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THE EFFECT OF FUNGICIES APPLIED DURING THE GROWING SEASON ON THE HEALTH STATUS OF POTATO TUBERS AFTER STORAGE

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K e y w o r d s: potato tubers, fungicidal control, diseases, fungi.

A b s t r a c t

In an exact micro-plot experiment, potato plants of three cultivars were sprayed at 10-day intervals with the following fungicides: Sandofan Manco 64 WP, Penncozeb 80 WP and Tanos 50 WG; Tanos 50 WG applied three times; Tanos 50 WG, Penncozeb 80 WP and Tanos 50 WG (control treatment without fungicides). After five-month storage, the incidence of common scab (*Streptomyces scabies*) was determined on 100 tubers selected randomly of particular treatments, according to a nine-point scale (percentage infection index). The symptoms of late blight (*Phytophthora infestans*) and dry rot (*Fusarium* spp.) were evaluated in 5 kg samples for each treatment (percentage of the mass of infected tubers). Fungi were isolated from tubers at the laboratory.

The applied fungicidal control insignificantly affected the severity of infection caused by *S. scabies* only in the last year of the study. Potato tubers from fungicide-treated plants showed weaker symptoms of infections caused by *P. infestans* and fungi of the genus *Fusarium*. The abundance of pathogens colonizing potato tubers was lower in fungicide treatments.

WPŁYW FUNGICYDÓW STOSOWANYCH W OKRESIE WEGETACJI ZIEMNIAKA NA ZDROWOTNOŚĆ PRZECHOWYWANYCH BULW

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S ł o w a k l u c z o w e: bulwy ziemniaka, ochrona chemiczna, choroby, grzyby.

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Abstrakt

W ścisłym doświadczeniu mikropoletkowym w Tomaszkowie rośliny trzech odmian ziemniaka: 'Aster', 'Tara' i 'Salto' opryskiwano co 10 dni fungicydami: Sandofanem Manco 64 WP, Penncozebem 80 WP, Tanosem 50 WG; 3-krotnie Tanosem 50 WG; Tanosem 50 WG, Penncozebem 80 WP, Tanosem 50WG (kombinacja kontrolna bez stosowania fungicydów). Po 5-miesięcznym przechowywaniu na 100 bulwach oceniano nasilenie parcha zwykłego wg 9º skali (Ip w %). Nasilenie objawów zarazy ziemniaka i suchej zgnilizny szacowano w 5-kilowej próbie bulw (% masy chorych bulw). W laboratorium wyizolowano grzyby z bulw.

Zastosowana ochrona chemiczna tylko w ostatnim roku badań nieznacznie różnicowała porażenie *S. scabies*. Na bulwach wyrosłych z roślin opryskiwanych fungicydami zanotowano słabsze objawy infekcji *P. infestans* i grzybów rodzaju *Fusarium*. Stwierdzono ograniczenie liczebności patogenów zasiedlających bulwy ziemniaka w kombinacjach chronionych.

Introduction

The health status of potato tubers is determined by a variety of factors. The results of studies investigating the effect of fertilization on the incidence of tuber diseases are inconclusive. Mineral and organic fertilizers had no impact on tuber infection rates (RÓŻYŁO and PAŁYS 2006). Sulfur fertilizers reduced the severity of infections caused by *Streptomyces scabies* and *Rhizoctonia solani* (KLIKOCKA 2005, PAVLISTA 2005). Increasing nitrogen levels (from 0 to 180 kg ha^{-1}) decreased the number of tubers affected by late blight and black scurf, but they had no effect on the number of tubers infected by *S. scabies* (RĘBARZ and BORÓWCZAK 2007). The differences in tuber infection levels between potato cultivars suggest that cultivars with improved resistance to *S. scabies* and *Fusarium* fungi can be selected (SADOWSKI et al. 2004). Potato tubers can be protected against pathogens with the use of biocontrol agents (SLININGER et al. 2007, KURZAWIŃSKA and MAZUR 2008) and fungicides (SINGH 2008). In a study by LOBATO et al. (2008), phosphite compounds reduced disease severity in potato seed tubers infected by *Phytophthora infestans* and *F. solani*.

The aim of this study was to determine the effect of fungicides applied to potato plants on the severity of common scab, late blight and dry rot in tubers stored for five months, and on the composition of fugal communities colonizing potato tubers.

Materials and Methods

The experimental materials comprised potato tubers of three cultivars, Aster, Tara and Salto, stored for five months. The tubers were harvested in 2000–2002 in experimental microplots in Tomaszkowo (NE Poland). Potato

plants were sprayed three times at 10-day intervals with the following fungicides: – Sandofan Manco 64 WP, Penncozeb 80 WP and Tanos 50 WG; Tanos 50 WG; Tanos 50 WG, Penncozeb 80 WP and Tanos 50 WG. Plots with unprotected plants served as the control treatment. The experiment was carried out in a randomized block design, with cultivars as blocks and fungicide treatments as sub-blocks. Every treatment comprised six plants per plot, in four replications. Certified seed potato tubers were planted. Winter wheat was grown as a forecrop. Agricultural practices recommended by the Institute of Soil Science and Plant Cultivation in Puławy were applied. Weather conditions are presented in Table 1. High, above-average rainfalls were noted in the first two years of the study, and July was found to be particularly wet. In 2002, July was dry, with temperatures exceeding the long-term average. The summer was dry and hot. A similar temperature distribution in the summer months was noted in 2001, but due to abundant rainfalls the growing season was only relatively warm. The growing season of 2000 was the coldest.

Table 1

Weather conditions (Meteorological Station in Tomaszkowo)

Month	2000	2001	2002	Mean for 1961–1995
Temperature [°C]				
May	14.0	12.8	16.2	12.7
June	16.0	13.9	16.5	15.9
July	15.9	20.0	20.1	17.7
August	16.9	18.1	19.8	17.2
Monthly mean	15.7	16.2	18.2	15.9
Rainfall [mm]				
May	53.5	33.2	81.5	49.1
June	34.8	77.9	48.6	82.9
July	98.7	148.6	27.5	71.3
August	110.8	53.0	61.0	67.1
Monthly total	297.8	312.7	218.6	270.4

The health status of potato tubers was studied after five-month storage. The rates of tuber infection by *Streptomyces scabies* were estimated on 100 tubers selected randomly of particular treatments, according to a nine-point scale (1 – no symptoms, 9 – most severe symptoms, ROZTROPOWICZ 1999). Results were presented as a percentage infection index. The symptoms of late blight (*P. infestans*) and dry rot (*Fusarium* spp.) were evaluated in 5 kg samples for each treatment. The results were expressed as a percentage of the

mass of infected tubers. The results were processed statistically and subjected to an analysis of variance (STATISTICA® 8.0 2007–2008 software). Means were compared by Duncan's test ($p = 0.05$).

Laboratory samples consisted of 24 tubers collected randomly in four replications per treatment, after five-month storage at 5°C. Following disinfection with 50% ethanol and 1% sodium hypochlorite, blocks (0.5 x 0.5 x 1.5 cm) were cut from tubers and placed on PDA medium. After seven days of incubations, fungal colonies were inoculated onto agar slants for later microscopic identification according to the relevant keys (ARX 1970, ELLIS 1971, SKIRGIEŁŁO et al. 1979, NELSON et al. 1983).

Results and Discussion

The applied fungicides had no effect on the incidence of common scab in the first two years of the study – wet with moderate temperatures (Table 1). According to SADOWSKI et al. (2004), SZUTKOWSKA and LUTOMIRSKA (2002), a high soil moisture content due to potato watering during tuberization may reduce the severity of common scab symptoms on tubers.

In 2000 and 2001, potato tubers from fungicide-sprayed plants were characterized by lower intensity of common scab than unprotected tubers, but the differences in infection index values between the treatments were statistically non-significant (Table 2). In all cultivars, the strongest symptoms of common scab were observed in the last year of the experiment. The highest infection index values were reported in cv. Salto in the control treatment (Ii = 16.5%). One of the common disease management strategies is chemical disinfection of seed potatoes, which prevents pathogen translocation in the plant (POWELSON et al. 2002). BANYAL (2002) demonstrated that seed potato treatment with fungicides (carbendazim, tiuram, captan, mancozeb and chlorothalonil) reduced the severity of common scab on tubers. The following fungicides were found to provide effective control of common scab in Poland: Vitavax 200, Monceren 12,5 DS, Monceren 250 FS (Osowski 2002) and Vitavax 2000 FS (KURZAWIŃSKA and MAZUR (2008). According to some authors (INGLIS and POWELSON 2001), contact fungicides used for seed potato disinfection could offer tuber protection against infection caused by *Phytophthora infestans*.

The rate of infection caused by *P. infestans* was significantly higher in cv. Tara than in the other cultivars (Table 3). The highest share (6%) of affected tubers was noted in the first two years of the study. Disease symptoms were absent or sporadic on potato tubers harvested in the last growing season, except in the control treatment. This resulted from low infection rates on the aboveground parts of plants in the dry and warm year 2002. Previous research

Table 2

Infection of potato tubers by *S. scabies* after 5-month storage [Ii, %]

Treatments	'Aster'	'Tara'	'Salto'	Mean for treatments
2000				
Control	10.1^{ab}	9.2^{b}	11.7^{a}	10.3^{a}
S, P, T	8.9^{b}	10.3^{ab}	12.3^{a}	10.5^{a}
3 x *T*	8.7^{b}	8.8^{b}	10.4^{ab}	9.3^{a}
Mean for cultivar	9.2^{b}	10.6^{b}	12.4^{a}	
2001				
Control	11.3^{ab}	8.7^{bcd}	12.0^{a}	10.7^{a}
S, P, T	11.0^{abc}	7.4^{d}	9.7^{a-d}	9.4^{a}
3 x *T*	9.4^{a-d}	8.5^{cd}	11.3^{ab}	9.7^{a}
T, P, T	11.4^{ab}	8.0^{d}	10.8^{abc}	10.1^{a}
Mean for cultivar	10.8^{a}	8.2^{b}	11.0^{a}	
2002				
Control	14.2^{abc}	13.2^{a-d}	16.5^{a}	14.6^{a}
S, P, T	12.8^{bcd}	10.5^{cd}	15.5^{ab}	12.9^{ab}
3 x *T*	10.3^{d}	11.9^{bcd}	13.6^{a-d}	11.9^{b}
T, P, T	11.7^{cd}	10.8^{cd}	13.2^{a-d}	11.9^{b}
Mean for cultivar	12.3^{b}	11.6^{b}	14.7^{a}	

Explanations: *S, P, T* – Sandofan Manco 64 WP, Penncozeb 80 WP, Tanos 50 WG, 3 x *T* – Tanos 50 WG applied three times, *T, P, T* – Tanos 50 WG, Penncozeb 80 WP, Tanos 50 WG, Values denoted by the same letters in years do not differ significantly at 5% error (Duncan's test).

results (RĘBARZ and BORÓWCZAK 2007) show that heavy precipitation in the summer (June – August) supports the development of *P. infestans* infections. In the present experiment, fungicide application significantly contributed to reducing the rate of potato tuber infections caused by *P. infestans*. BASU et al. (2003) reported that mancozeb and mancozeb with metalaxyl offered the best control of late blight on potato leaves and tubers. As demonstrated by MATKOWSKI et al. (2002), potato plant spraying with fungicides (Tattoo C 750 SC, Curzate Cu 49.5 WP, Bravo Plus 500 SC, Dithane M-45, Altima 500 SC oraz Brestanid 72 WP0) reduced the severity of *P. infestans* infections and provided control against other pathogens. Recent studies (SHAILBALA and PUNDHIR 2008, SINGH 2008) show that the following fungicides were effective in controlling late blight and early blight of potato tubers: iprovalicarb with propineb, propineb, iprovalicarb with propineb, and metalaxyl M with mancozeb.

Table 3

Percentage mass of potato tubers infected by *P. infestans* after 5-month storage

Treatments	'Aster'	'Tara'	'Salto'	Mean for treatments
2000				
Control	3.1^{bc}	5.8^{a}	3.5^{b}	4.1^{a}
S, P, T	0.9^{e}	2.2^{cd}	1.0^{e}	1.4^{b}
3 x *T*	1.0^{e}	2.0^{d}	0.8^{e}	1.3^{b}
Mean for cultivar	1.7^{b}	3.3^{a}	1.8^{b}	
2001				
Control	3.4^{b}	5.2^{a}	3.0^{b}	3.8^{a}
S, P, T	1.1^{de}	2.2^{b}	1.1^{de}	1.5^{b}
3 x *T*	0.8^{e}	1.7^{cd}	1.1^{de}	1.3^{b}
T, P, T	1.2^{de}	2.0^{b}	0.9^{e}	1.4^{b}
Mean for cultivar	1.6^{b}	2.8^{a}	1.5^{b}	
2002				
Control	1.8^{c}	3.1^{a}	2.3^{b}	2.4^{a}
S, P, T	0.1^{d}	0^{d}	0^{d}	0.03^{b}
3 x *T*	0^{d}	0^{d}	0^{d}	0^{b}
T, P, T	0.2^{d}	0.2^{d}	0^{d}	0.13^{b}
Mean for cultivar	0.5^{b}	0.8^{a}	0.6^{b}	0.6^{b}

Explanations as in Table 2

Over the experimental period, potato tubers cv. Salto were infected by *Fusarium* fungi to a significantly higher degree than other cultivars, except for cv. TARA in 2001 (Table 4). The health and storage life of potato tubers are determined, among others, by the proper selection of cultivars (GAWIŃSKA-URBANOWICZ 2007). Based on the results of field experiments, storage tests and laboratory analyses, the author of the present study reported significantly higher susceptibility to dry rot in medium-late and late potato cultivars than in early cultivars. In this experiment, the severity of dry rot was reduced in cv. Salto in all fungicide treatments during the three-year experimental period. Tanos 50 WG applied three times inhibited disease development in potato tubers cv. Tara in the last growing season. The mean values of the infection index show that the percentage of tubers affected by dry rot was significantly lower in fungicide treatments than in unprotected plants. In the first two growing seasons, tuber infection rates were similar in all fungicide treatments.

The fungal community isolated from potato tubers harvested in three consecutive years (2000–2002) comprised 33 species of filamentous and yeast-like fungi and non-sporulating cultures (Table 5). The highest number of pathogens were isolated from unprotected tubers. The predominant fungal

Table 4

Percentage mass of potato tubers infected by *Fusarium* spp. after 5-month storage

Treatments	'Aster'	'Tara'	'Salto'	Mean for treatments
2000				
Control	1.0^{cd}	1.2^{c}	2.6^{a}	1.6^{a}
S, P, T	0.7^{cd}	0.9^{cd}	1.7^{b}	1.1^{b}
3 x *T*	0.5^{d}	0.7^{cd}	2.1^{b}	1.1^{b}
Mean for cultivar	0.73^{b}	0.93^{b}	2.13^{a}	
2001				
Control	1.8^{abc}	2.0^{ab}	2.4^{a}	2.1^{a}
S, P, T	1.2^{cd}	1.3^{bcd}	1.7^{bcd}	1.4^{b}
3 x *T*	1.0^{c}	1.5^{bcd}	1.6^{bcd}	1.4^{b}
T, P, T	1.4^{bcd}	1.6^{bcd}	1.4^{bcd}	1.5^{b}
Mean for cultivar	1.35^{b}	1.6^{ab}	1.8^{a}	
2002				
Control	1.5^{cd}	1.7^{c}	3.5^{a}	2.2^{a}
S, P, T	1.2^{cde}	1.3^{cde}	2.5^{b}	1.7^{b}
3 x *T*	1.0^{de}	0.8^{e}	2.3^{b}	1.4^{c}
T, P, T	1.0^{de}	1.5^{cd}	2.6^{b}	1.7^{b}
Mean for cultivar	1.18^{b}	1.33^{b}	2.73^{a}	

Explanations as in Table 2

species were *Alternaria alternata* (10.5% of all isolates in this treatment) in 2001 and *Colletotrichum coccodes* (15.5%) in 2002. A decrease in the population size of pathogens colonizing potato tubers from fungicide-treated plants was noted, particularly in the abundance of *C. coccodes* during the entire experimental period. The most substantial reduction in the abundance of *A. alternata* was observed in the growing seasons of 2001 and 2002 in plants sprayed three times with Tanos 50 WG, and with Tanos 50 WG and Penncozeb 80 WP used alternately. The high efficacy of Tanos 50 WG and Curzate 72.5 WP against late blight and early blight has been previously reported by KUCIŃSKA (2005). The causal agents of dry rot and black scurf were isolated from potato tubers in small numbers, and *Helminthosporium solani* was isolated sporadically.

Fungicide treatment had no effect on the abundance of the above pathogens. Fungi of the genus *Fusarium* produce toxins that pose a threat to the health of humans and animals. These dangerous pathogens attack potato tubers over storage. According to VAUGH and SPENCER (1994), KURZAWIŃSKA (1997) and PETERS et al. (2008), the following species are the most common disease agents: *F. avenaceum, F. culmorum, F. oxysporum, F. sambucinum* and

F. solani. FRAZIER et al. (1998) pointed to the inhibitory effect of mancozeb with thiophanate-methyl and mancozeb with captan on tuber infections by *H. solani*.

