UNIVERSITY OF WARMIA AND MAZURY IN OLSZTYN

Polish Journal of Natural Sciences

(2/2012) 27

PUBLISHER UWM
OLSZTYN 2012

EDITORIAL BOARD
Janusz Falkowski (Editor-in-chief), Eugeniusz Biesiadka (Biology), Jerzy Czapla (Agriculture), Jan Glogowski (Reproductive Biology), Ryszard Zadernowski (Food Science), Małgorzata Jankun-Woźnicka (Fishery), Józef Szarek (Veterinary Science), Julita Dunalska (Environmental Protection), Vaclav Matoušek (Animal Science, Czech Republic), Juraj Mlynek (Animal Behavior, Slovak Republik)

Statistical editor Anna Wiśniewska

Executive editor Agnieszka Orłowska-Rachwał

The Polish Journal of Natural Sciences is indexed and abstracted in Biological Abstracts and Biosis Previews

The print edition is the primary version of the Journal

The Journal is also available in electronic form on the web site http://wydawnictwo.uwm.edu.pl (subpage *Czytelnia*)

PL ISSN 1643-9953

© Copyright by Wydawnictwo Uniwersytetu Warmińsko-Mazurskiego
Olsztyn 2012

PUBLISHER UWM OLSZTYN

Address
ul. Jana Heweliusza 14
10-718 Olsztyn-Kortowo, Poland
tel.: +48 89 523-36-61
fax: +48 89 523-34-38
e-mail: wydawca@uwm.edu.pl

Ark. wyd. 9,5, ark. druk. 8, nakład 125 egz.
Druk – Zakład Poligraficzny UWM w Olsztynie
zam. nr 375

TABLE OF CONTENTS

Agriculture

Biology

Environmental Protection

Fishery

Food and Nutrition Sciences

SPIS TREŚCI

Rolnictwo

Biologia

Ochrona środowiska

Rybactwo

Nauka o żywności i żywieniu

POLISH JOURNAL OF NATURAL SCIENCES
Abbrev.: Pol. J. Natur. Sc., Vol 27(2): 125–134, Y. 2012

THE EFFECT OF MULTI-COMPONENT FERTILIZERS ON THE YIELD AND MINERAL COMPOSITION OF WINTER TRITICALE*

Anna Nogalska, Stanisław Sienkiewicz, Jerzy Czapla, Małgorzata Skwierawska

Department of Agricultural Chemistry and Environmental Protection
University of Warmia and Mazury in Olsztyn

K e y w o r d s: winter triticale, yield, macronutrients, multi-component fertilizers, uptake.

A b s t r a c t

Multi-component fertilizers are increasingly used due to their easy application, solubility and complex composition. A clear advantage of multi-component fertilizers over simple fertilizers is that the former supply a combination of nutrients at a time. The objective of this study was to determine the effect of multi-component fertilizers, Amofosmag 4 and Amofosmag 3, on winter triticale yield, and the content and uptake of macronutrients. A three-year field experiment (2008–2010) was carried out in a randomized block design at the Research and Experimental Station in Tomaszkowo, at the University of Warmia and Mazury in Olsztyn (NE Poland). The experiment comprised three fertilization treatments in four replications: control treatment (simple fertilizers) and two treatments with mixed multi-component fertilizers, Amofosmag 4 and Amofosmag 3. The tested crop was winter triticale cv. Grenado. Wet mineralized plant samples were assayed for the content of: total nitrogen – by the hypochlorite method, phosphorus – by the vanadium-molybdenum method, calcium and potassium – by atomic emission spectrometry (AES), and magnesium – by atomic absorption spectrometry (AAS). In most cases, the application of Amofosmag 4 and Amofosmag 3 increased the yield of winter triticale grain and straw, in comparison with simple fertilizers. The concentrations of the analyzed macronutrients in triticale were similar in all fertilization treatments, thus pointing to a comparable effect of the applied fertilizers, except for the nitrogen content of triticale grain which was highest in plots fertilized with simple fertilizers, compared with the other treatments. Differences in the chemical composition of triticale plants were observed between successive years of the study. The highest total uptake of phosphorus, potassium and magnesium by winter triticale was noted in plots fertilized with Amofosmag 3. Nitrogen uptake was higher in the control treatment, and calcium uptake in the Amofosmag 4 treatment.

Address: Anna Nogalska, University of Warmia and Mazury, ul. Michała Oczapowskiego 8, 10-718 Olsztyn, Poland, phone: +48 (89) 523 32 50, e-mail: anna.nogalska@uwm.edu.pl
* This study was financed by Agrochem Ltd., Dobre Miasto.

WPŁYW NAWOZÓW WIELOSKŁADNIKOWYCH NA PLONOWANIE I SKŁAD MINERALNY PSZENŻYTA OZIMEGO

Anna Nogalska, Stanisław Sienkiewicz, Jerzy Czapla, Małgorzata Skwierawska

Katedra Chemii Rolnej i Ochrony Środowiska
Uniwersytet Warmińsko-Mazurski w Olsztynie

S ł o w a k l u c z o w e: pszenżyto ozime, plon, makroelementy, nawozy wieloskładnikowe, pobranie.

A b s t r a k t

Nawozy wieloskładnikowe są coraz powszechniej stosowane ze względu na łatwość aplikacji, rozpuszczalność i kompleksowy skład pierwiastkowy. Możliwość wprowadzenia jednocześnie kilku składników czyni je konkurencyjnymi w porównaniu z jednoskładnikowymi. Celem pracy było zbadanie wpływu nawozów wieloskładnikowych Amofosmagu 4 i Amofosmagu 3 na plon, zawartość i pobranie makroskładników przez pszenżyto ozime. Trzyletnie doświadczenie polowe (2008–2010) przeprowadzono w Ośrodku Dydaktyczno-Doświadczalnym w Tomaszkowie należącym do Uniwersytetu Warmińsko-Mazurskiego w Olsztynie. Doświadczenie, założone metodą losowanych bloków, obejmowało trzy obiekty nawozowe w czterech powtórzeniach: obiekt kontrolny (nawozy jednoskładnikowe), Amofosmag 4 i Amofosmag 3. Rośliną testowaną było pszenżyto ozime odmiany 'Grenado'. W zmineralizowanych „na mokro" próbkach roślinnych oznaczono: azot ogólny – metodą podchlorynową, fosfor – metodą wanadowo-molibdenową, wapń i potas – metodą emisyjnej spektometrii atomowej (ESA) oraz magnez – metodą absorpcyjnej spektometrii atomowej (ASA). Z przeprowadzonego doświadczenia wynika, że nawożenie Amofosmagiem 4 i Amofosmagiem 3 miało na ogół wpływ na zwiększenie plonu ziarna i słomy pszenżyta ozimego w porównaniu z nawozami jednoskładnikowymi. Koncentracja badanych makroelementów w pszenżycie w poszczególnych obiektach nawozowych była na ogół zbliżona, zastosowane nawozy wykazywały działanie równorzędne. Wyjątek stanowiła zawartość azotu w ziarnie pszenżyta, gdzie po zastosowaniu nawozów jednoskładnikowych wystąpiła największa zawartość tego składnika w porównaniu z pozostałymi obiektami nawozowych. Zróżnicowanie w składzie chemicznym badanej rośliny wystąpiło między poszczególnymi latami badań. Największe łączne pobranie fosforu, potasu i magnezu przez pszenżyto ozime stwierdzono w obiektach z Amofosmagiem 3. Azot był nieco lepiej pobierany przez pszenżyto w obiekcie kontrolnym, zaś wapń – w obiekcie z Amofosmagiem 4.

Introduction

The use of multi-component mineral fertilizers, supplying a balanced mixture of major nutrients, allows to address environmental concerns in agricultural ecosystems (ŁABUDA 1994). Since the 1990s, there has been a steady increase in the share of multi-component fertilizers in total mineral fertilizer consumption. Numerous fertilizer manufacturers offer a wide variety and range of mixed fertilizers, blends and compound fertilizers (POTARZYCKI and LEWICKA 2002). Mineral fertilizers currently available on the market differ considerably with respect to quality and price. The most important characteristics of fertilizers include their easy application and storage, solubility and

complex composition. A clear advantage of multi-component fertilizers over simple fertilizers is that the former supply a combination of nutrients at a time (GLABISZ et al. 1992). Compound fertilizers provide crops with essential nutrients in adequate amounts and proportions, and they help prevent or reduce nutrient leaching (ZAWARTKA and SKWIERAWSKA 2004a). Fertilization rates should be adapted to the requirements of a given plant species, and they have to be determined in view of crop yield and quality, fertilizer efficiency, and environmental issues.

The objective of this study was to determine the effect of mixed component fertilizers, Amofosmag 4 and Amofosmag 3, on winter triticale yield, and the content and uptake of macronutrients.

Materials and Methods

A three-year field experiment (2008–2010) was carried out in a randomized block design at the Research and Experimental Station in Tomaszkowo, at the University of Warmia and Mazury in Olsztyn. The experiment, which comprised three fertilization treatments in four replications: control treatment (simple fertilizers), Amofosmag 4 and Amofosmag 3, was established on proper brown soil developed from sandy loam, of quality class III b and very good rye complex. The physicochemical properties of soil in each year of the study are presented in Table 1. The tested crop was winter triticale (*Triticosecale Wittm L.)* cv. Grenado. The preceding plants were winter triticale. Plot surface area was 10 m^2.

Table 1

Selected physicochemical properties of soil used in the experiment [mg kg^{-1}]

Year	pH w 1 M KCl	Available forms		
		P	K	Mg
2008	6.2	72	207	28
2009	7.0	84	149	35
2010	5.7	70	244	96

Based on the average levels of available phosphorus in the soil, 350 kg ha^{-1} Amofosmag 3 (NPKMg 3:14:20:2 + 22% CaO + 9% SO3: 10.5 kg N, 21.5 kg P, 58 kg K, 55 kg Ca, 4 kg Mg, 12.5 kg S on pure ingredient basis) and Amofosmag 4 (NPKMg 4:15:15:2 + 24% CaO + 9% SO3 : 12 kg N, 23 P, 43.5 kg K, 60 kg Ca, 4 kg Mg, 12.5 kg S on pure ingredient basis) were applied pre-sowing. The nitrogen rate of 80 kg per ha was supplemented with two

doses of ammonium nitrate applied by top-dressing in all treatments, including control. In the control treatment, the following fertilizers were applied presowing: 14 kg N in the form of urea, 23 kg P in the form of triple superphosphate and 43.5 kg K kg ha^{-1} in the form of potash salt.

Samples of winter triticale were collected at the stage of full maturity. The grain and straw harvested in each plot was dried and weighed individually. Wet mineralized samples were assayed for the content of: total nitrogen – by the hypochlorite method, phosphorus – by the vanadium-molybdenum method, calcium and potassium – by atomic emission spectrometry (AES), and magnesium – by atomic absorption spectrometry (AAS). The results of chemical analyses were verified statistically by a two-factorial analysis of variance for a randomized block design. The experimental factors were as follows: a – fertilization, b – duration of the experiment. The least significant difference was assumed at $p = 0.05$.

Results and Discussion

The distribution of air temperatures in the growing season of 2008 differed insignificantly from the long-term average (Table 2). Precipitation total in May and June was substantially lower than the multiannual average, which could have reduce the number and size of triticale ears. In 2009, mean monthly temperatures were similar to the long-term average. The highest temperature was recorded in July. April was relatively dry, while in June precipitation levels considerably exceeded the long-term average. In 2010, air temperatures during the growing season were slightly above the long-term average. Precipitation total in May was over 2.5-fold higher than the long period average. May was wet, with a difference of 80.0 mm between mean monthly rainfall and the long period average. Weather conditions could have affected the yield of winter triticale.

Table 2
Weather conditions in 2008–2010 – data provided by the Meteorological Station in Tomaszkowo

Month	Mean monthly temperature [°C]				Precipitation total [mm]			
	2008	2009	2010	1970–2000	2008	2009	2010	1970–2000
April	7.7	9.4	8.1	6.9	31.4	4.8	18.2	36.1
May	12.3	12.4	12.0	12.7	27.0	52.9	131.9	51.9
June	16.9	14.9	16.4	15.9	32.7	136.9	84.8	79.3
July	18.5	20.4	21.1	17.7	57.7	48.3	80.4	73.8
August	18.4	17.6	19.3	17.2	102.1	19.3	95.3	67.1
September	15.1	14.2	12.0	12.5	22.9	25.7	40.5	59.0

In 2008, the yield of winter triticale grain ranged from 9.90 to 10.57 t ha^{-1}, and it was not significantly affected by the type of fertilizers (Table 3). The highest average yield of winter triticale grain was noted in the Amofosmag 3 treatment. Straw yield corresponded to grain yield, and it was not significantly influenced by the fertilizers applied in the study. In an experiment with spring wheat conducted by NOGALSKA et al. (2010), multi-component fertilizers had a more desirable yield-forming effect than simple fertilizers. In the second year of the study (2009), the yield of winter triticale grain varied from 3.82 to 3.97 t ha^{-1}, and it was lower than in 2008 and 2010, which could be due to less favorable weather conditions. Precipitation total in April was very low, which could have reduce the number and size of triticale ears. As demonstrated by ALARU et al. (2003) and JACZEWSKA-KALICKA (2008), grain crops are highly sensitive to weather conditions. The experimental factors had no significant effect on straw yield. In the third year of the experiment (2010), Amofosmag 4 had the most beneficial influence on triticale grain yield, which was found to increase by around 7%, compared with the control treatment. Wheat straw yield was affected by the applied fertilizers to a lower degree.

Table 3

Winter triticale yield after the application of Amofosmag 4 and Amofosmag 3 [t ha $^{-1}$]

Treatment	Grain				Straw			
	2008	2009	2010	mean for *a*	2008	2009	2010	mean for *a*
NPK	10.04	3.82	6.94	6.93	8.94	7.54	7.76	8.08
Amofosmag 4	9.90	3.97	7.44	7.10	9.07	7.81	8.86	8.58
Amofosmag 3	10.57	3.91	7.28	7.25	9.04	8.73	8.72	8.82
Mean for *b*	10.17	3.90	7.22		9.01	8.02	8.44	
$LSD_{p=0,05}$ for *a*	n.s.				n.s.			
b	0.56				n.s.			
ab	n.s.				n.s.			

Explanations: *a* – fertilization, *b* – duration of the experiment, *ab* – interaction, n.s. – non-significant difference

The results of the present study show that Amofosmag 3 caused an approximately 5% and 9% increase (on average) in the yield of triticale grain and straw, respectively, compared with simple fertilizers. An increase in the yield of different cereal species in response to the application of mixed fertilizers was also reported by ZAWARTKA and SKWIERAWSKA (2004b), TRAWCZYŃSKI and SOCHA (2006) and NOGALSKA et al. (2010, 2011), whereas in an experiment by WINIARSKI et al. (2002) the yield-forming effects of multi-component and simple fertilizers were comparable.

Table 4
Macronutrient content of winter triticale after the application of Amofosmag 4 and Amofosmag 3 [g kg^{-1} d.m.]

Macro-nutrient	Treatment	Grain				Straw			
		2008	2009	2010	mean for *a*	2008	2009	2010	mean for *a*
Nitrogen	NPK	17.30	11.15	16.80	15.08	3.17	3.97	5.55	4,23
	Amofosmag 4	15.22	10.42	16.12	13.92	3.62	4.96	6.75	5,11
	Amofosmag 3	9.32	11.71	16.35	12.46	2.75	5.85	5.64	4,74
Mean for *b*		13,94	11.09	16.42		3.18	4.92	5.98	
$LSD_{p=0.05}$ for *a*		1.285				n.s.			
b		1.299				0.739			
ab		2.226				n.s.			
Phosphorus	NPK	1.79	4.81	3.86	3.48	1.04	2.65	1.67	1.78
	Amofosmag 4	1.77	5.47	3.74	3.66	1.08	2.72	1.79	1.86
	Amofosmag 3	1.73	4.76	3.89	3.46	1.07	3.07	1.67	1.93
Mean for *b*		1.76	5.01	3.83		1.06	2.81	1.71	
$LSD_{p=0.05}$ for *a*		n.s.				n.s.			
b		0.359				0.282			
ab		n.s.				n.s.			
Potassium	NPK	3.82	5.75	5.47	5.01	14.85	17.22	22.40	18.15
	Amofosmag 4	3.47	5.95	5.24	4.88	13.90	13.80	19.34	15.68
	Amofosmag 3	3.70	5.85	5.60	5.05	13.85	15.70	22.63	17.39
Mean for *b*		3.66	5.84	5.43		14.20	15.57	21.45	
$LSD_{p=0.05}$ for *a*		n.s.				1.511			
b		0.309				1.600			
ab		n.s.				n.s.			
Calcium	NPK	0.72	0.62	0.79	0.71	5.78	2.50	4.25	4.17
	Amofosmag 4	0.73	0.67	0.75	0.71	6.06	2.65	4.07	4.26
	Amofosmag 3	0.72	0.63	0.93	0.76	4.69	2.47	4.16	3.77
Mean for *b*		0.72	0.64	0.82		5.51	2.54	4.16	
$LSD_{p=0.05}$ for *a*		n.s.				n.s.			
b		0.111				0.537			
ab		n.s.				n.s.			
Magnesium	NPK	0.85	0.94	0.88	0.89	0.41	0.49	0.44	0.44
	Amofosmag 4	0.82	0.95	0.89	0.88	0.43	0.58	0.43	0.48
	Amofosmag 3	0.92	0.94	0.87	0.91	0.39	0.63	0.37	0.46
Mean dla *b*		0.86	0.94	0.88		0.41	0.56	0.41	
$LSD_{p=0.05}$ for *a*		n.s.				n.s.			
b		0.066				0.035			
ab		n.s.				0.061			

Explantations as in Table 3

Triticale is used as a feed grain, therefore its macronutrient content is equally important as yield. Cereal grains serve as the main source of mineral substances for animals (BRZOZOWSKA 2006). The results of chemical analyses of winter triticale grain and straw (Table 4) suggest that the concentrations of the analyzed macronutrients varied between the years of the study. In 2008, the nitrogen content of winter triticale grain ranged from 9.32 to 17.30 g kg DM, and it was highest in the control treatment. The lowest nitrogen content of triticale kernels was noted in 2009, and the highest nitrogen concentrations in triticale grain (16.42 g kg^{-1} DM) were observed in 2010. In a study by GROMOVA and POLACK (1995), the average nitrogen content of triticale grain was 23.70 g kg^{-1} DM. The applied fertilizers had no influence on nitrogen concentrations in triticale straw. The highest nitrogen content of triticale straw was reported in the third year of the experiment, in the Amofosmag 4 treatment.

In 2008, triticale grain contained significantly less phosphorus and potassium than in 2009 and 2010. In the second and third year of the experiment, triticale kernels were more abundant in phosphorus and potassium (significant differences). The calcium content of winter triticale grain and straw was not determined by the type of fertilizers. Differences between treatments were minor. The lowest calcium concentrations in triticale grain were noted in 2009. The magnesium content of winter triticale remained stable throughout the experiment, reaching the highest level in the second year. The findings of numerous authors (FILIPEK 2001, TRAWCZYŃSKI and GRZEŚKIEWICZ 2006, MAZUR et al. 2001, NOGALSKA et al. 2010, 2011) indicate that multi-component fertilizers have no significant effect on the macronutrient content of tested plant species.

Macronutrient uptake was estimated based on the yield and macronutrient content of winter triticale grain and straw. The highest nitrogen uptake by winter triticale plants (215.92 kg N ha^{-1}) was noted in the first year of the experiment, in the control treatment (Table 5). Nitrogen uptake was correlated with the percentage content of nitrogen in plants (Table 4). Similar results were reported by FOSSATI et al. (1993). Nitrogen uptake was substantially lower in the second year of the study. A similar, albeit less pronounced, trend was noted with regard to calcium and magnesium concentrations. Phosphorus uptake levels were comparable in all treatments, and they tended to increase in response to Amofosmag 3. Phosphorus uptake varied considerably between years, due to differences in triticale yield and the percentage content of macronutrients. Phosphorus uptake was highest in 2009 (38.35–45.41 kg P ha^{-1}), and lowest in 2008 (over two-fold lower than in 2009 and 2010) when triticale kernels were least abundant in phosphorus. Potassium uptake by winter triticale plants was highest in the third year of the

experiment, in particular after the application of Amofosmag 3. Such a trend was also observed with respect o the total potassium uptake (Figure 1). The highest total (mean values of three years) nitrogen uptake was noted in the control treatment (Figure 1). The highest uptake of phosphorus, potassium and magnesium was observed in treatments with Amofosmag 3, while the highest calcium uptake was observed in the Amofosmag 4 treatment. Partially different results were reported by NOGALSKA et al. (2010, 2011).

Table 5

Nutrient uptake by winter triticale grain and straw [kg ha^{-1}]

Treatment	Nitrogen	Phosphorus	Potassium	Calcium	Magnesium
2008					
NPK	215.92	27.26	171.10	58.59	12.19
Amofosmag 4	183.50	27.31	160.42	62.18	12.01
Amofosmag 3	123.37	27.86	164.30	50.00	13.24
2009					
NPK	72.52	38.35	151.79	21.21	7.28
Amofosmag 4	80.09	42.95	131.39	23.34	8.29
Amofosmag 3	96.85	45.41	159.93	24.02	9.16
2019					
NPK	159.65	39.73	211.78	38.46	9.51
Amofosmag 4	179.73	43.67	199.40	41.61	10.42
Amofosmag 3	168.20	42.87	238.08	43.04	9.55

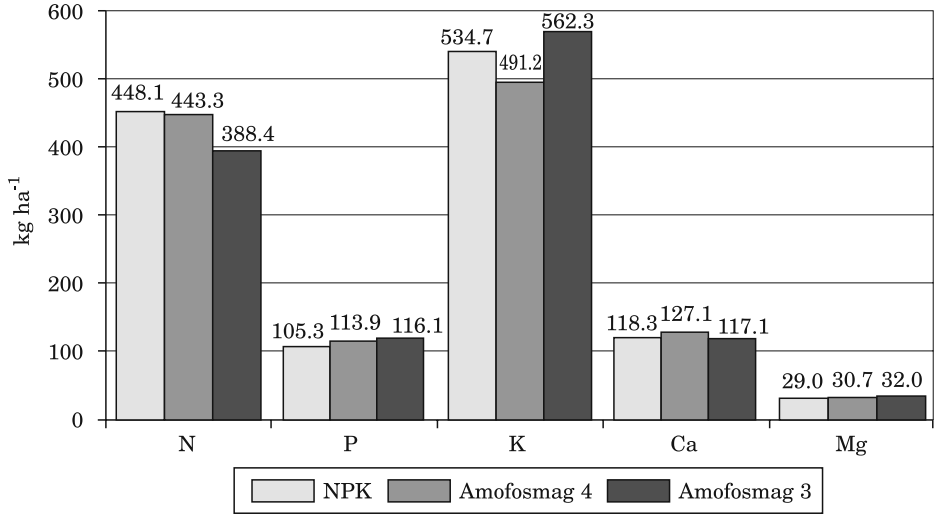

Fig. 1. Total macronutrient uptake by winter triticale over a three-year experimental period

Conclusions

1. The most beneficial effect was reported for Amofosmag 3 which increased the yield of winter triticale grain by 5% on average, compared with the control treatment.

2. The concentrations of the analyzed macronutrients in winter triticale grain and straw varied insignificantly between fertilization treatments. Simple and multi-component fertilizers exerted a comparable effect on the mineral composition of the tested crop. Significant differences were observed in this respect between successive years of the study. The only exception was the nitrogen content of triticale grain which was highest in plots fertilized with simple fertilizers.

3. The highest total uptake of phosphorus, potassium and magnesium by winter triticale was noted in plots fertilized with Amofosmag 3, which may suggest that the nutrients contained in this product were more readily available to plants. Nitrogen uptake was higher in the control treatment, and calcium uptake in the Amofosmag 4 treatment.

Translated by ALEKSANDRA POPRAWSKA

Accepted for print 31.01.2012

References

ALARU M., LAUR Ü., JAAMA E. 2003. *Influence of nitrogen and weather conditions on the grain quality of winter triticale.* Agronomy Research, 1: 3–10.

BRZOZOWSKA I. 2006. *Wpływ herbicydów i sposobu nawożenia azotem na zawartość makroelementów w ziarnie pszenżyta ozimego.* Pam. Puł., 142: 9–17.

FILIPEK T. 2001. *Zawartość składników pokarmowych a zastosowanie nawozów wieloskładnikowych z KIZPS „Siarkopol".* Folia Univ. Agric. Stetin., Agricultura, 223 (89): 41–46.

FOSSATI D., FOSSATI A., FEIL B. 1993. *Relationship between grain yield and grain nitrogen concentration in winter triticale.* Euphytica, 71: 115–123.

GLABISZ U., KIC B., GRZMIL B. 1992. *Manufacture of low chloride multicomponent fertilizers based on conversion in aqueous solution.* J. Agric. Food Chem., 40: 1393–1397.

GROMOVA Z., POLAĆEK M. 1995. *Uptake of nutrients in triticale.* Rostlinná výroba, 41: 71–75.

JACZEWSKA-KALICKA A. 2008. *Wpływ zmian klimatycznych na plonowanie i ochronę zbóż w Polsce.* Progress in Plant Protection, 48(2): 415–425.

ŁABUDA S. 1994. *Skład pierwiastkowy nawozów w Polsce.* Annales UMCS, Sec. E, 49 Suppl., 133–147.

MAZUR T., MAZUR Z., WOJTAS A., GRZEŚKOWIAK A. 2001. *Wpływ nawozów wieloskładnikowych na wielkość i jakość plonów roślin uprawianych w 4-polowym zmianowaniu.* Folia Univ. Agric. Stetin., Agricultura, 223(89): 113–120.

NOGALSKA A., CZAPLA J., SKWIERAWSKA M. 2010. *The effect of multi-component fertilizers on spring wheat yield, the content and uptake of macronutrients*. Pol. J. Natur. Sc., 25(4): 323–331.

NOGALSKA A., CZAPLA J., SKWIERAWSKA M. 2011. *The effect of multi-component fertilizers on spring barley yield, the content and uptake of macronutrients*. Pol. J. Natur. Sc., 26(2): 89–97.

POTARZYCKI J., LEWICKA L. 2002. *Efektywność plonotwórcza nawozów wieloskładnikowych w uprawie buraka cukrowego.* Biul. IHAR, 222: 111–118.

ZAWARTKA L., SKWIERAWSKA M. 2004a. *Wpływ nawozów wieloskładnikowych na wymywanie fosforu i innych makroelementów z gleby.* Prace Nauk. AE Wrocław, Chemia, 1017: 69–77.

ZAWARTKA L., SKWIERAWSKA M. 2004b. *Wpływ nawozów wieloskładnikowych na plon i zawartość fosforu i innych makroelementów w jęczmieniu jarym.* Prace Nauk. AE Wrocław, Chemia, 1017: 149–157.

WINIARSKI A., PODLEŚNA A., NOWAK R. 2002. *Technologia wytwarzania i badania agrochemiczne nawozów wieloskładnikowych USP.* Nawozy i nawożenie, 1(10): 19–29.

TRAWCZYŃSKI C., GRZEŚKIEWICZ H. 2006. *Effect of the agravita multicomponent fertilizer under conditions of different nitrogen doses on the yield and some quality features of potato tubers.* Zesz. Prob. Postęp. Nauk Roln., 511: 149–155.

TRAWCZYŃSKI C., SOCHA T. 2006. *The influence of multicomponent fertilizers (agrafoska, amofoska, amofosmag) on the yield and chemical composition of potato tubers.* Zesz. Prob. Postęp. Nauk Roln., 511: 157–164.

POLISH JOURNAL OF NATURAL SCIENCES
Abbrev.: Pol. J. Natur. Sc., Vol 27(2): 135–150, Y. 2012

THE LICHEN BIOTA OF "SKAŁKI PIEKŁO POD NIEKŁANIEM" NATURE RESERVE – CURRENT STATE AND CHANGES IN SPECIES COMPOSITION OVER THE PAST 100 YEARS

Anna Łubek

Institute of Biology
Jan Kochanowski University in Kielce

K e y w o r d s: lichenized fungi, lichenicolous fungi, rare species, species diversity, Świętokrzyskie Mountains, Central Poland.

A b s t r a c t

The lichenological study was conducted in the "Skałki Piekło pod Niekłaniem" Nature Reserve and its surroundings in the Świętokrzyskie Mts. The aim of the study was to present the current state of lichen biota and changes that have occurred in the species composition in the last 100 years. A total of 168 species of lichens and lichenicolous fungi were found in the investigated area. There are currently 20 unconfirmed species of lichens and lichenicolous fungi.

BIOTA POROSTÓW REZERWATU „SKAŁKI PIEKŁO POD NIEKŁANIEM" – STAN OBECNY I ZMIANY W SKŁADZIE GATUNKOWYM W CIĄGU OSTATNICH STU LAT

Anna Łubek

Instytut Biologii
Uniwersytet Jana Kochanowskiego w Kielcach

S ł o w a k l u c z o w e: grzyby zlichenizowane, grzyby naporostowe, gatunki rzadkie, różnorodność gatunkowa, Góry Świętokrzyskie, Polska Centralna.

A b s t r a k t

Badania lichenologiczne przeprowadzono w rezerwacie przyrody „Skałki Piekło pod Niekłaniem" i w jego otoczeniu w Górach Świętokrzyskich. Celem badań było przedstawienie aktualnego stanu bioty porostów oraz zmian, jakie zaszły w jej składzie gatunkowym na przestrzeni ponad 100 lat. Na badanym terenie stwierdzono łącznie 168 gatunków porostów oraz grzybów naporostowych. Obecnie nie potwierdzono 20 gatunków porostów i grzybów naporostowych.

Address: Anna Łubek, Jan Kochanowski University, ul. Świętokrzyska 15, 25-406 Kielce, Poland, phone: +48 (41) 349 63 47, e-mail: alubek@ujk.edu.pl

Introduction

On the north-western rim of the Świętokrzyskie Mountains at the top of Piekło Mt., is a large outcrop of rock accumulation built of Lower Jurassic sandstone. The rocks are of various and interesting forms, such as: thresholds, pulpits, towers, mushrooms, etc., as a result of various factors of erosion. This area was, and still is, attractive to many researchers, mainly geologists (LINDNER 1972, URBAN 1996) and botanists (BŁOŃSKI 1890, MASSALSKI, KAZNOWSKI 1928). The first accounts of lichens of the area date back to works of BŁOŃSKI (1890) and BERDAU (1876). These historic studies are at present the only source of information about the occurrence of lichens in the whole region of the Świętokrzyskie Mts in the late 19th century. Lichenological research in this area was resumed only one hundred years later. A short report on the lichens of the "Skałki Piekło pod Niekłaniem" reserve includes the work of TOBOROWICZ (1992). At present the study on the state of conservation of lichen biota and changes in the species composition has been carried out at the top of Piekło Mt. and the neighbouring areas. Preliminary results of the research on the species that have disappeared from the area since the late 19th century include the work of CIEŚLIŃSKI and CZYŻEWSKA (2006). This work complements the previous report. The historic study (BŁOŃSKI 1890) documented the species composition of lichens from the late 19th century in the region of Niekłań, and especially about natural sandstone rocks located in this area. This is a very suitable location for following the dynamics of lichen biota over a long time period.

The aim of this study is to characterize the current state of lichen biota in positions that BŁOŃSKI (1890) investigated, and to trace its degree of extent and direction of change that have occurred during more than one hundred years. Particular attention was paid to population changes of rare and endangered species in the region of the Świętokrzyskie Mts and in Poland.

