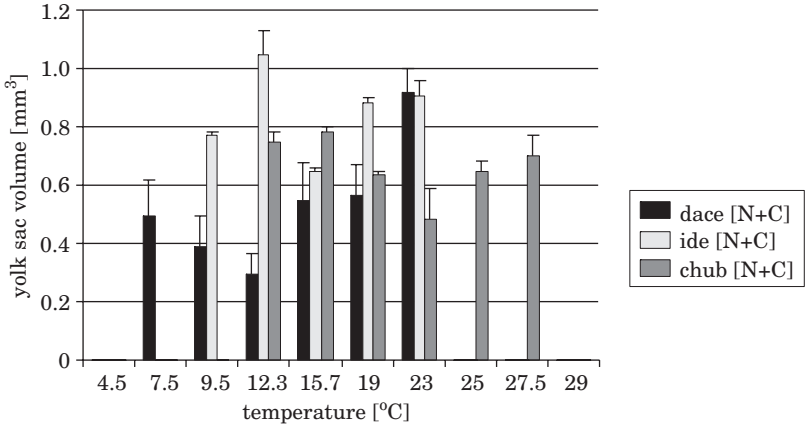

Fig. 4. Mean volume of the yolk sacs of dace, ide and chub larvae at the moment of hatching (50% individuals hatched). Means (± SD) from two studied populations

recorded in the larvae hatched from eggs incubated at 9.5 and 15.7°C. The lowest yolk sac volume was recorded in the individuals hatched from eggs kept at 15.7°C (Table 3).

The total length of chub from northern Poland reached from 4.20 to 6.28 mm at hatching time, with yolk sac volumes varied from 0.38 to 0.80 mm^3. In fish from central Poland respective size ranged from 5.24 to 6.22 mm, with yolk sac volumes ranged from 0.59 to 0.77 mm^3. Generally larvae obtained from 27.5°C were the smallest, while the fish hatched from eggs kept at 19.0°C and 23.0°C showed the highest mean total length. and the smallest yolk sac volume (Table 3).

Generally there were a significant decline of both hatching success and total length ($P < 0.05$) in all tested groups in extreme (sublethal) temperatures (Figure 1, Figure 3, Table 2, Table 3). The volume of yolk and incidence of abnormalities at hatching showed inverse but usually less clear tendency (Figure 2, Figure 4,Table 2 and Table 3). Inter-population differences in hatching success after incubation at the same water temperature were the most visible in the case of young ide (Table 2). Besides newly hatched ide and also chub from two different parts of Poland were characterized by very similar mean total length and yolk sac volume. Dace from north and central Poland were far more differentiated (Table 3).

Table 3
Mean total length and volume of the yolk sacs dace, ide and chub larvae at the moment of hatching (50% individuals hatched). Means (± SD) in the same column with different letters are significantly different (Tukey test $P < 0.05$).

Species	Temperature (°C)	Total lenght (mm)	Yolk sac volume (mm^3)
Dace (N)	7.5	$6.94 + 0.20^{e}$	$0.62 + 0.22^{b}$
	9.5	$8.06 + 0.36^{a}$	$0.45 + 0.15^{c}$
	12.3	$8.30 + 0.41^{a}$	$0.33 + 0.07^{c}$
	15.7	$7.66 + 0.11^{b}$	$0.61 + 0.14^{b}$
	19.0	$6.63 + 0.28^{d}$	$0.65 + 0.08^{b}$
	23.0	$5.78 + 0.25^{e}$	$0.92 + 0.08^{a}$
Dace (C)	7.5	$6.76 + 0.10^{e}$	$0.37 + 0.03^{c}$
	9.5	$7.42 + 0.14^{b}$	$0.33 + 0.06^{c}$
	12.3	$7.39 + 0.41^{b}$	$0.26 + 0.07^{c}$
	15.7	$6.99 + 0.20^{e}$	$0.49 + 0.11^{bc}$
	19.0	$6.37 + 0.27^{d}$	$0.48 + 0.13^{bc}$
Ide (N)	9.5	$7.23 + 0.37^{ab}$	$0.78 + 0.22^{b}$
	12.3	$6.33 + 0.49^{e}$	$1.13 + 0.12^{a}$
	15.7	$7.13 + 0.26^{b}$	$0.66 + 0.13^{c}$
	19.0	$6.01 + 0.15^{d}$	$0.90 + 0.18^{b}$
	23.0	$6.18 + 0.20^{cd}$	$0.96 + 0.07^{b}$
Ide (C)	9.5	$7.52 + 0.24^{a}$	$0.76 + 0.27^{b}$
	12.3	$6.39 + 0.29bc$	$0.97 + 0.15^{b}$
	15.7	$6.95 + 0.20^{b}$	$0.63 + 0.24^{c}$
	19.0	$6.50 + 0.29^{bc}$	$0.87 + 0.09^{b}$
	23.0	$5.89 + 0.42^{d}$	$0.85 + 0.12^{b}$
Chub (N)	12.3	$5.01 + 0.15^{d}$	$0.78 + 0.08^{a}$
	15.7	$5.60 + 0.17^{c}$	$0.80 + 0.08^{a}$
	19.0	$5.90 + 0.23^{b}$	$0.62 + 0.07^{b}$
	23.0	$6.28 + 0.22^{a}$	$0.38 + 0.07^{c}$
	25.0	$5.29 + 0.19^{d}$	$0.68 + 0.13^{b}$
	27.5	$4.20 + 0.06^{e}$	$0.63 + 0.13^{b}$
Chub(C)	12.3	$5.26 + 0.32^{d}$	$0.71 + 0.09^{ab}$
	15.7	$5.73 + 0.19^{c}$	$0.77 + 0.11^{ab}$
	19.0	$6.18 + 0.20^{a}$	$0.65 + 0.04^{b}$
	23.0	$6.22 + 0.22^{a}$	$0.59 + 0.07^{b}$
	25.0	$6.01 + 0.14^{b}$	$0.62 + 0.10^{b}$
	27.5	$5.24 + 0.17^{d}$	$0.77 + 0.09^{ab}$