Table 5

Fungi isolated from potato tubers after 5-month storage (number of isolates)

Species	2000			2001				2002			
	k	*S, P, T*	3 x *T*	*k*	*S, P, T*	3 x *T*	*T, P, T*	*k*	*S, P, T*	3 x *T*	*T, P, T*
Pathogens (*A. alternata, C. coccodes, Fusarium avenaceum, F. equiseti, F. culmorum, F. oxysporum, F. poae, F. solani, F. sporotrichioides, H. solani, R. solani*)	26	14	11	24	12	12	10	36	18	13	14
Antagonists: (*Gliocladium* spp., *Paecilomyces* spp., *Trichoderma* spp.)	2	2	1	3	4		3	3	6	1	
Mucorales (*Mortierella* spp., *Mucor* spp., *Rhizopus* spp., *Zygorhynchus* spp.)	20	29	24	26	12	13	9	24	24	18	24
Penicillium spp.	33	30	31	31	27	22	32	27	20	30	24
Yeast-like fungi, non-sporulating	8	8	2	2	7	5	8	4	2	6	
Other: (A. *strictum, C. cladosporioides, Coniothyrium* spp., *Endothia* spp., *G. murorum, M. glauca, S. olivaceum*)	15	5	44	9	15	33	16	23	13	19	25
Total	104	88	113	95	77	85	78	117	83	87	87

Explanations as in Table 2

Conclusions

1. Potato tubers from fungicide-treated plants showed weaker symptoms of late blight and dry rot.

2. The applied fungicides had no effect on the incidence of common scab.

3. The abundance of potential pathogens colonizing potato tubers was lowest in fungicide treatments.

Translated by ALEKSANDRA POPRAWSKA

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MICROPROPAGATION OF *CHAMAEDAPHNE CALYCULATA* (L.) MOENCH BY DIRECT SHOOT ORGANOGENESIS

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K e y w o r d s: *Chamaedaphne calyculata* (L.) Moench (leather leaf), the heath family (*Ericaceae*), micropropagation, plant species conservation.

A b s t r a c t

The objective of this experiment was to investigate the effect of various concentrations of sucrose, 6-(γ,γ-dimethylallylamino)-purine (2iP) and pH values of Lloyd's and McCown's medium (1981, WPM) on the induction of lateral shoot growth in *Chamaedaphne calyculata* (L.) Moench. The explants were 2–3 cm nodal sections without the apex, with preserved leaves, from plants grown *in vitro*. The highest regenerative capacity was observed in culture media without cytokinin, with 58 mM sucrose content and pH 5.0. The lowest capacity for shoot organogenesis was reported in media with pH 5.6 with a higher sucrose content (88 mM) and 25 μM of 2iP. 80% of rooted explants were successfully transferred to *ex vitro* conditions. The survival rate of plantlets reached around 60% after three months of greenhouse cultivation.

MIKROROZMNAŻANIE *CHAMAEDAPHNE CALYCULATA* (L.) MOENCH METODĄ BEZPOŚREDNIEJ ORGANOGENEZY PĘDOWEJ

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S ł o w a k l u c z o w e: *Chamaedaphne calyculata* (L.) Moench (chamedafne północna), mikrorozmnażanie, ochrona gatunkowa, wrzosowate.

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Abstrakt

Badania podjęte w tej pracy miały na celu zbadanie wpływu różnych stężeń sacharozy, 6-(γ,γ-dimetyloallioamino)-puryny (2iP) oraz odczynu pH pożywki Lloyda i McCowna (1981,WPM) na efektywność procesu indukcji rozwoju pędów bocznych u *Chamaedaphne calyculata* (L.) Moench. Eksplantatami były fragmenty węzłowe pozbawione wierzchołka wzrostu pędu o długości od 2 do 3 cm, z zachowanymi liśćmi, pochodzące z roślin hodowlanych w kulturach *in vitro*. Najwyższy stopień regeneracji stwierdzono na podłożu bez dodatku cytokininy, zawierającym sacharozę w stężeniu 58 mM, o pH 5,0. Najsłabszą organogenezę pędową obserwowano na pożywkach o wyższym stężeniu sacharozy (88 mM), z 2iP o stężeniu 25 μM oraz o pH 5,6. 80% ukorzenionych eksplantatów pomyślnie przeniesiono do warunków *ex vitro*. Przeżywalność roślinek w warunkach szklarniowych po 3 miesiącach wynosiła około 60%.

Introduction

Chamaedaphne calyculata (L.) Moench is one of the rarest species of the family *Ericaceae* in Poland. It is a relic postglacial species characteristic of the boreal climate zone. The global distribution of the species covers the boreal and subarctic zones of Europe, Asia and North America. In Europe and Asia, it is widespread in Siberia and Scandinavia (Kloss 1999, Kruszelnicki 2001). Poland marks the south-western boundary of the species' European range, and the plant is mostly encountered in lakelands and the Masovian-Podlachian Lowland (Kloss 1999) in marshy forests and peatlands. In Europe, the species inhabits raised bogs of the *Oxycocco-Empetrion hermaphroditi* association in the subarctic and boreal zone (Kruszelnicki 2001). In Poland, the plant grows on raised bogs with acidic soils (pH below 5) within the *Sphagnetum magellanici* association and, less frequently, in *Vaccinio uliginosi-Pinetum* marshy coniferous forests (Kloss 1999, Kruszelnicki 2001). There are nine (10) leather leaf localities in Poland (out of the 13 localities known historically) which are seriously threatened by human activity, mainly peatland drainage (Kruszelnicki 2001). *Ch. calyculata* is a wildlife native species in Poland, and it remains under full legal protection.

Biotechnology offers advanced plant conservation methods which are increasingly often applied in the protection of biological diversity. In Poland, such methods are deployed to protect species of the genera *Drosera*, *Gentiana, Epipactis, Gladiolus, Asplenium* and *Polysticum*. It has been found that the best effects are produced by combining *in vitro* culture methods, which support intensive propagation of plant material, with cryopreservation techniques that allow for long-term material storage (Mikuła and Rybczyński 2006, Rybczyński and Mikuła 2007). Under laboratory conditions, plants can be propagated on a large scale and various species cultures can be preserved for many years, thus enabling the creation of tissue banks of selected plant species.

This paper investigates the micropropagation of *Chamaedaphne calyculata* (L.) Moench by direct organogenesis. The development of a highly effective micropropagation method supporting the production of a large number of seedlings over a short period of time would significantly contribute to species preservation in the natural habitat. Such a method would also enable the colonization of well-preserved raised bogs that offer an ideal habitat for this endangered species.

Materials and Methods

Plant material

Secondary explants were 2–3 cm shoot sections with 4–8 nodes, without the apex and with preserved leaves, isolated from stabilized *in vitro* cultures of *Chamaedaphne calyculata* (L.) Moench shoots on the WPM medium (LLOYD and MCCOWN 1981). Input material for maternal cultures comprised sections of *Ch. calyculata* shoots grown naturally. They were sampled from two selected individuals of a single population at a locality in the Masurian Landscape Park, municipality of Piecki.

Medium composition for inducing the formation of lateral shoots

Each secondary explant was transferred to one of twelve induction media (Table 1). All growth media contained salts, vitamins and amino acids, according to the methodology proposed by LLOYD and MCCOWN (1981, WPM), as well as 8 g l^{-1} of agar. Medium variants differed with regard to their sucrose concentrations (58 and 88 mM), 2iP concentrations (0, 10 and 25 μM) and pH (5.0 and 5.6).

Explants were cultured at a temperature of 20°C (+/– 2°C) under a fluorescent light (OSRAM L36W/77 Fluora, Flora type) photoperiod (16 h light and 8 h dark). The cultures were passaged every six weeks.

The experiment was performed in a completely randomized block design. Every experimental treatment was represented by at least five replications. Every replication consisted of 10 test tubes (50 ml each) with 10 ml of the medium where individual explants were placed vertically (the bottom internode without the lateral bud was inserted into the medium). A total of 50 explants were cultured in each medium type throughout the experiment.

The formation of lateral shoots and aseptic culture conditions were monitored every 5–7 weeks. The development of lateral shoots was observed

Table 1
Differences in the composition of Lloyd and McCown's medium variants (WPM, 1981) used in the experiment. The underlined growth media were also applied in the rooting process

Medium	Sucrose [mM]	2iP [μM]	pH
111	58	0	5.0
112	58	0	5.6
121	58	10	5.0
122	58	10	5.6
131	58	25	5.0
132	58	25	5.6
211	88	0	5.0
212	88	0	5.6
221	88	10	5.0
222	88	10	5.6
231	88	25	5.0
232	88	25	5.6

based on the following scale (RPB): 1° – swollen bud; 2° – shoot with folded leaves in a bud, 3° – shoot shorter than 3 cm with unfolded leaves, 4° – shoot longer than 3 cm with unfolded leaves.

Rooting and acclimatization in *ex vitro* conditions

To induce rooting, explants were cultured in each of the four WPM variants which did not contain phytohormones and differed in their sucrose content and pH (Table 1).

After 18 weeks (three growth cycles), plants with branching roots, where the length of the main root exceeded 1.5 cm, were transferred to *ex vitro* conditions. During the first six weeks, the plantlets were grown on perlite saturated with a 50% solution of WPM salts (LLOYD and MCCOWN 1981). After the successive six weeks, the plantlets were transferred to a 3:1 mix of acidic peat and perlite, and they were watered with a 50% solution of WPM salts (LLOYD and MCCOWN 1981). After the following six weeks, the 50% WMP salt solution was replaced with deionized water (pH 5.0–5.2). Prior to transfer, the plantlets were rinsed in distilled water to remove medium residues. The *ex vitro* culture was carried out in conditions identical to the *in vitro* culture. The vessels containing the plantlets were covered to maintain high levels of air humidity.

Statistical analysis

The obtained data were processed by an analysis of variance and Duncan's test at a significance level of $\alpha=0.05$ using Microsoft Excel 2007 and STATISTICA 8.0 software.

Results and Discussion

The highest rate of lateral shoot induction was observed in medium 111 without 2iP with sucrose content of 58 mM and pH 5.0. The data presented in Table 2 suggest that higher sucrose concentrations, the presence of 2iP and higher pH values had a detrimental effect on the induction and growth of lateral shoots in *Ch. calyculata*. The noted differences were statistically significant.

Carbohydrates added to in vitro culture media (mainly disaccharides – sucrose, maltose, and monosaccharides – glucose, fructose, galactose and maltose) are the main source of carbon for the cultured plant material. They also stabilize the substrate's osmotic equilibrium, thus enhancing nutrient uptake and the growth of explant cells (STEFANIAK 2004, DEBNATH 2005). Research results show that the sugar which is most often applied in the micropropagation of plants of the family *Ericaceae* is sucrose at a concentration level of 88 mM, although lower doses of 44–58 mM are recommended by some authors (e.g. KYTE and KLEYN 2003, CAO et al. 2003, LITWIŃCZUK and WADAS 2008). In this study, culture media were enriched with 58 mM sucrose (this concentration level is generally applied to the media proposed by LLOYD and MCCOWN 1981, as well as ZIMMERMAN and BROOM 1980) and 88 mM sucrose (most popular concentration for ANDERSON'S medium, 1975). After 18 weeks (three growth cycles), higher sucrose concentrations limited the number of induced lateral shoots and impaired their growth (Table 2). The above could be attributed to the fact that the presence of sugar in culture media affects the substrate's osmotic pressure. The sucrose content of 88 mM could impair nutrient uptake in comparison with substrates characterized by lower sugar concentrations, thus inhibiting the growth of lateral shoots. The fact that infection rates were twice lower in media with lower sucrose concentrations (30% and 60%, respectively; data not presented) additionally contributes to the above hypothesis. The obtained results showed statistically significant differences in the number of explants induced on culture media with varied sucrose concentrations.

Axillary buds are usually dormant under *in vivo* conditions, but their development can be induced when the bud is separated from the shoot apex

Table 2
Percentage share of lateral buds and stems at various development stages (on the RPB scale) in variants of the WPM medium after three growth cycles (18 weeks). Data presented in columns were analyzed

Medium	Number of explants/ number of explants with inducted lateral buds [%]	Percentage of inducted lateral buds/explant	Percentage of lateral buds at various development stages on the RPB scale/ explant			
			1^0	2^0	3^0	4^0
111	50/36 [72%]	39.68^{a}	25.61^{a}	15.85^{a}	18.29^{a}	40.24^{a}
112	50/24 [49%]	25.37^{b}	5.88^{b}	9.80^{b}	54.90^{b}	29.41^{b}
121	50/17 [35%]	27.89^{b}	11.32^{c}	11.32^{a}	41.51^{c}	32.07^{b}
122	50/15 [30%]	20.30^{b}	15.00^{c}	17.50^{a}	50.00^{b}	17.50^{c}
131	50/13 [27%]	26.46^{b}	18.64^{c}	11.86^{a}	52.54^{b}	16.95^{c}
132	50/12 [25%]	16.37^{c}	16.22^{c}	18.92^{a}	62.16	$2.70^{d,e}$
211	50/31 [62%]	22.54^{b}	14.58^{c}	12.50^{a}	56.25^{b}	16.67^{c}
212	50/20 [40%]	15.24^{c}	31.25^{a}	25.00^{c}	34.37^{d}	9.37^{d}
221	50/15 [30%]	20.19^{b}	16.28^{c}	20.93^{c}	$46.51^{b,c}$	$11.63^{c,d}$
222	50/11 [23%]	9.09^{d}	31.58^{a}	26.32^{c}	42.10^{c}	0.00^{e}
231	50/11 [23%]	14.56^{c}	40.00^{d}	30.00^{c}	30.00^{d}	0.00^{e}
232	50/10 [20%]	10.55^{c}	17.39^{c}	26.09^{c}	$47.83^{b,c}$	8.69^{d}

(elimination of apical dominance). For this reason, culture media that do not contain phytohormones are suitable for the propagation of plants via shoot apices and axillary buds. Nevertheless, the addition of phytohormones, in particular cytokinins, speeds up the elimination of apical dominance, it stimulates shoot branching and increases the propagation rate (e.g. KIRKORIAN 1995, MOK et al. 2000, CZERPAK and PIOTROWSKA 2003, STEFANIAK 2004, SACHS 2005). The most popular cytokinins applied in *in vitro* propagation of ericaceous plants are 2iP and zeatin, used in combination or separately. The selection of optimal concentrations for each species is a vital consideration because the above compounds may exhibit phytotoxic effects (LITWIŃCZUK 2007). According to reference data, the preferred 2iP concentrations for plants of the family *Ericaceae* range from 1 to 73.82 μM, and the range of 10–30 μM is most highly recommended (e.g. MCCOWN 2000, TOMSONE and GERTNERE 2003, LITWIŃCZUK 2007). In view of the above, we decided to test 2iP concentrations of 10 and 25 μM in our experiment. The reported results (Table 2) indicate that although 2iP is popularly used in the micropropagation of other species of the family *Ericaceae*, it does not effectively stimulate direct shoot organogenesis in *Ch. calyculata*.

The pH of the substrate significantly affects ion bioavailability (e.g. WILLIAMS 1993), and it may exert a powerful influence on organogenesis *in vitro* (e.g. ANTHONY et al. 2004). According to most researchers, pH 4.6 to 5.8 is the

optimum range for ericaceous plants (e.g. LITWIŃCZUK 2007). In this experiment, culture media had the pH of 5.0 and 5.6. In general, a higher rate of lateral shoot induction was observed in media with a lower pH (5.0), and statistically significant differences were reported in the number of explants induced in media with different pH values (Table 2). Nutrient availability is lower in media with a lower pH, nevertheless, *Ch. calyculata* is naturally habituated for peatlands with a low pH, and the obtained results should not be surprising.

Effective rooting of regenerated shoots and high plant survival rates in soil are the critical final stages of micropropagation. The shoots of ericaceous plants grown *in vitro* on media enriched with auxins (indole-3-butanoic acid, IBA) and the shoots grown *ex vitro* in a controlled environment chamber without the addition of phytohormones develop roots relatively easily. The effectiveness of rooting is often similar in both environments, therefore, the *in vitro* rooting phase may be omitted, and the shoots produced by tissue cultures may be rooted directly in the greenhouse (ALMEIDA et al. 2005, CANTOS et al. 2007, MEINERS et al. 2007). In several referenced studies, shoots developed weak root systems on agar media, and more satisfactory results were reported *in vitro* (e.g. ORLIKOWSKA 1986, LYRENE and PERRY 1988). The existing body of research on *Ch. calyculata* describes only the rooting of shoot cuttings from mature plants growing in the Drawa National Park (MALINOWSKA et al. 2004). Our previous experiments have demonstrated that *Ch. calyculata* roots equally well in media without phytohormones and media enriched with auxins. The shoots grown *ex vitro* develop significantly weaker root systems (data not presented). For this reason, in this experiment, the rooting process was carried out *in vitro* on media not containing phytohormones. Adventitious root formation was observed during six weeks of culture in 75% shoots. The roots of plantlets grown on media with a higher sucrose content (211 and 212) were notably shorter and less branched than the roots of plants cultured in media with lower sucrose concentrations (111 and 112) – Figure 1. No differences were reported in the root systems of explants cultured on media with various pH (Figure 1).

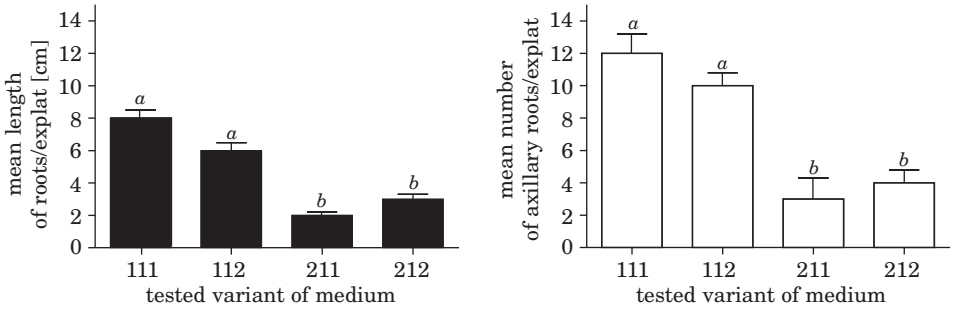

Fig. 1. *In vitro* rooting and root development in explants grown on different media after 18 weeks

80% of rooted shoots were successfully transferred to *ex vitro* conditions, and the remaining 20% died in the first two weeks of acclimatization. The survival rates of plantlets grown *in vitro* reached around 60% after three months.

Conclusions

Increasing 2iP concentrations in the culture medium had an inhibitory effect on the induction of lateral shoots and their growth. Higher sucrose concentrations reduced the efficiency of *Ch. calyculata* micropropagation. The rate of lateral shoot induction was higher in media with lower pH values (5.0). The applied growing regime supported the growth of *Ch. calyculata* under *in vitro* conditions.