Characteristics of the research area

The elevation called Piekło Mt. (368 m asl), with a vast hilltop, is distinguished by large accumulations of natural rock outcrop. These rocks are composed of Lower Jurassic sandstone. In total there are over 70 separate rocks (URBAN 1996). The height of some of them is from 5 up to 8 meters, not counting a sandy cone line surrounding their foothills. The surface of the rocks is very diverse with interesting microrelief in the form of slats, grooves, ribs, cornices, cavities, etc., shaped by various processes of chemical and physical

weathering. Sandstone, which is the basic building material for the rocks, especially yellow sandstone, crumbles easily, making plant and lichen settlement difficult. The unequal size of the rocks, their diverse formation, and varied exhibition create a wide variety of habitats. Moreover, forest communities surrounding them affect their further differentiation, especially in the scope of moisture and light relations. The high humidity of the rocks, especially their northern area of exposure, favours the occurrence of only a few species of lichens. Mosses prevail in the area. The flat tops of the rocks are covered by a coat of silicate-ferruginous mineral, a few millimetres thick. The coat is mostly covered by mosses, with the rare appearance of vascular plants, and even more rarely spotted with some species of lichens of the *Cladonia* genus.

Part of the top of the Piekło Mt., with the greatest accumulation of rocks, with the most prominent forms and all features of natural beauty, has been under legal protection as a nature reserve since 1959 under the name of "Skałki Piekło pod Niekłaniem" ("Hell Rocks under Niekłań"). The reserve's area is 6.3 hectares. The subjects of protection are sandstone rocks and the rare relict fern *Asplenium septentrionalne*, which grew abundantly in the past, forming dense turfs in the crevices of rock outcrops (MASSALSKI, KAZNOWSKI 1928). In recent years, the fern has been found only in a single specimen. Outside the reserve, inside the forest communities are also found less imposing sandstone outcrops of rocks.

Forest communities in the reserve surrounding the rocks belong to two groups: fresh pine forest *Leucobryo-Pinetum* and mixed pine-oak forest *Querco-Pinetum*. Forest communities surrounding the reserve are commercial forests, with a floristic composition and structure similar to those that are inside the reserve. Some specimens of *Quercus petrea* and *Pinus sylvestris* in the reserve reach the age of 140–150 years, so they were already growing in the late 19th century. In addition, frequent tree species found in the reserve and outside are *Betula pendula*, more seldom *Abies alba*, *Fagus sylvatica* and *Larix decidua.* However, the trees in the village of Niekłań (hamlet of Kałuża), along the road leading to Szydłowiec, are venerable trees: *Betula pendula*, *Tilia cordata* and *Acer platanoides*. These old specimens of trees are being removed at the moment.

Materials and Methods

Field studies carried out in the reserve "Skałki Piekło pod Niekłaniem" and its surroundings were focused on as accurate a reference to the work of BŁOŃSKI (1890) as possible. The author mentions the following name of sites: "Niekłań", "Piekło pod Niekłaniem", and "Piekło", but still, the most com-

monly mentioned name is "Niekłań". Of all these sites BŁOŃSKI listed 45 species of lichens and one species of lichenicolous fungus. It is assumed that the author conducted the research on: the top of Piekło Mt, where there are numerous natural outcrops, in the village of Niekłań and probably along the forest road leading from Niekłań (Kałuża) to the top of Piekło Mt. The ecological character of some lichen species mentioned by BŁOŃSKI (1890) confirms the assumption that the author conducted the research outside forest communities, and thus in Niekłań. Therefore, four sites in the field were chosen. They could most likely have been places of BŁOŃSKI'S (1890) research:

– "Skałki Piekło pod Niekłaniem" reserve, within the limits laid down in the regulation establishing the reserve. The reserve area does not include all rocks;

– forest communities and sandstone rocks around the reserve. In the late 19th century, when there was no reserve outlined, BŁOŃSKI (1890) was able to conduct the research on the hilltop across the top of Piekło Mt., where outcrops of rocks are found;

– forest communities along the forest road leading from Niekłań to the reserve. Heavily eroded rocks overgrown with young pine trees, with a large amount of terrestrial lichen of the *Cladonia* genus are found here;

– trees by the road in Niekłań.

Only a few herbarium specimens have been preserved that were collected from this area by BŁOŃSKI in 1889. They are: *Cladonia squamosa* var. *squamosa, Dibaeis baeomyces, Hypogymnia physodes, Parmelia saxatilis, Platismatia glauca, Pseudevernia furfuracea* and *Umbilicaria polyphylla.*

The study included, as in the work of BŁOŃSKI (1890), all ecological groups of lichens, such as epiphytes, epixylithes, epilythes and epigeithes. Field studies were conducted between 2005–2007 (compare CIEŚLIŃSKI, CZYŻEWSKA 2006) and supplemented in 2010. Sterile lichens were determined by thin layer chromatography (TLC) in accordance with the procedures set out in the work of ORANGE et al. (2001).

A list of lichen species found in the past and present in the above sites is presented in Table 1.

Categories of threats to species in Poland were taken from CIEŚLIŃSKI et al. (2006) and in the Świętokrzyskie Mts from CIEŚLIŃSKI and ŁUBEK (2003). Nomenclature of lichen species follows SMITH et al. (2009) (compare FAŁTYNOWICZ 2003), KUKWA (2009) and lichenicolous fungi: CZYŻEWSKA and KUKWA (2009).

All sites are located in the large Ee34 square of the ATPOL system (compare CIEŚLIŃSKI, FAŁTYNOWICZ 1993). The specimens are deposited in the herbarium at Jan Kochanowski University in Kielce. The specimens collected by BŁOŃSKI are deposited in the herbarium at University of Warsaw.

Table 1
List of species of lichens and lichenicolous fungi found in the "Piekło pod Niekłaniem" reserve and its surroundings

No	Species	Data from Błoński (1890)	Data from 2006 and 2010, number of sites (substrate)	Status of threat in Świętokrzyskie Mts/Poland
1	2	3	4	5
1.	**Abrothallus parmeliarum* (Sommerf.) Arnold	+ on the thallus of *Parmelia saxatilis*	–	–
2.	*Absconditella lignicola* Vězda & Pišút	–	1 (l)	–/DD
3.	*Acarospora fuscata* (Nyl.) Arnold	–	1 (r)	–
4.	*Agonimia repleta* Czarnota & Coppins	–	1 (Q)	–
5.	*Amandinea punctata* (Hoffm.) Coppins & Scheid.	–	1 (Q), 4 (A, B, Pt, Ti)	–
6.	*Anaptychia ciliaris* (L.) Körb. ex A. Massal.	+	–	CR/EN
7.	*Arthonia spadicea* Leight.	–	2 (Q)	–
8.	*A. vinosa* Leight.	–	1 (Q), 2 (Q)	CR/NT
9.	*Arthothelium ruanum* (A. Massal.) Körb.	–	3 (F)	VU/NT
10.	*Aspicilia gibbosa* (Ach.) Körb.	+	–	DD/EN
11.	*A. caesiocinerea* (Nyl. ex Malbr.) Arnold	–	1 (r)	DD/–
12.	*Bacidia phacodes* Körb.	–	1 (Q), 4 (A)	LC/–
13.	*B. rubella* (Hoffm.) A. Massal.	+	4 (Ti)	EN/VU
14.	*B. subincompta* (Nyl.) Arnold	–	3 (F)	CR/EN
15.	*Baeomyces rufus* (Huds.) Rebent.	–	1 (r), 2 (r), 3 (r)	–
16.	*Bryoria fuscescens* (Gyeln.) Brodo & D. Hawksw.	–	1 (Q)	CR/VU
17.	*Bryoria jubata* auct. [*Bryopogon jubatum* (L.) *α prolixum* (Ach.)]	+	–	RE/RE
18.	*Buellia griseovirens* (Turner & Borrer ex Sm.) Almb.	–	1 (Q), 2 (Q, B), 4 (A, B, Pt, Ti)	–
19.	*Calicium adspersum* Pers.	–	2 (Q)	–
20.	*C. glaucellum* Ach.	–	3 (Q)	EN/VU
21.	*C. salicinum* Pers.	–	1 (Q), 3 (Q)	EN/VU
22.	! *Caloplaca chrysodeta* (Vain. ex Räsänen) Dombr.	–	1 (r)	–
23.	*C. holocarpa* (Hoffm.) A.E. Wade	–	4 (A, B, Ti)	–
24.	*Candelariella vitellina* (Hoffm.) Müll. Arg.	(+)	4 (B, Ti)	–
25.	*C. xanthostigma* (Pers. ex Ach.) Lettau	–	4 (A, Fr, Ti)	–
26.	*Cetraria aculeata* (Schreb.) Fr.	+	3 (s)	–
27.	*C. sepincola* (Ehrh.) Ach.	+	–	CR/EN
28.	! *Cetrelia monachorum* (Zahlbr.) W.L. Culb. & C.F. Culb.	–	1 (Q)	–

cont. table 1

1	2	3	4	5
29.	*Chaenotheca chrysocephala* (Turner ex. Ach.) Th. Fr.	–	1 (Q)	–
30.	*Ch. ferruginea* (Turner ex Sm.) Mig.	–	1 (Q)	–
31.	*Ch. furfuracea* (L.) Tibell	+	–	CR/NT
32.	*Cladonia cervicornis* (Ach.) Flot. subsp. *verticillata* (Hoffm.) Ahti	–	3 (s)	–
33.	*C. coccifera* (L.) Willd.	+	–	NT/–
34.	*C. coniocraea* (Flörke) Spreng.	–	1 (Q, r, s, l), 2 (Q), 3 (Ti)	–
35.	*C. deformis* (L.) Hoffm.	–	3 (s)	–
36.	*C. digitata* (L.) Hoffm.	+	1 (Q, r), 3 (T)	–
37.	*C. fimbriata* (L.) Fr.	+	1 (r), 3 (s, l), 4 (B)	–
38.	*C. floerkeana* (Fr.) Flörke	–	3 (s)	–
39.	*C. furcata* (Huds.) Schrad.	–	1 (r), 3 (s)	–
40.	*C. grayi* G. Merr. ex Sandst.	–	3 (s, l)	–
41.	*C. gracilis* (L.) Willd.	+	3 (s)	–
42.	*C. macilenta* Hoffm.	+	1 (s), 4 (B)	–
43.	*C. mitis* Sandst.	–	3 (s)	–
44.	*C. ochrochlora* Flörke	–	1 (B, Q, s)	–
45.	*C. phyllophora* Hoffm.	–	3 (s)	–
46.	*C. pleurota* (Flörke) Schaer.	–	1 (r), 3 (s)	–
47.	*C. polydactyla* (Flörke) Spreng.	–	1 (s)	DD/–
48.	*C. pyxidata* (L.) Hoffm.	+	3 (s)	–
49.	*C. rangiferina* (L.) F.H. Wigg.	+	3 (s)	–
50.	*C. squamosa* (Scop.) Hoffm. var. *squamosa*	+	1 (br, s), 3 (s)	–
51.	*C. subulata* (L.) F.H. Wigg.	–	3 (s)	–
52.	*C. uncialis* (L.) F.H. Wigg.	(+)	3 (s)	–
53.	**Clypeococcum hypocenomycis* D. Hawksw.	–	on the thallus of *Hypocenomyce scalaris* 1 (P, l)	–
54.	*Cornicularia normoerica* (Gunn.) Du Rietz	+	–	absent/VU
55.	*Dibaeis baeomyces* (L. f.) Rambold & Hertel	+	–	EN/NT
56.	*Dimerella pineti* (Ach.) Vězda	–	1 (P, Q), 3 (F)	–
57.	*Diploschistes scruposus* (Schreb.) Norman	+	1 (r)	–
58.	*Evernia divaricata* (L.) Ach.	+	–	RE/CR
59.	*E. prunastri* (L.) Ach.	+	1 (Q), 4 (Ti)	VU/NT
60.	*Graphis scripta* (L.) Ach.	+	1 (F)	NT/NT
61.	*Hypocenomyce anthracophila* (Nyl.) P. James & Gotth. Schneid.	–	1 (P, Q)	LC/–
62.	*H. scalaris* (Ach. ex Lilj.) M. Choisy	–	1 (B, l, P), 4 (B)	–

cont. table 1

1	2	3	4	5
63.	*Hypogymnia physodes* (L.) Nyl.	+	1 (B, P, Q), 2 (Q), 4 (B, Ti)	–
64.	*H. tubulosa* (Schaer.) Hav.	–	1 (Q)	NT/NT
65.	*Icmadophila ericetorum* (L.) Zahlbr.	+	–	RE/EN
66.	*Imshaugia aleurites* (Ach.) S.L.F. Meyer	(+)	1 (l)	–
67.	*Lecania cyrtella* (Ach.) Th. Fr.	–	4 (A)	–
68.	*Lecanora albella* (Pers.) Ach.	+	–	RE/EN
69.	*L. albellula* (Nyl.) Th. Fr.	–	4 (A)	–
70.	*L. carpinea* (L.) Vain.	+	4 (A)	–
71.	*L. chlarotera* Nyl.	–	4 (Ti)	–
72.	! *L. compallens* Herk & Aptroot	–	4 (Ti)	–
73.	*L. conizaeoides* Nyl. ex Cromb.	–	1 (P), 4 (B)	–
74.	*L. dispersa* (Pers.) Sommerf.	–	4 (B)	–
75.	*L. expallens* Ach.	–	4 (Ti)	–
76.	*L. glabrata* (Ach.) Malme	+	–	–
77.	*L. hagenii* (Ach.) Ach.	–	4 (A)	–
78.	*L. muralis* (Schreb.) Rabenh.	(+)	1 (r), 3 (r)	–
79.	*L. persimilis* (Th. Fr.) Nyl.	–	4 (Ti)	–
80.	*L. pulicaris* (Pers.) Ach.	–	1 (Q), 4 (B)	–
81.	*L. saligna* (Schrad.) Zahlbr.	–	4 (Ti)	–
82.	*L. swartzii* (Ach.) Ach.	–	1 (r)	–
83.	*L. varia* (Hoffm.) Ach.	–	4 (B)	–
84.	*Lecidea fuscoatra* (L.) Ach.	–	1 (r)	–
85.	*L. plana* (J. Lahm) Nyl.	–	3 (r)	NT/–
86.	*Lecidella elaeochroma* (Ach.) M. Choisy	(+)	4 (A, B, Pt, Ti)	–
87.	! *L. flavosorediata* (Vězda) Hertel & Leuckert	–	4 (A, Ti, Pt, Fr)	–
88.	! *Lepraria crassissima* (Hue) Lettau	–	1 (r)	–
89.	! *L. eburnea* J.R. Laundon	–	1 (Q), 2 (Q), 4 (Ti)	–
90.	*L. elobata* Tørnsberg	–	4 (Ti)	–
91.	*L. incana* (L.) Ach.	–	1 (r, s, Q, B), 2 (Q), 3 (F, Q), 4 (Ti)	–
92.	*L. jackii* Tørnsberg	–	1 (r), 2 (r)	–
93.	*L. lobificans* Nyl.	–	1 (r), 2 (Q)	–
94.	*L. membranacea* (Dicks.) Vain.	–	1 (r)	–
95.	*L. neglecta* (Nyl.) Lettau	–	1 (r)	–
96.	*L. rigidula* (B. de Lesd.) Tørnsberg	–	4 (Ti, Pt)	–
97.	*L. vouauxii* (Hue) R.C. Harris	–	1 (Q, B), 2 (Q), 4 (Ti)	–

cont. table 1

1	2	3	4	5
98.	**Lichenoconium erodens* M.S. Christ. & D. Hawksw.	–	on the thalus of *Platismatia glauca*, on apothecia of *Lecanora conizaeoides* 1 (Q), 4 (B)	–
99.	**Lichenoconium lecanorae* (Jaap) D. Hawksw.	–	on the apothecia of *Lecanora conizaeoides* 1 (P)	–
100.	! *Lichenomphalia umbellifera* (L.: Fr.) Redhead, Lutzoni, Moncalvo & Vilgalys	–	1 (l)	–/NT
101.	*Melanelixia fuliginosa* (Fr. ex Duby) O. Blanco, A. Crespo, Divakar, Essl., D. Hawksw. & Lumbsch	–	2 (Q), 4 (A, Ti)	–
102.	*M. subaurifera* (Nyl.) O. Blanco, A. Crespo, Divakar, Essl., D. Hawksw. & Lumbsch	–	4 (A)	–
103.	*Melanohalea exasperatula* (Nyl.) O. Blanco, A. Crespo, Divakar, Essl., D. Hawksw. & Lumbsch	–	4 (A, Ti)	–
104.	*M. olivacea* (L.) O. Blanco, A. Crespo, Divakar, Essl., D. Hawksw. & Lumbsch	+	–	RE/CR
105.	*Micarea botryoides* (Nyl.) Coppins	–	1 (r, s)	–
106.	! *M. lignaria* (Ach.) Hedl.	–	1 (B, r)	–
107.	*M. melaena* (Nyl.) Hedl.	–	1 (l)	VU/NT
108.	*M. micrococca* (Körb.) Gams ex Coppins	–	1 (Q, br), 2 (Q)	–
109.	*M. peliocarpa* (Anzi) Coppins & R. Sant.	–	1 (r)	DD/–
110.	*M. prasina* Fr.	–	1 (l, Q), 2 (Q)	–
111.	! *M. viridileprosa* Coppins & van den Boom	–	2 (br)	–
112.	*Miriquidica leucophaea* (Flörke ex Rabenh.) Hertel & Rambold	–	1 (r)	DD/VU
113.	! * *Monodictys epilepraria* Kukwa & Diederich	–	on the thallus of *Lepraria incana* 1 (r, Q)	–
114.	! *Mycobilimbia epixanthoides* (Nyl.) Vitik., Ahti, Kuusinen, Lommi & T. Ulvinen	–	1 (Q, br)	–
115.	*Mycoblastus fucatus* (Stirt.) Zahlbr.	–	4 (B)	–
116.	*Nephroma resupinatum* (L.) Ach.	+	–	RE/CR
117.	! *Ochrolechia bahusiensis* H. Magn.	–	1 (Q)	EN/VU
118.	*O. microstictoides* Räsänen	–	1 (Q), 2 (Q)	–
119.	*Opegrapha varia* Pers.	–	2 (Q)	VU/NT
120.	*Parmelia omphalodes* (L.) Ach.	+	1 (r)	–/EN
121.	*P. saxatilis* (L.) Ach.	+	1 (B, Q, r), 4 (B, Ti)	–

cont. table 1

1	2	3	4	5
122.	*P. sulcata* Taylor	+	1 (Q), 4 (Ti)	–
123.	*Parmelina tiliacea* (Hoffm.) Hale	–	4 (Ti)	CR/VU
124.	*Parmeliopsis ambigua* (Wulfen) Nyl.	–	1 (Q, l, r)	–
125.	*Peltigera canina* (L.) Willd.	+	–	CR/VU
126.	*Pertusaria albescens* (Huds.) M. Choisy & Werner	–	3 (Q), 4 (Ti)	LC/–
127.	*P. amara* (Ach.) Nyl.	–	3 (F)	LC/–
128.	*P. coccodes* (Ach.) Nyl.	–	1 (Q), 4 (Ti)	VU/NT
129.	*P. coronata* (Ach.) Th. Fr.	–	1 (Q), 2 (Q)	CR/VU
130.	*P. pertusa* (Weigel) Tuck.	+	1 (r), 2 (Q)	CR/VU
131.	*Phaeophyscia nigricans* (Flörke) Moberg	–	4 (A, Ti)	–
132.	*P. orbicularis* (Neck.) Moberg	+	4 (A)	–
133.	*Phlyctis argena* (Spreng.) Flot.	–	3 (F), 4 (B, Ti)	–
134.	*Physcia adscendens* H. Olivier	–	4 (A, B, Ti)	–
135.	*P. caesia* (Hoffm.) Fürnr.	(+)	4 (A)	–
136.	*P. dubia* (Hoffm.) Lettau	–	4 (B, Ti)	–
137.	*P. tenella* (Scop.) DC.	–	4 (A, B, Ti)	–
138.	*Physconia eneteroxantha* (Nyl.) Poelt	–	4 (A)	–
139.	*P. grisea* (Lam.) Poelt	–	4 (A, Fr)	–
140.	*Placynthiella dasaea* (Stirt.) Tørnsberg	–	1 (l)	–
141.	*P. icmalea* (Ach.) Coppins & P. James	–	1 (Q, l, br), 3 (s)	–
142.	*P. oligotropha* (J.R. Laundon) Coppins & P. James	–	3 (s)	–
143.	*Platismatia glauca* (L.) W.L. Culb. & C.F. Culb.	+	1 (P, Q, r)	NT/–
144.	*Pleurostica acetabulum* (Neck.) Elix & Lumbsch	–	4 (Ti)	CR/EN
145.	*Porina aenea* (Wallr.) Zahlbr.	–	3 (F)	–
146.	*Porpidia cinereoatra* (Ach.) Hertel & Knoph	–	1 (r)	LC/LC
147.	*P. crustulata* (Ach.) Hertel & Knoph	+	3 (r)	–
148.	*P. macrocarpa* (DC.) Hertel & A.J. Schwab	+	1 (r)	VU/LC
149.	*P. soredizodes* (Lamy ex Nyl.) J.R. Laundon	–	1 (r), 2 (r)	–
150.	*Pseudevernia furfuracea* (L.) Zopf	+	1 (Q), 2 (Q), 4 (B)	–
151.	*Psilolechia clavulifera* (Nyl.) Coppins	–	1 (r)	–/NT
152.	*Pycnothelia papillaria* Dufour	+	–	EN/EN
153.	*Ramalina farinacea* (L.) Ach.	–	1 (Q)	EN/VU
154.	*R. pollinaria* (Westr.) Ach.	+	1 (Q), 4 (Ti)	CR/VU
155.	*Scoliciosporum chlorococcum* (Graewe ex Stenh.) Vězda	–	4 (A, Ti)	–

cont. table 1

1	2	3	4	5
156.	*S. umbrinum* (Ach.) Arnold	–	1 (r)	–
157.	*Strangospora pinicola* (A. Massal.) Körb.	–	4 (A, Ti)	LC/LC
158.	*Trapelia obtegens* (Th. Fr.) Hertel	–	1 (r)	–
159.	*T. placodioides* Coppins & P. James	–	1 (r)	–
160.	*Trapeliopsis flexuosa* (Fr.) Coppins & P. James	–	1 (B, Q, P, s, l), 3 (s)	–
161.	*Tuckermanopsis chlorophylla* (Willd.) Hale	–	1 (Q)	EN/VU
162.	*Umbilicaria polyphylla* (L.) Baumg.	+	–	NT/LC
163.	*Usnea florida* (L.) Weber ex F.H. Wigg.	+	–	absent/CR
164.	*Vulpicida pinastri* (Scop.) J.E. Mattsson & M.J. Lai	+	–	VU/NT
165.	*Xanthoparmelia conspersa* (Ehrh. ex Ach.) Hale	+	1 (r)	–
166.	*Xanthoria candelaria* (L.) Th. Fr.	–	4 (B, Ti)	–
167.	*X. parietina* (L.) Th. Fr.	+	1 (Q), 4 (A, P, B)	–
168.	*X. polycarpa* (Hoffm.) Th. Fr. ex Rieber	–	4 (Ti)	–

Explanations: * – lichenicolous fungus; + – the species given by Błoński (1890) from the following sites: Niekłań, Piekło pod Niekłaniem, Piekło; (+) – species given by Błoński (1890) from neighbouring positions: Stąporków, Końskie; ! – species new to the Świętokrzyskie Mts; A – *Acer platanoides*; B – *Betula pendula*; F – *Fagus sylvatica*; Fr – *Fraxinus excelsior*; P – *Pinus sylvestris*; Pt – *Populus tremula*; Q – *Quercus* sp.; Ti – *Tilia cordata*; br – bryophytes; l – lignum; r – rocks of natural origin; s – soil.

Results and Discussion

Following the outcome of the study it was found that biota of the research area includes 168 species of lichens and lichenicolous fungi (Table 1). This area is distinguished by high species diversity of lichen biota in relation to other areas in Central Poland.

Currently, at the four researched sites, 148 species of lichens and lichenicolous fungi have been found. The biota is dominated by epiphytes – 95 species, including 78 found only on the bark of trees. Apart from forest communities on the bark of trees (site no 4) 57 species were found. On the outcrops of sandstone rocks 39 species (27 exclusively) grow. Terrestrial lichens include 24 species, lignicolous – 12 and bryophilous – 4. The biota is dominated by widespread and even common lichens, although species which are rare and endangered, both in the region and in the country, are also found there.

In comparison to the data provided by BŁOŃSKI (1890) the occurrence of 19 species of lichens – *Anaptychia ciliaris, Aspicilia gibbosa, Bryoria jubata, Cetraria sepincola, Chaenotheca furfuracea, Cladonia coccifera, Cornicularia normoerica, Dibaeis baeomyces, Evernia divaricata, Icmadophila ericetroum, Lecanora albella, L. glabrata, Melanohalea olivacea, Nephroma resupinatum, Peltigera canina, Pycnothelia papillaria, Umbilicaria polyphylla, Usnea florida,* and *Vulpicida pinastri,* and one species of lichenicolous fungus – *Abrothallus parmeliarum* has not been confirmed. Considering the number of lichen species (46) given by BŁOŃSKI (1890) it can be concluded that in the lichen biota unfavourable changes have taken place. Almost 44% of the species have disappeared from the study area within the last 100 years. Among them there are 6 species which currently belong to the category Regionally Extinct (RE) in the Świętokrzyskie Mts, and 4 to the category Critically Endangered (CR). Most of the species mentioned above belong to the high risk of extinction threat category in Poland (compare CIEŚLIŃSKI, CZYŻEWSKA 2006, CIEŚLIŃSKI et al. 2006).

The threat to the biota of lichens in the study area is a result of the general worsening of environmental conditions, mainly air pollution, and a reduction in moisture conditions. The condition of epiphytic lichen biota is negatively affected by cutting down old trees. Local threats to the nature reserve also arise from the high activity of tourists in the area. Their negative impact comes from excessive trampling of flora, even on the rock tops, and from the destruction of the rocks' surface by mechanically scratching inscriptions and drawings in soft sandstone, which had already been strongly emphasized by earlier investigators (MASSALSKI, KAZNOWSKI 1928).

The historic study of BŁOŃSKI (1890) provides information about 26 species, the presence of which has been confirmed. They have invariably been at their sites for over 100 years. Lichens which dominate here are now widespread, both in forest communities, as well as in open areas. In this group of species, among others, is *Parmelia omphalodes*, which grows on sandstone rocks in the reserve (site no 1). This is a very rare species in Poland. Its sites are known mainly from the 19th century, from the mountain areas and the Południowobałtyckie Lake District (FAŁTYNOWICZ 2003, BIELCZYK 2003). In the "Piekło pod Niekłaniem" reserve it is a very rare lichen nowadays. This species has been found only on one rock, and its thallus demonstrates decreased vitality. It is probably now disappearing from the area. At present a very rare species growing on sandstone rocks is *Xanthoparmelia conspersa*, mentioned by BŁOŃSKI (1890). A small residual lichen thallus was found on only one outcrop of rock. Among epiphytic lichens which are currently rare and threatened in the Świętokrzyskie Mts are: *Bacidia rubella, Graphis scripta, Pertusaria pertusa* and *Ramalina pollinaria*, which are reported in the historic study.

At the investigated sites 123 species that were not mentioned by BŁOŃSKI (1890) from "Skałki Piekło pod Niekłaniem" reserve and its surroundings (Table 1) were found. The author found some of them at sites in the neighbouring areas, i.e. Stąporków, and Końskie, without giving their exact location. They must have been widespread lichens in the region at the time, and with few exceptions must have had the same character they have at present. Among these species are: *Candelariella vitellina*, *Cladonia uncialis*, *Imshaugia aleurites*, *Lecidella elaeochroma*, *Physcia caesia* and *Lecanora muralis*.

Lichens new to the study area can be divided into two groups of species. The first group are the lichens (about 100 species) with a long-established and well-known place in taxonomic studies. It is difficult to determine whether these species occurred here in the late 19th century. Some species from this group were mentioned by BERDAU (1876) in other parts of the Świętokrzyskie Mts, mainly in the so-called "Łyse Mts". It cannot be stated clearly whether some of the species did not appear later in time than the study conducted by BŁOŃSKI (1890). Among them are species that are expansive, spreading especially in habitats with heavy anthropogenic factors e.g. *Amandinea punctata*, *Buellia griseovirens*, *Caloplaca holocarpa*, *Dimerella pineti*, *Lecanora conizaeoides, L. hagenii*, *L. pulicaris* and others. At present in this group are also interesting and rare species having the status of threatened and endangered lichens in the country. This group includes species which grow in forest communities, e.g. *Bacidia subincompta, Calicium adspersum, C. salicinum, C. glaucellum, Arthonia vinosa, Ramalina farinacea,* and *Pertusaria coronata*, and on aged lime trees in the village of Niekłań: *Parmelina tiliacea* and *Pleurosticta acetabulum*. These are very rare lichens, forming very small populations and growing only on old trees, mostly oaks and beeches. Their small size and residual thallus shows that these lichens are slowly disappearing from the studied area. With regard to rare species, even small environmental changes, or cutting down trees, lead to a complete disappearance of species in the area. The persistence of small populations of these species raise the natural values of the study area, particularly that of the, "Skałki Piekło pod Niekłaniem" reserve. The study of BŁOŃSKI (1890) also lacks a very interesting lichen *Lecanora swartzii*. It is now growing on sandstone rocks in the reserve (site no 1). This species probably appeared in this area some time later than when BŁOŃSKI'S study was conducted (compare CIEŚLIŃSKI, CZYŻEWSKA 2006).

The second (and large) group of species new to the investigated area are lichens which were not identified in the past and which have been listed in Poland only recently. This group includes about 20 species, mainly of the genera: *Lepraria* (*L. crassissima, L. eburnea, L. elobata, L. incana, L. jackii, L. lobificans, L. rigidula, L. vouauxi, L. membranacea, L. neglecta*), *Micarea*

(*M. botryoides, M. viridileprosa*) and *Placynthiella* (*P. dasaea, P. icmalea*), and also *Absconditella lignicola, Agonimia repleta, Cetrelia monachorum, Lecanora compallens, Lecidella flavosorediata, Mycobilimbia epixanthoides, Ochrolechia bahusiensis* and lichenicolous fungi: *Clypeococcum hypocenomycis, Lichenoconium erodens, L. lecanorae* and *Monodictys epilepraria*.

Particular attention should be put on the species of the genus *Lepraria* that occur on the bark of trees and highly overgrown sandstone rocks, creating specific layouts on them. On the bark of trees the following species have been recorded: *Lepraria eburnea, L. elobata, L. rigidula, L. vouauxi, L. incana* and *L. lobificans*. On the rocks, depending on the exhibition, various species of this genus occur with different abundance. On southern rocks grow: *Lepraria incana, L. jackii,* and *L. membranacea*, and on the north side: *L. crassissima, L. incana, L. jackii, L. lobificans,* and *L. membranacea*. These leprose lichens are often the only species which colonize the smooth, vertical walls of rocks. A specific habitat is occupied by *Lepraria neglecta*. The species has been recorded only on the top of rocks. The variety of epilithic lichens of the genus *Lepraria* enriches other crustaceous species such as *Caloplaca chrysodeta, Psilolechia clavulifera, Trapelia obtegens* and *T. placodioides*. Most of the species are mixed together, which often makes it difficult to identify them.