Discussion

The results presented in this paper are the first data showed full range of tolerated temperatures for embryonic development of dace, ide and chub connected with data described hatched embryos after incubation conducted under the same condition. These results showed that embryos from genus *Leuciscus* have similar range of tolerated temperatures (about 15°C) but they are shifted towards higher or lower temperatures with different lower and

upper temperature limit for incubation. Chub embryos tolerated treatments with highest water temperature (up to 27.5°C), while dace embryos with the lowest (up to 7.5°C). It is undoubtedly correlated with different temperatures which occur during time of spawning (KOKUREWICZ 1971, MANN 1996). Dace usually beginning reproduction during early spring (February – April) as a one of first cyprinid species. Chub can reproduce one or few times in the year but usually started when water temperature is about 18.0°C (KOKUREWICZ 1971, MANN 1996, KREJSZEFF et al. 2008, 2010). The results obtained in the present research generally agree with previous data which indicated ranges of tolerated temperature for ide (FLOREZ 1972) but not for dace (MILLS 1980, KUCHARCZYK et al. 2002) and chub embryos (ELLIOTT 1981). The lowest tolerated temperature (7.5°C) for dace embryos was similar as observed in nature at spawning grounds (KENNEDY 1969, MILLS 1980). Upper tolerated thermal conditions (19.0 and 23.0°C) were few degrees higher than registered for dace originated from English (MILLS 1980) and Polish (KUCHARCZYK et al. 2002) waters. For the chub embryos ELLIOTT (1981) set the lower boundary temperature at 16.0°C while in our study it was 12.3°C. These evident differences occurred among fish from various population (in our experiment also between embryos of dace originating from two parts of Poland) may be caused by adaptation to their particular habitat, especially to the different thermal conditions experienced by the parents before and during spawning season (KOKUREWICZ 1971, KUCHARCZYK et al. 1997, KUJAWA et al. 1997, BERMUDES and RITAR 1999). Another reason may be a significant methodological differences used in cited papers like exposure of fertilized eggs on extremely low or high temperatures without no adaptation period (e.g. KUCHARCZYK et al. 2002).

Incubation of fish eggs in not favourable conditions may also result in premature hatching of smaller less developed larvae (KOKUREWICZ 1969, KAMLER 1992, RECHULICZ et al. 2002, KORWIN-KOSSAKOWSKI 2008). Newly hatched dace, ide and chub larvae were generally the largest at temperatures, which were similar to thermal water conditions during spawning. In other treatments the individuals leaving the egg shells usually were relatively smaller and had larger yolk sac. BLAXTER (1969) and KOKUREWICZ (1969) suggested that ideal incubation conditions (optimal temperatures) resulted in relatively large larvae, which are expected to be stronger and better swimmers. At optimum temperatures morphogenesis, growth of hatching glands, activations of their enzymes are the most harmonious (KOKUREWICZ 1969, KAMLER 1992). Differences observed in morphological parameters between studied species and also dace populations at hatch (the largest body size of dace larvae and the smallest of the chub) were positively correlated to their eggs (Table 1) size and time of incubation (KUPREN et al. 2008a). These differences are

connected with different behavioural strategies of young fish during few first days of life (MANN 1996, KUPREN et al. 2008b).

The poor hatching percentage (< 50 %) and formation the highest level of malformed larvae at sublethal temperature (7.5 and 23.0°C for dace, 9.5 and 23.0°C for ide, 12.3 and 27.5°C for chub) suggest that this rearing temperature is well above the tolerance limit for development of their eggs or may be due to the lack of adequate enzymes involved in hatching (REDDY and LAM 1991). Higher hatching rate at others treatments suggest that these temperature ranges are most suitable for incubation.

Conclusions

Overall results suggest that 9.5–12.3°C, 15.7°C and 19–23.0°C are the optimal temperatures recommended for eggs incubation of dace, ide and chub, for better hatching percentage, lowest incidence of abnormalities, best developed larvae and harmonious embryonic development. These results also may help optimizing and reduced costs of production of stocking material. This study also reveals that embryos of studied species can adapt to increasing water temperature due to global warming up to 23.0°C (dace and ide) and 27.5°C (chub).

Translated by AUTHORS

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