The results of this study indicate that *Ch. calyculata* micropropagation by direct organogenesis yields the best results on a WPM medium (LLOYD and MCCOWN 1981) not enriched with 2iP, with a sucrose content of 58 mM and pH 5.0.

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THE DISTRIBUTION OF PSYCHROPHILIC AND MESOPHILIC BACTERIA IN LOBELIA LAKES NAWIONEK AND PIECKI LOCATED IN THE ZABORSKI LANDSCAPE PARK

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K e y w o r d s: bacterioplankton, psychrofilic bacteria, mesophilic bacteria, lobelia lakes, Zaborski Landscape Park.

A b s t r a c t

The research was carried out in Lake Piecki and Lake Nawionek, two lobelia lakes located within the Zaborski Landscape Park. Water samples were collected from the surface and bottom water layers in spring, summer and autumn 2009. The analysis reveals that the total number of planktonic bacteria (TNB) in Lake Nawionek ranged between 8.83 and $36.22 \cdot 10^5$ cells/cm^3 and between 15.77 and $44.93 \cdot 10^5$ cells/cm^3 in Lake Piecki. The highest counts of bacteria were noted in spring. In a majority of tests heterotrophic psychrophilic bacteria (CFU 22°C) were more abundant in the surface water layer while heterotrophic mesophilic bacteria were always more abundant in the bottom water layer. Rods were prevailing morphological forms in both lakes, followed by pleomorphic forms, whereas cocci and bacilli constituted the least abundant microbial groups. Furthermore, ammonifying and nitrifying bacteria were the most common physiological forms in both lakes. Higher physiological activities of bacterial strains were observed in Lake Piecki with the exception of pectinolytic and ureolytic bacteria, which were more active in Lake Nawionek.

WYSTĘPOWANIE BAKTERII PSYCHROFILNYCH I MEZOFILNYCH W WODZIE JEZIOR LOBELIOWYCH (JEZIORO NAWIONEK I JEZIORO PIECKI) W ZABORSKIM PARKU KRAJOBRAZOWYM

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S ł o w a k l u c z o w e: bakterioplankton, psychrofile, mezofile, jeziora lobeliowe, Zaborski Park Krajobrazowy.

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Abstrakt

Badaniami objęto dwa jeziora lobeliowe – Nawionek i Piecki, położone w Zaborskim Parku Krajobrazowym. Próbki wody z warstwy powierzchniowej i naddennej pobierano w cyklu sezonowym: wiosną, latem i jesienią 2009 r. Przeprowadzone badania mikrobiologiczne wykazały, iż ogólna liczba bakterii planktonowych (TNB) w jeziorze Nawionek wynosiła od 8,83 do $36{,}22 \cdot 10^5$ komórek/cm^3, a w jeziorze Piecki od 15,77 do $44{,}93 \cdot 10^5$ komórek/cm^3. Najliczniej bakterie w obu zbiornikach występowały wiosną. Heterotroficzne bakterie psychrofilne (CFU 22°C) w większości badanych stanowisk liczniej występowały w wodzie powierzchniowej niż naddennej. Z kolei heterotroficzne bakterie mezofilne (CFU 37°C) zawsze występowały liczniej w wodzie naddennej niż w powierzchniowej. Struktura morfologiczna bakterii heterotroficznych charakteryzowała się w obu badanych zbiornikach znaczną przewagą pałeczek. Drugą pod względem liczebności grupę stanowiły formy pleomorficzne, natomiast odsetek ziarniaków i laseczek był niewielki i podobny w obu jeziorach. Wśród badanych grup fizjologicznych w obu jeziorach najwięcej odnotowano bakterii amonifikacyjnych oraz nitryfikacyjnych. W jeziorze Piecki odnotowano większą niż w jeziorze Nawionek aktywność fizjologiczną wśród badanych szczepów bakteryjnych, poza bakteriami pektynolitycznymi i ureolitycznymi, które były aktywniejsze w jeziorze Nawionek.

Introduction

Lobelia lakes are unique water bodies distinguished according to a floristic (SZMEJA and CLÉMENT 1990) and limnological (SZMEJA 1998a, 1998b, 1998c, CHMARA 2007) criteria. As stated in the definition (KRASKA 2004), they are inhabited by one or all of the following plant species: Water Lobelia (*Lobelia dortmanna* L.), Lake Quillwort (*Isoëtes lacustis* L.) and American Shoreweed (*Littorella uniflora* L.)

Lobelia lakes are classified as soft water lakes with low concentration of mineral salts, typically characterised by low pH value and low electrolytic conductivity (SZMEJA 1997, MILECKA and BOGACZEWICZ-ADAMCZAK 2006). So far 177 lakes of this type have been registered in Poland (CHMARA 2007), a majority of which are located on the upland areas surrounding a terminal moraine ridge in Western Pomerania, on the outwash plains in the Tuchola Forest, on the Charzykowy Plain and in the Kashubian Lake District (KRASKA et al. 1996).

The proper functioning of lobelia lake ecosystems is vital for preserving rare lobelia species and thereby for preserving the unique character of these water bodies. Over the decades researchers have made attempts to characterise the trophicity of lobelia lakes on the basis of physico-chemical parameters of water and representative plants (SZMAL 1959, KRASKA et al. 1996, LEWICKA 2000, PACEWICZ and NOWIŃSKI 2007). Eutrophication in lakes is also hugely influenced by the distribution pattern of the bacterial population and types of bacteria in aquatic ecosystems (LEWICKA 2000).

This research was aimed at determining growth dynamics of planktonic bacteria in a seasonal cycle, their morphological diversity and selected physiological properties which have not been sufficiently recognised in lobelia lakes as yet.

Materials and Methods

Research area

Lake Nawionek is situated in the western part of the commune of Brusy, the District of Chojnice, near the border with the commune of Chojnice (BARAŃCZUK et al. 2010), approximately 6 km north-west of the village of Swornegacie and 2 km south-east of the village of Laska (BOIŃSKI 1985); its geographical coordinates are 17°31'57" E / 53°54'35" N (BOROWIAK et al. 2009a).

This endorheic lake, genetically classified as a lake formed by glacial meltwater, is located in a catchment area of a subglacial gully running north-south (BARAŃCZUK et al. 2010). It has a surface area of 10 ha, an average depth of 6.2 m and a maximum depth of 10 m. The lake, whose length is 546 m and breadth is 312 m at its widest point, has a slightly elongated shape (BARAŃCZUK et al. 2010). The relatively steep slopes of the lake basin are covered with a dense pine forest. The lake shores are sandy or muddy (BOIŃSKI 1985), the bottom is overgrown with a thick carpet of Water Lobelia (*Lobelia dortmanna* L) to a depth of approximately 2 metres, and is then gradually displaced by Lake Quillwort (*Isoëtes lacustis* L.) (BARAŃCZUK et al. 2010). Compared to other lobelia lakes, Lake Nawionek shows certain floristic peculiarities. It appears that with a higher amount of calcium and more neutral pH value, it constitutes a good habitat for Eurasian Water Milfoil (*Myriophyllum alternifolium* D. C.) (CHMARA 2003).

On 16th September 1974 Lake Nawionek was officially registered as a nature reserve by Minister of Forestry and Wood Industry. The reserve, which covers a surface area of 10.64 ha, was established for the protection and preservation of a lobelia lake ecosystem at the southern margin of Water Lobelia distribution in Europe.

Lake Piecki is situated in the commune of Brusy, the District of Chojnice, near the road joining Czernica, Asmus, and Laska, approximately 7 km from the intersection with the provincial road Brusy – Konarzyny (Wysocka and Grzempa 2007), 11 km north-west of the village of Brusy and approximately 7 km north-east of the village of Swornegacie (*Park Narodowy*... 2006). Its geographical coordinates are 17°33'22" E / 53°54'47" N (BOROWIAK et al. 2009b).

Lake Piecki is an endorheic water body located in the basin formed by glacial meltwater (WYSOCKA 2007). It has a surface area of 6.43 ha, the maximum depth of 7.6 m and the average depth of 4.2 m. Its maximum length is 432 m while its maximum width is 217 m (BOROWIAK et al. 2009b). Steep slopes of the lake basin are almost entirely covered with

a thick pine forest, with a predominance of fresh pine forest. Peat bog formations developed along its southern shore and an alder patch spreads along the south-western shore. The bottom is carpeted with Water Lobelia (*Lobelia dortmanna* L) and Lake Quillwort/Merlin's Grass (*Isoëtes lacustis* L.) (WYSOCKA and GRZEMPA 2007).

Lake Piecki is situated within the Nature Reserve Piecki established by Pomeranian Governor on the 21st November 2001. It covers an area of 19.42 ha and the surrounding belt covers 92.42 ha. The reserve was established to protect and preserve plant communities typical of lobelia lakes, transitional peat bogs, and fresh and marsh pine forests, which are habitats for numerous rare or endangered protected plant species (WYSOCKA and GRZEMPA 2007).

Methods

Water sampling was conducted in a seasonal cycle in spring, summer and autumn 2009. In each lake two sampling sites were established in the littoral zone, namely near the northern shore (Sampling Site I) and the southern shore (Sampling Site III). In each lake one sampling site was established in the pelagic zone (Sampling Site II).

At each site water samples were collected from the surface water layer (approx. 30 cm below the surface) and from the bottom water layer (approx. 50 cm above the sediment) with the use of Schindler-Patalas plankton trap. Water was poured into sterile one-litre glass bottles, sealed tightly with a ground stopper. The bottles were filled to 3/4 of their volume. Additionally, water samples of 50 cm^3 were poured into sterile glass bottles with a capacity of 100 cm^3, and subsequently fixed with 1 cm^3 of 40% formaldehyde. The bottles with the collected water samples were then placed in a portable refrigerator filled with ice (the temperature never rose above 7°C) and transported to the laboratory. The time lapse between the sampling and the beginning of the microbiolocal analysis never exceeded 5 hours.

At the same time measurements of selected physicochemical parameters were obtained from each sampling site. The temperature of water was measured with the use of HI 9143 field thermometer (Hanna Instruments – Deutschland GMBH), pH value was measured with the use of N 5110 field pH meter (Hanna Instruments – Deutschland GMBH), electrolytic conductivity was measured with the use of CM 204 Slandi conductivity meter (Hanna Instruments – Deutschland GMBH), and the concentration of dissolved oxygen was measured with the use of HI 9143 dissolved oxygen meter (Hanna Instruments – Deutschland GMBH).

The microbiological analysis of water included determining the total number of planktonic bacteria (TNB) at all sampling sites, determining the number of heterotrophic bacteria capable of growing at 22°C (psychrophilic) as well as determining the number of heterotrophic bacteria capable of growing at 37°C (mesophilic), investigating their morphology, and identifying their selected physiological properties.

The total count of planktonic bacteria was determined with the use of the ZIMMERMAN and MEYER-REIL (1974) method, which involves counting bacteria on membrane filters. The water samples, previously fixed with formalin, were filtered through Nucleopore polycarbonate membrane filters with 0.22 μm pores. After that bacterial cells, stained with DAPI reagent, were counted under the NIKON ECLIPSE E 600 fluorescence microscope, with the application of Ex 450–490 nm, BA 520 nm, DM 505 nm filters, 100X lens and an apparatus 1.30, and CFI 10X eyepiece. An automated image analysis based on LUCIA G programme was applied for counting bacterial cells.

The numbers of heterotrophic bacteria (CFU) capable of growth at 22°C and at 37°C in both lakes were determined with the spread plate technique in accordance with ISO norm 6222:1998(E), with the use of iron-peptone agar medium after FERRER at al. (1963). Before inoculating water samples were repeatedly diluted with sterile saline solution (0,85% NaCl). Inoculations were then performed from each dilution in three parallel replicates The plates were incubated in a thermostat for 3 days at 22°C and for 2 days at 37°C.

The morphology of the bacteria was investigated in Gram stained preparations (RODINA 1968). Thirty random strains were transferred from iron-peptone agar medium into liquid medium, where they were subsequently incubated. After two and then again after three days of incubation at 22°C stained preparations with bacteria were viewed under a microscope at 1000x magnification with the aim of identifying bacilli, rods, cocci and pleomorphic forms.

Two hundred random strains incubated at 22°C were used for the investigation of the physiological and biochemical properties of the bacteria. Tests included investigating the following features: the bacteria's ability to break down fat, protein, starch, DNA, pectin, cellulose, urea, the bacteria's ability to produce hydrogen sulphide of organic combinations, the bacteria's ability to perform ammonification and nitrification (oxidation NH_4^- to NO_2^-), and the bacteria's ability to reduce nitrates to nitrites. Test media were prepared after DONDERSKI (1983), LALKE-PORCZYK (1999) and WALCZAK (2002).

Results and Discussion

The analysis of the average values of the selected physicochemical parameters of water reveals that the temperatures of surface water layer and bottom water layer in both lakes were comparable (Table 1), with the exception of summer, when more significant differences between the temperatures of the surface and the bottom water layers were observed. The difference was 4.25°C for lake Nawionek and 2.67°C for lake Piecki.

Two types of lakes can be distinguished in the Tuchola Forest, namely lakes with sediments poor or rich in $CaCO_3$ (SZMEJA et al. 1998), i.e. with acidic or alkaline pH. The studied lakes show differences in pH values – pH values for lake Nawionek ranged between 7.89 and 8.41 during the research (similar results were also obtained by PACEWICZ and NOWIŃSKI (2007) and BARAŃCZUK et al. (2010) while pH values for lake Piecki ranged between 5.11 and 7.26 (slightly acidic); a similar result was obtained by PACEWICZ and NOWIŃSKI (2007).

Moreover, the investigated lakes show differences in electrolytic conductivity. Notably, both lakes are characterised by poorly mineralised water. The average value of electrolytic conductivity for Lake Nawionek (94 $\mu S\ cm^{-1}$) was three times as high as for Lake Piecki (30 $\mu S\ cm^{-1}$). Furthermore, the values of electrolytic conductivity for Lake Piecki did not undergo considerable fluctuations throughout the entire research period whereas the values for Lake Nawionek varied significantly. The highest values (100 $\mu S\ cm^{-1}$) were noted in summer while the lowest values (87 $\mu S\ cm^{-1}$) were noted in autumn. Remarkably, similar results were obtained by BARAŃCZUK et al. (2010).

Aerobic conditions in both lakes may be described as favourable and are similar in the surface and bottom water layers. In Lake Nawionek the average concentration of oxygen dissolved in water was 7.47 mg dm^{-3}, while the oxygen saturation was around 83.61%. In Lake Piecki the average concentration of oxygen dissolved in water was 6.85 mg dm^{-3}, while the oxygen saturation was around 75.98%. In Lake Piecki the differences between the values of dissolved oxygen concentration and the oxygen saturation were higher and amounted to 7.06 mg dm^{-3} and 78.09% for the surface water layer, and 6.63 mg dm^{-3} and 74.5% for the bottom water layer respectively. This phenomenon may be related to high transparency of water in lobelia lakes, where sunlight can easily penetrate lakes even at considerable depths (KRASKA i in. 1998) therby stimulating photosynthesis in planktonic algae.

Determining the number of bacteria provides information about lake trophicity (RHEINHEIMER 1987), so any changes in the concentration of bakterioplankton may indicate early stages of imbalance in aquatic ecosystems.