Some interesting species of lichens and lichenicolous fungi

Some rare species in Poland, which have recently been distinguished in lichenological studies, deserve special consideration. New sites in the investigated area contribute new information about their occurrence and habitat preferences. Some of them are new to the area of Central Poland.

Agonimia repleta – the second recording in Central Poland. So far it has also been recorded in Chęcińsko-Kielecki Landscape Park (ŁUBEK 2012).

Caloplaca chrysodeta – Leprose species characterized by a diffuse, unlimited, powdery, grey-yellow to orange thallus. It is easily identified by the reaction of the thallus with K+ purple. Known in Poland from the Beskid Sądecki Mts (ŚLIWA 1998) and the Tatra Mts (ALSTRUP, OLECH 1992). New to the Świętokrzyskie Mts and Central Poland.

Cetrelia monachorum – the species separated from the group of *Cetrelia olivetorum*. Based on its secondary metabolites (imbricaric acid – major and perlatolic acid – minor) identified by TLC. New to the Świętokrzyskie Mts.

Cladonia polydactyla – in Central Poland known from Spalski Landscape Park (CZYŻEWSKA 1972) and Załęczański Landscape Park (CZYŻEWSKA 1986).

Lecanora swartzii – the site in the investigated reserve is the only one known in Central Poland. The other sites are located in mountain areas: the Tatra Mts, Góry Stołowe Mts, and Karkonosze Mts (compare CIEŚLIŃSKI, CZYŻEWSKA 2006).

Lichenomphalia umbellifera – one of only a few lichens in Poland in which the mycobiont is *Basidiomycota*. Characterized by a dark green, granulate thallus, *Botrydina*-type, which grows yellow-brown cap-fruiting bodies. New to the Świętokrzyskie Mts and Central Poland. Known from southern and northern parts of the country (FAŁTYNOWICZ 2003).

Lepraria crassissima – new to the Świętokrzyskie Mts. In Poland known mainly from mountainous areas (KUKWA 2006).

Lepraria eburnea – new to the Świętokrzyskie Mts. In Poland known from both mountainous and lowland areas (KUKWA 2006).

Micarea lignaria – new to the Świętokrzyskie Mts and Central Poland. In Poland known from mountainous areas (CZARNOTA 2007).

Micarea viridileprosa – in the Świętokrzyskie Mts so far known from Świętokrzyski National Park (ŁUBEK 2007).

**Monodictys epilepraria* – Lichenicolous fungus given for the first time from the Central Poland area, from Wysoczyzna Bełchatowska Upland Plain and Niecka Włoszczowska Basin (CZYŻEWSKA et al. 2008). New to the Świętokrzyskie Mts.

Ochrolechia bahusiensis – the species separated from the group of *Ochrolechia androgyna* s. lat. based on its secondary metabolites (gyrophoric acid, trace of lecanoric acids and 3 substances of murolic acid complex) separated by TLC. Known from the Świętokrzyskie Mts under the name *O. androgyna* C., from Świętokrzyski National Park and Świnia Góra Mt. reserve (JABŁOŃSKA, KUKWA 2007, compare KUKWA 2009). *Ochrolechia bahusiensis* is a typical lowland lichen with very few mountain records.

Parmelia omphalodes – new to the Świętokrzyskie Mts and Central Poland. In Poland known from the southern part of the country (FAŁTYNOWICZ 2003).

Psilolechia clavulifera – in the Świętokrzyskie Mts known from the Świętokrzyski National Park so far (ŁUBEK 2007). From Central Poland known from Spalski Landscape Park (ŁUBEK 2007).

Conclusions

1. The study of lichen biota changes in the same sites over more than a hundred years provides reliable data with which to determine the extent and trends of lichen biota impoverishment, as well as the sensitivity of selected species to changes in the natural environment.

2. The results of the present study of lichen biota in the area of Piekło Mt. confirms the legitimacy of the classification of many species to appropriate categories of endangered species, as accepted in the studies as red lists.

3. The degree of adverse change in lichen biota in the analyzed time interval is high. The losses are about 44% of the total species recorded in the late 19th century in the same area.

4. Investigated sites are distinguished by the presence of rare and endangered species not only in the region of the Świętokrzyskie Mts., but in many other areas of the Polish lowlands, for example, *Parmelia omphalodes, Lecanora shwartzii, Bacidia subincompta, Calicium adspersum, C. salicinum, C. glaucellum, Pleurosticta acetabulum, Arthonia vinosa, Pertusaria coronta, Ramalina farinacea,* and *Cetraria chlorophylla*. With the exception of *Lecanora shwartzii* these lichens are very rare in the investigated sites, forming small, isolated populations with clearly reduced viability, and showing signs of disappearing lichens.

5. Outcrops of sandstone rocks in the Piekło Mt. and the surrounding forest communities, currently under reserve protection, are a good subject for conducting research on the dynamics of lichen biota. Permanent monitoring of the inventory of lichens in the area is necessary.

6. The studies provide material with which to assess the threat to lichens in north-western parts of the Świętokrzyskie Mts, in the region of a large forested area in the Świętokrzyska forest. Due to the absence of adequate data, this area was not included in the study *Red list of threatened lichens in the Świętokrzyskie Mountains* (CIEŚLIŃSKI, ŁUBEK 2003).

Acknowledgements

I would like to thank Prof. Stanisław Cieśliński (Jan Kochanowski University, Kielce) for valuable suggestions on the manuscript and to Dr Martin Kukwa (University of Gdańsk) for revising the determination of some sterile taxa.

Translated by PETER FOULDS

Accepted for print 23.03.2012

References

ALSTRUP V., OLECH M. 1992. *Checklist of the lichens of the Tatra National Park, Poland*. Zeszyty Naukowe Uniwersytetu Jagiellońskiego, Prace Botaniczne, 24: 185–206.

BERDAU F. 1876. *Lišajniki isledovannyje do sich por w oblasti Varšavskogo Učebnogo Okruga z ukazaniem do morfologiu i fizjologiu lišajnikov*. Tipogr. K. Kovalevskego, Warszawa, pp. 1–125.

Bielczyk U. 2003. *The lichens and allied fungi of the Polish Western Carpathians*. [In:] *The lichens and allied fungi of the Polish Western Carpathians – an annotated checklist*. Ed. U. Bielczyk, Institute of Botany, Polish Academy of Sciences, Kraków, pp. 23–232.

Błoński F. 1890. *Wyniki poszukiwań florystycznych skrytokwiatowych dokonanych w ciągu lata r. 1889 w obrębie 5 powiatów Królestwa Polskiego*. Pam. Fizjogr., 10: 129–190.

Cieśliński S., Czyżewska K. 2006. *Changes in the lichen biota of the „Skałki Piekło pod Niekłaniem" Nature Reserve and its surroundings* (*Central Poland*) *during the past 100 years*. [In:] *Central European lichens*. Eds. A. Lackovičová, A. Guttová, E. Lisická, P. Lizoň, Institute of Botany, Slovak Academy of Sciences. Mycotaxon, Ithaca, pp. 259–269.

Cieśliński S., Czyżewska K., Fabiszewski F. 2006. *Red list of the lichens in Poland*. [In:] *Red list of plants and fungi in Poland*. Eds. Z. Mirek, K. Zarzycki, W. Wojewoda, Z. Szeląg, W. Szafer Institute of Botany, Polish Academy of Sciences, Kraków, pp. 71–89.

Cieśliński S., Fałtynowicz W. 1993. *Note from editors*. [In:] *Atlas of the geographical distribution of lichens in Poland 1*. Eds. S. Cieśliński, W. Fałtynowicz, W. Szafer Institute of Botany, Polish Academy of Sciences, Kraków, pp. 7–8.

Cieśliński S., Łubek A. 2003. *Czerwona lista porostów zagrożonych w Górach* Świętokrzyskich. Monogr. Bot., 91: 143–158.

Czarnota P. 2007. The lichen genus *Micarea* (*Lecanorales, Ascomycota*) in Poland. Polish Botanical Studies, 23: 1–199.

Czyżewska K. 1972. *Porosty rezerwatu leśnego „Spała"*. Zesz. Nauk. UŁ, 51, Nauki Mat.-Przyr., 2: 145–158.

Czyżewska K. 1986. *Flora porostów naziemnych w Załęczańskim Parku Krajobrazowym* (*Wyżyna Wieluńska*). Acta Univ. Lodz., Folia Sozol., 2: 315–341.

Czyżewska K., Kukwa M. 2009. *Lichenicolous fungi of Poland. A catalogue and key to species*. [In]: *Biodiversity of Poland 11*. Ed. Z. Mirek. W. Szafer Institute of Botany, Polish Academy of Sciences, Kraków, pp. 1–133.

Czyżewska K., Hachułka M., Łubek A., Zaniewski P. 2008. *Distribution of some lichenicolous fungi in Poland.* II. Acta Mycol., 43(2): 193–206.

Fałtynowicz W. 2003. *The lichens, lichenicolous and allied fungi of Poland. An annotated checklist*. Institute of Botany, Polish Academy of Sciences, Kraków, pp. 1–435.

Jabłońska A., Kukwa M. 2007. *The lichen genus Ochrolechia in Poland I. O. androgyna s. lat. and O. arborea*. Herzogia, 20: 13–27.

Kukwa M. 2006. *The lichen genus Lepraria in Poland*. Lichenologist, 28(4): 293–305.

Kukwa M. 2009. *The lichen genus Ochrolechia in Poland III with a key and notes on some taxa*. Herzogia, 22: 43–66.

Lindner L. 1972. *Geneza i wiek skałek piaskowcowych góry Piekło koło Niekłania*. Acta Geol. Polon., 22(1): 169–179.

Łubek A. 2007. *Antropogeniczne przemiany bioty porostów Świętokrzyskiego Parku Narodowego*. Fragm. Flor. Geobot. Polonica. Suppl., 10: 3–94.

Łubek A. 2012. *Nowe dane o interesujących gatunkach porostów z Gór Świętokrzyskich i terenów przyległych*. Fragm. Flor. Geobot. Polonica 19(1) (in print).

Massalski E., Kaznowski K. 1928. *Piaskowcowe skałki góry Piekło koło Niekłania*. Ochr. Przyr., 8: 29–33.

Orange A., James P.W., White F.J. 2001. *Microchemical methods for the identification of lichens*. British Lichen Society, London, pp. 1–101.

Smith C.W., Aptroot A., Coppins B.J., Fletcher A., Gilbert O.L., James P.W., Wolseley P.A. 2009. *The lichens of Great Britain and Ireland.* The British Lichen Society, London, pp. 1–1046.

Śliwa L. 1998. *Antropogeniczne przemiany lichenoflory Beskidu Sądeckiego*. Prace Botaniczne, 31: 1–158. Kraków.

Toborowicz K. 1992. *Flora porostów rezerwatu „Skałki Piekło koło Niekłania"* (*Wyżyna Kielecko-Sandomierska*). [In:] *49 Zjazd Polskiego Towarzystwa Botanicznego*. Ed. S. Cieśliński, Kielce, 1–5.09.1992. Streszczenia referatów i plakatów. p. 168.

Urban J. 1996. *Jaskinie pseudokrasowe w piaskowcach liasowych Piekła pod Niekłaniem*. Prace Naukowe Uniwersytetu Śląskiego, 1527: 113–123.

POLISH JOURNAL OF NATURAL SCIENCES
Abbrev.: Pol. J. Natur. Sc., Vol 27(2): 151–164, Y. 2012

MICROALGAE – CULTIVATION METHODS*

Marcin Dębowski, Marcin Zieliński, Mirosław Krzemieniewski, Magda Dudek, Anna Grala

Department of Environmental Protection Engineering
University of Warmia and Mazury in Olsztyn

K e y w o r d s: microalgae, cultivation, open ponds, photobioreactors, dark systems.

A b s t r a c t

Investigations into the use of algae for energy production have been carried out for many years. A key issue is the selection of technology for culture and acquisition of algae biomass for energetic purposes. The proliferation and culture of algae may be conducted with a variety of methods, beginning from strictly monitored methods in closed laboratory systems, to less predictable methods in open systems. Though many systems have been developed so far, unfortunately none of them may be found cost-effective. Photobioreactors are expensive and require high exploitation inputs (lighting, supply of carbon dioxide), they additionally pose some difficulties in exploitation e.g. due to overgrowing and restricted light penetration. In contrast, some technological systems are applied in the technical scale that merge certain elements of open and closed systems. There is also a possibility of algae biomass proliferation and culture in dark systems.

MIKROALGI – METODY HODOWLI

Marcin Dębowski, Marcin Zieliński, Mirosław Krzemieniewski, Magda Dudek, Anna Grala

Uniwersytet Warmińsko-Mazurski w Olsztynie
Katedra Inżynierii Ochrony Środowiska

S ł o w a k l u c z o w e: mikroglony, hodowla, stawy otwarte, fotobioreaktory, systemy ciemne.

Address: Marcin Dębowski, University of Warmia and Mazury, ul. Romana Prawocheńskiego 1, 10-719 Olsztyn, Poland, phone: + 48 (89) 523 41 24, e-mail: marcin.debowski@uwm.edu.pl.
* This research was carried out under the Key Project No. POIG.01.01.02-00-016/08 titled: *Model agroenergy complexes as an example of distributed cogeneration based on local and renewable energy sources*. Project financed under the OP Innovative Economy.

Abstrakt

Badania nad możliwością wykorzystania glonów do celów energetycznych prowadzone są od wielu lat. Jedną z najważniejszych kwestii, warunkujących opłacalność produkcji glonów, jest wybór odpowiedniego systemu hodowlanego. Stosowane są zarówno reaktory zamknięte, jak również technologie oparte na systemach otwartych. Testowane w warunkach laboratoryjnych oraz w skali technicznej rozwiązania technologiczne są zwykle mało opłacalne. Fotobioreaktory są drogie i stwarzają trudności eksploatacyjne, np. ze względu na zarastanie oraz ograniczenie dostępu światła. Systemy otwarte charakteryzują się niską efektywnością i produktywnością. W skali technicznej stosowane są również rozwiązania, które łączą pewne elementy systemów otwartych i zamkniętych. Istnieje również możliwość namnażania biomasy glonów w systemach ciemnych.

Introduction

Investigations into the use of algae for energy production have been carried out for many years. The first attempts to culture and exploit algae for fuel production purposes have been undertaken during the II World War by German researchers. The possibility of using algae biomass in processes of methane fermentation was discovered in the fifties of the XXth century by, among others (*Algae culture*. 1953). In the sixties, algae biomass production has been started in a technical scale in open systems. In the eighties the USA Department of Energy has initiated a research on the use of algae in energy production (Aquatic Species Program). In the successive years technologies have been developed for biodiesel production and algae culture in photobioreactors, and commercial bio-refineries have been established in, among others, Turkey and the United States. Nowadays, a number of research and implementation programs have been underway worldwide, including the EU Member States, that are aimed at boosting the effectiveness of algae biomass production and conversion into biofuels (LO et al. 2010, MUSSATTO et al. 2010, VIJAYARAGHAVAN 2009). A few thousands of patents linked with technologies of algae biomass production, separation and conversion into biofuels are registered annually, which indicates a great global interest of scientists in this respect (GALLAGHER 2011, STEPHENS et al. 2010).

A key issue is the selection of technology for culture and acquisition of algae biomass for energetic purposes. The proliferation and culture of algae may be conducted with a variety of methods, beginning from strictly monitored methods in closed laboratory systems, to less predictable methods in open systems (MOLINA-GRIMA 1999).

Owing to the site the process is being run at, systems are divided into:

– open systems (outdoor systems), making use mainly of open aquifers, ponds, though under favorable environmental conditions (light exposure and high temperature) they may also refer to photobioreactors (PBRs). Applied

mainly for economic concern, they provide no possibility for monitoring conditions of the culture process, and are sensitive to environmental pollution, predators and competitive species. The open systems include (BOROWITZKA 1999):
  – traditional ponds, ground or concrete with large (up to 250 ha) area and depth of up to 0.5 m;
  – circular ponds with mechanical or convective mixing;
  – race track-type ponds with a paddle wheel;
  – cascade ponds.
– closed systems, making use mainly of various types of photobioreactors or small tanks. Such cultures assure, most of all, the possibility of constant monitoring over lighting and temperature, protection against predators, parasites and competitive species of algae. The closed systems include:
  – the sack system of "large bags", operating in a sequential or semi-continuous mode,
  – tubular photobioreactors with horizontal or vertical orientation, or inclined at any angle. A technological solution of this type may be arranged in parallel or spirally as the Biocoil type reactor,
  – plate photobioreactors.

Owing to the duration and method of running a culture, systems may be divided into:
– batch systems, where the culture is inoculated in a single dose into the culture medium and cultivated until the moment when the population of cultured organisms reaches its maximum or when cell density in the culture approximates the maximum. Then harvest occurs, and the cultured biomass is separated from the culture medium;
– continuous systems with continuous inflow and outflow of culture medium and continuous reception of biomass produced;
– semi-continuous systems with partial reception of biomass and continuous addition of culture media so as to maintain algae growth rate close to the maximum.

Open systems

A very important issue is the choice of technology for culture and production of algae biomass for energy purposes. Owing to economic concerns, the systems used currently in the industrial scale are open ponds. In most cases, their design is very simple. These are usually ground tanks with a large surface area and depth of up to 0.5 m, stirred mechanically by means of a paddle agitator. They are built in the form of a round pond or a racetrack. The culture

media applied usually include chemical substances or sewages containing appropriate quantities of biogenic compounds, if necessary supplemented with microelements. Carbon dioxide is acquired directly from atmospheric air through simple diffusion. An advantage of this technological solution is simple and inexpensive construction, its drawbacks however include high water losses as a result of evaporation, low yield of biomass production, limited possibilities of culture of specific algae species susceptible to various infections, diseases and parasites. Systems of this type prove successful in regions with high insolation and unlimited access to water, hence at the seaside areas. These technologies are commonly applied in the Asian countries, Mexico, the USA (e.g. in Arizona) as well as in Europe, including e.g. Italy, Spain and even the Netherlands, mainly for cultures of Spirulina and Chlorella genera algae (DEMIRBAS et al. 2011).

Due to the very good results are obtained as breeding ponds in a race track that we decided to just describe the type of open systems. This type of technology not only high productivity but also is probably the most reasonable choice in economic terms.

The system consists of shallow open ponds racetrack type with a width of 2.0–3.0 m and depth 0.1–0.3 m, made of PVC, clay or paved area from 1000 to 5000 m^2 (MOLINA-GRIMA 2003). Pond type of race track design is based on

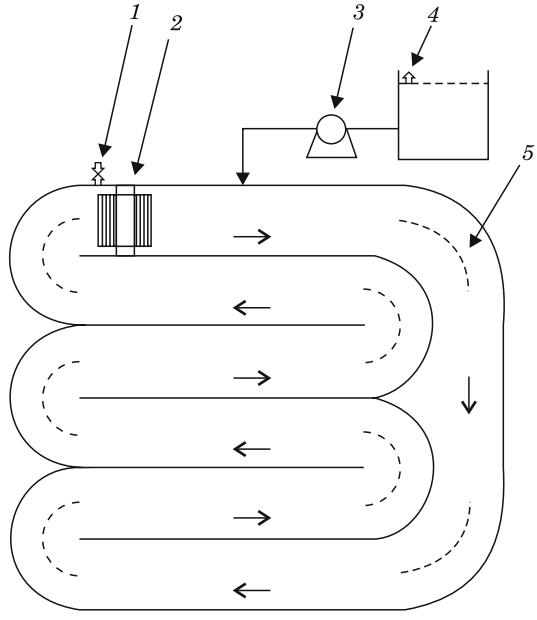

Fig. 1. Rays ways reactor: *1* – discharge of biomass; *2* – puddlewheel; *3* – nutrient dosing pomp; *4* – medium tank; *5* – baffle

a series made in a closed loop recirculation duct. Excitation processes of mixing and flow in such a device there, using the paddle wheel. Channels of the pond can be constructed of concrete or compressed earth and are lined with white plastic. The medium can be fed into the system on a continuous basis during the day when the culture is efficiently radiated. Nutrients are administered before the paddle wheel, where the stream begins breeding. Grown biomass is received from the device before the paddle wheel at the end of the loop. To prevent falling of algae stirrer is maintained constantly in motion. Rays ways reactor is shown in Figure 1.

If the wastewater system are introduced, it can remove up to 35 g BZT/m^2 d (175 g BZT/m^3 d pond with a depth of 0.2 m) compared to 5–10 g BZT/m^2 d (5–10 g BOD/m^3 d pond at a depth of 1 m) achieved in conventional stabilization ponds (RACAULT and BOUTIN 2005). This project also requires a much shorter hydraulic retention time (HRT) in the system, amounting to 2–6 d (MARA and PEARSON 1998) compared to 10–40 d in traditional ponds (CRITES, TCHOBANOGLOUS 1998). Despite a much better cleaning efficiency, few such systems are currently used for wastewater treatment in the world.

Photobioreactors

A completely different approach to the problem of proliferation and culture of alga biomass is the application of closed systems, the so-called photobioreactors. A variety of these systems have been developed so far, including: horizontal tubular photobioreactors, horizontal tubular photobioreactors or sloping under any angle, biocoil type reactors, continuous or semi-continuous big bag systems, or flat-plate photobioreactors (BOROWITZKA 1999, AMIN 2009) – Figure 2.

Photobioreactors are much more universal devices that may be applied under various climatic conditions. The closed character of bioreactors restricts evaporation, eliminates the problem of parasites and predators, whilst artificial lighting assures optimal conditions for photosynthesis. Such conditions afford the possibility for running cultures of specific algae species, e.g. these with a high concentration of oil in biomass (CUARESMA et al. 2011, DEMIRBAS 2011).

The first closed photobioreactor in use was a big gags photobioreactor (BAYNES et al. 1979, WATSON 1979). It consisted of large sterile plastic bags ca. 0.5 m in diameter with an adjusted aeration system. Most of those systems have been designed to operate in the batch mode, however semi-continuous systems do happen as well. This variant of the system was developed by COHEN and ARAD (1989) and its modification consisted in using bags with a smaller

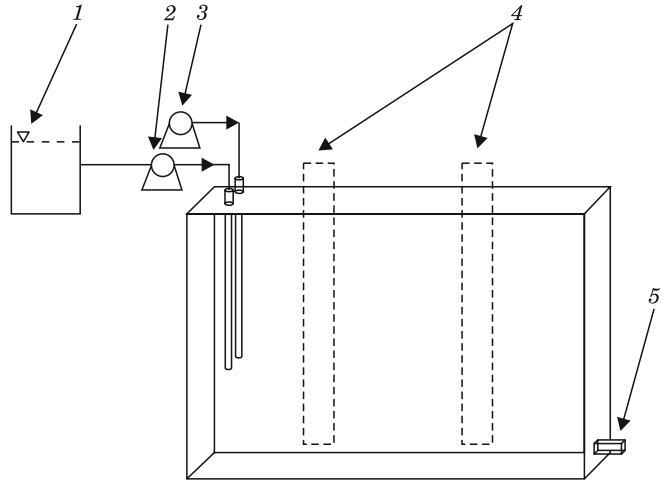

Fig. 2. Plate photobioreactor: *1* – medium tank; *2* – nutrient dosing pomp; *3* – air pomp; *4* – light source; *5* – discharge of biomass

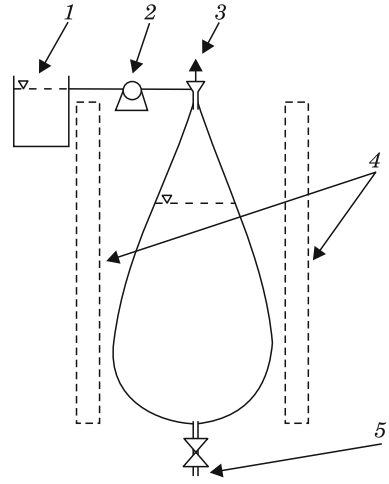

Fig. 3. Big bags photobioreactor: *1* – medium tank; *2* – nutrient dosing pomp; *3* – gas outlet; *4* – light source; *5* – discharge of biomass

diameter. So far, however, a few companies have been exploiting this system (Figure 3).

The key problem linked with its exploitation is the necessity of running the process indoor, for there is no possibility of controlling temperature. In addition, a relatively large diameter of the bags poses problems with culture lighting, which in turn constitutes a factor diminishing system's productivity.

A reactor of this type requires high workload and does not assure thorough mixing, which may cause the collapse and reduction of process effectiveness.

Most of tubular reactors are made of glass or polycarbonate (PC), and the flow of medium and supply of gases proceed through pumps or, preferably, by means of the airlift system. They may be constructed in the horizontal (MOLINA et al. 2001), vertical or inclined (TREDICI and ZITTELLI 1998, UGWU et al. 2002), and conical orientation (WATANABE and SAIKI 1997). The aeration and mixing of culture in a tubular photobioreactor is usually conducted with the use of air pumps or the airlift system. This reactor may be exploited also outdoor, as it possesses a vast illumination surface area. In contrast, one of its main drawbacks is a low mass transfer. This phenomenon occurs as a result of an increasing level of oxygen along with increasing sizes of this type of reactors. Investigations have shown that a very high level of dissolved oxygen (DO) may easily be reached in the tubular reactors (MOLINA et al. 2001). Furthermore, of key significance is the process of photoinhibition ongoing in the tubular reactor under external (outdoor) conditions (VONSHAK and TORZILLO 2004). Once the system is scaled up by increasing tubes' diameter, the ratio of illumination surface area to system volume is decreasing. In this case, the cells at the lower part of the tube will not receive enough light for cell growth (due to light shading effect) unless there is a good mixing system. Then, the effectiveness of providing light to cells may be achieved through the improvement of the mixing system (UGWU et al. 2003, UGWU et al. 2005).

An additional difficulty in a tubular photobioreactor is temperature control. Though it is feasible to apply a thermostat, it is an expensive and difficult to implement solution. Worthy of notice is also the possibility of adherence of algae cells to the walls of the reactor. In addition, a long tubular reactor is characterized by gradients of oxygen and CO_2 transported alongside the tubes (CAMACHO RUBIO et al. 1999, UGWU et al. 2003). An increase in pH value would lead to the necessity of frequent re-carbonization, which in turn would increase algae production expenditures.

A Biocoil type photobioreactor is a tubular photobioreactor composed of a transparent, plastic tube with a small diameter (2.4–5.0 cm), which is screw-wrapped around a vertical tube with a large diameter (BOROWITZKA 1999). A few parallel systems of tubes are coupled through collectors with a pumping system that may be realized through the airlift system or a variety of pumps. The type of the pump applied depends on algae type. The reactor may be equipped in a gas exchange system. Temperature may be controlled manually or automatically. Reactor's design assures uniform mixing and minimizes the adherence of algae cells to the internal walls of the tubes. Reactor's operation may be fully automated, which enables reducing costs of the production process. The system may also be designed so as to assure the axenic conditions (Figure 4).

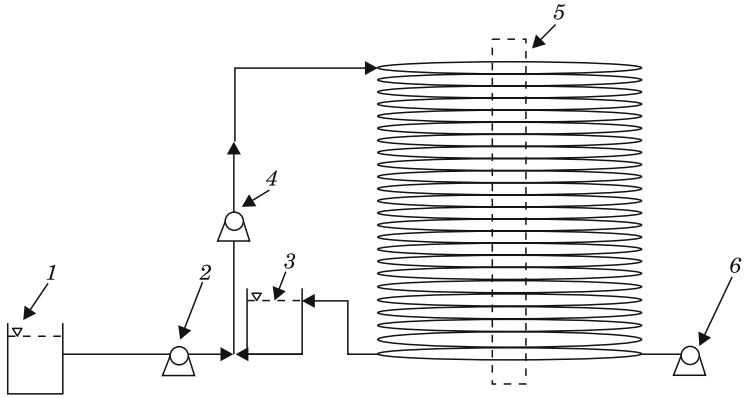

Fig. 4. Biocoil photobioreactor: *1* – medium tank; *2* – nutrient dosing pomp; *3* – discharge of biomass; *4* – circulation pump; *5* – light source; *6* – air pomp

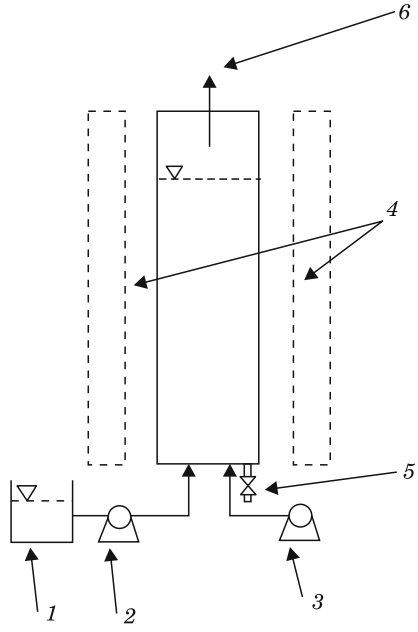

Fig. 5. Column photobioreactor: *1* – medium tank; *2* – nutrient dosing pomp; *3* – air pomp; *4* – light source; *5* – discharge of biomass; *6* – gas outlet

Various configurations of vertical-column photobioreactors have been extensively studied in view of their applicability for cultivation of algae (CHOI et al. 2003, VEGA-ESTRADA et al. 2005, GARCIA MALEA LOPEZ et al. 2006, KAEWPINTONG et al. 2007). They are compact, low-cost, and easy to operate monoseptically (SANCHEZ MIRON et al. 2002). In addition, they are very promising for

large-scale cultivation of algae. Studies have shown that bubble-column and airlift photobioreactors (up to 0.19 m in diameter) may attain the final biomass concentration and specific growth rate that are comparable to values typically reported for narrow tubular photobioreactors (SANCHEZ MIRON et al. 2002). Some bubble column photobioreactors are either equipped with two draft tubes or constructed as split cylinders. In the first case, the mixing occurs between the riser and the downcomer zones of the photobioreactor through the walls of the draft tubes (Figure 5).

Some photobioreactors may be equipped in external lighting with fluorescence lamps or light-emitting diodes (LED), which has become the focus of interest in recent studies (WANG et al. 2007). Those reactors are also equipped in stirrers to assure the transfer of algae cells in the whole volume of the system. Air and CO_2 are supplied to the reactor from the bottom, so as to assure the maximally long time of algae cells contact with gases. This type of photobioreactor may also be equipped in automated sensors measuring the intensity of lighting, to enable the exploitation of solar energy of both natural and artificial origin (OGBONNA et al. 1999). In this case, the artificial source of solar energy is switched on only at diminished intensity of the natural source (in cloudy whether or at night). There is also a possibility of applying light pipes for distribution of radiation in cylindrical photobioreactors (MATSUNAGA t al. 1991). One of the main advantages of this type of bioreactors is the feasibility of hot- or pressure-sterilization of their contents. Furthermore, solar energy may be supplied to the reactors in a continuous mode (both in daytime and at night) by coupled application of artificial and natural sources of light.

Though many systems have been developed so far, unfortunately none of them may be found cost-effective. Photobioreactors are expensive and require high exploitation inputs (lighting, supply of carbon dioxide), they additionally pose some difficulties in exploitation e.g. due to overgrowing and restricted light penetration. In contrast, some technological systems are applied in the technical scale that merge certain elements of open and closed systems. For instance, in Turkey the racetrack-type ponds were located in greenhouses, which has a positive impact on reduced evaporation and restricted access of predators, enables achieving temperature stability and applying additional lighting if necessary. It also affords the possibility of introducing an additional source of CO_2 in the form of e.g. combustion gases, to the greenhouse's interior. It seems that these types of solutions may prove successful also in Poland (UGWU et al. 2008). The table 1 shows advantages and disadvantages of commonly used systems for the cultivation of algae.