Table 1

An average values of the selected physicochemical parameters in water of the Nawionek Lake and the Piecki Lake

–	Date of sampling		Temperature [°C]	pH	Electrolytic conductivity [μS cm^{-1}]	Concentration oxygen [mg dm^{-3}]	Dissolved oxygen [%]
Lake Nawionek	Spring 23 IV 2009	surface water layers	6.73 [6.0–7.2]	8.0 [7.9–8.1]	95.0 [94.0–98.0]	6.97 [6.67–7.23]	86.03 [83.0–90.0]
		bottom water layers	6.03 [4.9–6.7]	8.0 [7.8–8.3]	91.0 [85.0–94.0]	7.57 [7.09–8.09]	90.61 [83.5–99.0]
	Summer 29 VII 2009	surface water layers	21.93 [21.8–22.0]	8.23 [8.1–8.4]	100.0 [99.0–102.0]	8.25 [8.12–8.47]	87.38 [85.3–88.7]
		bottom water layers	17.67 [12.9–20.1]	8.17 [8.0–8.41]	100.0 [98.0–102.0]	7.19 [6.9–7.46]	83.58 [81.6–87.5]
	Autumn 23 IX 2009	surface water layers	17.07 [16.9–17.3]	7.89 [7.89–8.0]	87.0 [85.0–87.0]	7.28 [6.84–7.8]	75.53 [72.7–80.57]
		bottom water layers	16.27 [15.9–16.6]	8.0 [7.8–8.2]	90.0 [87.0–94.0]	7.54 [7.01–7.89]	78.52 [74.77–85.1]
Lake Piecki	Spring 23 IV 2009	surface water layers	6.43 [6.0–6.8]	5.17 [5.0–5.5]	30.0 [29.0–31.0]	6.76 [6.31–6.61]	79.01 [77.73–81.30]
		bottom water layers	5.92 [5.1–6.45]	5.17 [5.0–5.5]	30.0 [29.0–30.0]	6.32 [5.11–7.23]	75.33 [56.20–88.90]
	Summer 29 VII 2009	surface water layers	21.83 [21.7–22.0]	7.07 [6.9–7.3]	30.0 [29.0–31.0]	7.39 [7.20–7.50]	82.54 [77.60–87.67]
		bottom water layers	19.16 [14.2–21.9]	7.26 [6.9–7.6]	33.0 [33.0–34.0]	6.50 [5.55–7.0]	76.45 [69.85–82.0]
	Autumn 23 IX 2009	surface water layers	14.64 [14.32–14.9]	5.13 [4.9–5.3]	29.0 [28.0–31.0]	7.04 [7.02–7.08]	72.69 [71.67–73.47]
		bottom water layers	14.10 [13.0–14.73]	5.11 [4.8–5.6]	29.0 [28.0–31.0]	7.07 [6.18–8.16]	71.71 [66.90–75.33]

Table 2
Total number of planktonic bacteria (TNB) in water of the Nawionek Lake and the Piecki Lake

Date of sampling		Lake Nawionek Number of bacteria · 10^5 cells/cm^3			Lake Piecki Number of bacteria · 10^5 cells/cm^3		
		Site I	Site II	Site III	Site I	Site II	Site III
Spring 23 IV 2009	surface water layers	10.79	36.22	14.42	29.44	20.06	33.15
	bottom water layers	31.35	34.55	16.84	26.0	44.93	26.66
Summer 29 VII 2009	surface water layers	8.83	29.88	28.46	22.95	30.47	18.86
	bottom water layers	13.54	15.88	22.91	25.63	25.57	15.85
Autumn 23 IX 2009	surface water layers	30.09	25.58	17.60	30.75	15.77	32.71
	bottom water layers	23.93	21.16	18.21	26.12	25.02	25.94

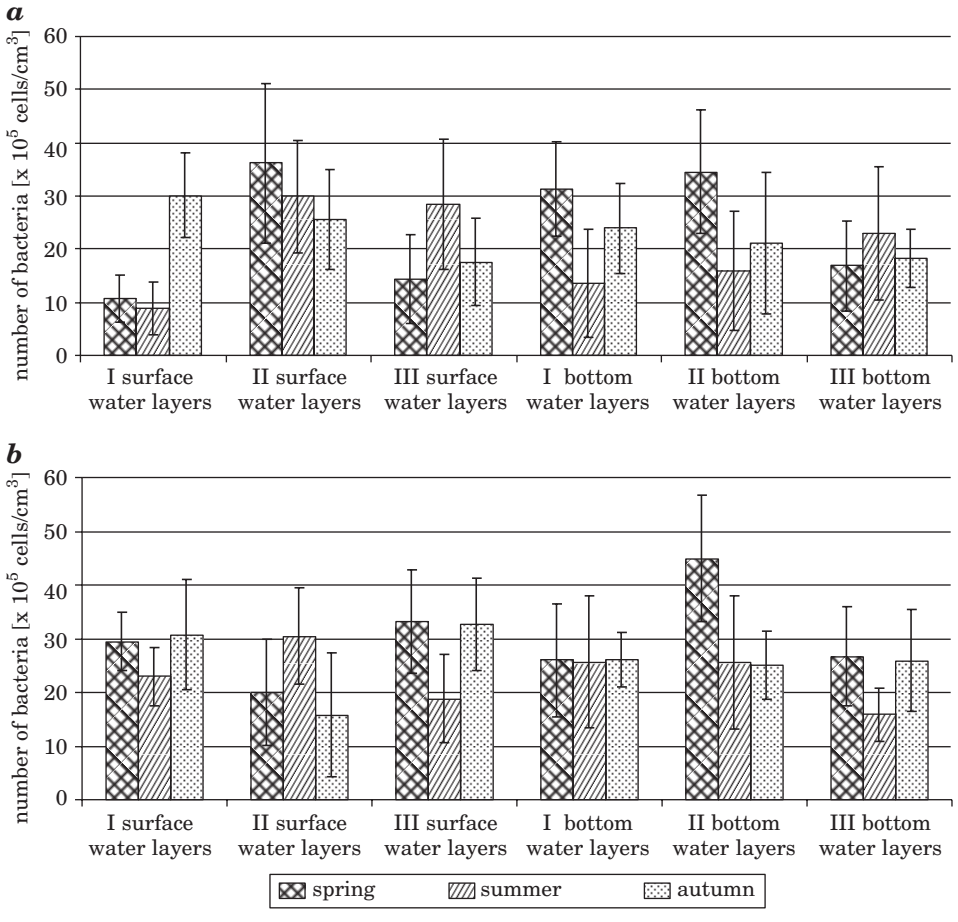

Fig. 1. Total number of planktonic bacteria (TNB): *a* – in water of the Nawionek Lake, *b* – in water of the Piecki Lake

The results of the investigation into the number of planktonic bacteria in the studied lakes are presented in Table 2 and Figures 1a and 1b. The total number of planktonic bacteria (TNB) in the surface water layer of Lake Nawionek was the highest in spring at Sampling Site II ($36.22 \cdot 10^5$ cells/cm^3) and the lowest – in summer at Sampling Site I ($8.83 \cdot 10^5$ cells/cm^3). In the bottom water layer the highest number of planktonic bacteria was observed in spring at Sampling Site II ($34.55 \cdot 10^5$ cells/cm^3), and the lowest – in summer at Sampling Site I ($13{,}54 \cdot 10^5$ cells/cm^3).

Similarly to Lake Nawionek, the total number of planktonic bacteria in the surface layer if Lake Piecki reached its maximum in spring at Sampling Site III ($33.15 \cdot 10^5$ cells/cm^3) and the minimum – in autumn at Sampling Site II ($15.77 \cdot 10^5$ cells/cm^3). In the bottom water layer the highest number of planktonic bacteria was also noted in spring at Sampling Site II ($44.93 \cdot 10^5$ cells/cm^3) while the lowest number was noted in summer at Sampling Site III ($15.83 \cdot 10^5$ cells/cm^3).

Comparable results were obtained by MARCHLIK et al. (2003) during his studies on the number of planktonic bacteria in lobelia lakes in the Tuchola Forest, and ranged between $6.0 \cdot 10^5$ and $36.0 \cdot 10^5$ cells/cm^3. Much higher total counts of planktonic bacteria were recorded by KALWASIŃSKA (2003) in eutrophic Lake Chełmżyńskie, where they varied from $1 \cdot 10^5$ to $34.90 \cdot 10^7$ cells/cm^3. The above results support the thesis that the degree of lake trophicity contributes greatly to the number of bacteria. As stated by PALUCH (1973) and CHRÓST and SIUDA (2006), the number of planktonic bacteria is much lower in oligotrophic lakes than in eutrophic lakes. According to DONDERSKI (1983) minor differences in vertical distribution of bacterial population may be related to good mixing of water masses resulting from shallow depths of the studied lakes.

A majority of bacteria inhabiting lakes are heterotrophic (RHEINHEIMER 1987). In hydrobiological studies the determining of the number of heterotrophic bacteria is considered extremely significant for recognising lake trophicity, the dynamics of changes, and the contamination level (ŚWIĄTECKI 1997). The results concerning the number of heterotrophic bacteria capable of growing at 22°C and 37°C in lakes Nawionek and Piecki are presented in Table 3 and Figures 2a, 2b, 3a and 3b. In the surface water layer of Lake Nawionek the number ranged between $0.03 \cdot 10^2$ and $27.43 \cdot 10^2$ cells/cm^3, and between $3.0 \cdot 10^2$ and $5.73 \cdot 10^2$ cells/cm^3 in the bottom water layer. In the surface water layer of Lake Piecki the number fluctuated from $0.57 \cdot 10^2$ to $24.03 \cdot 10^2$ cells/cm^3, and from $7.33 \cdot 10^2$ to $22{,}23 \cdot 10^2$ cells/cm^3 in the bottom water layer.

Table 3

Number of psychrophilic bacteria (CFU 22°C) and mesophilic bacteria (CFU 37°C) in water of the Nawionek Lake and the Piecki Lake

–	Date of sampling	–	Number of bacteria 10^2 cells/cm^3							
			Site I		Site II		Site III		Average	
			22°C	37°C	22°C	37°C	22°C	37°C	22°C	37°C
Lake Nawionek	Spring 23 IV 2009	surface water layers	3.90 [1.47–10.33]	0.90 [0.14–5.66]	3.90 [1.47–10.33]	0.30 [0.02–4.58]	1.20 [0.23–6.17]	2.40 [0.71–8.09]	3.0	1.20
		bottom water layers	21.0 [13.62–32.38]	156.0 [132.94–183.06]	45.0 [33.44–60.56]	204.0 [185.51–226.49]	8.57 [4.38–16.76]	243.0 [213.76–276.24]	24.86	201.0
	Summer 29 VII 2009	surface water layers	4.20 [1.64–10.76]	6.81 [3.22–14.4]	3.57 [1.29–9.85]	7.56 [3.72–15.44]	9.42 [4.96–17.88]	10.20 [5.51–18.89]	5.73	8.19
		bottom water layers	11.25 [6.25–20.25]	11.79 [6.64–20.94]	10.97 [6.05–19.89]	15.57 [9.43–25.71]	7.89 [3.93–15.85]	16.50 [10.13–26.87]	10.03	14.62
	Autumn 23 IX 2009	surface water layers	4.50 [1.81–11.19]	2.04 [2.55–9.53]	6.04 [2.73–13.35]	1.41 [0.31–6.51]	3.33 [1.17–9.49]	1.44 [0.32–6.56]	4.62	1.63
		bottom water layers	52.63 [39.98–69.28]	68.30 [53.65–86.95]	15.70 [9.53–25.87]	19.95 [12.8–31.1]	13.95 [8.22–23.68]	7.03 [3.36–14.7]	27.43	31.76
Lake Piecki	Spring 23 IV 2009	surface water layers	48.70 [36.6–64.8]	18.0 [11.28–28.72]	10.70 [5.86–19.54]	17.10 [10.59–27.61]	12.70 [7.3–22.1]	8.40 [4.27–16.53]	24.03	14.5
		bottom water layers	45.0 [33.44–60.56]	21.0 [13.62–32.38]	18.60 [11.75–29.45]	23.0 [15.2–34.8]	3.10 [1.05–9.15]	12.40 [7.08–21.72]	22.23	18.8
	Summer 29 VII 2009	surface water layers	12.50 [7.15–21.85]	8.60 [4.40–16.80]	5.40 [2.34–12.46]	6.60 [3.09–2.34]	3.60 [1.37–9.83]	23.10 [15.28–34.92]	7.17	12.77
		bottom water layers	26.90 [18.34–39.46]	25.70 [17.37–38.03]	15.90 [9.68–26.12]	13.70 [8.03–23.37]	9.60 [4.69–17.71]	33.80 [24.0–47.60]	17.47	24.4
	Autumn 23 IX 2009	surface water layers	0.50 [0.05–4.95]	6.70 [3.15–14.25]	0.40 [0.03–4.77]	9.30 [4.88–17.72]	0.80 [0.12–5.48]	0.40 [0.03–4.77]	0.57	5.47
		bottom water layers	8.40 [4.27–16.53]	26.80 [18.26–39.34]	0.80 [0.12–5.48]	27.40 [18.74–40.06]	4.70 [1.93–11.47]	19.40 [12.37–30.43]	7.33	24.53

Explanations: [] – 95% confidence interval

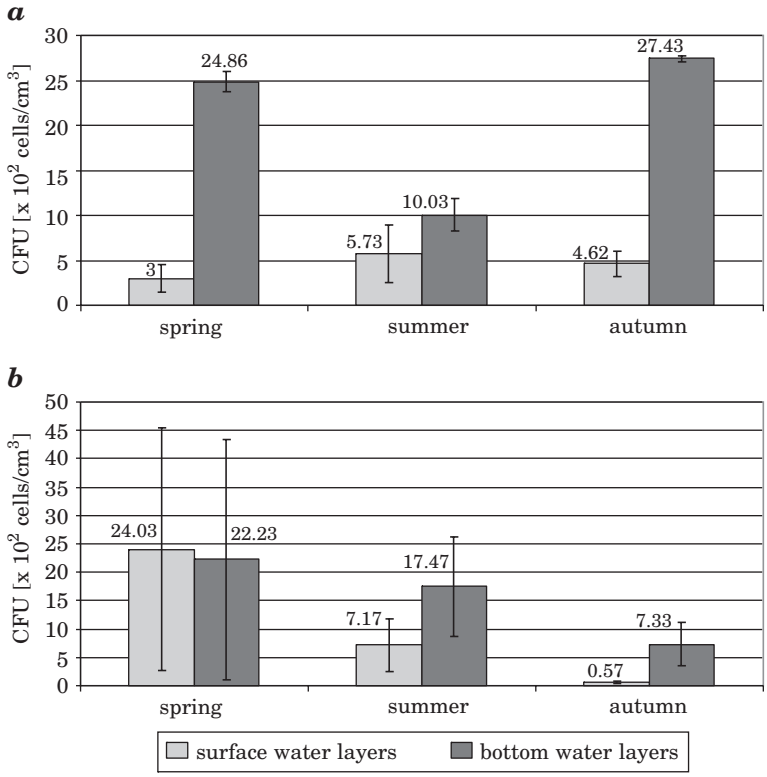

Fig. 2. An average number of psychrophilic bacteria (CFU 22°C): *a* – Nawionek Lake, *b* – Piecki Lake

Similar results were obtained by DANIELAK (1998) during her research into the number of psychrophilic bacteria in lakes Piecki and Nawionek. She noted $3.4 \cdot 10^2$ cells/cm^3 on average in the surface water layer and $6.2 \cdot 10^2$ cells/cm^3 in the bottom water layer in Lake Nawionek. In Lake Piecki, however, considerably smaller counts of psychrophilic bacteria were noted, namely $0.8 \cdot 10^2$ cells/cm^3 in the surface water layer and $6.2 \cdot 10^2$ cells/cm^3 in the bottom water layer. An increase in the number of heterotrophic bacteria observed by the author of this paper in the studied lakes may indicate a higher contamination level. Additionally, the number of heterotrophic bacteria growing at 22°C in both lakes was subject to seasonal fluctuations, which, as stated by FJERDINGSTAD et al. (1975), is a natural phenomenon. According to ŚWIĄTECKI (1997) fluctuations in the number of heterotrophic bacteria are smaller in eutrophic lakes than in oligotrophic lakes. In Lake Nawionek the number of heterotrophic bacteria was always higher in the surface water layer than in the bottom water layer. Moreover, the highest numbers of heterotrophic bacteria

were noted in spring and autumn. According to MUDRYK (2003) the autumn increase is correlated with the decay of hydrobionts.

In the Polish climate heterotrophic bacteria growing at 37°C (mesophilic) found in natural surface waters are allochthonous organisms and are considered contaminants (ŚWIĄTECKI 1997). In both investigated lakes the number of mesophilic bacteria was higher in the bottom water layer than in the surface water layer and was subject to significant seasonal fluctuations. In Lake Nawionek the number of mesophilic bacteria in the surface water layer ranged between $1.2 \cdot 10^2$ and $8.19 \cdot 10^2$ cells/ml (Figure 3a).

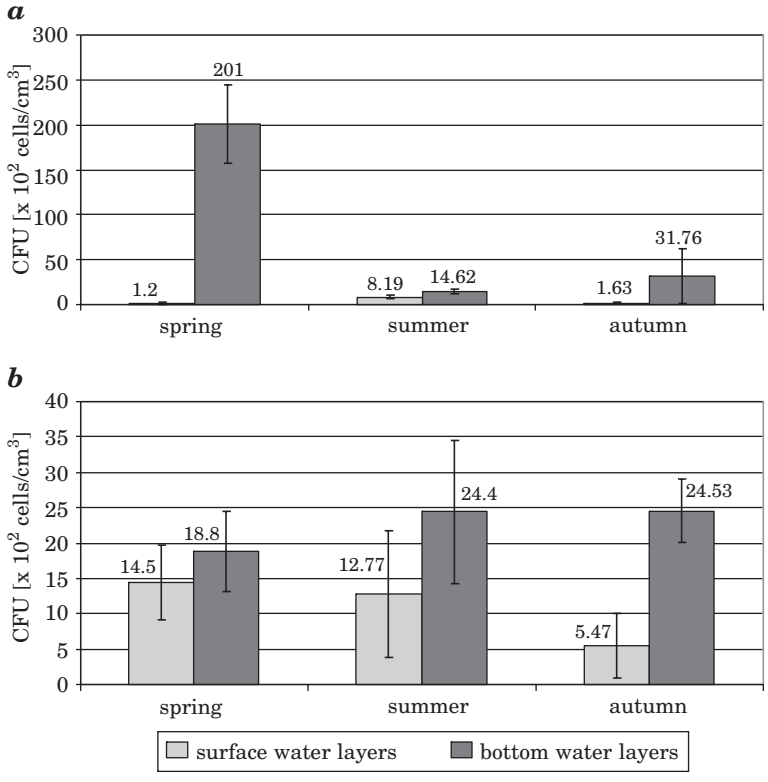

Fig. 3. An average number of mesophilic bacteria (CFU 37°C): *a* – Nawionek Lake, *b* – Piecki Lake

In Lake Piecki the number of mesophilic bacteria in the bottom water layer did not vary significantly and ranged from $18.8 \cdot 10^2$ to $24.53 \cdot 10^2$ cells/cm^3. In the surface water layer the number fluctuated significantly between $5.47 \cdot 10^2$ and $14.5 \cdot 10^2$ cells/cm^3 (Figure 3b); which is probably connected with temporary contamination (mostly in spring). Located in the forest, the lakes

may constitute watering places for animals. Additionally, both lake basins have relatively high and steep slopes, which may facilitate the flow of contaminants into water. The research conducted in the River Brda flowing through the Tuchola Forest demonstrated that the number of mesophilic bacteria was subject to major fluctuations and ranged between $0.9 \cdot 10^2$ and $35.77 \cdot 10^4$ cells/cm^3 (MAŁECKA and DONDERSKI 2006).

The results of the investigation into the morphological structure of heterotrophic psychrophilic bacteria capable of growing at 20°C found in the studied lakes are presented in Figures 4a and 4b. Rods were predominant morphological forms in both lakes and constituted 70% of all bacterial forms in Lake Nawionek and 74% in Lake Piecki. Pleomorphic forms formed the second largest group in both lakes and constituted 4% of all bacterial forms in Nawionek Lake and 11% in Piecki Lake.

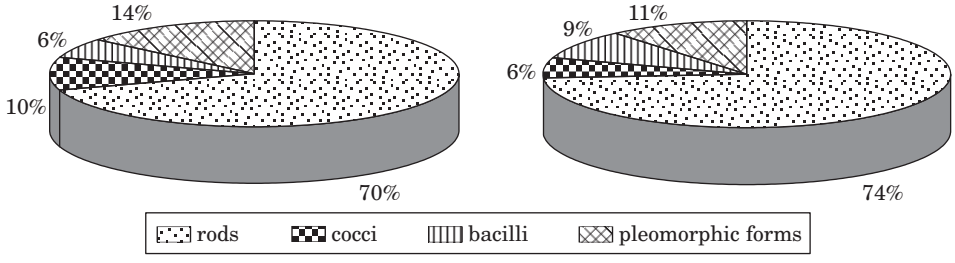

Fig. 4. Occurrence of morphological forms in water: *a* – Nawionek Lake, *b* – Piecki Lake

The percentage of spherical forms and bacilli was similar and equalled 10% and 6% respectively in Lake Nawionek and 6% and 9% in Lake Piecki. According to JÜRGENS et al. (1999) spherical forms are predominant in oligo-mesotrophic lakes. As stated by BILLEN et al. (1990) an increase in lake trophicity leads to an increase in the percentage of rods and bacilli among bacterial morphological forms. It may be concluded that both studied oligotrophic lobelia lakes show changes indicating their increased trophicity. The results of DONDERSKI'S (1983) investigation suggest that a majority of heterotrohic bacteria found in water bodies are Gram-negative and that pleomorphism in this community is a common feature. The predominance of bacilli over other morphological forms was also noted by MAŁECKA and DONDERSKI (2006) and ŚWIĄTECKI (1997).

Ammonifying bacteria constituted the most abundant group among the studied groups distinguished by physiological properties (Figure 5), which complies with the observations of DONDERSKI and STRZELCZYK (1992) and MUDRYK (1994) stating that this bacterial group forms as much as 80% to 90%

of the total number of heterotrophic bacteria in lakes of different trophycity. Ammonifying bacteria are one of the largest microbial groups in aquatic environment (ZO BELL 1946, cited after WALCZAK 2002), which is also confirmed by DANIELAK (1998). Their abundant distribution is directly connected to the prevalence of amino acids in water.

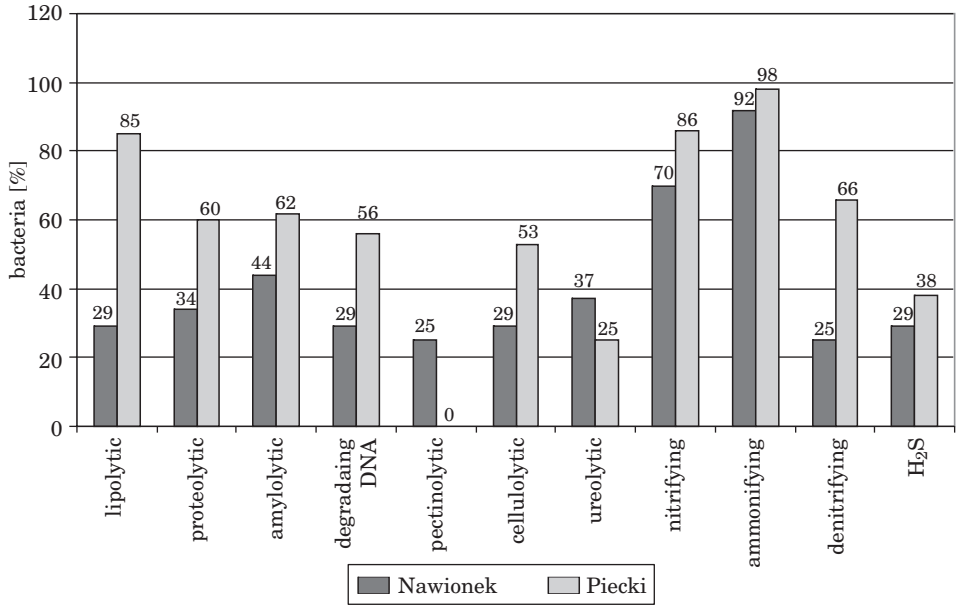

Fig. 5. Occurrence of physiological groups among bacteria isolated from water: *a* – Nawionek Lake, *b* – Piecki Lake

The second largest group was formed by heterotrophic nitrifying bacteria, which require large amounts of ammonia and oxygen. They constituted 86% of the total number of heterotrophic bacteria in Lake Piecki and 70% in Lake Nawionek. Remarkably, the number of bacteria responsible for the oxidation NH_4^+ to NO_2^- is considered to be a very sensitive indicator of water quality (ZMYSŁOWSKA 1987, cit. MAŁECKA-ADAMOWICZ 2006). According to ŚWIĄTECKI (1994) low number of nitrifying bacteria indicates a high level of water contamination.

In Lake Piecki more than 50% of the total number of heterotrophic bacteria were bacteria which hydrolyse fat, proteins, starch, DNA, cellulose and which perform the denitrification process. The second largest group (over 20%) was constituted by bacteria which decompose urea and produce hydrogen sulphide from organic combinations. No bacteria hydrolysing pectin were observed

in the studied lakes. Generally, the activity of particular physiological groups was lower in Lake Nawionek than in Lake Piecki.

Conclusions

1. The total number of planktonic bacteria was higher in Lake Piecki than in Lake Nawionek.
2. Psychrophilic bacteria were more abundant than mesophilic bacteria.
3. Higher numbers of bacteria were observed in the surface water layer than in the bottom water layer.
4. The highest numbers of bacteria were noted in spring while the lowest numbers were noted in summer.
5. Rods were prevailing morphological forms in both lakes, followed by pleomorphic forms.
6. Ammonifying bacteria were the most common physiological groups in both lakes, which indicates progressive water mineralisation.
7. Higher physiological activity of bacteria was observed in Lake Piecki than in Lake Nawionek.

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THE EFFECTIVENESS OF SELECTED HORMONAL PREPARATIONS IN STIMULATING THE SPERMATION OF THE CHUB *LEUCISCUS CEPHALUS* (L.)

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K e y w o r d s: *Leuciscus cephalus* (L.), milt, spermatozoa, Ovopel, Ovaprim, LHRHa, CPE.

A b s t r a c t

We analysed the effects of [(D-Ala^6, Pro^{9}NEt)-mGnRH+metoclopramide] i.e. Ovopel at the dose of 0.5 granule kg^{-1} of body weight (b.w.), [(D-Arg^6, Pro^{9}NEt)-sGnRH+domperidone] i.e. Ovaprim at the dose of 0.25 ml kg^{-1} b.w., luteinizing hormone releasing hormone (LHRHa) at the dose of 50 μg kg^{-1} b.w. and carp pituitary extract (CPE) at the dose of 2 mg kg^{-1} b.w. on the total volume of milt (TVM, ml), volume of milt per kg of body weight (VOM, kg^{-1} b.w.), spermatozoa motility [%], osmolality of seminal plasma (mOsm kg^{-1}), spermatozoa concentration ($\times 10^9$ ml^{-1}), total sperm produced (TSP, $\times 10^9$) and total number of spermatozoa per kg of b.w. (TNS, $\times 10^9$ kg^{-1} b.w.) in the chub *Leuciscus cephalus* (L.). Milt was collected 24 hours after injection from individuals (n=9) belonging to each group. The control group (control, n=9) consisted of the males which were given 0.9% NaCl at 0.5 ml kg^{-1} b.w. The highest values of TVM and VOM were observed after Ovaprim (5.88±1.62 ml and 19.24±3.56 ml kg^{-1} b.w. respectively) and CPE stimulation (5.39±1.19 ml and 19.61±3.32 ml kg^{-1} b.w. respectively). The lowest TVM and VOM values were observed after LHRHa injection (2.46±0.89 ml and 8.95±3.13 ml kg^{-1} b.w. respectively) and those values were statistically different from values recorded after Ovopel (P<0.05), Ovaprim (P<0.001) and CPE (P<0.001). A significant increase in spermatozoa motility (P<0.05) was observed following hormonal stimulation as compared to the control, regardless of the type of hormonal agent applied. The values of osmolality were similar in all the groups with no significant differences between them (288–300 mOsm kg^{-1}, P>0.05). The highest spermatozoa concentrations in the control were $10.26\pm1.70 \cdot 10^9$ ml^{-1}, and the lowest values were $5.47\pm0.99 \cdot 10^9$ ml^{-1}, P<0.001, observed after Ovaprim stimulation. The highest TSP and TNS values were recorded after CPE injection ($42.84\pm11.72 \cdot 10^9$ and $160.4\pm65.67 \cdot 10^9$ kg^{-1} b.w. respectively), while the lowest were obtained following stimulation with LHRHa ($23.57\pm8.56 \cdot 10^9$

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and 85.72±30.56 · 10^9 kg^{-1} b.w. respectively). Those differences were statistically significant ($P<0.05$). Considering the large volume of milt (TVM and VOM) and the large number of spermatozoa (TSP and TNS), chub spermation can be successfully stimulated with Ovaprim or CPE injections.

EFEKTYWNOŚĆ WYBRANYCH PREPARATÓW HORMONALNYCH W STYMULOWANIU SPERMACJI KLENIA *LEUCISCUS CEPHALUS* (L.)

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S ł o w a k l u c z o w e: *Leuciscus cephalus* (L.), mlecz, plemniki, Ovopel, Ovaprim, LHRHa, CPE.

A b s t r a k t

Przeanalizowano wpływ Ovopelu [(D-Ala^6, Pro^{9}NEt)-mGnRH+metoklopramid] w dawce 0.5 granulki kg^{-1} m.c., Ovaprimu [(D-Arg^6, Pro^{9}NEt)-sGnRH+domperidon] w dawce 0.25 ml kg^{-1} m.c., analogu gonadoliberyny (LHRHa) w dawce 50 μg kg^{-1} m.c. oraz ekstraktu przysadki mózgowej karpia (CPE) w dawce 2 mg kg^{-1} m.c. na całkowitą objętość pozyskanego mlecza (TVM, ml), objętość w przeliczeniu na kg masy ciała (VOM, kg^{-1} m.c.), ruchliwość plemników [%], osmolalność plazmy nasienia (mOsm kg^{-1}), koncentrację plemników (×10^9 ml^{-1}), całkowitą ilość plemników (TSP, ×10^9) oraz ilość plemników w przeliczeniu na kg masy ciała klenia *Leuciscus cephalus* (L.). Mlecz pobierano po 24 godz. od wykonania iniekcji od każdego osobnika ($n=9$) z każdej grupy. Grupę kontrolną (kontrola, $n=9$) stanowiły samce, którym aplikowano 0.9% NaCl w dawce 0.5 ml kg^{-1}. Najwyższe wartości TVM i VOM stwierdzono po podaniu samcom Ovaprimu (odpowiednio: 5.88±1.62 ml i 19.24±3.56 ml kg^{-1}m.c.) oraz CPE (odpowiednio: 5.39±1.19 ml i 19.61±3.32 ml kg^{-1}m.c.), natomiast najniższe po stymulacji LHRHa (odpowiednio: 2.46±0.89 ml i 8.95±3.13 ml kg^{-1}m.c). Stwierdzone wartości były istotnie niższe od wartości po stymulacji Ovopelem ($P<0.05$), Ovaprimem ($P<0.001$) i CPE ($P<0.001$). Po stymulacji hormonalnej zaobserwowano istotny wzrost odsetka plemników ruchliwych ($P<0.05$) w porównaniu z kontrolą i to bez względu na zastosowany środek hormonalny. Wartości osmolalności kształtowały się w każdej grupie na zbliżonym poziomie, nie różniąc się istotnie (288–300 mOsm kg^{-1}, $P>0.05$). Najwyższą koncentrację plemników stwierdzono w kontroli (10.26±1.70 · 10^9 ml^{-1}), natomiast najniższą po stymulacji Ovaprimem. Stwierdzone wartości 5.47±0.99 · 10^9 ml^{-1} były istotnie najniższe ($P<0.001$). Najwyższe wartości TSP i VOM stwierdzono po iniekcji CPE (odpowiednio: 42.84±11.72 · 10^9 i 160.4±65.67 · 10^9 kg^{-1}m.c.), natomiast najniższe po stymulacji LHRHa (odpowiednio: 23.57±8.56 · 10^9 i 85.72±30.56 · 10^9), a zaobserwowane różnice były istotne ($P<0.05$). Biorąc pod uwagę objętość pozyskanego mlecza (TVM i VOM) oraz ilość wyprodukowanych plemników (TSP i TNS), do indukowania spermacji klenia można polecać zarówno Ovoprim, jak i CPE.

Introduction

Biotechnology of rheophilic cyprinids from the genus *Leuciscus* has been successfully elucidated, and the ide *Leuciscus idus* (L.) is the best described

species, (KUCHARCZYK et al. 2008, TARGOŃSKA et al. 2008, JAMRÓZ et al. 2008, KUPREN et al. 2008, KREJSZEFF et al. 2009). Studies up to now have shown that the ide milt can be obtained both during the spawning period and outside it (KUCHARCZYK et al. 1999, 2000), with temperature being an important factor in out-of-season reproduction (KUCHARCZYK et al. 2008). Male spermation can be induced by gonadotropins, i.e. carp pituitary extract (CPE) or human chorionic gonadotropin (hCG), but much better results in terms of spermatozoa motility are achieved by treating males with Ovopel [(D-Ala^6,Pro^9-NEt)-mGnRH+metoclopramide] or Ovaprim [(D-Arg^9,Pro^9--NEt)-sGnRH+domperidon], (KUCHARCZYK et al. 1999, JAMRÓZ et al. 2008). The latest reports have also shown that the volume of milt and the amount and quality of spermatozoa depend on the length of period after the hormonal stimulation; the optimum for the ide has been found to be equal to 84 hours (CEJKO et al. 2010a). It is also noteworthy that water temperature does not need to be increased from 10°C to 12°C or 14°C after the hormonal stimulation of ide, because this does not result in any significant increase in the volume of milt, spermatozoa concentration, total number of spermatozoa and spermatozoa motility (CEJKO et al. 2010b).

Much less information about the biotechnology of reproduction can be found for the other species in genus *Leuciscus*, including dace *Leuciscus leuciscus* (L.) (ŻARSKI et al. 2009) and chub *Leuciscus cephalus* (L.) (KREJSZEFF et al. 2008, 2010). The advances in biotechnology is important for aquaculture, especially with respect to economically valuable species such as carp *Cyprinus carpio* L., tench *Tinca tinca* (L.) and species which act as water purity bioindicators, i.e. rheophilic species. Rheophilic species are a necessary feature of lotic ecosystems and they account for a considerable portion of food of preying fish. Concerning increasing degradation of the natural environment and decrease in their range, efforts aimed at developing a biotechnique for rheophilic fish reproduction are highly justifiable (TARGOŃSKA et al. 2010).

The chub is a ubiquitous species living in European rivers, most often rapids with gravelly bottoms (ECONOMOU et al. 1991, KOÇ et al. 2007, BOLLAND et al. 2007). Chub reach sexual maturity in the third or four year of its life and the spawning season in the waters of Central Europe starts from April to June (TADAJEWSKA 2000). Although a valuable fishing trophy, it does not play any important economic role due to its watery and bony meat, but its other features include high adaptability and such biological potential can be used to increase biodiversity with a resultant improvement of river conditions by stocking waters with fry (ARLINGHAUS and WOLTER 2003). Recently chub has disappeared from many habitats and has become an endangered species.

Methods of obtaining mature gametes under controlled conditions were developed in the 1930s; however, they involved only hypophysation and

administering gonadotropins to fish, which were not always effective (ZOHAR and MYLONAS 2001). Currently, there are a number of natural or synthetic hormonal agents available which can be given to fish to induce ovulation or spermation under controlled conditions. However, the effectiveness of commercial preparations varies (KREJSZEFF et al. 2008, 2010, TARGOŃSKA et al. 2010), which makes it necessary to experiment on them in the reproduction of rheophilic fish (CEJKO et al. 2008, JAMRÓZ et al. 2008, ŻARSKI et al. 2009). The tactics of chub reproduction are not certain yet so the aim of this study was to determine the effect of various hormonal preparations i.e. Ovopel, Ovaprim, luteinizing hormone releasing hormone (LHRHa) and CPE, on the volume of obtained milt, spermatozoa motility, seminal plasma osmotic pressure and the quantity and quality of spermatozoa obtained from the chub.

Materials and Methods

Origin of the fish, hormonal stimulation and manipulations with spawners

The breeding school of male chub was acquired from the Knieja Fishery Farm in south-west Poland. In mid-May 2008 the fish were caught from a pond and transported to the hatchery at the Department of Lake and River Fisheries University Warmia and Mazury in Olsztyn. In the hatchery the males were put into a tank (1000 dm^3) at a temperature of 16°C (14L: 10D) to acclimatise them to the thermal conditions. The fish were three-years-old and their mean body weight was 283 gram. After five days of adaptation the males were labelled and divided into five experimental group. After this the males were hormonally stimulated by an intraperitoneal injection with selected hormonal preparations i.e. Ovopel (Unic-trade, Hungary) at 1/2 granule kg^{-1} of body weight (b.w.), Ovaprim (Syndel, Canada) at 0.25 ml kg^{-1} b.w., LHRHa (Argent, USA) at 50 μg kg^{-1} b.w., and CPE (Argent, USA) at 2 mg kg^{-1} b.w. The males in the control group (Control) were given 0.9% NaCl at 0.5 ml kg^{-1}. Dosages of hormonal preparations determined the smallest and most effective dose used in hormonal stimulation of male chub.

Milt collection

Milt was taken from nine individuals ($n=9$) from each group 24 hours after hormonal stimulation through delicately massaging of the abdominal regions avoiding contamination of the samples with urine, faeces or blood. The males

were weighed before milt was taken from them and the amount of milt was measured with sterile syringes calibrated every 0.01 ml. To carry out the manipulations and milt collection the spawners were anaesthetised with 2-phenoxyethanol at 0.5 ml kg^{-1} b.w. (Sigma Aldrich, St. Louis, MO). The milt samples were transported on ice (±4°C) to the Department of Gamete and Embryo Biology (DG&EB), Institute of Animal Reproduction and Food Research Polish Academy of Sciences in Olsztyn.