Table 1

Advantages and disadvantages of commonly used systems for the cultivation of algae

Type of system		Advantages	Disadvantages
Open systems	round ponds, racetrack-type ponds	relatively economical, easy to use and clean after completion of culture, good for the cultivation of algae on a large scale	low ability to control culture conditions, difficulty with cultivation algae in the long term, low productivity, large size, limited number of culture species, cultivation susceptible to external factors (predators, disease, pollution)
Closed systems	photobioreactors column (vertical)	the high mass transfer, good mixing and low stress, low energy consumption, high potential for scalability, easy to ensure sterility, good for the immobilization of algae, reduce photoinhibition and photooxidation	a small area of exposure, their construction requires the use of sophisticated materials, the possibility of hydrodynamic stress, decrease in surface exposure with increasing diameter of the column
	photobioreactors plate	the large surface area exposure, suitable for outdoor culture, good for the immobilization of algae, good availability of light, good productivity, biomass, relatively cheap, easy to clean, low concentration of oxygen	increasing the size of the reactor requires the use of multiple chambers and supporting structures, the problems of controlling the culture temperature, the risk of fouling the walls, the possibility of hydrodynamic stress in some species of algae
	horizontal tubular photobioreactors	the large surface area exposure, suitable for outdoor culture, good productivity, biomass, relatively cheap	fluctuations in pH, dissolved oxygen and CO_2 in the pipe length, the risk of fouling the walls, requires a large surface

Technological parameters of autotrophic cultivation

Irrespective of the fact whether algae biomass is produced in open systems or in closed photobioreactors, appropriate technological parameters ought to be assured that determine fast development and growth of the algae biomass (COHEN 1991). For optimal growth algae need appropriate lighting at the level of 200–400 fmol photons m^{-2} s^{-1}, which corresponds to ca. 1/10 of the lighting provided directly from the Sun. Under conditions of intensive culture, the quantity of CO_2 that has to be supplied to the system reaches ca. 1.83 kg per 1 kg of produced biomass. In this case, wastewaters, fresh or salty water

supplemented with nitrate and phosphate fertilizers may serve as the culture medium. Significant microelements in such a culture include nitrogen, phosphorus, iron and in the case of selected species also silicon. Unlike that of nitrogen and phosphorus, the availability of carbon rarely suppresses the growth of algae. Production performance in fresh waters is often diminished by the availability of phosphates (CHISTI 2007). In turn, the availability of silicon is a factor reducing the growth of diatoms. A number of algae species require an external source of vitamins, often thiamine, biotin, B_{12} and riboflavin, purins, pyrimidine and other growth factors. The temperature of the culture should oscillate in the range of 20–30°C, whereas pH value in the range of 6 to 8. Water saturation with oxygen should not exceed 400% owing to the arrestment of photosynthesis and ongoing processes of photoinhibition. Worthy of notice is also that the life cycle of algae used for biofuels production reaches 7 days (TAMBURIC et al. 2011).

Dark systems

There is also a possibility of algae biomass proliferation and culture in dark systems (BOUARABA et al. 2004) (Figure 6). These are the so-called heterotrophic cultures, in which appropriate carbonic compounds are fed to bioreactors as a culture medium for the algae. Such cultures are run with acetate or glucose as a source of carbon. This system has for the first time been used in *Chlorella* culture, which was described by KAWAGUCHI and SOONG in 1980. As reported by LEE (1997), in the year 1996, approximately 550 t of this alga were produced in Japan. The Martek Inc. company (USA) runs a heterotrophic culture of *Crypthecodinium cohnii* to be used for the production of long-chain unsaturated fatty acids (KYLE et al. 1998). Typical conditions of the heterotrophic culture include:

- temperature: 26–28°C;
- no light;
- agitation with the rate of 200–480 rpm;
- pH from 6.1 to 6.5;
- culture medium containing ca. 20 g of glucose or acetate/L of culture;
- necessary supply of nitrogen and phosphorus compounds in a quantitative C:N:P ratio of 9:1.25:1.25.

A culture in the heterotrophic system has several advantages. Systems of fermentation are well recognized, much is known about their design and operation. Another advantage is a high concentration of biomass that may range from 20 to 100 g d.m./L (RADMER and PARKER 1994, RUNNING et al.

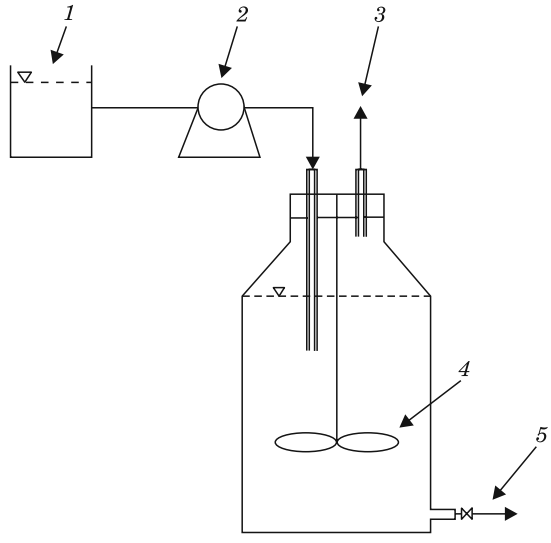

Fig. 6. Fermentation tank: *1* – medium tank; *2* – nutrient dosing pomp; *3* – gas outlet; *4* – stirrer; *5* – discharge of biomass

1994). The major drawbacks of this system are that it cannot be applied to all species of algae and that the process itself poses some technological complications (BOUARAB et al. 2004, OGBONNA 1996, PEREZ-GARCIA 2011).

Conclusion

Nowadays algae are perceived as one of the types of biomass with a very high energetic potential. In the technical scale, they are cultured mainly for feedstuff or foodstuff purposes, however there are also some biorefineries operating in the technical scale. The exploitation of this type of substrate in the systems of methane fermentation seems also promising, which has been proved by results of worldwide investigations.

Translated by JOANNA MOLGA

Accepted for print 9.03.2012

References

Algae Culture. 1953. Ed. Burlew, J.S. From Laboratory to Pilot Plant. Carnegie Institution of Washington, Washington, DC.

AMIN S. 2009. *Review on biofuel oil and gas production processes from microalgae.* Energy Convers. Manage., 50: 1834–1840.

BAYNES S.M., EMERSON L., SCOTT A.P. 1979. *Production of algae for use in the rearing of larval fish*. Fish. Res. Tech. Report., 53: 13–18.

BOROWITZKA M.A. 1999. *Commercial production of microalgae: ponds, tanks, tubes and fermenters*. J. Biotechnol., 70: 313–321.

BOUARAB L., DAUTA A., LOUDIKI M. 2004. *Heterotrophic and mixotrophic growth of Micractinium pusillum Fresenius in the presence of acetate and glucose: effect of light and acetate gradient concentration*. Water Res., 38: 2706–2712.

CAMACHO RUBIO F., ACIEN FERNANDEZ F.G., SANCHEZ PEREZ J.A., GARCIA CAMACHO F., MOLINA-GRIMA E. 1999. *Prediction of dissolved oxygen and carbon dioxide concentration profiles in tubular photobioreactors for microalgal culture*. Biotechnol. Bioeng., 62: 71–86.

CHISTI Y. 2007. *Biodiesel from microalgae*, Biotechnol. Adv., 25: 294–306.

COHEN E., ARAD S. 1989. *A closed system for outdoor cultivation of Porphyridium*. Biomass., 18: 59–67.

COHEN E., KOREN A., ARAD S.M. 1991. *A closed system for outdoor cultivation of microalgae*, Biomass and Bioenergy, 1(2): 83–88.

CHOI S.L., SUH I.S., LEE C.G. 2003. *Lumostatic operation of bubble column photobioreactors for Haematococcus pluvialis cultures using a specific light uptake rate as a control parameter*. Enzyme Microb. Technol., 33: 403–409.

CRITES R.C., TCHOBANOGLOUS G. 1998. *Small and decentralized wastewater systems*. McGraw-Hill, Boston.

CUARESMA M., JANSSEN M., VÍLCHEZ C., WIJFFELS R.H. 2011. *Horizontal or vertical photobioreactors? How to improve microalgae photosynthetic efficiency*. Bioresource Technol., 102: 5129–5137.

DEMIRBAS A., DEMIRBAS M.F. 2011. *Importance of algae oil as a source of biodiesel*. Energy Convers. and Manage., 52: 163–170.

GALLAGHER B.J. 2011. *The economics of producing biodiesel from algae*. Renew. Energ., 36(1): 158–162.

GARCIA-MALEA LOPEZ M.C., DEL RIO SANCHEZ E., CASAS LOPEZ J.L., ACIEN FERNANDEZ F.G., FERNANDEZ SEVILLA J.M., RIVAS J., GUERRERO M.G., MOLINA-GRIMA E. 2006. *Comparative analysis of the outdoor culture of Haematococcus pluvialis in tubular and bubble column photobioreactors*. J. Biotechnol., 123: 329–342.

KAEWPINTONG K., SHOTIPRUK A., POWTONGSOOK S., PAVASANT P. 2007. *Photoautotrophic high-density cultivation of vegetative cells of Haematococcus pluvialis in airlift bioreactor*. Bioresource Technol., 98: 288–295.

KAWAGUCHI K. 1980. *Microalgaeproduction systems in Asia*. G. Shelef, C.J. Soeder, Editors, Algae biomass production and use, Elsevier/North Holland Biomedical Press, Amsterdam, 25–33.

KYLE D.J., REEB S.E., SICOTTE V.J. 1998. *Dinoflagellate biomass, methods for its production, and compositions containing the same*. USA Patent 5: 711–983.

LEE Y.K. 1997. *Commercial production of microalgae in the Asia-Pacific rim*. J. Appl. Phycol., 9: 403–411.

LO Y.C., CHEN C.Y., LEE C.M., CHANG J.S. 2010. *Sequential darkephoto fermentation and autotrophic microalgal growth for high-yield and CO2-free biohydrogen production*. Int. J. Hydrogen Energ., 35: 10944–10953.

MARA D., PEARSON H. 1998. *Design manual for waste stabilization ponds in mediterranean countries*. Lagoon Technol. Int., Leeds, England.

MATSUNAGA T., TAKEYAMA H., SUDO H., OYAMA N., ARIURA S., TAKANO H., HIRANO M., BURGESS J.G., SODE K., NAKAMURA N. 1991. *Glutamate production from CO2* by marine cyanobacterium Synechococcus sp. using a novel biosolar reactor employing light diffusing optical fibers, Appl. Biochem. Biotechnol., 28/29: 157–167.

MOLINA GRIMA E., ACIEN FERNANDEZ F.G., GARCIA CAMACHO F., CHISTI Y. 1999. *Photobioreactors: light regime, mass transfer, and scaleup*, J. of Biotechnol., 70: 231–247.

MOLINA E., FERNÁNDEZ J., ACIÉN F.G., CHISTI Y. 2001. *Tubular photobioreactor design for algal cultures*. J. of Biotechnol., 92(2): 113–131.

MOLINA-GRIMA E., BELARBI E.H., ACIEN FERNANDEZ F.G., ROBLES-MEDINA A., CHISTI Y. 2003. *Recovery of microalgal biomass and metabolites: process options and economics*. Biotechnol. Adv., 20: 491–515.

Mussatto S.I., Dragone G., Guimarães P.M.R., Silva J.P.A., Carneiro L.M., Roberto I.C., Vicente A., Domingues L., José A. 2010. *Technological trends, global market, and challenges of bio-ethanol production.* Biotechnol. Adv., 28: 817–830.

Ogbonna J.C., Tanaka H. 1996. *Night biomass loss and changes in biochemical composition of cells during light/dark cyclic culture of Chlorella pyrenoidosa*. J. Ferment. Bioeng., 82(6): 558–564.

Ogbonna J.C., Soejima T., Tanaka H. 1999. *An integrated solar and artificial light system for internal illumination of photobioreactors.,* J. Biotechnol. 70: 289–297.

Perez-Garcia O., Froylan M.E. Escalante De-Bashan L.E., Bashan Y. 2011. *Heterotrophic cultures of microalgae: Metabolism and potential products*, Water Res., 45: 11–36.

Radmer R.J., Parker B.C. 1994. *Commercial applications of algae-opportunities and constraints*. J. Appl. Phycol., 6: 93–98.

Racault Y., Boutin C. 2005. *Waste stabilization ponds in France: state of the art and recent trends*. Water Sci. Technol., 12: 1–9.

Running J.A., Huss R.J., Olson P.T. 1994 *Heterotrophic production of ascorbic acid by microalgae*. J. Appl. Phycol., 6: 99–104.

Sanchez Miron A., Ceron Garcia M.C., Garcia Camacho F., Molina Grima E., Chisti Y. 2002. *Growth and characterization of microalgal biomass produced in bubble column and airlift photobioreactors: studies in fed-batch culture.* Enzyme Microb. Technol., 31: 1015–1023.

Soong P. 1980. *Production and development of Chlorella and Spirulina in Taiwan.* G. Shelef, C.J. Soeder, Editors , Algae Biomass, Elsevier/North Holland Biomedical Press, Amsterdam, 97–113.

Stephens E., Ross I.L., Mussgnug J.H., Wagner L. D., Borowitzka M.A., Posten C., Kruse O., Hankamer B. 2010. *Future prospects of microalgal biofuel production systems.* Trends Plant Sci., 15(10): 554–564.

Tamburic B., Zemichael F.W., Maitland G.C., Hellgardt K. 2011. *Parameters affecting the growth and hydrogen production of the green alga Chlamydomonas reinhardtii*. Int. J. Hydrogen Energ., 36(13): 7872–7876.

Tredici M.R., Zittelli G.C. 1998. *Efficiency of sunlight utilization: tubular versus flat photobioreactor*. Biotechnol. Bioeng., 57: 187–197.

Ugwu C.U., Ogbonna J.C., Tanaka H. 2002. *Improvement of mass transfer characteristics and productivities of inclined tubular photobioreactors by installation of internal static mixers.* Appl. Microbiol. Biotechnol., 58: 600–607.

Ugwu C.U., Ogbonna J.C., Tanaka H. 2003. *Design of static mixers for inclined tubular photobioreactors.* J. Appl. Phycol., 15: 217–223.

Ugwu C.U., Ogbonna J.C., Tanaka H. 2005. *Light/dark cyclic movement of algal cells in inclined tubular photobioreactors with internal static mixers for efficient production of biomass.* Biotechnol. Lett, 27: 75–78.

Ugwu C.U., Aoyagi H., Uchiyama H. 2008. *Photobioreactors for mass cultivation of alga*. Biores. Technol., 99: 4021–4028.

Wang C.Y., Fu C.C., Liu Y.C. 2007. *Effects of using light-emitting diodes on the cultivation of Spirulina platensis.* Biochem. Eng. J., 37: 21–25.

Vega-Estrada J., Montes-Horcasitas M.C., Dominigues-Bocanegra A.R., Canizares-Villanueva R.O. 2005. *Haematococcus pluvialis cultivation in split-cylinder internal-loop airlift photobioreactor under aeration conditions avoiding cell damage.* Appl. Microbiol. Biotechnol., 68: 31–35.

Vijayaraghavan K., Karthik R., Kamala Nalini S.P. 2009. *Hydrogen production by Chlamydomonas reinhardtii under light driven sulfur deprived condition*. Int. J. Hydrogen Energ., 34: 7964–7970.

Vonshak A., Torzillo G. 2004. *Environmental stress physiology*. [In:] *Handbook of microalgal culture*. Ed. A. Richmond. Blackwell Publishers, Oxford, 57–82.

Watanabe Y., Saiki H. 1997. *Development of photobioreactor incorporating Chlorella sp. for removal of CO_2 in stack gas.* Energy Convers. Manage., 38: 499–503.

Watson A.S. 1979. *Aquaculture and algae culture. Process and Production,* Noyes Data Corporation, NJ.

POLISH JOURNAL OF NATURAL SCIENCES
Abbrev.: Pol. J. Natur. Sc., Vol 27(2): 165–179, Y. 2012

PHYTOPLANKTON AS AN INDICATOR OF TROPHIC CHANGES IN A LAKE (LAKE KORTOWSKIE, NORTHERN POLAND)

***Bożena Jaworska*[1], *Bogusław Zdanowski*[2]**

[1] Department of Applied Ecology
University of Warmia and Mazury in Olsztyn
[2] Department of Hydrobiology
Institute of Fisheries in Olsztyn

Key words: phytoplankton, lake, taxonomic structure, biomass, trophic status.

Abstract

The aim of this study was to analyze multi-annual changes of the taxonomic structure and the intensity of the algal community development in phytoplankton of Kortowskie Lake, as a basis for determination of trophic state in the lake. There were observed qualitative and quantitative changes in the phytoplankton community. Species richness decreased in the subsequent years of the study. The number of Bacillariophyceae and Chlorophyta species declined, while the opposite trend was recorded in case of Cyanoprokaryota. The analysis of shifts in species composition revealed that the rate of species disappearance from the biocenosis was higher than appearance of new ones. The most intensive changes in the species composition affected the Bacillariophyceae and Chlorophyta associations. The stability of the Cyanoprokaryota biocenosis was remarkably higher and their constancy of occurrence increased. The rate of phytoplankton growth was high and was increasing in time. The intensity of phytoplankton development was determined by cyanoprokaryotes dynamics, which share in the total biomass was gradually increasing. The the blue-green algae limited the development of other plankton groups. The analysis of the multi-annual variations in the phytoplankton community of Kortowskie Lake indicated a trophic state in the ecosystem that were identified as progressing eutrophic status.

Address: Bożena Jaworska, University of Warmia and Mazury, Michała Oczapowskiego 5, 10-957 Olsztyn, Poland, phone: +48 (89) 523 35 95, e-mail: bozena.jaworska@uwm.edu.pl

FITOPLANKTON JAKO WSKAŹNIK ZMIAN TROFICZNYCH W JEZIORZE (JEZIORO KORTOWSKIE, POLSKA PÓŁNOCNA)

***Bożena Jaworska*[1], *Bogusław Zdanowski*[2]**

[1] Katedra Ekologii Stosowanej
Uniwersytet Warmińsko-Mazurski w Olsztynie
[2] Zakład Hydrobiologii
Instytut Rybactwa Śródlądowego w Olsztynie

Słowa kluczowe: fitoplankton, jezioro, struktura taksonomiczna, biomasa, stan trofii.

Abstrakt

Celem badań było przeanalizowanie wieloletnich zmian w strukturze taksonomicznej i intensywności rozwoju fitoplanktonu oraz określenie na tej podstawie stanu troficznego jeziora. Fitoplankton Jeziora Kortowskiego zmieniał się zarówno w aspekcie jakościowym, jak i ilościowym. Jego bogactwo taksonomiczne zmniejszało się w kolejnych latach. Malała liczba gatunków Bacillariophyceae i Chlorophyta, a zwiększała się Cyanoprokaryota. Wymianę gatunkową charakteryzowało większe tempo ustępowania gatunków niż pojawiania się nowych. Największe zmiany w składzie gatunkowym występowały w Bacillariophyceae i Chlorophyta, stabilność biocenozy Cyanoprokaryota była największa. Wzrastała również stałość występowania sinic. Rozwój fitoplanktonu był bardzo intensywny i z czasem wzrastał. O intensywności jego rozwoju decydowały Cyanoprokaryota, których udział w biomasie ogólnej był coraz większy. W warunkach postępującej dominacji sinic rozwój innych grup glonów był coraz słabszy. Analiza wieloletnich tendencji zmian w zbiorowisku fitoplanktonowym Jeziora Kortowskiego dała postawę do wnioskowania o stanie trofii jeziora i określeniu go jako stanu postępującej eutrofii.

Introduction

Lakes are relatively dynamic ecosystems that change over time. Their progressing trophic stage is a natural course of their development on condition that it runs in equilibrium state considering, both, physico-chemical and biotic features. The modification of factors, which combined acting determines the maintenance of the ecological equilibrium, may stimulate the increase in eutrophication rate (DILLON, RIGLER 1975, VEZJAK et al. 1998, ANNEVILLE et al. 2002). This process, understood as a set of symptoms resulting from an excessive supply of nutrients, causes significant changes in the structure and functioning of particular trophic units of the ecosystem (FORSBERG et al. 1978, CURRIE 1990, CARPENTER et al. 1997, BURGI et al. 1999, DANILOV and EKELUND 1999, REYNOLDS 2000). The phytoplankton community is one of them.

Algal associations respond sensitively to changes in water quality indicating even weak variations in, both, abiotic or biotic features. Phytoplankton development may modify physico-chemical properties of water and also, as a first link of a trophic chain, determine further trophic relations in the

environment (SCHINDLER 1978, MCQUEEN et al. 1986, DAUTA et al. 1990, LAFFORGUE et al., BAIRD et al. 2001, HAY and KUBANEK 2002). Analysing of the algal species composition, taxonomic structure and the intensity of phytoplankton development is highly useful in the assessment of the trophic state of the reservoir.

The aim of the research was to analyze multi-annual changes in the taxonomic structure and the estimation of the intensity of algal community development in phytoplankton of Kortowskie Lake as a basis for determination of trophic state in the lake.

Study area

The studies were conducted on Lake Kortowskie. It is located in north-eastern part of Poland, in the Mazurian Lakeland, within the limits of the town of Olsztyn. The lake has an elongated shape along the axis north-south. The reservoir is created by three distinct parts: the south basin of a maximal depth of 17.2 m, the north basin of a maximal depth of 15.7 m and the middle part that is relatively shallow, of a maximal depth of 6 m, that separates the two basins. The lake's surface area is 94 ha and its volume is 5 323 000 m^3 at water table of 103.3 m above the sea level (SYNOWIEC 1965). The total drainage basin of the lake is 38.0 km^2. The direct drainage basin of the lake is 102 ha. The lake is supplied by 5 inflows. There is only one river outflow – the Kortówka River – the tributary of the Łyna River, that is located in the southeastern part of the reservoir. The amount of waters flowing out of the lake is regulated by a weir that enables the removal of hypolimnion water by a pipeline installed in the southern part of the lake (Lake Kortowskie has been restored by the method of removal of nutrient-rich hypolimnion water to impede progressing eutrophication of the reservoir). The northern part of the lake is separated from the southern one (by shallows of a depth of 6 m) and is not indirectly influenced by pipeline activity similarly as the water layer from 0 to 6 m depth in the entire lake.

Materials and Methods

Studies of phytoplankton were carried out at two sites located in the deepest part of the two local basins: in the southern (S site) and northern (N site) part of the lake. Both stations had been recognized by CHUDYBA (1974, 1975) as representative. Phytoplankton analyses began in 1987, during spring mixing, and were finished in 1991 during winter water stagnation; next studies lasted from spring 1999 to winter 2000. The frequency of sampling depended

on the season. Samples were taken three times during summer periods, and two times in spring, autumn and winter periods. Preliminary qualitative phytoplankton analysis was conducted on living organisms collected by plankton net. Fundamental qualitative and quantitative plankton analyses were carried out basing on fixed samples obtained from water column (0–5 m) and concentrated by settling method. Qualitative analysis was performed according to STARMACH (1989) using drop method to calculate the number of individuals. Phytoplankton biomass was calculated basing on cell volume measurements (HEUSDEN 1972, STARMACH 1989). Qualitative and quantitative phytoplankton analyses were performed using the following microscope magnifications: 1.25 × 10 × 40 or 1.25 × 10 × 100.

Statistical characteristics of results included: minimal values (min.), maximal values (max.), mean values (x) and standard deviation (SD). Normality was assessed with the Shapiro-Wilks test. Significance of differences in mean phytoplankton biomass recorded at particular sites and years were tested using the Mann-Whitney test or Kruskal-Wallis test. Similarity of algal associations in the successive years was measured by hierarchical classification of cluster analysis. The relationships between changes of phytoplankton biomass and the content of chlorophyll *a* and the selected physico-chemical water properties were tested by means of the Pearson correlation. The following water features were taken into account: a Secchi disk visibility, water temperature, nitrogen and phosphorus contents.

Results

Phytoplankton qualitative structure

In the first period of the study (1987–1991), it was recorded from 233 to 251 species in Lake Kortowskie, but the species number was successively decreasing. Particularly low species richness of phytoplankton was found in 1999/2000 – 198 algal taxa. The differences between sites in taxonomic structure barely reached 4.4%. Bacillariophyceae and Chrysophyceae were the most taxonomically diversified groups which were represented by 97–103 species in 1987–1991. Their share in the total number of phytoplankton taxa ranged from 40.6 to 41.6%. Within this systematic groups the proportion of Bacillariophyceae amounted to 80%, while Chrysophyceae reached only 20%. Considering the limnological year 1999/2000, only 72 species from Bacillariophyceae and Chrysophyceae were present that constituted 36.4% of the total number of taxa. The flora of diatoms was predominated by the following genera: *Asterionella, Fragilaria, Aulacosiera, Stephanodiscus, Diatoma* and *Tabellaria*. *Dinobryon* was the most frequent taxon among chrysophytes.

Chlorophyta showed pattern of changes similar to Bacillariophyceae. Eighty-seven of their species were recorded in 1987/1988 while in the successive years – 87, 86 and 80 algal species, respectively. Chlorophyta constituted about 34–35% of the total number of algal taxa in the lake. In 1999/2000 their share decreased to 30.3%, while the number of their species dropped to 60. The most frequent were the species from the following genera: *Pediastrum, Coelastrum, Crucigenia, Kirchneriella, Oocystis, Scenedesmus, Tetraedron, Tetrastum* and *Dactylosphaerium* and *Pandorina*, *Eudorina, Phacotus*. In group of Cyanoprokaryota, 39, 40, 41 and 35 species were described in the lake in the successive years during the period 1987–1990. The proportion of cyanoprokaryotes was estimated at the level 15–16.4% of the total number. In the 1999/2000, the growth of Cyanoprokaryota proportion by about 10% (up to 24%) was recorded. Then, the number of their species reached 48. Among them, most often recorded species were the representatives of the following genera: *Microcystis, Anabaena, Aphanizomenon, Woronichinia, Planktothrix, Limnothrix, Aphanocapsa* and *Aphanothece.* The share of Euglenophyta and Dinophyta was low and did not exceed 8% and 4%. Twenty one species representing Euglenophyta belonged to the three genera i.e. *Euglena, Phacus* and *Trachelomonas*. There were recorded only 15 species from the Dinophyta and their contribution to the planktonic community was similar (Figure 1).

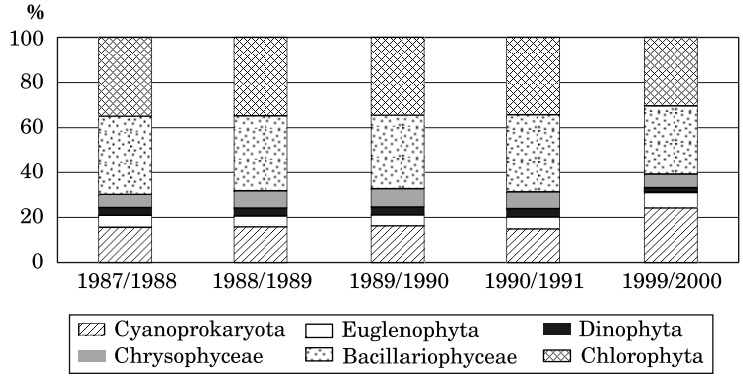

Fig. 1. The share of the numbers of species in particular systematic groups of phytoplankton in Lake Kortowskie in the years 1987–1991 and 1999–2000

Phytoplankton quantitative structure

The mean annual biomass reached 8.2 mg dm^{-3} (SD ± 4.6) in the years 1987–1991 and it varied in from 7.9 (SD ± 5.2) to 8.5 mg dm^{-3} (SD ± 4.4). This parameter value significantly increased up to 18.5 mg dm^{-3} (SD ± 9.6) in 1999/2000 ($H = 100.74$, $p < 0.001$). In Lake Kortowskie, phytoplankton showed

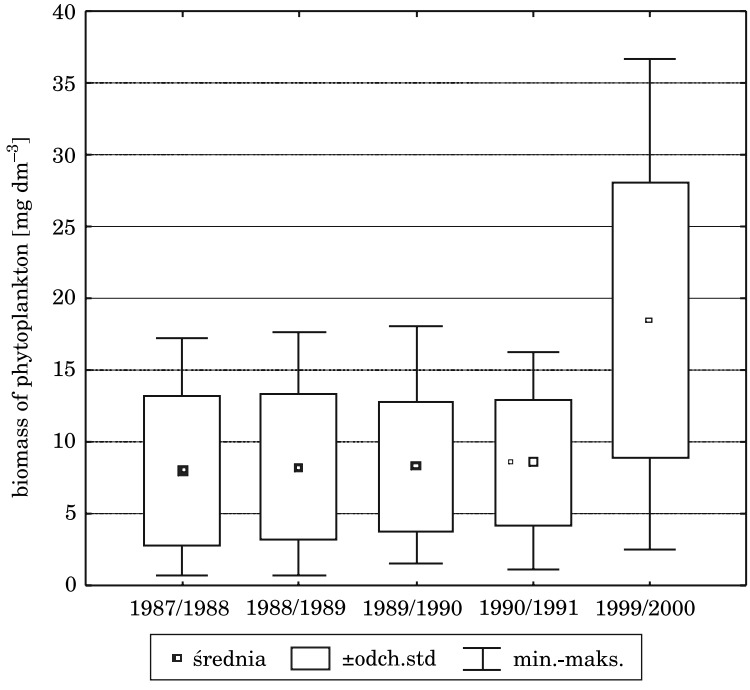

Fig. 2. Dynamics of algal biomass in the epilimnion of Lake Kortowskie in the years 1987–1991 and 1999–2000

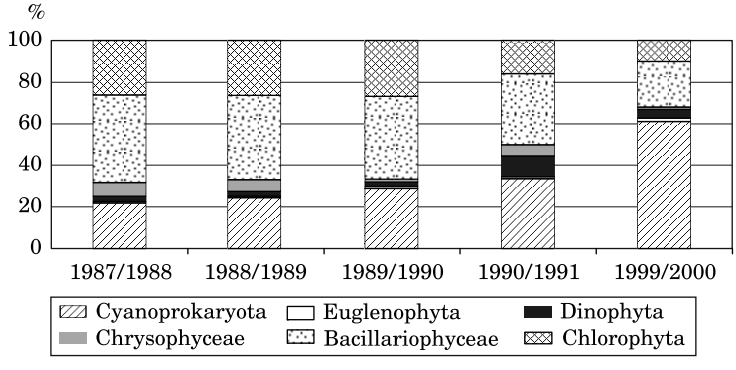

Fig. 3. The share of particular systematic groups in the biomass of phytoplankton in Lake Kortowskie in the years 1987–1991 and 1999–2000

similar quantitative structure at particular sites ($p > 0.001$, during the whole period of the study). In 1987–1991, phytoplankton biomass dynamics was determined by the development of Bacillariophyceae and Chrysophyceae. The most important species among them were: *Asterionella formosa* Hass.,

Fragilaria crotonensis Kitt. and *Fragilaria capucina* Des. and also, at the end of the period of study, *Aulacosiera granulata* (Ehr.) Sim., *Aulacosiera islandica* (O. Müll.) Sim. and *Stephanodiscus rotula* (Kutz.) Hend.. Then, the biomass of this algal group and its share in the total phytoplankton biomass slightly dropped from 4.8 mg dm^{-3} (SD ± 4.2) to 3.5 mg dm^{-3} (SD ± 2.6) on average, and from 48.8% to 39.5%. In 1999/2000, the contribution of Bacillariophyceae and Chrysophyceae declined to 23.1%. At one time, the Chlorophyta mean biomass reached 2.5 mg dm^{-3} (SD +2.6) in 1987-1989 constituting about 26% of the total. This proportion decreased to 15.9% in 1990/1991 and the mean biomass dropped to 1.4 mg dm^{-3} (SD ± 1.5). Then a slight increase was recorded, to 1.9 mg dm^{-3} (SD ± 1.8), which value constituted barely 10% of the total algal biomass in 1999/2000. The share of Chlorophyta gradually decreased and the same was recorded in case of dominants i.e. *Pandorina morum* (O.F. Müll.) Bory, *Pediastrum boryanum* (Tur.) Men., *Coelastrum microporum* Näg. A. Braun and *Phacotus lenticularis* (Her.) Stein. Considering Cyanoprocaryota, their mean annual biomass showed the trend to rise from 2.1 mg dm^{-3} (SD ± 2.4) to 3.0 mg dm^{-3} (SD ± 2.5) and their share in the community went up from 21.9 to 33.6% considering the time between 1987 and 1991. More intensive growth of this algal group was detected in 1999/2000 when their mean annual biomass reached 12.1 mg dm^{-3} (SD ± 8.5) and their proportion in the algal community increased up to 61%. Among Cyanoprokaryota, the most abundant were the following taxa: *Microcystis aeruginosa* (Kütz.) Kutz., *Anabaena spiroides* Kleb., *Anabena flos-aquae* (Lyng.) Breb. and additionally *Aphanizomenon flos-aquae* (L.) Ralfs, *Woronichinia naegeliana* (Unger) Elen., *Limnothrix planctonica* (Wolosz.) Meff. and *Limnothrix redeckei* (Van Goor) Meff., and their contribution was gradually increasing. The biomass of Euglenophyta did not reach 0.7 mg dm^{-3} and the proportion – 1.6% of the total. The biomass of Dinophyta usually did not exceed 0.4 mg dm^{-3} and 4% by share, except for the year 1999/2000 when it reached 0.8 mg dm^{-3} (SD ± 1.0), on average, and constituted about 10% of the total algal biomass. *Ceratium hirundinella* (O.F. Müll.) Duj. was the taxon that was responsible for a sudden development of this algal group (Figure 2, Figure 3).