Determination of the milt volume and number of spermatozoa

The total volume of milt expressed in ml (TVM, ml) was measured directly after it was collected, and the value, together with the males' body weight, was subsequently used to calculate the volume of milt per kg of body weight (VOM, kg^{-1} b.w.). Spermatozoa concentration ($\times 10^9$ ml^{-1}) was determined by the spectrophotometric method (CIERESZKO and DĄBROWSKI 1993) after dilution of the milt ×2000 with 0.7% NaCl (Sigma Aldrich, St. Louis, MO). Absorbance of the samples was measured with a Beckman DU-640 spectrophotometer (Analytical Instruments, LLS, USA) at α=530 nm. The concentration values were substituted in the formula for the standard curve, made earlier for the chub with the cytometric method (BIELAŃSKI 1979). The TVM and concentration were then used to calculate the total sperm production by the males (TSP, $\times 10^9$). The latter TSP and the males' b. w. were used to calculate the number of spermatozoa per kg of the b.w. of fish (TNS, $\times 10^9$ kg^{-1} b.w.).

Determination of spermatozoa motility and seminal plasma osmotic pressure

Immediately after the milt was brought to the DG&EB, the spermatozoa motility was determined by the subjective method for each individual with the use of an optical microscope and with magnification of 400×. Spermatozoa were activated by mixing 1 µl of milt with 30 µl of activation solution containing 86 mM NaCl (Sigma Aldrich, St. Louis, MO) with 0.5% bovine serum albumin (Sigma-Aldrich, St. Louis, MO). Motility was determined immediately after activation by one observer and the value of motility spermatozoa was subjectively estimated in per cent (CEJKO et al. 2010b). Seminal plasma was obtained by centrifuging samples of milt (10 000 *g*) for 10 minutes; this was followed by decantation of the plasma and determination of its osmolality (mOsm kg^{-1}). The operation was done with a Vapor Pressure Osmometer 5520 (WESCOR, Logan, UT, USA).

Statistical analysis

The results were analysed by means of the arithmetic average and standard deviation (±SD). The significance of differences between the groups for an analysed characteristic was tested by single-factorial analysis of variance (ANOVA) and Tukey's post-hoc test ($P<0.05$). All the analyses were done with GraphPad Prism software (GraphPad Software Inc., USA).

Results

The highest values of TVM and VOM were found in the group of males stimulated with Ovaprim (5.88±1.62 ml and 19.24±3.56 ml kg^{-1} b.w. respectively) and CPE (5.39±1.19 ml and 19.61±3.42 ml kg^{-1} b.w. respectively), whereas the lowest ones were for the males given LHRHa (2.46±0.89 ml and 8.95± ±3.13 ml kg^{-1} b.w. respectively) and in the control (2.88±1.23 ml and 10.80±4.93 ml kg^{-1} b.w. respectively). Significant differences in TVM values were observed between control and the males stimulated with Ovaprim ($P<0.001$) and also with CPE ($P<0.01$), and between the group stimulated with LHRHa, and the group which had been given Ovopel ($P<0.05$), Ovaprim ($P<0.001$) and CPE ($P<0.001$) (Figure 1a). The significant differences in VOM values were recorded between control and Ovaprim ($P<0.001$) or CPE treated groups ($P<0.001$), and also between the group stimulated with LHRHa and the groups which had been given Ovopel ($P<0.05$), Ovaprim ($P<0.001$) and CPE ($P<0.001$) (Figure 1b). The hormonal stimulation resulted in a significant increase in spermatozoa motility from 36% in control to 50–60% in the other groups (Figure 1c). Osmolality of seminal plasma ranged from 287.5 (LHRHa) to 299.8 (control) mOsm kg^{-1} with no significant differences between the groups ($P>0.05$).

The highest spermatozoa concentration was found in the control ($10.26±1.70 \cdot 10^9$ ml^{-1}), whereas the lowest was found following the administration of Ovaprim ($5.47±0.99 \cdot 10^9$ ml^{-1}). The concentrations were similar in the other groups i.e. after CPE, Ovopel and LHRHa administration. Significant differences were observed between Ovaprim and, control ($P<0.001$) and LHRHa ($P<0.01$), (Figure 1d). The TSP and TNS values found after giving Ovopel ($36.38±15.33 \cdot 10^9$ and $124.9±50.6 \cdot 10^9$ kg^{-1} b.w. respectively) and Ovaprim ($33.08±13.21 \cdot 10^9$ and $108.79±31.6 \cdot 10^9$ kg^{-1} b.w. respectively) were similar with no significant difference between them ($P>0.05$). Much more spermatozoa was found following stimulation with CPE ($42.84±11.72 \cdot 10^9$ and $160.4±65.6 \cdot 10^9$ kg^{-1} b.w. respectively), and the values differed significantly from those found after LHRHa administration ($23.57±8.56 \cdot 10^9$ and $85.7±30.6 \cdot 10^9$ kg^{-1} b.w. respectively), ($P<0.05$), (Figure 1e, f).

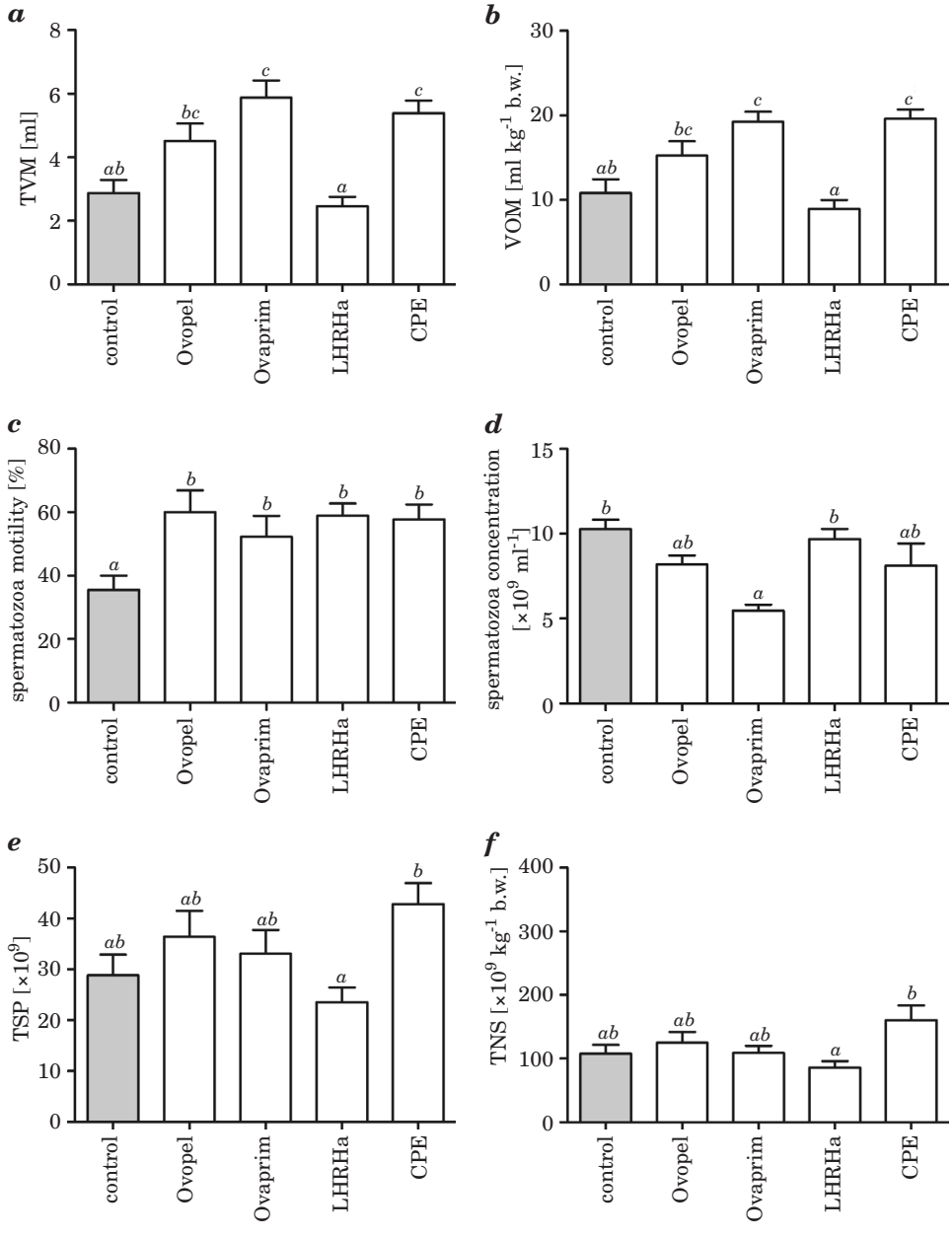

Fig. 1. The main milt parameters of the chub *Leuciscus cephalus* (L.) obtained from the control group (control) and after hormonal stimulation with Ovopel [(D-Ala6, Pro9NEt)-mGnRH+metoclopramide], Ovaprim [(D-Arg6, Pro9NEt)-sGnRH+domperidone], LHRHa and CPE, (n=9): *a* – total volume of milt (TVM); *b* – volume of milt per kg of body weight (VOM); *c* – spermatozoa motility; *d* – spermatozoa concentration; *e* – total sperm production (TSP); *f* – total number of spermatozoa per kg of body weight (TNS). Boxes labeled with different superscripts are statistically different from each other

Discussion

Owing to hormonal stimulation, the volume of milt obtained from cyprinid fish significantly increases as compared to the control and such an increase is observed soon after treatment (KUCHARCZYK et al. 2005, CAILLE et al. 2006, CEJKO et al. 2010a). Considering the hormonally stimulated chub, it may be claimed that the highest milt volume (TVM) is obtained in fish following stimulation with Ovaprim and CPE, and the lowest after the administration of LHRHa. The results are consistent with the findings of the study conducted by KREJSZEFF et al. (2008), in which the highest VOM was found in the males which were given CPE (3.7±0.3 ml kg^{-1} b.w.), whereas the lowest was in the group stimulated with hCG (2.5±0.4 ml kg^{-1} b.w.). The lowest TVM and VOM values were also found in the dace after hCG stimulation (0.41±0.16 ml and 4.78±2.06 ml kg^{-1} b.w.) compared to fish stimulated with Ovaprim (1.01±0.46 ml and 11.50±4.43 ml kg^{-1} b.w.) (CEJKO, unpublished data). The absence of any increase in the volume of milt following stimulation of spermation with LHRHa as compared with control shows that the application of the compounds without dopamine receptor antagonist is ineffective due to a strong mechanism which inhibits the secretion of gonadoliberins from the hypothalamus. This situation is typical of cyprinids (MIKOŁAJCZYK et al. 2004, YARON 1995), catfish (SILVERSTEIN et al. 1999) and mullets (GLUBOKOV et al. 1994). In our research LHRHa stimulation was also ineffective in comparision to Ovaprim injection which confirms the uselessness of LHRHa in the stimulation of chub male spermation.

The determination of the biological value of milt is usually based on spermatozoa motility because it is generally accepted that this feature enables one to forecast the effect of spawn fertilisation (LAHNSTEINER et al. 1998, RURANGWA et al. 2001). Spermatozoa do not acquire the ability to move until they reach the spermatic ducts, where their ultimate maturation takes place (SCHULZ et al. 2010). Gonadotropins regulate this process by stimulating Leydig cells to synthesise and secrete 11-ketotestosterone, the main androgen which affects the changes in the male reproductive system. Therefore, hormone administration speeds up the maturation of spermatozoa in the spermatic duct, synchronises this process and in some cases has a positive effect on spermatozoa motility (CLEARWATER and CRIM 1998). Our results show that chub stimulation with Ovopel, Ovaprim, LHRHa and CPE significantly increases spermatozoa motility as compared to Control, but – like in the study by KREJSZEFF et al. (2008) – the motility of spermatozoa in the groups following hormonal stimulation did not differ significantly.

Hormonal stimulation results in hydration which is a necessary step in the process of spermatozoa maturation in testicles and their release from the

spermatic duct. It also facilitates milt collection when the fertilization process occurs in controlled conditions (VERMEIRSSEN et al. 2004). Hydrations resulted in a decrease of spermatozoa concentrations, and simultaneously, in an increased amount of spermatozoa which manifests itself in TSP values. We reported those situations in the males stimulated with Ovaprim and Ovopel compared to control and LHRHa. Chub hypophysation (stimulation with CPE) is not likely to bring about milt hydration, although it results in the highest TSP values. Probably it was as a consequence of a high LH concentration which is responsible for secreting androgens by Leydig cells. Androgens, including 11-ketotestosteron, are responsible for spermatogenesis, whereas MIS (17α20β-dihydroxy-4-pregnen-3-one), which is synthesised in spermatozoa, activates specific enzymes and increases seminal plasma pH and brings about morphological changes in spermatozoa. Consequently, it makes them fully mature with full abilities to move and fertilise (MIURA and MIURA 2003).

To analyse the TVM and VOM values Ovaprim and CPE were the best preparations for stimulating the milt volume of chub. According to scarce reports, Ovaprim, although used less frequently in fish reproduction, is more effective in some species than the commonly used Ovopel or CPE. The treatment of asp *Aspius aspius* (L.) with Ovaprim resulted in a significant increase in spermatozoa motility and concentration as compared to the group stimulated with Ovopel (CEJKO et al. 2008). The TVM, concentration and TSP obtained for the smelt *Osmerus eperlanus* (L.) were also higher than in the fish stimulated with Ovopel, although the differences were not significant (KRÓL et al. 2009). The differences may stem from the fact that the salmon GnRH analogue, which is a component of Ovaprim, is much more similar in structure to the LHRH naturally occurring in cyprinids (PODHOREC and KOURIL 2009, TARGOŃSKA et al. 2010). Since it is necessary to apply dopamine antagonists, it cannot be ruled out that both preparations contained those substances.

It can be concluded that gonadotropins, i.e. CPE, similary like Ovaprim are effective in stimulating spermation in the chub. However, in light of the facts that during the hypophysation procedure a foreign protein can by introduced into the recipient body and that there is no standarization of CPE (YARON 1995), Ovaprim can be recommended in the biotechnology of chub reproduction, especially since the results of earlier studies on ovulation and spermation induction in rheophilic fish with Ovaprim (CEJKO et al. 2008, JAMRÓZ et al. 2008, ŻARSKI et al. 2009, TARGOŃSKA et al. 2010) show that this propagation gives the best results in controlled reproduction of rheophilic cyprinids.

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ARTIFICIAL REPRODUCTION OF THE IDE *LEUCISCUS IDUS* (L.) BRED UNDER CONTROLLED CONDITIONS

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K e y w o r d s: ide, hormonal stimulation, GnRHa, farm fish school.

A b s t r a c t

The aim of the study was to compare the effectiveness of hormonal stimulation of the female ide (*Leuciscus idus* L.), following the application of two commercial preparations concerning different GnRHa combined with dopamine inhibitors: Ovopel and Ovaprim. Ide spawners were bred under controlled conditions. The examined parameters included the percentage of ovulating females, time of ovulation and the rate of embryo survival to the eyed-egg stage. The growth and survival rates for larvae produced by farm and wild fish were compared. Stimulation with Ovopel resulted in a shorter latency time (36), whereas stimulation with Ovaprim resulted in a higher percentage of live embryos at the eyed-egg stage (79.7 %). Eggs was obtained from 20% of females in the control group, whereas in treated groups it was at level 90–100%. No differences were found between the growth rate of the ide larvae produced by the farm fish and that obtained from wild fish.

SZTUCZNY ROZRÓD JAZIA *LEUCISCUS IDUS* (L.) WYHODOWANEGO W WARUNKACH KONTROLOWANYCH

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S ł o w a k l u c z o w e: jaź, stymulacja hormonalna, GnRHa, stado hodowlane.

A b s t r a k t

Celem pracy było porównanie efektywności stymulacji hormonalnej samic jazia (*Leuciscus idus* L.) po zastosowaniu dwóch różnych preparatów zawierających analog GnRH i inhibitor dopaminy (Ovopel i Ovaprim). Eksperyment przeprowadzono w warunkach kontrolowanych. W trakcie

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jego trwania notowano odsetek owulujących samic, czas owulacji oraz przeżywalność embrionów do stadium zaoczkowania. Porównano również tempo wzrostu i przeżywalność larw uzyskanych od ryb hodowlanych i ryb dzikich. Stymulacja Ovopelem poskutkowała krótszym okresem wystąpienia owulacji u samic (36), natomiast Ovaprimem – wyższym odsetkiem żywych embrionów w stadium zaoczkowania (79,7%). Od 20% samic z grupy kontrolnej uzyskano także ikrę, podczas gdy od ryb doświadczalnych od 90–100%. Nie stwierdzono różnic w tempie wzrostu larw jazia pozyskanych ze stada hodowlanego i od ryb dzikich.

Introduction

For many years, artificial reproduction of non-commercial cyprinids was outside the scope of interest of breeders and scientists (KREJSZEFF et al. 2008, ŻARSKI et al. 2009, TARGOŃSKA et al. 2010). However, an increase in the body of research into different aspects of reproduction biotechnique, such as obtaining gametes, their quality and effects of incubation (KUCHARCZYK et al. 1997a, b, c, 2005, SZABO et al. 2002, TARGOŃSKA et al. 2010, ŻARSKI et al. 2011), sperm biology (GLOGOWSKI et al. 1997, 1999, KOWALSKI et al. 2003, 2004, CEJKO et al. 2010), genomic manipulations (KUCHARCZYK et al. 1997d) and sperm cryopreservation (BABIAK et al. 1998) has been observed in recent years. The results of reproduction are greatly affected by the origin of spawners and their breeding conditions (KREJSZEFF et al. 2009, 2010). Frequently, the origin of fish, spawner rearing technique and the degree of their domestication affect the economic profitability of reproduction (HAKUĆ-BŁAŻOWSKA et al. 2009, 2010).