The relationships between phytoplankton and selected physico-chemical parameters

In order to estimate the relationships between physico-chemical water properties and phytoplankton dynamics, the following features were selected: the Secchi disc visibility, water temperature, nitrogen and phosphorous contents in lake water. It was shown that phytoplankton biomass was growing

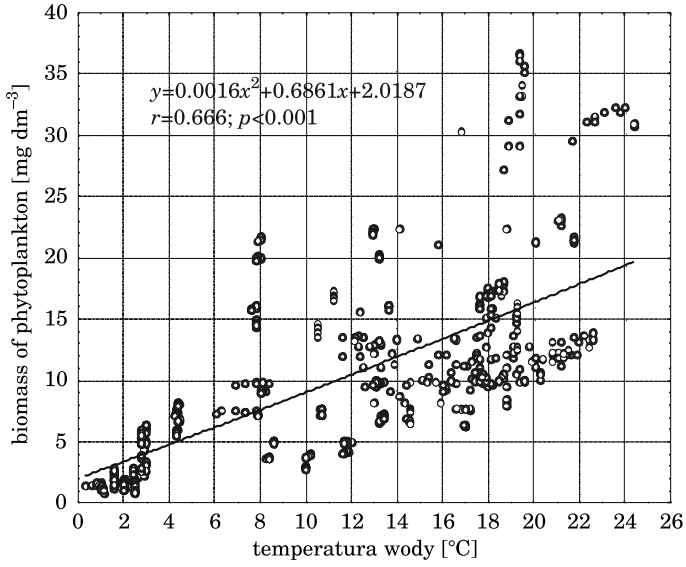

Fig. 4. The correlation coefficient between phytoplankton biomass and water temperature in the epilimnion of Lake Kortowskie in the years 1987–1991 and 1999–2000

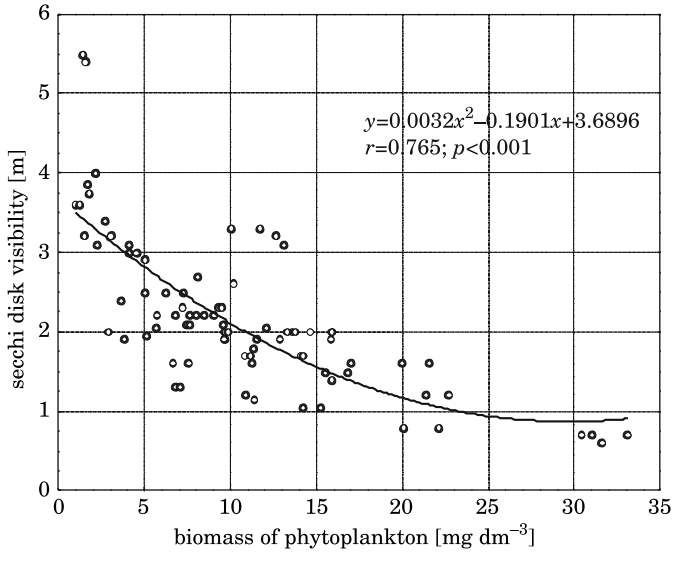

Fig. 5. The correlation coefficient between the Secchi disk visibility and phytoplankton biomass in the epilimnion of Lake Kortowskie in the years 1987–1991 and 1999–2000

together with increasing water temperature ($r = 0.666$, $p < 0.001$) – Figure 4. This rise of phytoplankton biomass significantly influenced water turbidity ($r = -0.765$; $p < 0.001$) causing its decrease (Figure 5). Algal growth was also

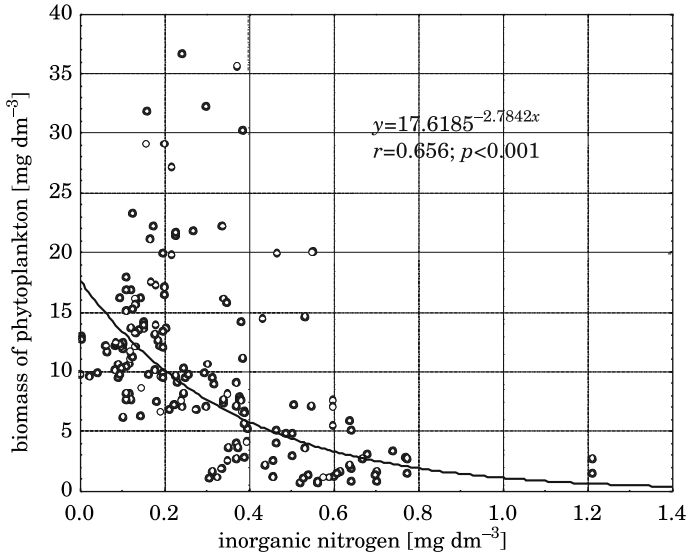

Fig. 6. The correlation coefficient between algal biomass and inorganic nitrogen contents in the epilimnion of Lake Kortowskie in the years 1987–1991 and 1999–2000

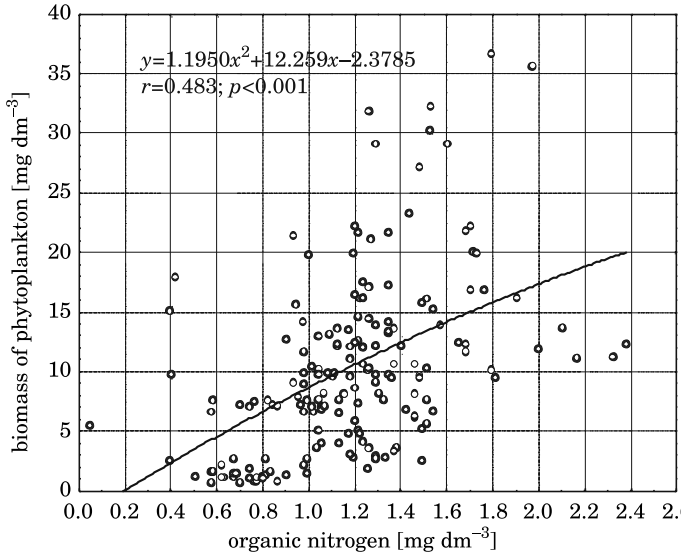

Fig. 7. The correlation coefficient between algal biomass and organic nitrogen contents in the epilimnion of Lake Kortowskie in the years 1987–1991 and 1999–2000

correlated with a decline in the content of the sum of inorganic nitrogen (r = -0.656; p < 0.001) – Figure 6 as well as with the increase in organic nitrogen concentrations in the water (r = 0.483; p < 0.001) – Figure 7 and

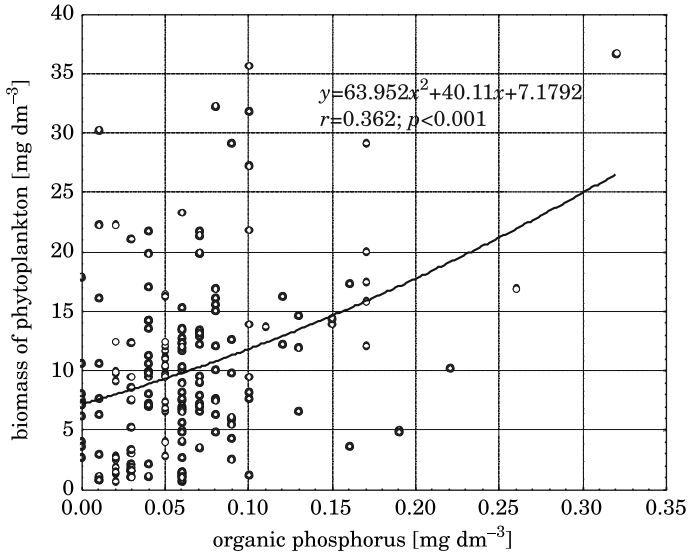

Fig. 8. The correlation coefficient between algal biomass and organic phosphorous contents in the epilimnion of Lake Kortowskie in the years 1987–1991 and 1999–2000

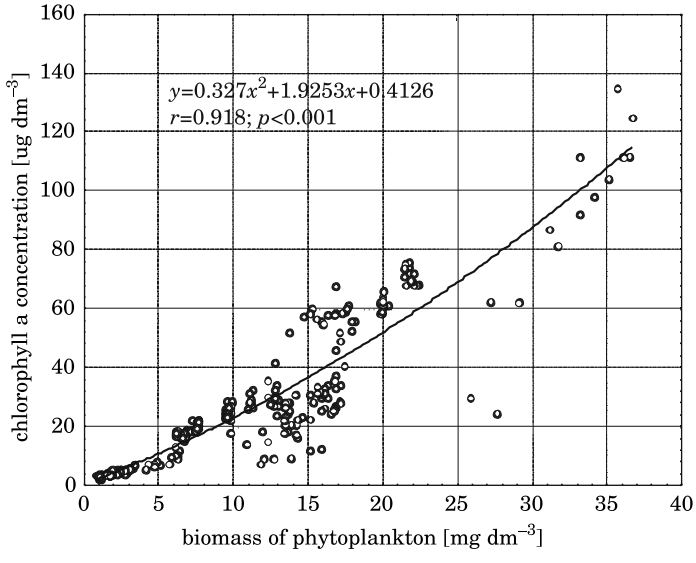

Fig. 9. The correlation coefficient between phytoplankton biomass and chlorophyll *a* levels in the epilimnion of Lake Kortowskie in the years 1987–1991 and 1999–2000

organic phosphorous (r = 0.362; p < 0.001) – Figure 8. The relationships between algal biomass and total phosphorous, total nitrogen contents as well as available form of phosphorous (orthophosphates) were not statistically

important. It was recorded that chlorophyll a concentration in epilimnion water was significantly correlated with algal biomass ($r = 0.918$; $p < 0.001$) – Figure 9.

Discussion

Species richness, shifts in composition and taxonomic structure and dynamics of phytoplankton development may indicate, beside other ecosystem components, a direction of trophic changes in lakes (DILLON and RIGLER 1975, MCQUEEN et al. 1986, BURCHARDT 1993, BURCHARDT and ŁASTOWSKI 1999, REYNOLDS 2000, REYNOLDS et al. 2002).

All of the algae community changes are the result of compositional shifts in phytoplankton (LAFFORGUE et al. 1995, SNIT'KO and ROGOZIN 2002). In Lake Kortowskie, the highest rate of taxonomic variations was recorded in case of the Bacillariophyceae and Chlorophyta, whereas Cyanoprokaryota showed the highest stability of their biocenosis. The rate of species exchange within this phylum was relatively low but the number of their taxa was permanently increasing. It was also shown that in the remaining taxonomic groups the proportion of the number of taxa retreating from the environment was higher than species appearing for the first time. Therefore, the rate of taxa extinction was higher than the rate of their emergence. It is noteworthy that new species usually represented taxa typical of eutrophic waters (STARMACH 1989, BURCHARDT 1993, REYNOLDS 2000). Despite the fact that the number of Cyanoprokaryota species increased, their species diversity was lower in comparison to diatoms and chlorophytes retreating from the environment what additionally enhanced the observed decrease in the overall taxa number. Such a direction of phytoplankton changes resulted in lowering of the species richness in Lake Kortowskie. Species richness usually rises together with a gradual growth of trophic state. HEINONEN (1980) documented that a moderate increase of a trophic level, with a corresponding biomass not exceeding 3.5 mg dm^{-3}, usually caused variations in algal number only in narrow limits. In turn, remarkable impoverishment of species resources takes place, when a progress in eutrophication is significant (CRONBERG 1999, DANILOV and EKELUND 1999, REYNOLDS et. al. 2002). Compositional modifications in phytoplankton and shifts in taxonomic structure coincided with intensified phytoplankton development. This increasing rate of algal growth did not exceed 10% in 1987–1991; however, in 1999/2000 it was higher by 50%. Recorded in the earlier period slight but progressing growth of phytoplankton development might have been the first symptom of a slow, trophic shift that manifested itself in a much more distinct way in 1999/2000. The most important group which conditioned the pattern of phytoplankton dynamics were Cyanoprokaryota, which proportion

in the total algal biomass was permanently increasing. Cyanoprokaryota occurred in large numbers and achieved very high biomass not only in summer, but also in spring and autumn. The blue-green algae decided of earlier regression of the diatoms in spring and their weaker development in autumn, also succeeded in competition with the remaining phytoplankton taxa in summer. The species that dominated were *Microcystis aeruginosa* (Kütz.) Kütz., *Anabaena spiroides* Kleb. or *Anabaena flos-aqae* (Lyng.) Breb. and *Aphanizomenon flos-aqae* (L.) Ralfs., which are typical of eutrophic waters (STARMACH 1989, BURCHARDT et al. 1994, REYNOLDS 2000). The community of the blue-green algae limited the development and stability of other plankton groups. In turn, the development of Bacillariophyceae and Chlorophyta was limited in the successive years. It was recorded a 3-fold drop of Bacillariophyceae biomass and 6-fold in case of Chlorophyta. The comparison of the values of algal biomass in Lake Kortowskie with the biomass recorded in other temperate lakes of different trophic stage (KAJAK 1983, HILLBRICHT-ILKOWSKA, KAJAK 1986) revealed that in 1987–1991 the level of the lake trophic state was moderate, while in 1999/2000 the obtained results indicated that the lake was highly eutrophicated. SPODNIEWSKA (1983) documented, basing on the studies on a series of Mazurian lakes, that the eutrophy occurred when the phytoplankton biomass exceeded 8 mg dm^{-3}. Whereas in pursuance of the trophic classification of Finnish lakes by HEINONEN (1980), the indicative to eutrophy value of algal biomass ranged from 3.5–10.0 mg dm^{-3}. In Lake Kortowskie, chlorophyll *a* levels, commonly regarded as an indicator of algal biomass (SCHMID et al. 1998), varied in limits indicative of eutrophic waters (KUFEL 2001) and phytoplankton biomass explained 84% of variations in that pigment content.

Multi-annual shifts in composition and taxonomic structure of phytoplankton together with intensified algal development that characterize phytoplankton communities subject to variations in trophic state usually reflect changes in physico-chemical water properties (HILLBRICHT-ILKOWSKA and ZDANOWSKI 1983, SPODNIEWSKA 1983, DAUTA et al. 1990, VEZJAK et al. 1998, BAIRD et al. 2001). The community of phytoplankton in Lake Kortowskie also responded to modifications in physico-chemical features of water among which the most important were: water turbidity, temperature and availability of inorganic forms of nutrients. Water temperature rise stimulated phytoplankton biomass growth and that, in turn, resulted in significant increase of water turbidity. About 60% of the biomass variations can be explained by water turbidity fluctuations. The Secchi disc visibility decreased proportionally to phytoplankton growth (DAUTA et al. 1990). In Lake Kortowskie, the most intensive growth of water turbidity was recorded when phytoplankton biomass exceeded 10 mg dm^{-3}. However, light conditions were mainly determined by intensive

development of cyanoprokaryotes (SPODNIEWSKA 1986), which occurred even in spring when water temperature suddenly arose. Weak correlation between algal biomass and water turbidity occurred only then, when Cyanoprokaryota appeared in large quantities forming colonies. BROOKES'A et al. (1999) documented that this sort of algal aggregations absorbed and dispersed light weaker than the suspension of fine planktonic organisms. The development of phytoplankton caused a significant drop in contents of the sum of inorganic nitrogen forms and increase in organic nitrogen and phosphorous concentrations. Variations in inorganic nitrogen contents determined 43% of phytoplankton biomass changes, whereas organic nitrogen and phosphorous concentrations were responsible for only 23% and 13% (respectively) of the recorded changes. It was probably due to the fact that their contents that corresponded to the particular biomass values varied in wide limits (CURRIE 1990, MIENTKI and DUNALSKA 2001). In a consequence, the correlation coefficients between phytoplankton biomass and total phosphorous and nitrogen were not significant. The same was recorded in case of the content of soluble reactive phosphorus (orthophosphates) which contribution to total phosphorous was always high (about 60%). In turn, the N/P ratio might have had a significant input to the development of phytoplankton and its composition, which dropped even to 5 in the studied lake. Average values of this ratio varied from 8 to 11 in 1987–1991 and to about 14 in 1999/2000. Such N/P values suggest accumulation or regeneration of phosphorus in epilimnion (SCHINDLER 1978, ZDANOWSKI 1982, UCHMAŃSKI 1988, MIENTKI and DUNALSKA 2001). The recorded values might have also had impact on phytoplankton composition and structure. Relatively low nitrogen contents and high phosphorus concentrations in water stimulate cyanoprokaryotes growth (SCHINDLER 1977, LAFFORGUE et al. 1995, HAY and KUBANEK 2002). Another important factor that should be considered when explaining the rate and character of phytoplankton changes in Lake Kortowskie is the hydrological regime of the lake. Limited inflow of waters (from Lake Ukiel by the main tributary) could cause that amounts of water flowing out of the were higher than overall water supply from all sources. In a consequence, in summer, water table in the lake dropped by 40 cm and the periods of low water table lasted from June to November (DUNALSKA et al. 2001, DUNALSKA 2002). Such hydrological conditions can stimulate phytoplankton growth and may also have an impact on taxonomic structure and the rate of phytoplankton development affecting pace and direction of trophic changes in the epilimnion of lake.

The multi-annual variations in the taxonomic structure and the estimation of the intensity of algal community development in phytoplankton of Kortowskie Lake indicated a trophic changes in the ecosystem that were identified as progressing eutrophic status.

Acknowledgements

Authors thank prof. dr hab. Czesław Mientki from Department of Environment Protection Engineering, who provided the data of physico-chemical analyses of water.

Translated by Magdalena Bowszys

Accepted for print 20.01.2012

References

Anneville O., Ginot V., Druat J.C., Angeli N. 2002. *Long-term study (1974–1998) of seasonal changes in the phytoplankton in Lake Geneva: a multi-table approach.* J. Plank. Res., 24(10): 993–1008.

Baird M., Emsley S.M., Meglade J.M. 2001. *Modelling the interacting effects of nutrient uptake, light capture and temperature on phytoplankton growth*. J. Plank. Res., 23(8): 829–840.

Brooks J., Ganf D., Green D., Whittington J. 1999. *The influence of light and nutrients on buoyancy, filament aggregation and flotation of Anabaena circinalis.* J. Plank. Res., 21: 327–341.

Burchardt L., 1993. *Bioindication in the assessment of lake ecosystem*. [In:] *Theory and practices in ecosystems research*. Ed.: L. Burchardt. Idee Ekol., 3(2): 39–44.

Burchardt L., Łastowski K. 1999. *The problem of using common species in bioindication: Basis term*. Acta Hydrobiol., 41(3/4): 231–234.

Burchardt L., Łastowski K., Szmajda P. 1994. *Różnorodność ekologiczna a bioindykacja.* [In:] *Teoria i praktyka badań ekologicznych.* Ed. L. Burchardt. Idee Ekol., 4(3): 27–44.

Burgi H., Heller C., Gaebel S., Mookerji N., Wand J. 1999. *Strength of coupling between phytoplankton and zooplankton in Lake Lucerne (Switzerland) during phosphorus abatement subsequent to a weak eutrophication*. J. Plank. Res., 21: 485–507.

Carpenter S.R., Cole J.J., Kitchell J., Pace M. 1997. *Impact of dissolved organic carbon, phosphorus and grazing on phytoplankton biomass and production in experimental lakes*. Limnol. Oceanogr., 43(1): 73–80.

Chudyba H. 1974. *Wpływ usuwania hypolimnionu na fitoplankton Jeziora Kortowskiego.* Zesz. nauk. ART Olszt. 2(119): 1–53.

Chudyba H. 1975. *Struktura i dynamika rozwoju fitoplanktonu Jeziora Kortowskiego.* Zesz. nauk. ART Olszt., 5(147): 1–70.

Cronberg G. 1999. *Qualitative and quantitative investigations of phytoplankton in Lake Ringsjon, Scania, Sweden.* Hydrobiologia, 404: 27–40.

Currie D.J. 1990. *Large-scale variability and interactions among phytoplankton, bacterioplankton and phosphorus*. Limnol. Oceanogr., 35(7): 1437–1455.

Danilov R., Ekelund N.G.A. 1999. *The efficiency of seven diversity and one similarity indices on phytoplankton data for assessing the level of eutrophication in lakes in central Sweden*. Sci. Tot. Environ., 234: 15–23.

Dauta A., Devaux J., Piquemal F., Boumnich L. 1990. *Growth rate of four freshwater algae in relation to light and temperature.* Hydrobiologia, 207: 201–226.

Dillon P.J., Rigler F.H. 1975. *A simple method for predicting the capacity of a lake for development based on lake trophic status.* J. Fish. Res. Board Can., 32(9): 1519–1531.

Dunalska J. 2002. *Influence of limited water flow in a pipeline on the nutrients budget in a lake restored by hypolimnetic withdrawal method.* Pol. J. Environ. Stud., 11(6): 631–637.

Dunalska J., Wiśniewski G., Mientki C. 2001. *Water balanse as factor determining the Lake Kortowskie restoration*. Limnol. Rev., 1: 65–72.

Forsberg C., Ryding S., Claesson A., Forsberg A. 1978. *Water chemical analyses and / or algal assay? Sewage effluent and polluted lake water studies.* Mitt. Int. Ver. Limnol., 21: 352–363.

Hay M.E., Kubanek J. 2002. *Community and ecosystem level consequences of chemical cues in the plankton.* J. Chem. Ecol., 28(10): 201–214.

HEINONEN P. 1980. *Quantity and composition of phytoplankton in Finnish island waters.* Publ. Wat. Res., 37: 1–91.

HEUSDEN G.P.H. 1972. *Estimation of the biomass of plankton.* Hydrobiologia, 39(2): 165–208.

HILLBRICHT-ILKOWSKA A., KAJAK Z. 1986. *Parametry i wskaźniki przydatne do kontroli zmian funkcjonalnych i strukturalnych w ekosystemach jeziornych ulegających procesowi eutrofizacji.* [In:] *Monitoring ekosystemów jeziornych.* Ed. A. Hillbricht-Ilkowska. Ossolineum, Wrocław-Warszawa-Kraków-Gdańsk-Łódź, pp. 23–45.

HILBRICHT-ILKOWSKA A., ZDANOWSKI B. 1983. *Sensitivity of lakes to inorganic enrichment stress – some results of experimentally induced fertilization*. Int. Rev. Hydrobiol., 68(2): 153–174.

KAJAK Z. 1983. *Ecological characteristic of lakes in north-eastern Poland versus their trophic gradient. XII. Dependence of chosen indices of structure and functioning of ecosystems on trophic status and mictic type of 42 lakes.* Ekol. pol., 31: 495–530.

KUFEL L. 2001. *Uncoupling of chlorophyll and nutrients in lakes – possible reasons, expected consequences.* Hydrobiologia, 443: 59–67.

LAFFORGUE M., SZELIGIEWICZ W., DEVAUX J., POULIN M. 1995. *Selective mechanisms controlling algal succession in Aydat Lake.* Wat. Sci. Tech., 4: 117–127.

MCQUEEN D.J., POST R., MILLS W.L. 1986. *Trophic relationships in freshwater pelagic ecosystems.* Can. J. Fish. Aquat. Sci. 43: 1571–1581.

MIENTKI C., DUNALSKA J. 2001. *Phosphorus balanse at various water flow in a lake restoired by hypolimnetic withdrawal.* Ecohydrol. Hydrobiol., 1(4): 417–422.

REYNOLDS C.S. 2000. *Phytoplankton designer – or how to predict compositional responses to trophic-state change*. Hydrobiologia, 424: 123–132.

REYNOLDS C.S., HUSZAR V., KRUK C., NASELLI-FLORES L., MELO S. 2002. *Towards a functional classification of the freshwater phytoplankton*. J. Plank. Res., 24(5): 417–428.

SCHINDLER D.W. 1977. *Evolution of phosphorus limnitation concept in lakes.* Science, 195: 260–262.

SCHINDLER D.W. 1978. *Factors regulating phytoplankton production and standing crop in the world's freshwaters*. Limnol. Oceanogr., 23: 478–486.

SCHMID H., BAUER F., STICH H. 1998. *Determination of algal biomass with HPLC pigment analysis from lakes of different trophic state in comparison to microscopically measured biomass*. J. Plank. Res., 20: 1651–1661.

SNIT'KO L.V., ROGOZIN A. G. 2002. *On the assessment of the structural organization of phytoplankton (Lake Bol'shoe Miassovo, the Southern Urals).* Russ. J. Ecol., 33(6): 402–406.

SPODNIEWSKA I. 1983. *Ecological characteristics of lakes in north-eastern Poland versus their trophic gradient.* VI. *The phytoplankton of 43 lakes.* Ekol. pol., 31: 353–381.

SPODNIEWSKA I. 1986. *Planktonic blue-green algae of lakes in north-eastern Poland.* Ekol. Pol., 34(2): 151–183.

STARMACH K. 1989. *Plankton roślinny wód słodkich. Metody badania i klucze do oznaczania gatunków występujących w wodach Europy Środkowej*. PWN, Warszawa-Kraków, pp. 496.

SYNOWIEC A. 1965. *Morfologia Jeziora Kortowskiego*. Zesz. nauk. WSR Olszt., 19: 3–16.

UCHMAŃSKI J. 1988. *Simulation model of phosphorus cycling in the epilimnion of an eutrophic lake*. Ekol. Pol., 36: 347–386.

VEZJAK M., SAVSEK T., STUHLER E.A. 1998. *System dynamics of eutrophication processes in lakes*. Europ. J. Operat. Res., 109: 442–441.

ZDANOWSKI B. 1982. *Variability of nitrogen and phosphorus contents and lake eutrophication*. Pol. Arch. Hydrobiol., 29(3/4): 541–597.

POLISH JOURNAL OF NATURAL SCIENCES
Abbrev.: Pol. J. Natur. Sc., Vol 27(2): 181–192, Y. 2012

EFFICIENCY OF THE ANAEROBIC DIGESTION PROCESS FOR PLANT SUBSTRATES USING A FERMENTATION REACTOR WITH A CAGE MIXING SYSTEM*

Marcin Zieliński, Anna Grala, Marcin Dębowski, Mirosław Krzemieniewski, Magda Dudek

Department of Environmental Protection Engineering
University of Warmia and Mazury in Olsztyn

K e y w o r d s: cage reactor, cage mixing system, anaerobic digestion, biomass, biogas.

A b s t r a c t

The aim of the presented research was to determine the effect of the applied mixing system on the efficiency of the methane fermentation process. The effect of the substrate dosing method (in batches, continuous) on the efficiency of biogas production was simultaneously analysed. The presented research was carried out on a pilot scale using a fermentation reactor equipped with an innovative mixing system. The research on the operational efficiency of the fermentation reactor equipped with a cage mixing system was carried out in 10 series differing in the rotational speed of the mixer: 0.5; 1.0; 1.5; 2.0; 2.5; 3.0; 3.5; 4.0; 4.5; 5.0 rev/min. The obtained research results indicate effective operation of the cage mixer in the fermentation reactor.The amount of obtained biogas grew with increasing rotational speed of the mixer from 0.5 to 2.5 rev/min. The value of the biogas production coefficient also grew with increasing rotational speed only to 2.5 rev/min.

Address: Anna Grala, University of Warmia and Mazury, ul. Romana Prawocheńskiego 1, 10-719 Olsztyn, Poland, phone: +48 (89) 523 38 46
* The study was carried out under a Key Project No. POIG.01.01.02-00-016/08 entitled: *Model agroenergetic complexes as an example of dispersed cogeneration based on local and renewable sources of energy*. The Project was financed under Innovative Economy Operational Programme.

EFEKTYWNOŚĆ PROCESU BIOGAZOWANIA SUBSTRATÓW ROŚLINNYCH Z ZASTOSOWANIEM REAKTORA FERMENTACYJNEGO Z KLATKOWYM SYSTEMEM MIESZAJĄCYM

Marcin Zieliński, Anna Grala, Marcin Dębowski, Mirosław Krzemieniewski, Magda Dudek

Katedra Inżynierii Ochrony Środowiska
Uniwersytet Warmińsko-Mazurski w Olsztynie

S ł o w a k l u c z o w e: reaktor klatkowy, klatkowy system mieszania, fermentacja beztlenowa, biomasa, biogaz.

A b s t r a k t

Celem prezentowanych badań było określenie wpływu zastosowanego systemu mieszania na efektywność procesu fermentacji metanowej. Jednocześnie analizowano wpływ sposobu dozowania substratu (okresowy, ciągły) na wydajność produkcji biogazu. Badania przeprowadzono w skali pilotującej z użyciem reaktora fermentacyjnego wyposażonego w nowatorski system mieszania. Badania nad efektywnością pracy reaktora fermentacyjnego wyposażonego w klatkowy system mieszający przeprowadzono w 10 seriach różniących się prędkością obrotową mieszadła: 0,5; 1,0; 1,5; 2,0; 2,5; 3,0; 3,5; 4,0; 4,5; 5,0 obr./min. Wyniki badań wskazują na skuteczne funkcjonowanie mieszadła klatkowego w reaktorze fermentacyjnym. Ilość uzyskiwanego biogazu rosła wraz ze zwiększaniem prędkości obrotowej mieszadła od 0,5 do 2,5 obr./min Wartość współczynnika produkcji biogazu również rosła wraz ze zwiększaniem prędkości obrotowej, ale jedynie do 2,5 obr./min.