Reproduction of freshwater fish is often impossible without any hormonal stimulation and this applies mainly to commercial fish, such as the carp, *Cyprinus carpio* L. (BRZUSKA 2000, 2005, KUCHARCZYK et al. 2008), the tench *Tinca tinca* L. (MAMCARZ et al. 2006, KUJAWA et al. 2011) or goldfish *Carassius auratus* L. (TARGOŃSKA, KUCHARCZYK 2011) and rheophilic cyprinids, such as the asp *Aspius aspius* L. (TARGOŃSKA et al. 2010), the ide *Leuciscus idus* L., the dace *Leuciscus Leuciscus* L. (ŻARSKI et al. 2009), the nase *Chondrostoma nasus* L. (SZABO et al. 2002) and the chub *Leuciscus cephalus* L. (KREJSZEFF et al. 2008). Hormonal stimulation is not necessary to start reproduction in percidae and the burbot *Lota lota* L., but it positively affects spawning synchronisation and increases the percentage of fish which start reproduction (KUCHARCZYK et al. 1996, 1998, SZCZERBOWSKI et al. 2009, ŻARSKI et al. 2010). Only in some domesticated fish is it possible to obtain gametes without hormonal stimulation (KREJSZEFF et al. 2009, KUCHARCZYK et al. 2010). Examination of a hormonal agent for common use in fish reproduction takes into account its effect on quantitative parameters (the number of fish ready to spawn, working and relative fertility, volume of milt) as well as qualitative ones (post-spawn

mortality) for spawners and its products (spermatozoa motility, embryo survival rate). Convenience of use and simplicity of the procedure are also important. So, there is need a possibility to prepare new spawning protocols for domesticated stock of fish, especially when the fish stock was a 'live gene bank'. There is no data about artificial reproduction of ide bred under controlled conditions. Such stimulation in fish can be effected at the level of the hypothalamus, the pituitary gland or gonads. The method of reproduction used initially was based on introducing exogenous gonadotropins to the body (YARON 1995). Spawning success was frequently affected by the quality of pituitary homogenate and concentration of the hormones contained in it and, consequently, by the choice of the dose for injection. The necessity, both economic and practical, to improve the effectiveness of fish reproduction has forced breeders to seek more effective methods. Stimulation has been effected with human chorionic gonadotropin hCG, and synthetic analogues of GnRH (PETER 1993, KUCHARCZYK et al. 1996, 1998). However, due to the fact that secreting gonadotropin by the pituitary gland may be inhibited by dopamine in many fish species (PETER and YU 1997, MYLONAS, ZOHAR 2001), it has become necessary to administer dopamine antagonists (metoclopramide or domperidone) with the hormone (HORVATH et al. 1997, YARON et al. 2009), which has greatly complicated the injections, especially on a mass scale. It was a breakthrough in controlled reproduction when preparations acting on endogenous gonadotropins with the ready mixtures of GnRH analogues (mammalian or piscine) and dopamine antagonists appeared on the market.

The aim of this study was to compare the effectiveness of selected hormonal preparations in controlled reproduction of the ide, *Leuciscus idus* (L.) bred under controlled conditions.

Materials and Methods

Spawners breeding

Ide spawners with individual weight ranging from 123 to 170 g (220 fish) were reared from larvae. The spawners' parents (breeding generation F_1) had been brought to the Aquarium Hall of the Department of River and Lake Fisheries, University of Warmia and Mazury, Olsztyn from the Fishing Farm Knieja near Częstochowa, and their reproduction was carried out by the method described by ŻARSKI et al. (2009) with the use of Ovopel at total dose 1.2 pellet kg^{-1}. The larvae produced as a result were reared at the temperature of 25°C and were initially fed with Artemis nauplii followed by granulated feed. When the fish had reached the length of 4 cm, the water temperature was lowered to

14–15°C and maintained until the end of the rearing period. The fish were given mixed feed: granulated trout feed produced by Aller Aqua and frozen chironomids larvae in the proportion of 1:1. The daily feeding dose ranged from 1 to 1.5% of the fish biomass.

Fish handling

Before the experiment started, the water temperature was lowered to 10°C for 14 days. Before being transferred to the tanks, the fish were divided in groups according to their sex. The 1000 dm^3 tanks in which the spawners were placed were fitted out with aerating equipment and devices for controlling water temperature and photoperiod (KUJAWA et al. 1999). The water temperature in the tanks was 10°C on the day when spawners were transferred into it. Hormonal injections were preceded by a several days of adaptation to the breeding conditions. The photoperiod was constant throughout the experiment and equal to 12 h (12 L : 12 D). Oocyte samples were taken from females using a catheter and the maturity stage was determined based on the position of the germinal vesicle according to the 4-degree scale (BRZUSKA 1979):
– stage 1 – the germinal vesicle is in the oocyte centre;
– stage 2 – the germinal vesicle is in less than half the oocyte radius;
– stage 3 – the germinal vesicle is outside half of the oocyte radius;
– stage 4 – the germinal vesicle is on the cell fringes or on the wane (germinal vesicle break-down – GVBD).

Hormonal stimulation

Hormonal stimulation was performed when the oocytes were at 2–2/3 maturity stage. Before the first injection, the fish were labelled using floy-tags and divided into groups according to the hormonal preparation used for stimulation. Fish which were given physiological saline (0.9% NaCl) were used as control. Hormonal stimulation of the fish in the study groups was performed with: Ovopel (Unic-Trade, Hungary) (contains mammalian analogue of GnRH [(D-Ala^6, Pro^9-Net)-mGnRH] and dopamine antagonist – metoclopramide) (HORVATH et al. 1997) and Ovaprim (Syndel, Canada) (a complex of the salmon analogue of GnRH [(D-Arg^6, Pro^9-Net) sGnRH] and domperidone – dopamine antagonist) (PETER et al. 1993). Injections in females were performed intraperitoneally, under the ventral fin, at the doses presented in Table 1. After the injection, the water temperature in the spawner tank was raised to 12°C for the next 12 hours. After 12 hours, the fish were subjected to another hormonal

injection. Injections in males were performed at the time of the second injection in females at the same doses as females. After the second injection, the water temperature was raised to 14°C. After 30 hours from the second injection ovulation control was started. The females in each group were checked for the next 16 hours every three-four hours.

Table 1
The hormonal agents used in stimulation of the ide reproduction per kg of the spawner's body weight. The time between injections was 24 hours

Group	Control	Ovaprim	Ovopel
I injection	0.9% NaCl	0.1 cm^3	0.2 granule
II injection	0.9% NaCl	0.5 cm^3	1.0 granule

Gametes were obtained from spawners by delicate abdominal massage and pressing. Eggs from each experimental group were collected to separate plastic bowls. In order to determine the effect of the stimulant on the biological quality of gametes in each experimental group, 3 egg samples were taken in each of the groups (100–150 eggs), and subsequently incubated on Petri dishes in water at 16–18°C. The embryo survival rate was determined on the day when the spawn achieved the eyed-egg stage. All the manipulations on fish were preceded by anaesthetising them by immersion in 0.5 cm^3 dm^{-3} solution of 2-phenoxyethanol (Sigma-Aldrich, Germany).

Larvae rearing

Larvae obtained from farm and wild fish, reproduced by the same method with the use of Ovaprim (ŻARSKI et al. 2009) (the spawners were caught in the Pisa river) were reared for 21 days in 20 dm^3 tanks at the density of 50 fish dm^{-3}. They were fed *ad libitum* 3 times a day with Artemis nauplii (groups *A*) or with Artemis nauplii for 12 days, followed by trout starter (groups *P*). The temperature of rearing was 25°C. Thirty fish were taken from each of the variants in weekly intervals and were anaesthetised in 2-phenoxyethanol solution (Sigma-Aldrich, Germany) at 0.4 cm^3 dm^{-3}. The fish were photographed and then returned to the tanks from which they were taken. The documentation thus accumulated was used to measure the total length of the larvae. The measurements were made within an accuracy of 0.01 mm (ProgRes® Capture Pro 2.5, Jenoptic, Germany). Larvae mortality and percentage of developmental deformities were also recorded during the rearing period. The experiment was performed in three replications.

Statistical analysis

The statistical differences between groups were analysed with the analysis of variance (ANOVA), and the Tukey's post hoc test was used after obtaining significant values ($P < 0.05$). Before the statistical analysis, the data expressed in percentage were subjected to arcsine transformation.

Results

90–100% of females in the study groups started the reproduction process, whereas only 20% of females in the control group ovulated (Table 2). The time of ovulation in the study was longer after Ovaprim was used as compared to the other study group and lasted 38–48 hours. Ovulation was observed after 36 hours in the fish stimulated with Ovopel. The females in the control group were ready to spawn after 36 to 50 hours. The rate of embryo survival at the eyed-egg stage in the Ovaprim group was significantly higher than that in the Ovopel group and was close to 80% (Table 2). The rate of embryo survival was the lowest in the control group, but it was not statistically different from the Ovopel group. No mortality among the spawners was found.

Table 2

The results of the ide reproduction under controlled conditions

Attribute/group	Control	Ovaprim	Ovopel
Number of males	10	10	10
Spermiation	100%	100%	100%
Number of females	20	20	20
Ovulation	20%	100%	90%
Latency time (h)	36–50	38–48	36
Rate of embryo survival to the eyed-egg stage (%)	46.5 ± 6.5^{c}	79.7 ± 2.1^{a}	67.3 ± 3.1^{b}
Spawner mortality (%)	0	0	0

The groups in lines with the same letter index are not statistically different

No differences in the final length of larvae from spawners of different origin were observed (Table 3). Nor were there any differences in the rate of embryo survival in groups *A*, whereas a higher survival rate was observed in fish in groups *P* obtained from farm spawners, compared to that observed in groups *A*.

Table 3
The results of ide rearing when larvae were obtained from spawners from different breeding systems

Fish school	Farm		Wild	
Group	*A*	*P*	*A*	*P*
Initial length	8.12 ± 0.24^a	8.12 ± 0.24^a	8.27 ± 0.27^a	8.27 ± 0.27^a
Final length	19.11 ± 0.87^a	19.08 ± 1.02^a	19.05 ± 1.12^a	19.01 ± 1.26^a
Survival	98.8 ± 0.83^a	98.4 ± 1.05^a	98.6 ± 1.00^a	92.4 ± 1.34^b
Deformations	0	0.1 ± 0.1	0	0.1 ± 0.1

The groups in lines with the same letter index are not statistically different

Discussion

With the growing demand for fry-stocking material of many fish species, it is becoming necessary to improve the techniques of stimulation and reproduction control, and to develop such methods from scratch for some species. It is particularly important when it applies to endangered species (PHILIPART 1995). Also, when spawners from wild, farm or even domesticated fish schools can be used for reproduction, it is important to develop proper procedures of hormonal stimulation (KREJSZEFF et al. 2009, 2010). Developing detailed reproduction biotechniques makes it possible to implement restoration programmes and to carry out the relevant research (BABIAK et al. 1998, TARGOŃSKA et al. 2010). A number of hormonal preparations have become available during the past several years, which can be used in controlled fish reproduction (YARON 1995, BRZUSKA 2000, 2005, SZABO et al. 2002). However, this has required many studies into determining the optimal conditions for the procedure as well as the type of the preparations to be used and its dosage. This study confirms earlier reports on the effectiveness of the hormonal preparations in reproduction of rheophilic cyprinids, used in order to induce ovulation in fish, including the ide (ŻARSKI et al. 2009). A positive effect of stimulation with such agents has been observed in the nase (SZABO et al. 2002), the chub (KREJSZEFF et al. 2008) and the asp (TARGOŃSKA et al. 2010). A high percentage of ovulations observed in the study groups in this experiment (90–100%) was close to the results of reproduction of the ide living in natural conditions and farm fish during the reproduction period (KREJSZEFF et al. 2009, ŻARSKI et al. 2009). Ovulation observed in the fish in the control group is proof of the progressive domestication and likely increasing resistance of fish to stress (KREJSZEFF et al. 2009).

Different latency time after injection of hormonal agents has been previously reported (YARON 1995, BRZUSKA 2000, 2005, KREJSZEFF et al. 2009). This

frequently applies to differences of body reaction to carp pituitary homogenate (CPH) as compared to preparations which contain GnRH analogues (KUCHARCZYK et al. 2005). Two different analogues of GnRH have been used in this experiment, whose action is the same and which contain different dopamine antagonists. Application of Ovaprim in the present paper elongated latency time in comparison to females which was stimulated with Ovopel. A different reaction of the body, which manifested itself in the time of ovulation, and which was the result of injections of different GnRH analogues, has been observed, for example, in the carp (BRZUSKA 2000). A similar reaction in the ide was reported by ŻARSKI et al. (2009), who observed the highest synchronisation of ovulation following administration of Ovopel (36 h) as opposed to the group stimulated with Ovaprim, in which ovulation was observed between 36 and 44 hours after the hormone-releasing injection. A longer time of latency following the use of Ovaprim as compared to Ovopel has been observed in the asp (TARGOŃSKA et al. 2010). One of the main parameters of effectiveness of controlled reproduction with hormonal stimulation is the biological quality of gametes, expressed as the percentage of live embryos at the eyed-egg stage. This study has revealed a higher embryo survival rate after using Ovaprim as compared to Ovopel. A similar relationship has been reported by JAMRÓZ et al. (2008) for the ide and ŻARSKI et al. (2009) for the ide and the dace.

The growth rate of the ide larvae, observed in this study, is similar to that observed by other authors (KWIATKOWSKI et al. 2008, ŻARSKI et al. 2008). The absence of differences between the growth rate of ide larvae from different environments shows the full usability of ide spawners bred under controlled conditions as material for reproduction. This provides the possibility of keeping fish schools under controlled conditions as live gene pools.

The results have shown that much better results can be achieved in controlled reproduction of the ide with Ovaprim. This is indicated by better quality of gametes, expressed in this experiment as survival of embryos to the eyed-egg stage. Better synchronisation of ovulation after using the preparation can be achieved by making the first (initiating) injection of Ovopel. However, both the hormonal agents can be successfully used in controlled reproduction of fish from a school bred under controlled conditions. Although the larvae produced were slightly smaller than those obtained from wild fish, no negative effect of the fish origin on the results of initial larvae growth rate was observed. This means that fish schools bred under controlled conditions can be used as live genome pools of valuable populations or species.

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EFFECT OF DIFFERENT HORMONAL TREATMENTS ON SPAWNING EFFECTIVENESS AND ECONOMIC PROFITABILITY IN WILD NASE, *CHONDROSTOMA NASUS* (L.), UNDER CONTROLLED CONDITIONS

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K e y w o r d s: hormonal stimulation, GnRH analogue, economic profitability, cyprinids, reproduction.

A b s t r a c t

This study determined the effect of hormonal stimulation of the wild female nase, *Chondrostoma nasus* (L.), on its basic reproduction indices (percentage of ovulations, latency time, embryo survival) and the economic profitability of its use. Two commercial preparations were used in the experiment: Ovopel and Ovaprim. They were used separately (group 1 and 2 for Ovopel and Ovaprim, respectively) and in combination (group 3), where Ovopel was given in initial and Ovaprim in resolving injection. The study found a high effectiveness of all the hormonal treatments applied (ovulation rate 90–100%, latency time 36 h, embryo survival rate 78.6–81.2%) ($P>0.05$), which may be evidence of the greater susceptibility of the nase to stimulation with the less active mammalian analogue of GnRH as compared to other species of rheophilic cyprinids. In consequence, the lowest cost of hormonal stimulation (0.59 EUR per 10,000 viable embryos) was achieved with Ovopel. Using the hormonal agents in combination (in group 3) reduced the cost of stimulation by 0.17 EUR as compared to Ovaprim (group 2), where the cost was the highest (1.57 EUR per 10,000 viable embryos). The results presented in this study are providing useful information for fish breeders who manage wild populations of the nase and other species of rheophilic cyprinids.

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WPŁYW RÓŻNYCH PREPARATÓW HORMONALNYCH NA EFEKTYWNOŚĆ ROZRODU I EKONOMICZNĄ OPŁACALNOŚĆ ICH STOSOWANIA U ŚWINKI, *CHONDROSTOMA NASUS* (L.), W WARUNKACH KONTROLOWANYCH

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S ł o w a k l u c z o w e: stymulacja hormonalna, analog GnRH, efektywność ekonomiczna, ryby karpiowate, rozród.

A b s t r a k t

Celem pracy było określenie wpływu rodzaju stymulacji hormonalnej dzikich samic świnki, *Chondrostoma nasus* (L.), na podstawowe wskaźniki rozrodowe (odsetek owulacji, czas latencji, przeżywalność embrionów) oraz ekonomiczną opłacalność ich stosowania. Zastosowano dwa komercyjne preparaty: Ovopel (zawierający ssaczy analog gonadoliberyny [GnRH]) i Ovaprim (zawierający łososiowy analog GnRH). Były one stosowane osobno (grupa 1 – Ovopel i grupa 2 – Ovaprim, odpowiednio) oraz w kombinacji (grupa 3), gdzie Ovopel był podany w iniekcji wstępnej, a Ovaprim w wyzwalającej. Uzyskane wyniki wskazują na wysoką efektywność wszystkich zastosowanych wariantów hormonalnych (odsetek owulacji 90–100%, czas latencji – 36 h, przeżywalność embrionów średnio – 78.6–81.2%) (P>0.05). Może to świadczyć o tym, że świnka jest podatniejsza na stymulację mniej aktywnym ssaczym analogiem GnRH w porównaniu z innymi gatunkami karpiowatych ryb reofilnych. Dzięki temu najniższy koszt stymulacji hormonalnej (0.59 EUR na 10000 sztuk żywotnych embrionów) uzyskano po zastosowaniu Ovopelu. Zastosowanie kombinacji środków hormonalnych (w grupie 3) obniżyło koszt stymulacji o 0,17 EUR w porównaniu z Ovaprimem (grupa 2), gdzie koszt stymulacji był najwyższy (1.57 EUR na 10000 sztuk żywotnych embrionów). Wyniki przedstawione w pracy mogą być bardzo przydatne dla hodowców zarządzających dzikimi populacjami świnki oraz innymi gatunkami karpiowatych ryb reofilnych.

Introduction

Transformations of running waters due to melioration works and dams built on rivers have brought about degradation of natural habitats of many endemic fish species. This applies to both spawning grounds and specific habitats for offspring growth. In consequence, it has reduced the size of many populations (PENCZAK and KRUK 2000, SCHIEMER et al. 2003). Rheophilic cyprinids, which require access to various habitats during their lifetime, are especially exposed to such threats (MANN 1996, PENCZAK et al. 1998). This also applies to species which used to form large and stable populations, such as the nase, *Chondrostoma nasus* (L.) (LUSK and HALACKA 1995, PENAZ 1996, KECKEIS et al. 1997). As a consequence, recent years have seen a considerable increase in scientific research into the production of stocking material of rheophilic cyprinids for restoration purposes under controlled conditions

(KAMLER et al. 1998, WOLNICKI and MYSZKOWSKI 1999, SZABO et al. 2002, SPURNY et al. 2004, KREJSZEFF et al. 2008, TARGOŃSKA et al. 2010). This, in turn, has enabled the development of fish restoration pilot projects by river managers which has improved the quality of many populations. However, adequate abundance of many populations still may only be maintained by supporting natural recruitment through restocking (COWX 1994, PHILIPPART 1995, KUCHARCZYK 2002, PONCIN and PHILIPPART 2002).

Production of larvae and broodstock under controlled conditions enables the production of high quality stocking material (KWIATKOWSKI et al. 2008, KUJAWA et al. 2010). Proper planning may help to effectively manage production outcome (KUPREN et al. 2008, TURKOWSKI et al. 2008). However, controlled reproduction, which directly affects the effectiveness of further procedures, is the basis of success (KREJSZEFF et al. 2008, ŻARSKI et al. 2011). Its effectiveness, in turn, depends on the stock origin (KREJSZEFF et al. 2010a, KUJAWA et al. 2011), thermal manipulations (TARGOŃSKA et al. 2010, ŻARSKI et al. 2010) and the type of the hormonal preparation used (ŻARSKI et al. 2009). The latter factor is an essential condition for producing high quality gametes in wild cyprinid populations (KREJSZEFF et al. 2009).

The effectiveness of controlled reproduction of some wild cyprinids, such as the barbel, *Barbus barbus* (L.), and the chub, *Leuciscus cephalus* (L.), is very low (KREJSZEFF et al. 2008, 2010a) compared to domesticated fish (KREJSZEFF et al. 2009, TARGOŃSKA and KUCHARCZYK 2011). Hence, fish produced in captivity are frequently the basis of a broodstock (KREJSZEFF et al. 2009, 2010a). This allows fish farmers to lower production costs and reduce the interference in the natural spawning course because it is during the reproduction season that spawners are most frequently caught (KUCHARCZYK et al. 2008, TARGOŃSKA et al. 2008). However, it is very important in a restoration programme to manage the gene pool properly in order to maintain a low level of inbreeding (HARADA et al. 1998). Therefore, the reproduction of wild fish is still a very important element of stocking material production. On the other hand, an increase in its effectiveness makes it possible to reduce the intensity of the exploitation of wild spawners (KUCHARCZYK et al. 2008) and considerably lower production costs. This largely applies to economic profitability which arises from using different kinds of hormonal preparations to stimulate final oocyte maturation (FOM) and to synchronise ovulation (HAKUĆ-BŁAŻOWSKA et al. 2009, 2010). It should be stressed that data on the controlled reproduction of the nase is scarce (TARGOŃSKA et al. 2008).

The aim of the study was to determine the effectiveness of the controlled reproduction of the nase with the use of two commercial hormonal preparations (Ovopel and Ovaprim) and their combination, taking into account its economic profitability.

Materials and Methods

Broodstock management

Nase spawners (40 females, 15 males) were caught in autumn (October) 2009 with the use of electrofishing in the Oder river near Opole (in the south-west of Poland). After being caught, the fish were transferred to the flow-through earthen pond in the Paliwoda Fish Farm (PZW Opole), where they were kept through winter. In the early spring, when the water temperature in the pond reached 9°C, the fish were caught and transported to the hatchery of the Knieja Fishery Farm (near Częstochowa, south-east of Poland) where they were put into 1,000 l tanks with controllable thermal and light conditions (KUJAWA et al. 1999). The males and females were kept separately. The water temperature in the tanks was 9°C. After another 24 h, the FOM stimulation procedure started. All the manipulations were preceded by anaesthetising the fish in 2-phenoxyethanol solution (0.5 ml l^{-1}).

Stimulation of FOM and the spawning procedure

Before the hormonal stimulation, oocyte samples were taken from the females with a catheter. They were subsequently put into clarifying solution (ethanol 70%, 36% formaldehyde and 95% acetic acid at the proportion of 6:3:1). The position of the germinal vesicle was determined after 3–5 minutes; it was used as the basis for determination of the oocyte maturity stage according to the classification described by BRZUSKA and BIENIARZ (1977). All the females were in the 2/3 stage of maturity, which was regarded as the best moment for carrying out hormonal stimulation (KUCHARCZYK 2002).

The fish were randomly divided into four groups: three experimental ones (1–3) and a control one. Group 1st comprised fish stimulated with Ovopel (Unic-Trade, Hungary) (a preparation containing 18–20 μg of mammalian analogue of gonadoliberine (GnRH) [(D-Ala^6, Pro^9-Net)-mGnRH] and 8–10 mg dopamine antagonist – metoclopramide) (HORVATH et al. 1997), group 2nd – ones stimulated with Ovaprim (Syndel, Canada) (a complex containing 20 μg of salmon analogue of GnRH [(D-Arg^6, Pro^9-Net) sGnRH] and 10 mg dopamine antagonist – domperidone) (PETER et al. 1993); fish in group 3rd were stimulated with a combination of those preparations, which was described as very effective in the reproduction of rheophilic cyprinids (ŻARSKI et al. 2009). The doses of the hormonal preparations used were the smallest doses which were effective in the reproduction of rheophilic cy-

prinids (e.g. KUCHARCZYK et al. 2008). These doses are shown in Table 1. Injections in each group of fish were made twice in a 12-hour interval. After the first (initial) injection (when fish in group 2 were given a placebo with 0.9% NaCl, the same as in the control group), the water temperature was raised to 11°C. After the second (resolving) injection, the temperature was raised to 12.5°C and then to 13.5°C after another 24 hours. The males were not stimulated. Ovulation control was started after 36 hours from the second injection. To that end, a fish was anaesthetised (2-phenoxyethanol, 0.5 ml l^{-1}), dried gently and the possibility of obtaining single eggs was checked by gently pressing its abdomen. When ovulation was certified, eggs were collected to separate dry plastic containers. Subsequently, three egg samples were collected from each female (100–150 eggs in each) and placed on separate Petri dishes. Samples were fertilised with 0.05 ml of sperm mixture (from at least 5 males). Eggs were incubated in closed water circulation at 14°C, as described by KREJSZEFF et al. (2010b). The embryo survival rate was determined at the eyed-egg stage. Ovulation control was carried out until 42 hours after the second injection. After that time, egg samples were taken with a catheter from the females which did not ovulate and the maturity stage was determined or the occurrence of egg atresia was checked. The ovulation rate and the latency time from the second injection was recorded.

Table 1

Kinds and the doses of spawning agents used for induction of final oocyte maturation in nase, *Chondrostoma nasus* (L.)

Specification	Initial injection	Resolving injection
–	Spawning agent (dose)	Spawning agent (dose)
Control group	NaCl (0.5 ml kg^{-1})	NaCl (0.5 ml kg^{-1})
Group 1	Ovopel (0.2 pellet kg^{-1})	Ovopel (1 pellet kg^{-1})
Group 2	NaCl (0.5 ml kg^{-1})	Ovaprim (0.5 ml kg^{-1})
Group 3	Ovopel (0.2 pellet kg^{-1})	Ovaprim (0.5 ml kg^{-1})

Calculation of economic profitability

The economic profitability of hormonal stimulation was determined based on ovulation rate, relative fecundity of fish, embryo survival rate and the price of the hormonal preparations. The cost of a hormonal injection for each 10,000 viable embryos obtained as a result was taken as the measure of the economic profitability of the procedure; it was calculated according to the following formula:

$$EPHS = ([100\ CHS\ (n\ s^{-1})]\ RF^{-1})\ (N_o\ N_1^{-1})^{-1}$$

where:
EPHS – economic profitability of hormonal stimulation (EUR),
CHS – the total cost of hormonal stimulation per 1 kg of a female (EUR),
n – the number of embryos for which the cost is calculated (here: 10,000),
s – embryo survival rate (%),
RF – relative fecundity (eggs kg^{-1}),
N_1 – total number of females in a group,
N_o – number of ovulating females in a group.

The actual prices of hormonal preparations in 2010, converted to euro (EUR), were taken for calculations. Conversion ratio used for calculation (from USD to EUR) was 1.35. The cost of purchase of 1 granule of Ovopel amounted to 0.4 EUR, while the cost of 1 ml of Ovaprim was 2.37 EUR.

Data analysis and statistic

All fish were weighted before first hormonal injection and prior to spawning (BW). Similarly weight of the stripped eggs (EGW) was estimated. The data were used to calculate the pseudo-gonado-somatic index expressed as percentage of the stripped eggs to the weight of the female prior to spawning (PGSI=[100 EGW] BW^{-1}). Relative fecundity (RF) was calculated on the basis of three subsamples of eggs taken from each female, where all eggs were counted and weighted within an accuracy of 0.001 g.

Statistical differences between groups were determined by an analysis of variance (ANOVA). Before the analysis, all percentage data were subjected to arc-sin transformation. The statistical analysis was performed with Statistica 9.0 (StatSoft, Inc.) software.

Results

Hormonal stimulation of the nase was necessary to induce FOM and ovulation because no eggs were obtained from any female in the control group (Table 2). After 42 hours from second injection, the oocyte maturity stage was the same as at the beginning of the experiment (stage 2/3). Ovulation was observed in at least 90% of females in the experimental groups. After 42 hours from the last injection, egg atresia was observed in non-ovulating females in groups 1 and 2. The latency time was identical in all the groups – 36 h. PGSI was similar ($P>0.05$) in all the groups

and it ranged from 10.7 to 22.1% BW. The average *RF* in all the experimental groups was also similar ($P>0.05$) and ranged from 6781 to 15 188 eggs kg^{-1}. No statistical differences between the groups ($P>0.05$) were found in the rate of embryo survival to the eyed-egg stage.

Table 2
Results obtained during induced spawning of nase, *Chondrostoma nasus*, after application of different spawning agents

Specification	Control	Group 1 (Ovopel)	Group 2 (Ovaprim)	Group 3 (Ovopel/Ovaprim)
Number of females	10	10	10	10
Initial weight of females [g]	366.8 ± 52.4	366.5 ± 57.4	341.7 ± 54.6	370.2 ± 43.3
Ovulation rate [%]	0	90	90	100
Latency time [h]	–	36	36	36
PGSI [% of BW]	–	18.5 ± 2.1	16.2 ± 3.2	18.6 ± 1.9
Relative fecundity [eggs kg^{-1}]	–	11 506 ± 2199	10 494 ± 2398	11 139 ± 1741
Embryos survival [%]	–	78.6 ± 5.6	79.8 ± 5.2	81.2 ± 4.6

Data presented as mean ± SD. No statistical differences were found between treatment groups ($P>0.05$)

An analysis of economic profitability has shown that the lowest cost of producing 10,000 viable embryos (0.59 EUR on average) was achieved when a double injection of Ovopel was applied (group 1). The other treatments required a higher cost, which amounted to 1.57 EUR in stimulation with Ovaprim, and 1.40 EUR in stimulation with a combination of hormonal preparations (Figure 1).

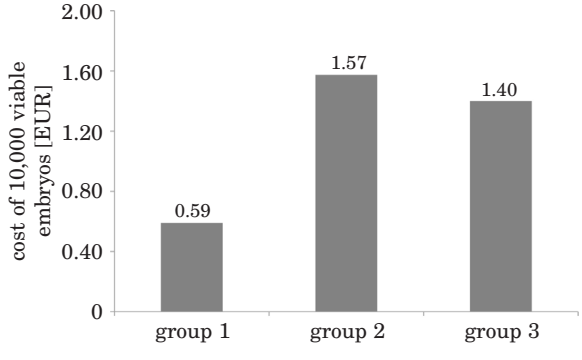

Fig. 1. Cost of 10,000 viable embryos of nase obtained after different hormonal stimulations. Females were injected with Ovopel, Ovaprim and combination of Ovopel and Ovaprim in groups 1, 2 and 3, respectively (for doses see Table 1)

Discussion

Gonadotropins contained in carp pituitary extract (CPE) have been used with success in the reproduction of cyprinids (YARON et al. 2009). However, GnRH analogues administered together with dopamine antagonist (e.g. Ovopel and Ovaprim) proved more effective in a number of cases (see KUCHARCZYK et al. 2008); they affect the pituitary gland and stimulate FOM with endogenous gonadotropins (YARON 1995, ZOHAR and MYLONAS 2001). Analogues of GnRH have also proven effective in rheophilic cyprinids (KREJSZEFF et al. 2008, TARGOŃSKA et al. 2010). There have been reports lately of very good results achieved when a combination of two preparations was used containing different GhRH analogues, where the initial dose of Ovopel improved the effectiveness of Ovaprim administered in a resolving dose (ŻARSKI et al. 2009).

The results of the nase reproduction achieved in this experiment are highly satisfactory when compared to the findings of other authors. SZABO et al. (2002) achieved comparable results (83% of ovulations, 83.5% embryo survival) after administering 20 μg of mammalian analogue of GnRH with 10 mg of dopmeridone in a single dose. The other treatments tested by the authors (CPE at 3 and 5 mg kg^{-1}) were much less effective (67% ovulation rate in both groups, 69.1 and 74.7% embryo survival rate for the CPE dose of 3 and 5 mg kg^{-1}, respectively). ŻARSKI et al. (2008) used the same hormonal preparations which were used in the present experiment and also achieved good results (89 and 100% ovulations following stimulation with Ovopel and Ovaprim, respectively). However, the RF observed in this experiment was more than three times lower than that reported by ŻARSKI et al. (2008) (40200 and 36800 eggs kg^{-1} for Ovopel and Ovaprim, respectively), which may have resulted both from the varied female size (those authors used female fish of more than 500 g) and population-related factors. However, more data are needed to examine the issue in more detail. Moreover, the latency time was also different (from 51 to 57 h), which was probably a result of thermal fluctuations, observed by ŻARSKI et al. (2008), when fish were kept under natural thermal conditions. It must be emphasised that the latency time observed in this study was exactly the same in all groups and the spawning was highly synchronised. Synchronisation of ovulation was possible only owing to the temperature control applied in the experiment. This is of great importance in commercial production, where the amount of labour (which grows as ovulation becomes less and less synchronised) needed to carry out the operation directly affects its cost effectiveness. However, the result is surprising considering the fact that Ovaprim was four times more effective in FOM stimulation in other rheophilic cyprinids, such as the asp, *Aspius aspius* (L.), the ide, *Leuciscus idus* (L.), and the dace, *Leuciscus leuciscus* (L.), inducing ovulation of the high quality eggs in a much

larger number of female fish than was the case with Ovopel (ŻARSKI et al. 2008, 2009, TARGOŃSKA et al. 2010). This resulted from the fact that the form of salmon analogue of GnRH is much closer to the natural form of salmon GnRH, which is a native form occurring in cyprinids (PODHOREC and KOURIL 2009). Therefore, the results suggest that the nase is much more susceptible to stimulation with less active mammalian analogue of GnRH as compared with other rheophilic cyprinids. However, the effect of using different dopamine antagonists contained in the preparations cannot be rejected.

The economic profitability of hormonal stimulation has not drawn much scientific attention to date (HAKUĆ-BŁAŻOWSKA et al. 2009, 2010), despite the fact that it is a factor which largely affects the type of hormonal preparations used in commercial hatcheries. It has been reported that the highest economic profitability in stimulating rheophilic cyprinids is achieved with Ovopel and Ovaprim. The cost of producing 1,000 viable embryos of the ide was exactly the same when either of the preparations was used. However, the relative cost of using Ovaprim in asp reproduction was lower by 30% (HAKUĆ-BŁAŻOWSKA et al. 2009), although Ovaprim itself was more expensive. This was caused by a much higher percentage of ovulations and embryo survival rate following the use of Ovaprim. On the other hand, the cost of stimulation with Ovaprim of cultured barbel was twice as high as when Ovopel was used (HAKUĆ-BŁAŻOWSKA et al. 2010). This resulted from the fact that the result of the reproduction was similar following the use of each of the preparations. A similar relationship was observed in this experiment: the result of the reproduction in each of the hormonal treatments was very good, hence the economic profitability was better in the group where the cheaper preparation – Ovopel – was used. It is noteworthy that administering a combination of hormonal agents resulted in higher ovulation rate which, in turn, reduced the cost of hormonal stimulation as compared to a single Ovaprim injection. However, it has to be pointed out that administration of hormonal preparation in single dose may positively affect economic effectiveness of single Ovaprim dosage using due to limitation of labor costs.

The results achieved in this study show that reproduction of wild nase spawners may be successfully carried out under controlled conditions. The very good spawning result achieved in each treatment of hormonal stimulation indicates that reproduction can be successfully carried out even on farms which have not done so with the nase, but which have experience with other cyprinids. In the view of the obtained results, one can recommend Ovopel stimulation of FOM and ovulation in the nase due to its high economic profitability.

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