Introduction

All the countries which have signed the Kyoto protocol are obliged to reduce greenhouse gas emission by 5.2% by 2012 compared to 1990 levels. Those which will continue to emit more greenhouse gases than the limits allow after 2012 will have to meet their commitments in the next period (HOLM-NIELSEN et al. 2009, FANTOZZI and BURATTI 2009). One of the greenhouse gas emission reduction strategies is the development of renewable energy sources, which causes a number of positive synergistic effects in the economy. Biomass, which can replace fossil fuels in energy and heat production, is a very promising renewable energy source. Energy produced from biomass in a sustainable manner is not only a valuable biofuel which reduces greenhouse gas emission, it is also a fuel which is more stable and safer with regard to energy supply than fossil fuels (KAPARAJU et al. 2009). Biomass is currently a substrate tested by researchers worldwide with regard to obtaining bioethanol, biodiesel or biogas.

Methane fermentation is the process of matter decomposition in an anaerobic environment by a microorganism consortium. This process occurs naturally in anaerobic environments, e.g. in deposits, wet soils or mammal

intestines. The fermentation can be effectively applied for both sewage treatment and anaerobic digestion of biomass (WARD et al. 2008). Biogas can be produced from almost all types of raw material and can be obtained from the agricultural sector and households (HOLM-NIELSEN et al. 2009). This affords the chance to use not only typical organic waste originating from agriculture, but also energy crops. Attempts are underway to optimize the methane fermentation process through the implementation of new reactor designs and modification of technological conditions for conducting the process.

Reactors for methane fermentation should be designed to allow the use of high organic loads with a short retention time, which reduces their volume. The reactor's shape should be selected so as not to hinder mixing and not to cause heat losses (WARD et al. 2008). Three main types of reactors used for the fermentation are distinguished. In batch reactors (the simplest type), the whole reactor volume is filled with substrate and the tanks are emptied after the retention time (CELIS et al. 2008). Single-stage reactors work in a continuous system, in which all biochemical reactions occur in one chamber (WARD et al. 2008). Two- and multistage reactors are also used, in which individual fermentation phases occur separately (LIU et al. 2006).

Besides the design basis, the mixing system plays a very important role in the reactor's construction. Mixing ensures appropriate distribution of fresh substrate in the reactor's active volume, thanks to which nutrition of the bacterial flora conducting the process takes place. Secondly, mixing is necessary because of heat distribution throughout the entire reactor volume (WAR et al. 2008). Mixing can take place both continuously and in batches (BURTON and TURNER 2003). A proper mixing system ensures sufficiently high efficiency of the methane fermentation process (WARD et al. 2008). The reactor mixing process can take place by means of different types of mechanical, hydraulic mixers located outside the reactor as well as pneumatic appliances blowing biogas into the reactor below the sediment level. In practice, mechanical mixers are of dominant importance in agricultural biogas plants (KHURSHEED et al. 2005).

The aim of the presented research was to determine the effect of the applied mixing system on the efficiency of the methane fermentation process. The effect of the substrate dosing method (in batches, continuous) on the efficiency of biogas production was simultaneously analysed.

Materials and Methods

Description of the cage reactor

The presented research was carried out on a pilot scale using a fermentation reactor equipped with an innovative mixing system. The reactor had the form of a cylindrical tank with an inside diameter $D = 1.2$ m and the height of the walls $H = 0.4$ m. The active height, filled with anaerobic sediment, was $H_{act} = 0.3$ m and the gaseous phase, in which biogas accumulated, lay above. In order to ensure anaerobic conditions, the reactor was closed with a dome, whose side walls were located below the liquid level in the reactor (Figure 1).

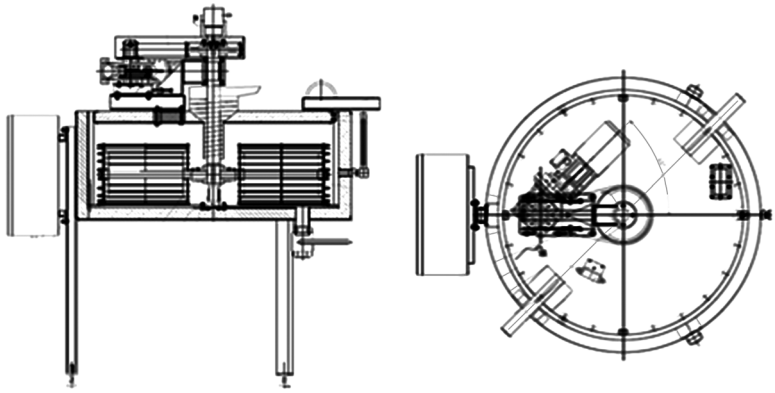

Fig. 1. Fermentation reactor with a cage mixing system

The side walls of the reactor, as well as the bottom and the dome, were thermally insulated with a 5.0 cm-thick polystyrene foam layer. A heating system was installed in the reactor's bottom, with the possibility of controlling the temperature inside the reactor. The closing dome contained a gas valve for carrying away biogas and a feeding worm for introducing organic substrate. The reactor's bottom had a valve allowing sediment to be drained. The mixing system of the reactor consisted of two cylindrical mixers in the form of a cage with a diameter of $D = 0.35$ cm. Each cage was welded together from steel bars with a diameter of 0.5 cm, located on the circumference of a circle. The cages rotated around the reactor's axis on a special track, simultaneously turning in relation to their own axes.

The drive of the mixer was ensured by a shaft which was at the same time the axis of rotation for the feeding worm. The rotational speed around the reactor's axis was regulated in the range from 0 to 5 revolutions per minute.

Parameters of the anaerobic reactor:
- inside diameter – ID = 1200 mm;
- outside diameter – OD = 1300 mm;
- active height – H_{act} = 300 mm;
- inside height – IH = 400 mm;
- active volume ca. – V_{act} = 339 l;
- gaseous phase volume – V_g = 113 l;
- mixing cage diameter – D_c = 350 mm;
- number of mixing cages – 2;
- rotational speed range – V = 0–5 rev/min.

Organization of research

The research on the operational efficiency of the fermentation reactor equipped with a cage mixing system was carried out in 10 series differing in the rotational speed of the mixer: 0.5; 1.0; 1.5; 2.0; 2.5; 3.0; 3.5; 4.0; 4.5; 5.0 rev/min. The research in each research series was conducted for the period of 21 days. The successive series were separated in time by a seven-day period of adaptation to new mixing conditions.

The fermentation reactor was fed with maize silage. The organic compound load for the reactor was ca. A' = 2 kg DOM/$m^3 \cdot$ d. Ca. M_s = 3.25 kg of maize silage hydrated to 85% was introduced into the reactor by means of the worm dosing system once a day. This corresponded to a volume of ca. 6.5 l. The hydraulic retention time HRT = 52.1 d was obtained this way. Before introducing a new batch of silage into the reactor, 6.5 l of post-fermentation sediment was drained each time. The sediment was drained through a draining valve in the reactor's bottom. The feeding of the reactor with silage lasted, depending on the applied rotational speed, from around 90 min at the rotational speed of 0.5 rev/min to around 15 min at the rotational speed of 5.0 rev/min.

The research was carried out under mesophilic conditions. The mean temperature indicated by the control system sensor was 35°C. This system turned on or off the heating plate built into the reactor's bottom with a hysteresis of ±1°C.

Measurement methods

The amount and composition of the forming biogas were analysed using the continuous mode. The biogas amount was measured by means of an Aalborg flowmeter, which allowed instantaneous and totalled measurement. The reading

of daily production was made once a day at the same time before the introduction of a new substrate batch. Measurement of the qualitative composition of biogas was made twice a day using a Gass Data LMF 430 meter. The percentage of methane CH_4, carbon dioxide CO_2, hydrogen sulfide H_2S as well as ammonia NH_2 and hydrogen H_2 was analysed. Measurements of temperature values inside the reactor above the bottom and near the surface of the fermentation liquid were made daily. The measurements were carried out using a Hanna four-channel temperature meter. Dry matter and dry organic matter contents in post-fermentation sediment and the introduced substrate were also analysed.

Statistical analysis of the obtained results

Statistical analysis of the obtained results was performed based on the STATISTICA 9.0 PL package. Verification of the hypothesis concerning the distribution of every examined variable was determined on the basis of the Shapiro-Wilk W test, with the zero hypothesis H_0: the distribution of the examined variable is normal.

In order to find the significance of differences between variables, a one-way analysis of variance (ANOVA) was carried out. The heating method for the model reactors was the grouping variable and the results of the conducted research were dependent variables. The application of ANOVA for one-way classification of parametric tests required the fulfilment of the following assumptions: the analysed variable is measurable, the considered k independent variables of the examined group have normal distributions and these distributions have identical variance.

Levene's test was used to check the homogeneity of variance in groups. Tukey's honestly significant difference (HSD) test was used to check the significance between the analysed variables. A significance level at $\alpha = 0.05$ was adopted in the tests.

Research results

The conducted research proved the operational efficiency of the cage mixing system. The amounts of biogas obtained in the methane fermentation process changed depending on the rotational speed. The least biogas flew away from the reactor at the rotational speed of the mixer 0.5 rev/min. On average, around 190 l of biogas was obtained in these conditions in a day. Increasing the rotational speed of the mixer to 1.0 rev/min caused an improvement in the conditions of the process, thanks to which the amount of the produced biogas

increased to 220 l/d on average. Statistically, there were no significant differences between these two speeds at the significance level of 0.05. Raising the rotational speed of the mixer to 2.0 rev/min caused the amount of the released biogas to be 315 l/d on average. Daily gas volume observed at this rotational speed was statistically different from that obtained in the series with the rotational speed of 1.0 rev/min, but was not significantly different than those found at higher rotational speeds. Further raising of mixing intensity did not cause a considerable increase in the amount of the forming biogas. The highest value was obtained in series 8 of the research with the rotational speed of 4.0 rev/min. However, this was not a value statistically significantly different from that observed in the series from 4 do 10 (Figure 2).

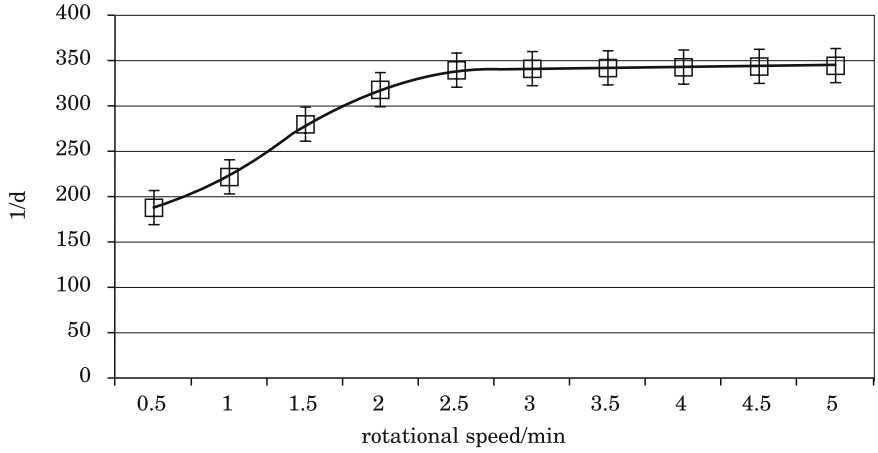

Fig. 2. Volume of the obtained biogas depending on the rotational speed of the mixer

An identical amount of organic substrate was introduced in all research series, hence differences in the value of the unit coefficient of biogas production y_B in successive series depended only on the volume of the captured biogas. In the series from 4 to 10, i.e. from rotational speed at the level of 2.0 to 5.0 rev/min, the value of the coefficient was similar. No statistically significant differences between the values of this coefficient were found in the successive series. The mean value of the y_B coefficient in series 3 (1.5 rev/min) was 411 l/kg DOM on average and was not statistically significantly different from series 4 (2.0 rev/min), where 464 l/kg DOM was observed on average. However, it differed significantly from series 5 (2.5 rev/min), where 498 l/kg DOM was observed on average. (Figure 3).

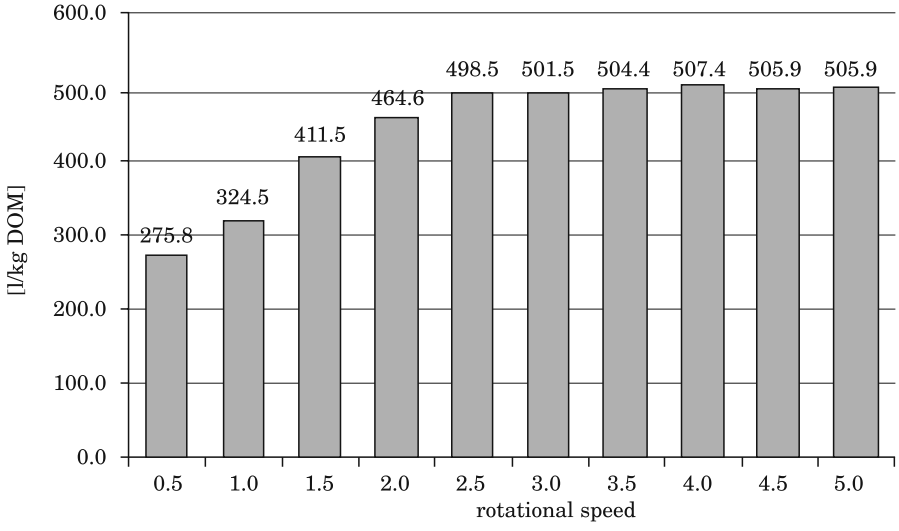

Fig. 3. Values of the unit coefficient of biogas production y_B

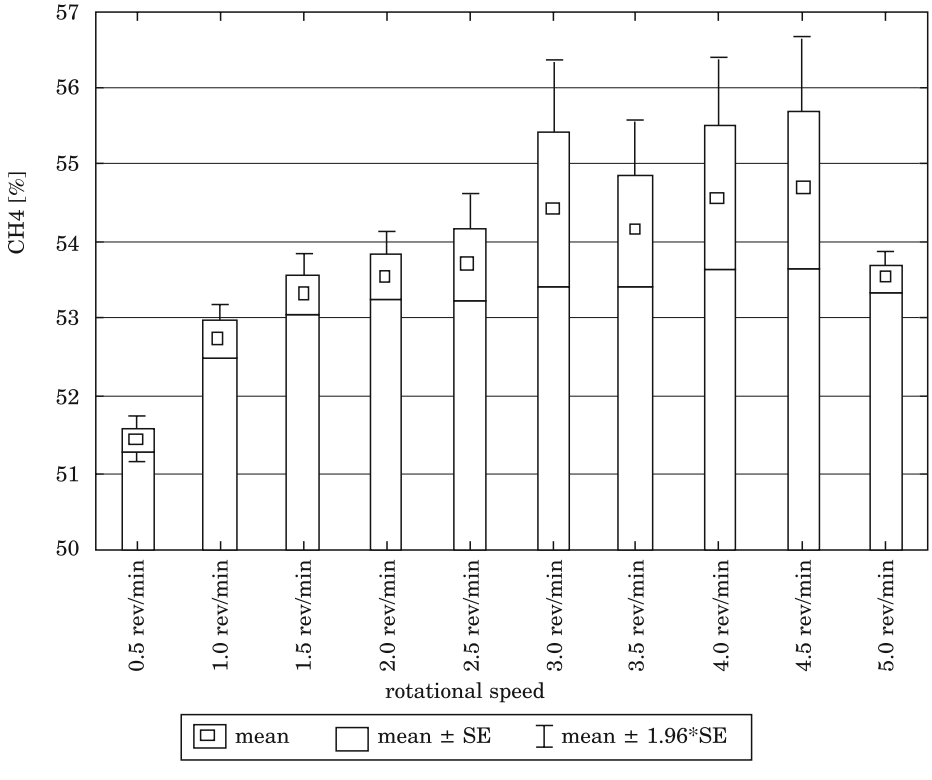

Fig. 4. Methane content in biogas in % depending on the rotational speed of the mixer

In analysing the composition of the forming biogas, no statistically significant differences were found between the series with rotational speeds above 1.0 rev/min. The highest methane concentration value was observed in series 6 (3.0 rev/min), 8 (4.0 rev/min) and 9 (4.5 rev/min), reaching maximally 56.7%. The methane content was lowest in series 1 (0.5 rev/min), where 51.5% of methane was found on average and it was statistically significantly lower than for the other series. The largest differences in biogas quality observed in a given series occurred in the case of series 9. The methane content in this series varied from 53.8% to 56.7%. The biogas composition was most stabilized in series 1. The maximum difference in the methane content between the highest and the lowest result was 0.5% (Figure 4)

Measurements of the temperature value were carried out in order to assess the homogeneity of liquid mixing in the reactor. The measurements were made above the reactor's bottom and at the surface of the liquid. Equalization of the temperature near the bottom and at the surface of the liquid in the fermentation reactor was observed with increasing rotational speed of the mixer. At the lowest rotational speed of 0.5 rev/min, the temperature difference reached Δ 7°C. At the rotational speed of 2.5 rev/min, the temperature value changed between the bottom and the liquid surface in the reactor by around 1°C. At the speed of 4.0 rev/min and higher, the difference was not greater than 0.5°C (Figure 5).

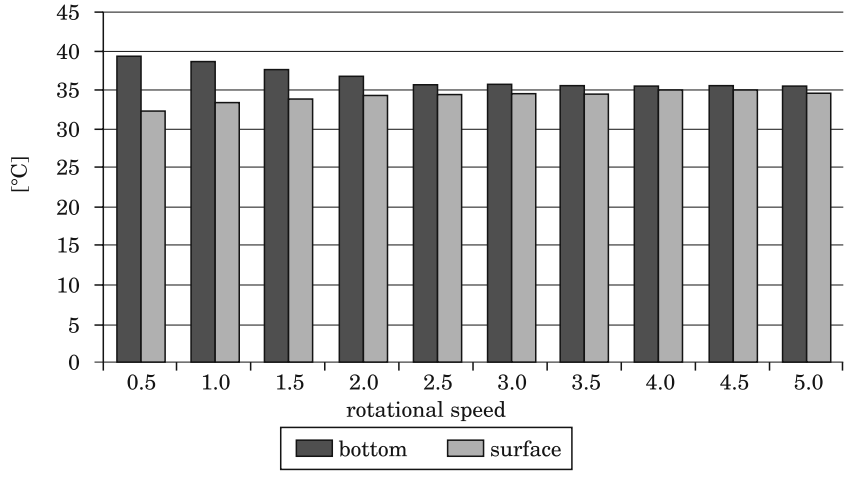

Fig. 5. Results of temperature measurements inside the fermentation reactor

Discussion and Results

According to Braun, maize silage is one of the most promising raw materials for biogas production (BRAUN et al. 2008). Generally, the literature gives values of the coefficient for biogas production from maize silage at a level from ca. 400 l/kg DOM. to 700 l/kg DOM. The methane content in the case of using only maize silage as the substrate ranges as a rule from 50% to 60%. The obtained values of the biogas production coefficient in the presented experiment are close to those presented in the literature. For example, Amon et al. obtained in mesophilic conditions 398 l CH_4/kg solid matter (AMON et al. 2007) ZAKI-UL-ZAMAN et al. compared methane production from maize silage to grass silage. They obtained 236 l CH_4/kg solid matter from maize silage and 3561 l CH_4/kg solid matter from grass silage (ASAM et al. 2011).

OSLAJ et al. studied the biogas potential of maize hybrids. Biogas production from maize hybrids (FAO 300–400) was from 455 to 544 l CH_4/kg solid matter. The methane amount ranged from 290 to 312 l CH_4/kg solid matter. The methane content varied between 55.3 and 57.3% (OSLAJ et al. 2010).

WUA et al. conducted similar research under mesophilic conditions. They used maize stems, oat and wheat straw in their research. The used biomass was subjected to the anaerobic fermentation process together with pig manure. The best results were reached for maize stems, where the percentage of methane in biogas was 68%, while the methane amount for oat straw was 57% (WUA et al. 2010). DAMIREL and SCHERERPR conducted mesophilic fermentation of sugar beet with a retention time from 9.5 d to 15 d and a load from 6.33 to 10 g DM/l · d. They obtained the best results at the shortest retention time and the load of 6.35 g DM/l · d, the methane content in biogas was 74% and the amount of produced methane was 670 l/kg DOM (DEMIREL and SCHERER 2009).

In our own research, the maximum methane content in biogas reached 56.7%. A lower concentration of this component in biogas compared to the research by WUA et al. could be caused by using as the substrate maize silage alone without a manure addition (WUA et al. 2010). This was confirmed by results from DINUCCIO et al. In this study, the methane concentration in biogas from maize silage was at the level of 50–60%, which is very close to the presented research (DINUCCIO at al. 2010).

Conclusions

The obtained research results indicate effective operation of the cage mixer in the fermentation reactor. The amount of obtained biogas grew with increasing rotational speed of the mixer from 0.5 to 2.5 rev/min.

Further raising of the rotational speed did not cause a significant increase in the amount of the obtained biogas. The value of the biogas production coefficient also grew with increasing rotational speed only to 2.5 rev/min. This means that full thorough mixing of the substrate and fermentation sediment was obtained at this mixing intensity. Further raising of the mixing rate and intensity did not improve the conditions of the process. Additionally, a small decrease in the methane content in the captured biogas was observed at the highest rotational speed.

The methane content in the captured biogas was at a similar level in all research series. This means that the applied retention time was sufficient and the higher biogas production in series with faster mixing resulted from easier gas release from the liquid and better availability of well-mixed substrate.

The effectiveness of the cage mixer's operation is attested by the analysed difference in liquid temperatures at the bottom and near the reactor's surface. It was just 1.0°C at the rotational speed of 2.5 rev/min. Because of this reactor operation parameter, further raising of the rotational speed was pointless.

Translated by JOANNA JENSEN

Accepted for print 9.05.2012

References

AMON T., AMON B., KRYVORUCHKO V., MACHMULLER A., HOPFNER-SIXT K., BODIROZA V., HRBEK R., FRIEDEL J., POTSCH E., WAGENTRISTL H., SCHREINER M., ZOLLITSCH W. 2007. *Methane production through anaerobic digestion of various energy crops grown in sustainable crop rotations*. Bioresource Technol., 98: 3204–3212.

ASAM Z., POULSEN T.G., NIZAMI A.S., RAFIQUE R., KIELY G., MURPHY J.D. 2011. *How can we improve biomethane production per unit of feedstock in biogas plants?* Appl. Energy., 88: 2013–2018.

BRAUN R., WEILAND P., WELLINGER A. 2008. *Biogas from energy crop digestion*. IEA Bionergy Task 37 – Energy from Biogas and Landfill Gas, in print, www.IEA-Biogas.net, access: 9.04.2010.

BURTON C.H., TURNER C. 2003. *Manure management treatment strategies for sustainable agriculture*, second ed. Silsoe Research Institute, Wrest Park, Silsoe, Bedford, UK.

CELIS E., ELEFSINIOTIS P., SINGHAL N. 2008. *Biodegradation of agricultural herbicides in sequencing batch reactors under aerobic or anaerobic conditions.* Water Res., 42: 3218–3224.

DEMIREL B., SCHERER P. 2009. *Bio-methanization of energy crops through mono-digestion for continuous production of renewable biogas.* Renew. Energy., 34: 2940–2945.

DINUCCIO E., BALSARI P., GIOELLI F. , MENARDO S. 2010. *Evaluation of the biogas productivity potential of some Italian agro-industrial biomasses.* Bioresource Technol., 101: 3780–3783.

FANTOZZI F., BURATTI C. 2009. *Biogas production from different substrates in an experimental Continuously Stirred Tank Reactor anaerobic digester*. Bioresource Technol., 100: 5783–5789.

HOLM-NIELSEN J.B, AL SEADI T., OLESKOWICZ-POPIEL P. 2009. *The future of anaerobic digestion and biogas utilization.* Bioresource Technol., 100: 5478–5484.

KAPARAJU P., SERRANO M., ANGELIDAKI I. 2009. *Effect of reactor configuration on biogas production from wheat straw hydrolysate.* Bioresource Technol., 100: 6317–6323.

KHURSHEED K., HOFFMANN R., KLASSON T.K., AL-DAHHAN M.H. 2005. *Anaerobic digestion of animal waste: Effect of mode of mixing*. Water Res., 39: 3597–3606.

LIU D.W., LIU D.P., ZENG R.J., ANGELIDAKI I. 2006. *Hydrogen and methane production from household solid waste in the two-stage fermentation process.* Water Res., 40, 2230–2236.

OSLAJ M., MURSEC B., VINDIS P. 2010. *Biogas production from maize hybrids*. Biomass and bioenergy, 34: 1538–1545.

WARD A.J., HOBBS P.J., HOLLIMAN P.J., JONES D.L. 2008. *Optimisation of the anaerobic digestion of agricultural resources*. Bioresource Technol., 99: 7928–7940.

WUA X., YAO W., ZHU J., MILLER C. 2010. *Biogas and CH4* productivity by co-digesting swine manure with three crop residues as an external carbon source. Bioresource Technol., 101: 4042–4047.

POLISH JOURNAL OF NATURAL SCIENCES
Abbrev.: Pol. J. Natur. Sc., Vol 27(2): 193–201, Y. 2012

TEMPORAL CHANGES IN MOTILITY PARAMETERS OF DACE *LEUCISCUS LEUCISCUS* (L.) SPERM OBTAINED FROM SPERMATIC DUCTS AND DIRECTLY FROM TESTICLES*

***Radosław Kajetan Kowalski*[1], *Beata Irena Cejko*[1], *Beata Sarosiek*[1], *Dariusz Kucharczyk*[2], *Katarzyna Targońska*[2], *Jan Glogowski*[1]**

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

Key words: dace, *Leuciscus leuciscus* (L.), CASA, spermatic duct milt, testicular milt.

Abstract

A Computer Assisted Sperm Analysis system, CASA, enables determination of numerous parameters characterizing sperm motion activity. This system allows for the examination of the effect of various environmental factors on spermatozoa motility parameters. The aim of this work was to compare time-dependent motility changes of sperm obtained from the dace, *Leuciscus leuciscus* (L.), by abdominal massage (sperm from spermatic ducts) and directly from gonads (testicular sperm). The analysis concerned such sperm motility parameters as: percentage of motile sperm (MOT, %), total sperm velocity (VAP, μm s^{-1}), straight line velocity (VSL, μm s^{-1}), curvilinear velocity (VCL, μm s^{-1}), linearity (LIN: VSL/VCL · 100%), straightness (STR: VSL/VAP · 100%), amplitude of the lateral head displacement (ALH, μm) and beat cross frequency (BCF, Hz). During 105 seconds of motility, no significant differences were found in the values of MOT between sperm originating from the spermatic ducts and from the testicles. Changes in MOT were only observed during seconds 120–135 of movement, when significantly higher values were found for testicular milt. Significant higher sperm velocities (VAP, VCL, VSL) at the 15s from activation were observed in the sperm originated from spermatic duct. On the other hand 120s after activation of movement, values of sperm velocity (VAP and VCL) of milt originating from spermatic duct significantly decreased in comparison to testicular milt. Our data showed that sperm obtained from spermatic duct have initially higher sperm motility speed compared to that obtained directly from the testis. However testicular sperm are able to swim longer than sperm obtained from spermatic duct.

Address: Beata Irena Cejko, Polish Academy of Sciences, ul. Bydgoska 7, 10-243 Olsztyn, Poland, phone: +48 (89) 539 31 33, e-mail: b.cejko@pan.olsztyn.pl
* This study was supported by the project *Innovation in finish aquaculture with special references to reproduction*. Operational Programme Sustainable Development of the Fishenies Sector and Coastal Fishing Areas 2007–2013 (OR14-61724-OR1400003/09/10/11).

ZMIANY W CZASIE PARAMETRÓW RUCHU PLEMNIKÓW JELCA *LEUCISCUS LEUCISCUS* (L.) POZYSKANYCH Z NASIENIOWODÓW ORAZ BEZPOŚREDNIO Z JĄDER

***Radosław Kajetan Kowalski*[1], *Beata Irena Cejko*[1], *Beata Sarosiek*[1], *Dariusz Kucharczyk*[2], *Katarzyna Targońska*[2], *Jan Glogowski*[1]**

[1] Zakład Biologii Gamet i Zarodka
Instytut Rozrodu Zwierząt i Badań Żywności, Polska Akademia Nauk w Olsztynie
[2] Katedra Rybactwa Jeziorowego i Rzecznego
Uniwersytet Warmińsko-Mazurski w Olsztynie

Słowa kluczowe: jelec, *Leuciscus leuciscus* (L.), CASA, mlecz nasieniowodowy, mlecz jądrowy.

Abstrakt

System komputerowy CASA (*Computer Assisted Sperm Analysis*) umożliwia oznaczanie wielu parametrów charakteryzujących motorykę ruchu plemników. Umożliwia badanie wpływu na ruchliwość plemników różnych czynników środowiskowych, jak również pozwala na szczegółową charakterystykę toru ich ruchu. Celem pracy było porównanie zmian w czasie parametrów ruchu plemników pozyskanych od jelca *Leuciscus leuciscus* (L.) za pomocą masażu powłok brzusznych (nasienie z nasieniowodów) oraz bezpośrednio z gonad (nasienie jądrowe). Analizowano takie parametry ruchu plemników jak: odsetek plemników ruchliwych (MOT, %), całkowita prędkość plemnika (VAP, μm s^{-1}), prędkość ruchu prostoliniowego (VSL, μm s^{-1}), prędkość ruchu krzywoliniowego (VCL, μm s^{-1}), liniowość ruchu (LIN: VSL/VCL · 100%), prostoliniowość ruchu (STR: VSL/VAP · 100%), amplituda odchyleń główki (ALH, μm) oraz częstotliwość uderzeń witki (BCF, Hz). W czasie 105 sekund obserwacji pomiaru parametrów ruchu nie stwierdzono istotnych różnic w wartościach MOT między plemnikami z mlecza pochodzącego z nasieniowodów a plemnikami pochodzącymi z jąder. Zmiany zaobserwowano dopiero w 120–135 sekundzie trwania ruchu, a istotnie wyższe wartości stwierdzono w mleczu jądrowym. W wartościach parametrów VAP, VCL i VSL również zaobserwowano istotne zmiany prędkości plemników w zależności od czasu po aktywacji. W 120 sekundzie trwania ruchu wartości prędkości plemników (VAP i VCL) mlecza nasieniowodowego, w porównaniu z mleczem jądrowym istotnie się obniżyły. W przeprowadzonych badaniach wykazano, że plemniki pozyskane z nasieniowodów charakteryzują się wyższą prędkością w porównaniu z plemnikami pozyskanymi bezpośrednio z jąder. Jednakże ruch plemników jądrowych trwać może dłużej niż plemników nasieniowodowych.

Introduction

In the 1990s, with the development of computer technology, a new method of determining sperm motility emerged, consisting in applying a computer analysis of sperm motility, i.e. CASA (Computer Assisted Sperm Analysis). This system was initially used in clinical laboratories for determining male fertility. Afterwards, it was applied for assessment of mammalian sperm motility (FARREL et al. 1998, MOORE and AKHONDI 1996), and used also in research on the fish semen quality (CHRIST et al. 1996, KIME et al. 1996, 2001, RAVINDER et al. 1997). The CASA system not only allows for the objective

measurement of the percentage of motile spermatozoa, but also for determination of the movement trajectory, head displacement or beat cross frequency (RURANGWA et al. 2004). CASA also offers the possibility of determining sperm velocity, including straight line, curvilinear and total velocity. The ability to determine numerous parameters characterizing spermatozoa movement made it possible to establish the impact of compounds that are toxic for fish, including heavy metals or xenobiotics (KIME et al. 1996, CHYB et al. 2000, 2001, JARMOŁOWICZ et al. 2010), and the effect of hormonal stimulation on sperm motility parameters (CEJKO et al. 2011a, 2012), or changes in the quality of milt after short-term refrigerated storage (RAVINDER et al. 1997, KOWALSKI et al. 2004).

In recent years, the CASA system has been used in the diagnostics of milt originating from species of slightly lower economic significance, i.e. rheophilic fish (CEJKO et al. 2011a, 2012). The decreasing area of their occurrence has forced researchers to seek solutions aimed at supporting their native populations by the reproduction of these fish under controlled conditions, the rearing of juvenile forms and, consequently, carrying out restocking with the material produced. A starting point for the production of the stocking material is optimization of reproductive biotechnology (KREJSZEFF et al. 2008, CEJKO et al. 2011b, TARGOŃSKA et al. 2011) and for particularly endangered species, i.e. the barbel, *Barbus barbus* (L.), nase, *Chondrostoma nasus* (L.), or vimba, *Vimba vimba* (L.), determination of quality markers, including milt quality markers, which are of direct significance for its diagnostics (CEJKO et al. 2012, SAROSIEK et al. 2011).

The latest research indicates that the reproductive success of salmon males is determined by total sperm velocity (GAGE et al. 2004). It is also assumed that ALH (amplitude of the lateral head displacement) can be one of the milt quality markers. It was also found that in barbel males, the value of ALH parameters was reduced in time after hormonal stimulation (CEJKO et al. 2012). Also in the smelt, *Osmerus eperlanus* (L.), significant differences were observed in the values of the ALH parameter between gonad milt (obtained from testicles) and "full milt", i.e. that obtained from spermatic ducts (HLIWA et al. 2009).

As the hormonal stimulation of wild fish not always allows to obtain sperm from spermatic duct, the aim of this study was to compare the changes in time in motility parameters of dace sperm obtained from spermatic ducts and gonads.

Materials and Methods

Fish originating from the Department of River Fishery of the Warmia and Mazury University in Olsztyn were used as material ($n=3$). Semen was collected by gentle abdominal massage. After collecting the semen, the gonads

were removed and milt was collected after gonadal tissue maceration. After collecting the milt, samples were transported on ice (+4°C) to the Department of Gametes and Embryos Biology of the Institute of Animal Reproduction and Food Research of the Polish Academy of Sciences in Olsztyn, where further analyses were carried out. In order to activate spermatozoa movement, a 0.5% NaCl solution with an addition of 2 mg ml^{-1} BSA was applied. The semen was diluted 200–400 times (depending on the original concentration), after which samples were placed on a microscopic slide. Sperm motility was analysed with the use of the CASA system, equipped in a black and white CCD camera, a microscope, a video and a computer with Hobson Vision software. The image of moving spermatozoa was recorded through a counter phase lens (10x magnification). The measurements were taken in 15-second intervals up to 135s after activation. The following sperm motility parameters were determined: percentage of motile sperm (MOT, %), total sperm velocity (VAP, μm s^{-1}), straight line velocity (VSL, μm s^{-1}), curvilinear velocity (VCL, μm s^{-1}), linearity (LIN: VSL/VCL · 100%), straightness (STR: VSL/VAP · 100%), amplitude of the lateral head displacement (ALH, μm) and beat cross frequency (BCF, Hz).

Results

In the first 15 seconds of movement, no statistically significant differences were observed in the values of MOT (46.3%) of sperm obtained from spermatic ducts in comparison to testicular sperm (49.7%), ($P>0.05$, Figure 1a). At the same time, sperm obtained from spermatic ducts was characterized by significantly higher values of VAP velocity (36.4 μm s^{-1}), VCL (41.7 μm s^{-1}) and VSL (28.1 μm s^{-1}) than sperm obtained from testicles (27.9; 34.3 and 20.5 μm s^{-1} for VAP, VCL and VSL, respectively), ($P<0.05$, Figure 1b–1d). Significant differences were also observed in the value of linearity (LIN) and straightness (STR) of sperm motility. The values of LIN of sperm from spermatic ducts oscillated about 60% while that of testicular sperm was around 50% ($P<0.05$, Figure 1e).

On the other hand, the values of STR were above 75% for sperm obtained from spermatic duct milt and 50% for sperm of testicular milt ($P<0.05$, Figure 1f). Within 30 seconds of movement, the values of ALH and BCF of sperm originating from testicles were significantly higher (2.3 μm s^{-1} for ALH and 1.47 Hz for BCF) in comparison to that from spermatic ducts (1.1 μm s^{-1} for ALH and 1.0 Hz for BCF) – Figure 1g, 1h). However, spermatozoa originating from testicles were characterized by significantly higher values of MOT in seconds 120–135 of movement as compared to spermatozoa originating from spermatic ducts ($P<0.05$; Figure 1b). The veloc-

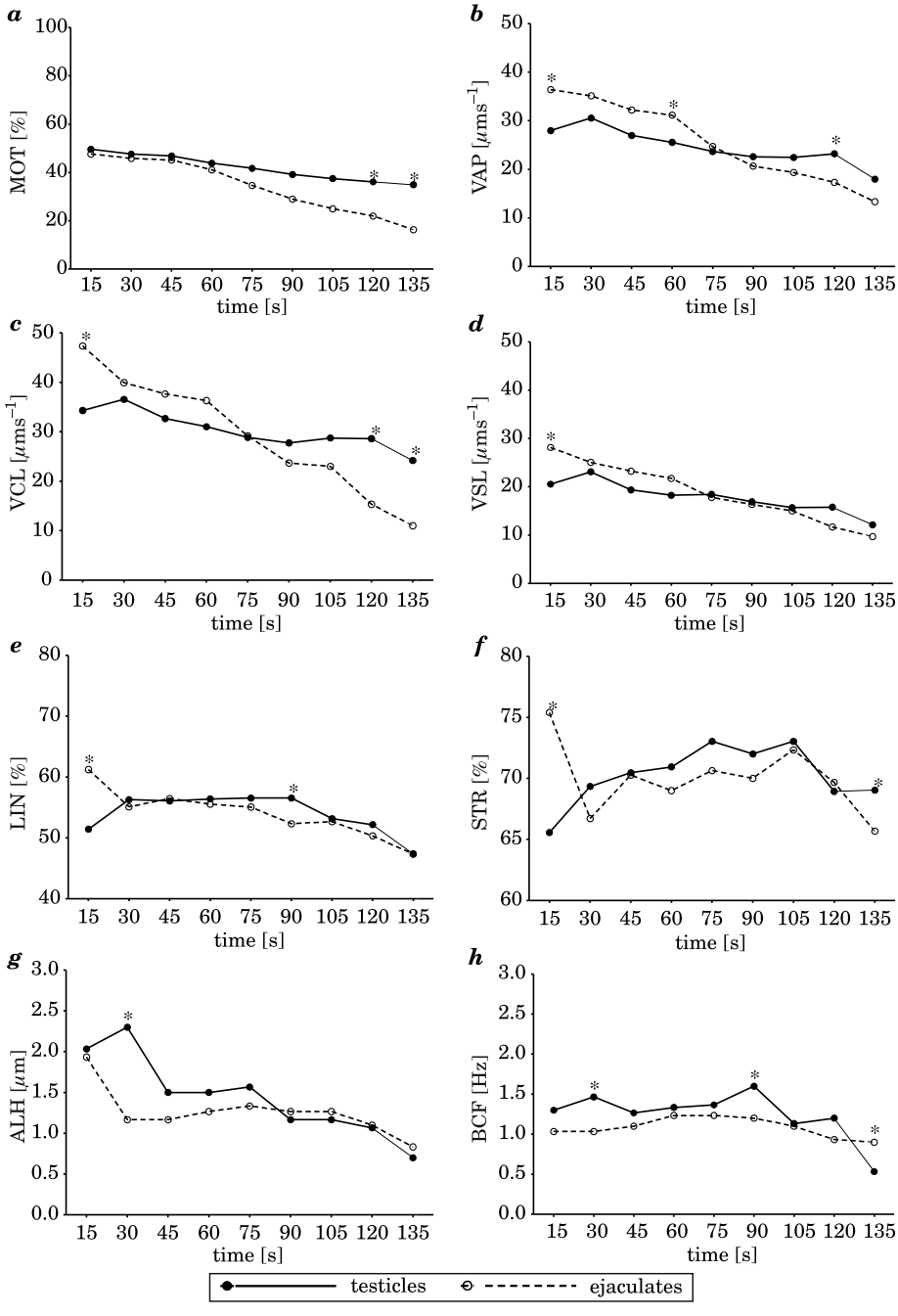

Fig. 1. Changes in the values of MOT (a), VAP (b), VCL (c), VSL (d), LIN (e), STR (f), ALH (g) and BCF (h) during the movement of dace *Leuciscus leuciscus* (L.) spermatozoa. Statistically significant differences in mean values of the analysed parameters are marked with an asterisk (n=3)

ity of spermatozoa from the milt obtained from spermatic ducts rapidly decreased at second 90 of movement (22.6; 27.7 and 15.4 μm s^{-1} for VAP, VCL and VSL, respectively), to finally, i.e. at second 135, evolve into the stage of vibration (17.9; 24.2 and 12.1 μm s^{-1} for VAP, VCL and VSL, respectively) – Figure 1b–1d.

Discussion

Sperm motility is one of the basic indicators of the quality of milt used in its diagnostics. Sperm movement corresponds with the reproductive strategy of a given species, and can last a few hours, e.g. in the Acipenseridae, several minutes in the Cyprinidae species, or a few seconds in the Salmonidae species (RURANGWA et al. 2004). Differences in values of specific sperm motility parameters have also been observed between closely-related species, i.e. rheophilic cyprinids of the genus Leuciscus (KOWALSKI et al. 2006). Consequently, the milt diagnostics on the basis of sperm motility was carried out for each species individually.

The mean initial velocity VCL of dace sperm (43 μm s^{-1}) was lower than the velocity of chub, *Squalius cephalus* (L.), sperm in which this parameter reached 70 μm s^{-1} (LAHNSTEINER et al. 2004) and carp, *Cyprinus carpio* (L.) sperm, with VCL between 80–90 μm s^{-1} (CHYB et al. 2001). The values of VCL were also lower than the values determined for the crucian carp, *Carassius carassius* (L.), in which the VCL velocity exceeded 50 μm s^{-1} (DIETRICH et al. 2003). Even lower values of the VCL parameter were found in gonadal milt (34 μm s^{-1}), which can indicate immaturity of the sperm in the testicles. It is interesting that our observations reveal that the values of dace sperm velocity can be definitely higher than the currently presented data. In the research on the effect of hormonal stimulation of the dace, we found that depending on the applied hormonal preparation, the velocity of dace sperm significantly differed. After stimulation with Ovopel [(D-Ala^6Pro^9 NEt)-mGnRH] + metaclopramide, the values of velocity were significantly lower (VCL: 96 μm s^{-1} and 76.5 μm s^{-1}) than the values after stimulation with LHRHa (VCL: 133 μm s^{-1} and VSL 107 μm s^{-1}), (CEJKO et al. 2011b). The lower values of the velocity could result from the individual variability and a different degree of sperm maturity.

The amplitude of lateral head displacement, i.e. the ALH parameter, can constitute an important quality indicator of milt. While analysing the motility of smelt sperm significantly lower values of ALH were found in gonadal milt as compared to milt from the spermatic duct (HLIWA et al. 2009). It was also found that 72 h after hormonal stimulation, the value of the ALH parameter decreased, which can be explained by sperm aging and loss of its fertilizing abilities (HLIWA et al. 2009). Similarly, in the case of barbel, *Barbus barbus* (L.),

a reduction in motility was observed with time after hormonal stimulation, including the ALH parameter, while a significant decrease was noted 36 h after stimulation (CEJKO et al. 2012). These results slightly differ from those previously presented, where ALH values amounted to: 0.9–1.1 μm (CEJKO et al. 2011a) and they indicate that dace sperm is rather similar to the crucian carp sperm (ALH about 3 μm) as regards the ALH parameter. Semen of the *Acipenseridae* (GLOGOWSKI et al. 2004; 10 μm), the *Salmonidae* (DIETRICH et al. 2005; 9–12 μm) and the *Percidae* (SAROSIEK et al. 2004, KOWALSKI et al. 2004, 6–15 μm) is characterized by significantly higher values of ALH. It should be emphasized that during the movement, the values of ALH decrease at a faster rate during the first 30 seconds (milt from spermatic ducts) and 45 seconds (milt from testicles). A slower decrease in the ALH parameter of testicular sperm, as compared to sperm from the spermatic ducts, can be related to their lower initial velocity, which makes them lose their energy reserves at a slower rate. Despite the sperm obtained directly from the testis is able to maintain their motility for longer time, it is not necessarily beneficial for the reproduction. Eggs of dace are able to be fertilized till 90s after contact with water (KUCHARCZYK, unpublished data). Therefore longer motility observed in sperm obtained from testis might not bring benefits in term of fertilization success.

The results presented indicated certain differences in the motility activity of dace sperm originating from spermatic ducts and from gonads. Spermatozoa originating from spermatic ducts are characterized by faster initial movement and a quite high rate of losing the ability to move (a sprinter type), while spermatozoa originating from gonads are characterized by lower initial velocities and a slower decrease of those parameters during the movement (a marathoner type). Those differences could result from immaturity of testicular spermatozoa. In view of the fact that for fish, a parameter determining reproductive success is sperm velocity (GAGE et al. 2004), the fertilizing capacity of testicular spermatozoa might be lower, although they move for longer time.

Translated by JOANNA JENSEN

Accepted for print 19.03.2012

References

CEJKO B.I., TARGOŃSKA K., KOWALSKI R.K., ŻARSKI D., SAROSIEK B., KUCHARCZYK D., GLOGOWSKI J. 2011a. *The effectiveness of selected hormonal preparations in stimulating spermiation in the common dace Leuciscus leuciscus* (L.). [In]: 3rd International Workshop on the Biology of Fish Gametes. Ed. R. Hohol, Diamond Congress, Budapest, Hungary, 128–129.

CEJKO B.I., KREJSZEF S., ŻARSKI D., KOWALSKI R.K., TARGOŃSKA K., KUCHARCZYK D., GLOGOWSKI J. 2011b. *The efectiveness of selected hormonal preparations in stimulating the spermation of the chub Leuciscus cephalus* (L.). Pol. J. Nat. Sci., 26(3): 235–245.

Cejko B.I., Kowalski R.K., Żarski D., Dryl K., Targońska K., Kucharczyk D., Glogowski J. (2012): *The influence of the length of time after hormonal treatment with [(D-Ala^6, Pro^9 NEt)-mGnRH+metoclopramide] i.e. Ovopel on barbel Barbus barbus* (L.) *milt quality and quantity indicators*. J. App. Ichthyol., 28: 249–253.

Christ S.A., Toth G.P., McCarthy H.W., Torsella J.A., Smith M.K. 1996. *Monthly variation in sperm motility in common carp assessed using computer-assisted sperm analysis (CASA).* J. Fish Biol., 48: 1210–1222.

Chyb J., Kime D.E., Mikołajczyk T., Szczerbik P., Epler P. 2000. *The influence of zinc on sperm motility of common carp – a computer assisted studies.* Arch. Pol. Fish., 8(1): 5–14.

Chyb J., Kime D.E., Szczerbik P., Mikołajczyk T., Epler P. 2001. *Computer-assisted analysis (CASA) of common carp Cyprinus carpio L. spermatozoa motility in the presence of cadmium*. Arch. Pol. Fish., 9(2): 173–181.

Dietrich G.J., Rzemieniecki A., Kowalski R., Targońska-Dietrich K., Glogowski J., Ciereszko A. 2003. *Zastosowanie komputerowej analizy ruchu plemników do wyboru optymalnego stymulatora dojrzewania karasia* (*Carassius auratus* L.). Mat. XIX Zjazdu Hydrobiologów Polskich, Warszawa, 9-12 września 2003, 34.

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

Farrell P.B., Presicce G.A., Brockett C.C., Foote R. H. 1998. *Quantification of bull sperm characteristics measured by computer-assisted sperm analysis* (CASA) *and the relationship to fertility.* Theriogenology, 49: 871–879.

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

Glogowski J., Kolman R., Rzemieniecki A., Dietrich G., Demianowicz W., Sieczyński P., Sarosiek B., Wysocka J., Kowalski R., Wojtczak M., Ciereszko A. 2004. *Biologia nasienia ryb jesiotrowatych i jego kriokonserwacja.* [W]: Rozród, podchów, profilaktyka ryb jesiotrowatych i innych gatunków. Red. Z. Zakęś, R. Kolman, K. Demska-Zakęś, T. Krzywosz, IRS, Olsztyn: 35–42.

Hliwa P., Kowalski R.K., Król J., Cejko B.I., Stabiński R., Ziomek E., Ciereszko A. 2009. *Morfologiczna i funkcjonalna asymetria jąder stynki* (*Osmerus eperlanus*). [W]: *Rozród, podchów, profilaktyka ryb łososiowatych i innych gatunków*. Red. Z. Zakęś, K. Demska-Zakęś, A. Kowalska, D. Ulikowski, Wyd. IRS, Olsztyn, 65–73

Jarmołowicz S., Demska-Zakęś K., Kowalski R.K., Cejko B.I., Glogowski J., Zakęś Z. 2010. *Impact of dibutyl phthalate and benzyl butyl phthalate on motility parameters of sperm from the European pikeperch Sander lucioperca* (L.). Arch. Pol. Fish., 18: 149–156.

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

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

Kowalski R., Sarosiek B., Zakęś Z., Szpyrka A., Cejko B.I., Ciereszko A., Glogowski J. 2004. *Zastosowanie komputerowej analizy ruchu plemników (CASA) oraz analizy komet w celu określenia przydatności nasienia sandacza do krótkookresowego przechowywania*. [In]: *Rozród, podchów, profilaktyka ryb jesiotrowatych i innych gatunków*. Red. Z. Zakęś, R. Kolman, K. Demska-Zakęś, T. Krzywosz, Wyd. IRS, Olsztyn, 113–118.

Kowalski R.K., Sarosiek B., Kucharczyk D., Targońska K., Glogowski J. 2006. *Zmiany parametrów ruchu plemników jazia (Leuciscus idus) i jelca (Leuciscus leuciscus) w zależności od czasu po aktywacji*. [In]: *Rozród, podchów, profilaktyka ryb jesiotrowatych i innych gatunków*. Red. Z. Zakęś, K. Demska-Zakęś, J. Wolnicki, Wyd. IRS, Olsztyn, 45–51.

Krejszeff S., Kucharczyk D., Kupren K., Targońska K., Mamcarz A., Kujawa R., Ratajski S. 2008. *Reproduction of chub Leuciscus cephalus* L. *under controlled condition*. Aquacult. Res., 39: 907–912.

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

MOORE H.D.M., AKHONDI M. A. 1996. *Fertilizing capacity of rat spermatozoa is correlated with decline in straight-line velocity measured by continuous computer-aided sperm analysis: epididymal rat spermatozoa from proximal cauda have a greater fertilizing capacity in vitro than those from the distal cauda or vas deferens*. J Androl., 17: 50–60.

RAVINDER K., NASARUDDIN K., MAJUMDAR K.C., SHIVAJI S. 1997. *Computerized analysis of motility, motility patterns and motility parameters of spermatozoa of carp following short-term storage of semen*. J. Fish Biol., 50: 1309–1328.

RURANGWA E., KIMED E., OLLEVIER F., NASH J. P. 2004. *The measurement of sperm motility and factors affecting sperm quality in cultured fish*. Aquaculture, 234: 1–28.

SAROSIEK B., KOWALSKI R., ZAKĘŚ Z., DIETRICH G., CEJKO B.I., GLOGOWSKI J. 2004. *Charakterystyka parametrów ruchu plemników sandacza wyznaczonych przy zastosowaniu komputerowej analizy ruchu plemników* (CASA). [In:] *Rozród, podchów, profilaktyka ryb jesiotrowatych i innych gatunków*. Red. Z. Zakęś, R. Kolman, K. Demska-Zakęś, T. Krzywosz, Wyd. IRS, Olsztyn, 119–126.

SAROSIEK B., CEJKO B.I., GLOGOWSKI J., KUCHARCZYK D., ŻARSKI D., TARGOŃSKA K., KOWALSKI R.K. 2011. *Cryopreservation of ide (Leuciscus idus) milt in the presence of selected antioxidants*. [In]: Aquaculture Europe, Rhodes, Greece, October 18–21 2011, pp. 976–977.

TARGOŃSKA K., KUCHARCZYK D., ŻARSKI D., CEJKO B.I., KREJSZEFF S., KUPREN K., KRÓL J., DRYL K., KOWALSKI R.K., GLOGOWSKI J. 2011. *Artificial reproduction of wild and cultured barbel Barbus barbus (Cyprinidae) under controlled conditions*. Acta Vet. Hung., 59(3): 363–372.

POLISH JOURNAL OF NATURAL SCIENCES
Abbrev.: Pol. J. Natur. Sc., Vol 27(2): 203–214, Y. 2012

FISHERY MANAGEMENT OF OMUL (*COREGONUS AUTUMNALIS MIGRATORIUS*) AS PART OF THE CONSERVATION OF ICHTHYOFAUNA DIVERSITY IN LAKE BAIKAL

***Vasily V. Smirnov*[1], *Natalia S. Smirnova-Zalumi*[2], *Lubov V. Sukhanova*[2]**

[1] Baikal Museum of Irkutsk Scientific Center SB RAS
[2] Limnological Institute SB RAS

K e y w o r d s: fisheries, ichthyofauna conservation, lake Baikal, *Coregonus autumnalis*.

A b s t r a c t

The ecosystem of Lake Baikal has been recognized for its uniqueness as well as the need to preserve its natural structure and functions. Commercial fishery, and especially large catches of Baikal omul, *Coregonus autumnalis migratorius*, influence the general biological diversity of the lake. The paper discusses main guidelines for managing commercial fishing and presents the population structure of Baikal omul. The idea of sustainable fishery that involves preservation of the entire community of Lake Baikal's ichthyofauna is proposed.

GOSPODAROWANIE RYBOSTANEM OMULA (*COREGONUS AUTUMNALIS MIGRATORIUS*) JAKO CZĘŚĆ OCHRONY RÓŻNORODNOŚCI ICHTIOFAUNY W JEZIORZE BAJKAŁ

***Vasily V. Smirnov*[1], *Natalia S. Smirnova-Zalumi*[2], *Lubov V. Sukhanova*[2]**
[1] Muzeum Bajkału Irkuckiego Rosyjskiej Akademii Nauk
[2] Instytut Limnologiczny Rosyjskiej Akademii Nauk w Irkucku

S ł o w a k l u c z o w e: rybołówstwo, ochrona ichtiofauny, jezioro Bajkał, *Coregonus autumnalis migratorius*.

Address: Vasily V. Smirnov, Baikal Museum of Irkutsk Scientific Center SB RAS, Akademicheskaya 1, Listvyanka, Irkutsk, 66601, Russia, phohe: +7(3952) 29 05 51, e-mail: bmsmirnov@mail.ru

Abstrakt

Ekosystem jeziora Bajkał jest znany ze swojej unikatowości, jak również z potrzeby ochrony jego naturalnej struktury oraz funkcji. Rybołówstwo przemysłowe, a w szczególności duże połowy omuli bajkalskich (*Coregonus autumnalis migratorius)*, wpływają na biologiczną różnorodność jeziora. W artykule omówiono główne wytyczne dotyczące gospodarowania rybostanem i przedstawiono strukturę populacji omuli bajkalskich. Zaproponowano strategię gospodarki zrównoważonej, która objęłaby ochronę całej ichtiofauny jeziora Bajkał.

Introduction

Technological progress and the associated gradual changes in the human environment have altered the hierarchy of priorities as regards the use of natural resources. The challenge of ensuring pure potable water has come to the forefront, along with the problem of preserving those areas which so far have not been modified by economic activities. In the 1970s and 1980s, Lake Baikal began to be regarded as a source of pure potable water of national and global significance. Since the 1990s, the lake has served this purpose. In 1996, in response to the application submitted by Russia, Lake Baikal and the adjacent territory were listed as one of the UNESCO World Natural Heritage sites. In 1999, the Federal Law on Protection of Lake Baikal was passed. The lake's ecosystem was recognized as a unique one and in need of the preservation of its natural structure and functions (DOBRETSOV 2003). Since then, it has been necessary to cope with the problem of how to reconcile the status of a special protected territory with plans for socioeconomic development of the region, in which commercial fishery has an important role.

Background: Fishing on Lake Baikal

Compared with other economic activities in the region, the exploitation of fish resources in Lake Baikal has the longest history. Lake Baikal has been known for its extremely rich resources of fish from time immemorial. The earliest written mentions on commercial catches date back to the 17th and 18th centuries. Most catches comprised omul (*Coregonus autumnalis migratorius* (Georgi)). During the above period, as much as 8–10 thousand tonnes of this fish were caught annually. At the turn of the 18th and 19th centuries, first reports on fluctuations in fish catches started to emerge.

A drastic and persistent decline in catches, down to 2.5–1.0 thousand year, was observed in the late 19th c. and early 20th c. It was suggested then that omul could disappear completely and therefore it was necessary to undertake protection and artificial breeding measures. A new increase in catches started

in the mid-1930s. As early as 1937–1942, the annual catch of omul reached 6–9 thousand tonnes. However, it was a short-lived increase. The mid-1940s witnessed another decline in catches. Towards 1968, the annual catches decreased to 1 000 t (Figure 1). The simultaneous drastic reduction in the spawning herds was responsible for the total cessation of commercial fishing of the omul in 1969 (SMIRNOV and SMIRNOVA-ZALUMI 1979).

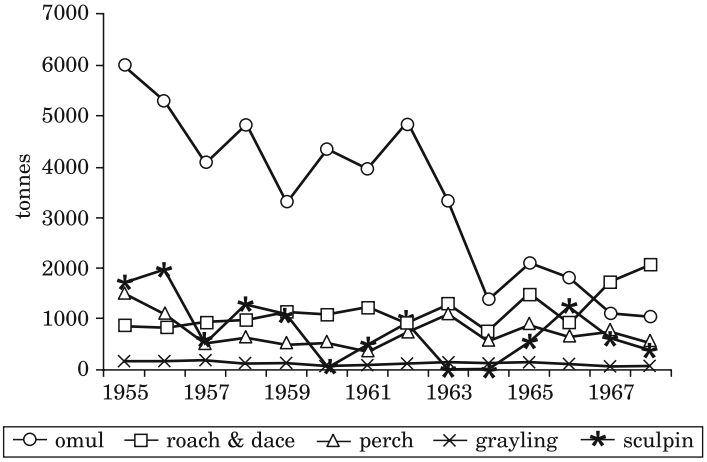

Fig. 1. Annual catches of the main commercial fishes during 1955–1968

The fishing companies, while struggling with lower Baikal omul catches in the 1950s and 1960s, were trying to maintain the previous level of the overall fish catch by harvesting more river perch (*Perca fluviatilis*), roach (*Rutilus rutilus*), dace (*Leuciscus leuciscus*) and other fish species living in Lake Baikal's gulfs and in the deltas of its large tributaries as well as the lakes within their basins (Figure 1). More intensive catches of the above fish, however, led to depleting their resources. Such near-shore species as Baikal whitefish (*Coregonus lavaretus*), grayling (*Thymallus arcticus*) and sturgeon (*Acipenser baeri*) have lost their commercial significance.

Main guidelines for managing commercial fishing

In 1982, when commercial exploitation of Baikal omul was resumed after the 13-year-long prohibition period, the underlying fishing regulations were modified. The ecological principles of managing fishing intensity, developed during the period of prohibition, were adopted. Thus, the intensity of commercial fishing was not regulated with quotas but through the spatiotemporal

structure of fishing expressed in terms of the number of fishing implements used, their distribution in areas of commercial fishing, and seasons of fishing (SMIRNOV 1977, 1979). The planned volume of total catch was achieved with the smallest possible number of fishing teams engaged in fishing operations (Figure 2), a solution which was accompanied by the enhanced economic efficiency of fishing operations and financial incentives offered to fishermen as well as by a decrease in unaccountable fish catches.

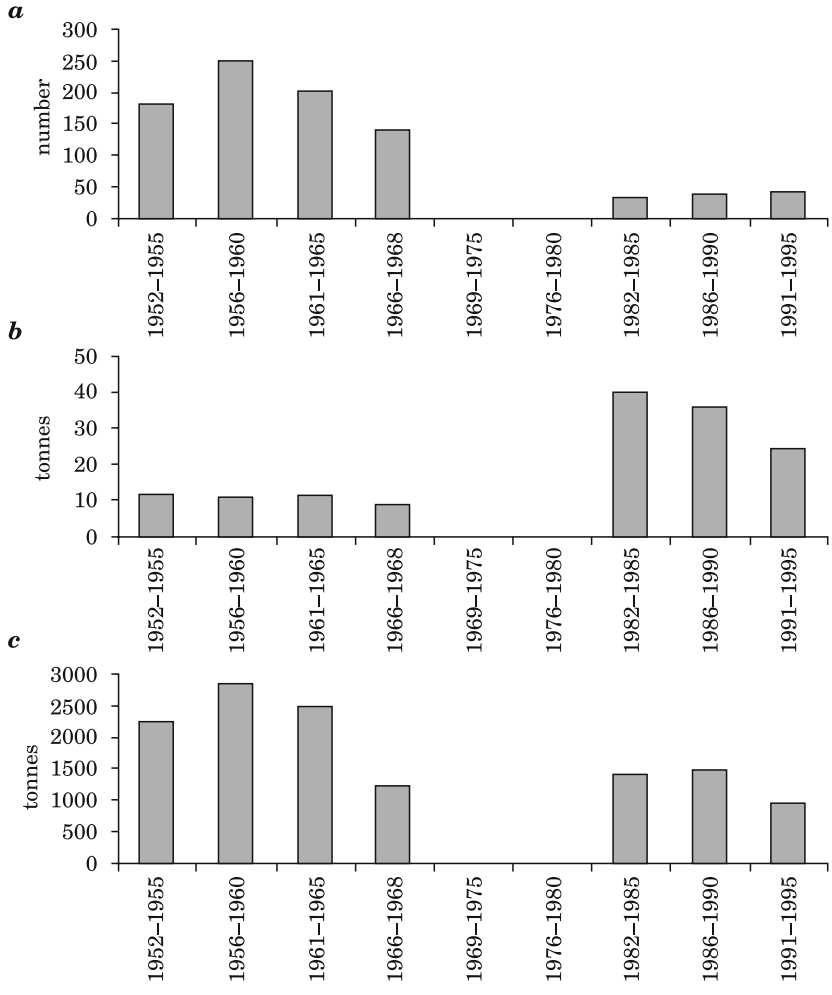

Fig. 2. Structure of commercial omul fishing prior to its prohibition by the fishery industry of the Republic of Buryatia, without limitation on catch intensity (1952–1968), and after it was resumed, with annual catch intensity planning expressed in terms of the quantity and composition of fishing implements, and areas and periods of fishing (1982–1995). The mean indices for periods covering 5 years are given: *a* – number of commercial fishing teams; *b* – annual catch of omul per team; *c* – total annual catch of omul by all teams

The transition of Russia to market economy coincided with a decreased significance of large fish factories on Lake Baikal during 1990–2000, and a growing number of smaller cooperatives engaged in fishing, processing and sales of omul and other fishes. As a result, a marked increase in the fishing seasons occurred and the quality of fish products improved substantially.

However, a very important question has been completely neglected, i.e. how does the fishing of omul (Figure 1) influence the resources of other fishes? Both prior to the prohibition of commercial omul fishing (before 1969) and at the present time, this fish was and still is captured in the near-shore waters, i.e. in habitats of other fish species, e.g. grayling, whitefish and sturgeon, which are less numerous, but equally valuable commercial fishes. During the 1950s–1960s, in shallow-water areas along the eastern shores of the lake, as many as 90 fixed seines and 40 haul seines, and up to 320 km of gill nets were installed in the summer season every year (Figure 3). The use of such a large number of fishing implements, designed for fishing omul specimens weighing on average 200–300 g, led to a mass destruction of sturgeon and whitefish fry. A single set of gill nets totalling 2000 m in length yielded a catch of 40–100 small sturgeon individuals up to 300 g in weight (YEGOROV 1960). From 1960 to 1980, seasonal distribution, abundance and biological state of Baikal omul were monitored by scientists, who noticed that up to 10 individuals of sturgeon fry were present in a catch performed in the lake's near-shore zone with standard 300-meter-long sets of gill nets of the mesh size ranging from 16 to 40 mm. However, it is only the mass catches of younger age groups of

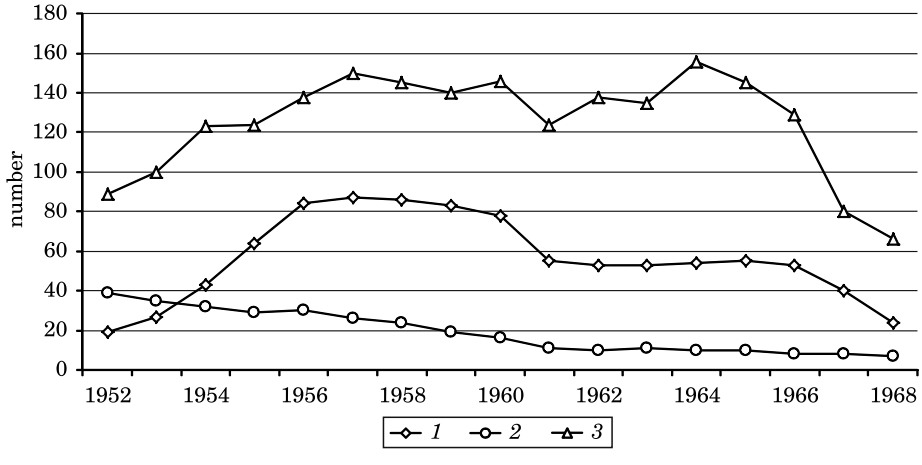

Fig. 3. Number of fishing implements as used in commercial omul fishing by the fishery industry of the Republic of Buryatia during 1952–1968 years: *1* – fixed seines; *2* – haul seines; *3* – gill seines two thousand meters long

sturgeon during the summertime fishing of omul that can be held responsible for a nearly complete ineffectiveness of fish-breeding operations aiming at increasing the abundance of sturgeon.

In the 1950s, a high number of Baikal whitefish *C. lavaretus* was caught while fishing omul in the shallow waters and bays of Lake Baikal (STERLYAGOVA 1958). In the 1960s, the fry of Baikal whitefish (from 1 to 5 years old) made up 30–90% of the total catch found in shutter and wind-seine (SKRYABIN 1969). Intensive fishing of omul in 1950–1960 had an adverse influence on the resources of Baikal grayling as well (TUGARINA 1981).

The threat to commercially valuable species inhabiting the near-shore waters (sturgeon, grayling, Baikal lake whitefish and lake-river whitefish) can only be overcome by relocating omul fishing operations from the near-shore zone to the main habitat of omul populations in the lake's open pelagic zone.

Population structure of Baikal omul

Most reports on protection and sustainable management of renewable natural resources use the term 'biological diversity' to refer to the composition of species. However, a water body is populated not by species as such, but by populations of species that have adapted themselves to its particular biotopes (BEKLEMISHEV 1951). The system of these populations treated as elementary self-reproducing units of a given species, i.e. the form of its existence (TIMOFEYEV-RESOVSKY 1958, SHVARTS 1967, MAYR 1970), is what the term 'biological diversity' covers more precisely.

The reproductive isolation of Baikal omul populations (SMIRNOV et al. 2009, 2011) was confirmed by biochemical (TALIYEV 1941, USHAKOV et al. 1962, MAMONTOV and YAKHNENKO 1987) and genetic investigations (SUKHANOVA 2004), as well as by labelling mature fishes in spawning rivers (MISHARIN and TYUMENTSEV 1965, SMIRNOV and SHUMILOV 1974). For their reproduction, the populations have colonized the river basins which are highly different in many parameters. The omul population in the Selenga River is the most numerous one because of a large area covered by the spawning habitats in this river (which is 1590 km long), where spawning herds migrate as far as 400–500 km upstream from the mouth (SELEZNEV 1942, SOROKIN 1981, VORONOV 1993). The omul of the North Baikal population is less numerous. The distance covered by spawning herds of this population in the Upper Angara and Kichera Rivers is 100–300 and 50–70 km, respectively (TYURIN and SOSINOVICH 1937, SMIRNOV and SHUMILOV 1974). Spawning sites of less numerous populations (the Posolsky, Chivyrkui, Kikinsky and some other populations reproducing in small rivers flowing into the lake)

are just 3–30 km from the river mouth (MISHARIN 1958, SMIRNOV and SHUMILOV 1974, STERLYAGOVA and KARTUSHIN 1980).

Reproducing in different zones (in the latitude and landscapes), the populations of Baikal omul differ not only in their number but also in the rhythms of their fluctuations. The river basins of the northern termination of Baikal (the Upper Angara and Kichera), and of the Selenga river are under the influence of opposing air masses (arriving from the North Atlantic and the Pacific, respectively) (AFANASYEV 1976, OBOLKIN 1977). The regime of atmospheric precipitation and the river runoff difffish in the early stages of ontogenesis (SMIRNOV and SMIRNOVA-ZALUMI 1999).

The dynamic characteristics of the abundance of populations, maturing age and age structure of populations are associated with the birth rate rhythms of generations and their foraging habitat. In the 1950s–1977s, the age when mass reproduction of individuals in omul populations occurred was 5–6 years in the North Baikal population (SMIRNOV and SHUMILOV 1974) and 10–11 years in the Selenga and Posolsky populations (SMIRNOVA-ZALUMI 1977, SMIRNOV et al. 1987).

The main biotope of populations. The adaptability of populations to the conditions for their reproduction in the river basins coincides with the adaptation to living in the conditions present in Lake Baikal's upper 350-meter water column (SMIRNOV 1969, 1992, SMIRNOVA-ZALUMI and SMIRNOV 1973, SMIRNOV et al. 2009):

1. Pelagic omul. The extensive spawning habitat of omul of the Selenga population is correlated with the exploitation by omul of food resources from Lake Baikal's largest biotope, i.e. the entire epipelagic zone. The highest concentrations of omul of the Selenga population occur in the layers of seasonal and deep thermocline. The seasonal thermocline in the summer-autumn period is observed at a depth of up to 10–20 m, and the deep thermocline occurs throughout the entire summer at a depth of 100–150 m from the water surface.

2. Coastal-pelagic omul. Feeding migrations of omul of the North Baikal population, having smaller areas of spawning sites, encompass the near-shore-pelagic biotope of Lake Baikal. During the daytime, North Baikal omul is distributed in the benthic water layers. At nightfall, omul ascends to the upper 5-meter layer following its food, i.e. zooplankton (AFANASYEVA 1977), young brood of benthic amphipods (BESSOLITSYNA 2002), and larvae and fry of yellowfin (*Cottocomephorus grewingki*) and longfin (*Cottocomephorus inermis*) sculpins (KORYAKOV 1972), thus forming nighttime concentrations.

3. Benthic-deepwater omul. The omul reproducing in small tributaries of the lake (of the Posolskaya, Chivyrkaya and Kikinskaya populations) has colonized the least extensive biotopes in the narrow slope area of the pelagic

zone adjoining the areas of reproduction. In these biotopes, in the zone where vertical hydrological fronts interact, in the benthic water layer at a depth of 50–350 m, vertical diurnal migrations result in considerable concentrations of food for omul, e.g. medium and large Macrohectopus (*Macrohectopus branizkii*), fry of little *Comephorus dybowskii* and big *Comephorus baicalensis* Baikal oilfish, yellowfin (*C. grewingki*) and longfin (*C. inermis*) sculpins (SMIRNOV 1974, KASTORNOV 1983, NAGORNY 1984).

Shutter and wind seines (principal modern industrial fishing gear) are used in the biotope of the Severobaikalskaya omul population. The Selenginskaya population and omul populations of the lake's small tributaries concentrate here for a short time in spring and during pre-spawning migrations in autumn (SMIRNOV et al. 2009). Basically, the fishing operations here focus on these concentrations of fish. During the year, most omul fish are not available to fishermen. At the same time, whitefish, sturgeon, and grayling are found in the zone where fishing takes place.

Variability in distribution of omul and in commercial catches

High variability of the seasonal distribution and associated fluctuations of commercial catches between years are another characteristic of Baikal omul (SMIRNOV et al. 2009). The main factor generating these fluctuations is the variability of temperatures of Lake Baikal's waters. The instability warming-up of the lake's different areas and zones in particular years is reflected by the distribution dynamics specific for each fish population. It mainly affects migrations of the most numerous (Selenga) omul population. Surveys from 1966–1968 showed that the yield of omul catches depended on the formation a layer of water where temperature jumped, and on the surface waters (epilimnion) being warmed up to 7–9°C (SMIRNOV and SHUMILOV 1974).

The importance of the degree of warming of the water masses in Lake Baikal's deep-water areas for omul's foraging behaviour is quite well illustrated by the data from surveys carried out in June in 1971–1982, which covered the main areas of springtime concentrations of the fish. The higher the values of water temperature and water heat content in the lake's deep-water areas, the higher the production of phytoplankton in the 0–25 m layer and the larger the total biomass of zooplankton in the 0–250 m layer (SHIMARAYEV 1971, 1977, SHIMARAYEV and AFANASYEVA 1977, BEKMAN and SMIRNOV 1977). This in turn means that this zone is more extensively colonized by omul in the summer season and therefore fewer omul individuals remain in shallow-water areas. Another analysis of the annual values of water temperature in May in

the Peschanaya Bay (the most representative of the thermal conditions of the Selenga area and the adjacent water masses of Central and South Baikal (SHIMARAEV 1971) compared to average catches of omul with experimental arrays of gill nets in the shallow-water areas in June showed contrary changes of these indicators in the series for 1972–1981 (r = 0.8). That is to say, the better the habitat conditions for omul in the lake's deep-water areas, the larger the numbers in which the populations are distributed in their biotopes and the lower its concentration in the shallow-water areas. A significant increase in commercial catches was observed during the years (1945, 1947, 1951, 1954–1958) with low enthalpy waters of the lake in spring, when more omul individuals would migrate from deep waters to the lake's littoral zone. Dislocation of fishing in the coastal-pelagic zone limits the capacity of fishing effort – omul fishing is effective here for 1–2 months, but not in every year.

Rational commercial fishing in general must take into consideration the interannual and seasonal changes in the distribution of populations in biotopes.

Fishery management of omul – recommendations

Biological diversity conservation in Lake Baikal is only possible by adopting the biocenotic approach to exploitation of natural resources, including fish stocks. In order to promote sustainable fishery management in Lake Baikal, the authors recommend:

– to restore the abundance and commercial fishing significance of such commercially valuable species as Baikal sturgeon and Baikal lake and lake-river whitefish through relocation of commercial fishing of omul from the near-shore zone to the pelagic zone of the lake's deep-water areas.

– to establish rational fishery which will involve maintenance of the natural structure of commercial fish populations.

– to plan catches of particular populations of omul, having regard to their importance in the formation of the overall number and biomass of the species, characteristics of their seasonal distribution, variability of foraging and spawning migrations.

– to resume omul catches with drifting nets in pelagic deep-water districts of the lake so as to promote rational distribution of fishery load on omul populations.

– to monitor continuously the fish stocks, which is necessary if the fishery industry on Lake Baikal is to progress. The monitoring should include observations of the species, population size, age and weight structure

of commercial catches. These indicators will form a basis for a method to assess the number and biomass of populations of commercial species.

– to use bio-statistical methods for assessment of the commercial stock of fish (DERZHAVIN 1922), which is not only economically advantageous estimate of commercial reserves, but also the most reliable method (RICKER 1971) under the conditions of rational (intensive) exploitation of fish stocks. The fishing operations will furnish the necessary information for planning their structure and intensity for each subsequent year and for the short- and long- term future.

Translated by G.J. NAGORNAYA

Accepted for print 23.03.2012

References

AFANASYEV A.N. 1976. *Water resources and water balance of the Lake Baikal Watershed Basin*. Novosibirsk, Nauka, 238 p.

AFANASYEVA E.L. 1977. *Biology of Baikal Epischura*. Novosibirsk, Nauka, 144 p.

BEKLEMISHEV V.N. 1951. *On the classification of biocenotic (symphysiological) associations*. Byul. MOIP. Otd-nie biol., 6: 3–30.

BEKMAN M.YU., SMIRNOV V.V. 1977. *Conclusion*. [In:] Biological productivity of Baikal's pelagic zone and its variability. Novosibirsk, Nauka, pp. 234–245.

BESSOLITSYNA I.A. 2002. *Nighttime vertical migrations of Baikal benthic amphipods*. Publ. by the Institute of Geography SB PAS, 160 p.

DERZHAVIN A.N. 1922. *Stellate Surgeon*. A biological essay. Baku, 393 p.

DOBRETSOV R.L. 2003. *The problems of Baikal and their legislative solution. Law of Russian Federatioǹ About the Protection of Lake Baikal'' as a factor of sustainable development in the Baikal Region*. International Scientific Conference Release. Irkutsk, September 16–19, 2003. Irkutsk: Izd-vo Instituta geografii SO RAN, pp. 3–6.

KORYAKOV E.A. 1972. *Pelagic Cottoidei of Lake Baikal*. Novosibirsk, Nauka, 156 p.

KOSTORNOV S.N. 1983. *Biological characterization of May activity of yellow-fin sculpins*. [In:] *Dynamics of production of fish in Lake Baikal*. Novosibirsk Nauka, pp. 15–23.

MAMONTOV A.M., YAKHNENKO V.M. 1987. *Biochemical polymorphism of omul*. [In:] *Fish morphology and Ecology*. Novosibirsk, Nauka, pp. 9–19.

MAYR E. 1970. *Populations, species, and evolution*. Cambridge: Belknap Press of Harvard University Press, 453 p.

MISHARIN K.I. 1958. *Baikal omul*. [In:] Fishes and fishery in the Lake Baikal Basin. Irkutsk, pp. 130–287.

MISHARIN K.I., TYUMENTSEV N.V. 1965. *Migration of Baikal omul according to ringing results*. Izv. biol.-geogr. nauch.-issled. in-ta pri Irkutskom gos. un-te, 18(1–2): C. 50–61.

NAGORNY V.K. 1984. *Comephorus and their role in the ecosystem of Lake Baikal*. [In:] The contribution of young biologists in Siberia solution of the food program and the environmental protection. Ulan-Ude, p.73.

OBOLKIN V.A. 1977. *Long-term fluctuations of atmospheric precipitation across the territory of East Siberia and their association with some types of macrosynoptic processes*. [In:] Long-term Forecasts of Natural Phenomena. Novosibirsk, Nauka, pp. 64–67.

RICKER W.E. 1971. *Derzhavin's biostatistical method of population analysis*. J. Fish. Res. Board Can., 28: 1666–1672.

SELEZNEV I.N. 1942. *Baikal omul, its natural reproduction and prospects for artificial breeding*. Izv. biol.-geogr. nauch.-issled. in-ta pri Irkutskom gos. un-te, 9(1–2): 24–38.

SHIMARAEV M.N. 1971. *Hydrometeorological factors and fluctuations in the population of Baikal zooplankton*. Tr. Limnolog. in-ta SO AN SSSR, 12(32): 259–267.

SHIMARAEV M.N. 1977. *Elements of the Thermal regime of Lake Baikal*. Novosibirsk, Nauka, p. 150.

SHIMARAEV M.N., AFANASYEVA E.L. 1977. *Influence of temperature conditions on interannual changes in summer zooplankton of the pelagic zone*. [In:] Biological productivity of Baikal's pelagic zone and its variability. Novosibirsk, Nauka, pp. 61–76.

SHVARTS S.S. 1967. *Populational structure of a species*. Zool. zhurn., 46(10): 1456–1469.

SKRYABIN A.G. 1969. *Baikal whitefish biology*. Moscov, Nauka, p. 112.

SMIRNOV V.V. 1969. *Age variability of Baikal omul Coregonus autumnalis migratorius (Georgi)*. Problems of Ichthyology, 9: 508–515.

SMIRNOV V.V. 1974. *Major trends in microevolution of Baikal omul Coregonus autumnalis migratorius (Georgi)*. Zoological studies of Siberia and the Far East. Vladivostok: 145–152.

SMIRNOV V.V. 1977. *Ecological principles of commercial fishing intensity management (exemplified by Baikal omul)*. [In:] Turnover of matter and energy in water bodies. Fishes and Fish resources. Listvennichnoye at Baikal, pp. 89–92.

SMIRNOV V.V. 1979. *The ecological intensity management system for commercial fishing of omul (a new approach in solving the fishery regulation problem)*. Rybnoye khozyaystvo 3: 9–11.

SMIRNOV V.V. 1992. *Intraspecific structure of Baikal omul Coregonus autumnalis migratorius (Georgi.)*. Pol. Arch. Hidrobiol., 39: 325–333.

SMIRNOV V.V., VORONOV M.G., VORONOV A.V. 1987. *On the intraspecific structure of Baikal omul Coregonus autumnalis migratorius (Georgi)*. Vopr. ikhtyologii 27: 342–345.

SMIRNOV V.V., SMIRNOVA-ZALUMI N.S. 1979. *Questions of forecasting, and main principles of urilization of Baikal Omul*. Novosibirsk, Nauka, pp. 138–143.

SMIRNOV V.V., SMIRNOVA-ZALUMI N.S. 1999. *Factors determining year-class strength in populations of Lake Baikal omul, Coregonus autumnalis migratorius (Georgi).* Arch. Hydrobiol. Spec. Issues Advanc. Limnol., 57: 65–75.

SMIRNOV V.V., SMIRNOVA-ZALUMI N.S., SUKHANOVA L.V. *Microevolution of Baikal omul Coregonus autumnalis migratorius (Georgi)*. Novosibirsk, Publishing House of Siberian Branch of RAS, 2009, p. 245.

SMIRNOV V.V., SMIRNOVA-ZALUMI N.S., SUKHANOVA L.V. 2011. *Dynamic structuring of water masses and speciation in Lake Baikal*. In: Water biodiversity assessment and protection. Eds. M. Jankun, G. Furgala-Selezniow, M. Wozniak, A.M. Wisniewska. Olsztyn, pp. 21–34.

SMIRNOV V.V., SHUMILOV I.P. 1974. *Omul of Lake Baikal*. Novosibirsk, Nauka, p. 160.

SMIRNOVA-ZALUMI N.S. 1977. *Structure of the spawning stock and the reproduction level of the Posolsky popualton of omul*. [In:] Biological productivity of Baikal's pelagic zone and its variability. Novosibirsk, Nauka, pp. 155–166.

SMIRNOVA-ZALUMI N.S., SMIRNOV V.V. 1973. *Omul populations in the Lake Baikal ecosystem*. [In:] Turnover of matter and energy in lakes and reservoirs. Irkutsk, pp. 92–95.

SOROKIN V.N. 1981. *Natural reproduction conditions for omul in the Selenga*. [In:] Ecology, diseases and breeding of baikal omul. Novosibirsk, Nauka, pp. 34–44.

STERLYAGOVA M.A. 1958. *The Biology and fishery of Baikal whitefish. B: Fish and fishery in the basin of Lake Baikal*. Irkutsk, pp. 288–310.

STERLYAGOVA M.A., KARTUSHIN A.I. 1980. *Natural reproduction and prospects for artificial breeding of Chivyrkui omul*. [In:] Fishes and fishery of east Siberia. Ulan-Ude, pp. 126–137.

SUKHANOVA L.V. 2004. *Molecular-phylogenetic study of Baikal omul Coregonus autumnalis migratorius (Georgi)*. Author's Abstract of Cand. Sci. Degree Dissertation. Novosibirsk, p. 17.

TALIYEV D.N. 1941. *Serological analysis of Baikal omul*. Tr. Zool. in-ta AN SSSR 6: 68–91.

TIMOFEYEV-RESOVSKY N.V. 1958. *Microevolution. Elementary phenomena, material and factors of the evolution process*. Bot. zhurn, 43: 317–336.

TUGARINA P.J. 1981. *Baikal grailings*. Novosibirsk, Nauka, p. 283.

TYURIN P.V., SOSINOVICH P.N. 1937. *Materials for understanding spawning of Baikal omul in the Kichera river*. Izv. biol.-geogr. nauch.-issled. in-ta pri Irkutskom gos. un-te, 7: 198–224.

USHAKOV B.P., VINOGRADOVA A.N., KUSAKINA A.A. 1962. *Cytophysiological analysis of the intraspecific differentiation of omul and grayling in Lake Baikal*. Zhurn. obshch. biologii, 23: 56–63.

VORONOV M.G. 1993. *The ecologo-biological foundations of the enhancement in reproduction efficiency of Omul in the Selenga River under Contemporary conditions*. Author's Abstract of Cand. Sci. (Biol.) Degree Dissertation. St. Petersburg, p. 18.

YEGOROV A.G. 1960. *Baikal Sturgeon*. Ulan-Ude, p. 122.

POLISH JOURNAL OF NATURAL SCIENCES
Abbrev.: Pol. J. Natur. Sc., Vol 27(2): 215–227, Y. 2012

REPRODUCTION OF THE MODEL FISH: ROSY BARB (*PUNTIUS CONCHONIUS*), UNDER CONTROLLED CONDITIONS

Katarzyna Targońska, Dariusz Kucharczyk

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

K e y w o r d s: rosy barb, model species, controlled reproduction, hatching, aquaristics.

A b s t r a c t

A study has been conducted of reproduction of the rosy barb (*Puntius conchonius*) under controlled conditions. This fish are commonly kept in aquaria and are also a model fish used in research studies. It has been shown that rosy barb spawners should be kept in water at 20°C before reproduction, with 23°C being the optimum temperature for reproduction and eggs incubation. The interval between successive spawnings should range from 20 to 40 days. If it is too long, reaching 60 days, the quality of the gametes, expressed in the number of reproduced fish, is significantly reduced. The effectiveness of reproduction increases when there are more males than females in the spawning shoal, with the sex ratio being not lower than 2:1. Despite the small size of the fish, eggs can be also obtained by a semi-artificial method. The quality of the eggs thus obtained is not lower than that of the eggs produced in spontaneous spawning.

ROZRÓD RYBY MODELOWEJ – BRZANKI RÓŻOWEJ (*PUNTIUS CONCHONIUS*) W WARUNKACH KONTROLOWANYCH

Katarzyna Targońska, Dariusz Kucharczyk

Katedra Rybactwa Jeziorowego i Rzecznego
Uniwersytet Warmińsko-Mazurski w Olsztynie

S ł o w a k l u c z o w e: brzanka różowa, gatunek modelowy, sztuczny rozród, wylęganie, akwarystyka.

Address: Katarzyna Targońska, University of Warmia and Mazury, ul. Michała Oczapowskiego 5, 10-957 Olsztyn, Poland, phone: +48 (89) 523 44 36, e-mail: targonska.k@uwm.edu.pl

Abstrakt

Przeprowadzono badania nad rozrodem brzanki różowej (*Puntius conchonius*) w warunkach kontrolowanych. Gatunek ten jest często hodowany w akwariach, jest również jedną z ryb modelowych do badań.

Wykazano, że przed rozrodem tarlaki brzanki różowej powinny być przetrzymywane w wodzie o temperaturze 20°C, a najlepsza temperatura w trakcie rozrodu oraz do inkubacji wynosi 23°C. Odstępy między kolejnymi tarłami nie powinny być krótsze niż 20 i dłuższe niż 40 dni. Gdy są zbyt długie i wynoszą 60 dni, jakość otrzymanych gamet, wyrażona ilością otrzymanego wylęgu, znacząco się obniża. Efektywność rozrodu jest zwiększona gdy w stadzie tarłowym jest większa liczebność samców niż samic, a stosunek płci wynosi nie mniej niż 2:1. Mimo małych rozmiarów ciała tych ryb możliwe jest pozyskanie ikry od samic metodą półsztuczną. Jakość tak pozyskanej ikry nie jest niższa niż uzyskana podczas tarła spontanicznego.

Introduction

Production and trading in ornamental fish is a large and profitable branch of agriculture (YANONG 1996, TLUSTY 2002, CEK and GOKCE 2005, CHELAPPA et al. 2005, WHITTINGTON and CHONG 2007). Its fastest growth has been observed in Asia, but aquarium fish are also bred in many other part of world, including Europe. Cyprinids are commonly bred as aquarium fish, but also as model fish for scientific research. These include e.g. the zebrafish (*Danio rerio*) (LAAN et al. 2002, PYRON 2003, KOC et al. 2008, SPENCE et al. 2008, SEGNER 2009) and the rosy barb (*Puntius conchonius*) (CEK and GOKCE 2005, TARGOŃSKA 2007). The latter species is found in natural conditions in the tropical waters of south-east Asia, including: Afghanistan, Pakistan, Nepal, India and Bangladesh. The popularity of domestic aquarium fish, including the rosy barb, is caused both by its attractive colouring and ease of breeding (CEKet al. 2001, CEK and GOKCE 2005, KUCHARCZYK et al. 2008a, KUPREN et al. 2008a, PRUSIŃSKA et al. 2008).

Some aquarium fish, such as medaka (*Ozyrias latipes*) and zebrafish, are commonly used as model fish in scientific research (e.g.: KOGER et al. 1999, PYRON 2003, SCHOLTZ et al. 2003, KOC et al. 2008, SPENCE et al. 2008, SEGNER 2009). They are popular because they are easy to breed; the biology of their reproduction has been elucidated in detail and they achieve sexual maturity very early. Laboratory experiments involving the fish have resulted in breeding lines in which the females can spawn every day, which, however, significantly affects the fertility (e.g.: LAAN et al. 2002, WITTBRODT et al. 2002, GERLACH 2006, SIMAO et al. 2007, BALASUBRAMANI and PANDIAN 2008). Final gamete maturity in these fish is achieved by stimulation through environmental conditions: temperature and photoperiod (YANONG 1996, KOC et al. 2008, SPENCE et al. 2008, SEGNER 2009).

Recently, model fish have come to include other species of ornamental and domestic aquarium fish. Cyprinids are represented in this group e.g. by the goldfish (*Carassius auratus auratus*) (e.g.: YUEN et al. 1997, STACEY et al. 2001, KOBAYASHI et al. 2002, BANDYOPADHYAY et al. 2005, TARGOŃSKA and KUCHARCZYK 2011) and the rosy barb. The latter species has been used to study the morphology and operation of micropyle in fish, ontogenesis, genome manipulations, including androgenesis and hormonal sex changes, freezing embryos and clusters of cells (e.g. AMAZE and IYENGAR 1990, ADAM et al. 1995, CEK et al., 2001, KIRANKUMAR et al. 2003, PANDIAN and KIRANKUMAR 2003). However, no reports have been published on the comprehensive technology of rosy barb reproduction under controlled conditions. The data published to date have been related to selected fragments of reproduction biology of the species (CEK et al. 2001, BHATTACHYARA et al. 2005, 2006, CEK and GOKCE 2005, TARGOŃSKA 2007). The majority of the data available on this species include amateur observations from domestic culture rather than scientific publications. Data on reproduction under controlled conditions are absent not only for the rosy barb, but also for many other species of aquarium and ornamental fish, even if final maturation of gametes is induced by the administration of hormonal agents (YANONG 1996).

Aim of the study

The aim of this study was to determine the optimum conditions for effective reproduction of the rosy barb under controlled conditions without hormonal stimulation.

Materials and Methods

About 2000 larvae of rosy barb were obtained from a private aquarium-fish breeding farm in Olsztyn. Initial rearing was conducted for the first three weeks in a 1 L closed water circulation (stocking density – 200 fish L^{-1}), for another 5 weeks – in 50 L circulation with a density of 50 fish L^{-1}. After that time, the broodstock was transferred to 1000 L tanks with a controllable environment, where the broodstock density was 10 fish L^{-1}. While the fish were being reared, the temperature was maintained at between 20 and 21°C, and the rosy barb were fed feed for aquarium fish: *Artemia* nauplii, "Supervit" produced by Tropical, trout pellets "Safir" produced by Aller-Aqua and frozen chironomid larvae (TARGOŃSKA 2007, BALASUBRAMANI and PANDIAN 2008) – Table 1.

Table 1
A listing of the basic components of the feeds offered to the barb during their rearing, according to the producer's data; data on chironomid larvae were from biochemical analysis published by TARGOŃSKA (2007)

Specification	Artemia	Frozen chironomids larvae	Tropical® "Supervit"	Aller Aqua® Safir"
Protein	42	9	48	45
Fat	20	8	8	20
Carbohydrates	11–23	Nd	Nd	16
Ash	Nd	8	8.5	8
Fibre	Nd	Nd	3.5	2

Nd – no data

Initial (mass) spawn

After 10 months of rearing and after the fish had grown to be 4–5 cm long, most of them had reached sexual maturity, which made it possible to effect mass spawning. Spawn grates, made of a 5 mm plastic mesh, were put at the bottom of each 10 L spawning tank, which operated in a closed water circulation. The grates were to protect the eggs from being eaten by the spawners. The tanks were filled with tap water mixed with water subjected to reversed osmosis so that carbonate hardness was lower than 2°n, and the total hardness did not exceed 8°n (BEKASIAK 2000). The temperature was set at 20°C (± 0.1°C). Ten males and fifteen females were put into each of the 53 tanks and the fish were stimulated to spawn. To this end, spawning substrate (*Fontinalis sp.*) was put on the grate, the tanks were totally darkened and the heating of the water to 23°C was started (at a rate not exceeding 1°C h^{-1}). After 12 hours the light was switched on and the fish were checked to see if they were starting to spawn. Since the spawning act is spread over time and usually lasts from 2 to 4 hours, checks were made three times (every 4 hours) whether the spawn had been laid, and the fish were then caught.

After the first (control) spawn, the fish which started reproduction were divided into three groups and five separate experiments were carried out. The fish which had not started reproduction were transferred to a separate tank and were not used in further experiments.

Experiment 1. Determination of the effect of the number of males in a spawning shoal on the reproduction results. The first experiment involved examination of the effect of the number of males in a spawning shoal on the percentage of females which start reproduction and on the rate of embryo survival at the eyed-egg stage. Forty spawning teams were used to this end. A spawning team consisted of 1 female and 1, 2, 3 or 4 males. Each

of the teams was put in a separate 10 L aquarium, whose size and accessories were the same as those described above. The protocol of spawning was the same as described above. Additionally, three Petri dishes were put under the grate in each aquarium, onto which part of the eggs was to fall, which would enable determining the rate of embryo survival at the eyed-egg stage (PYRON 2003, TARGOŃSKA 2007). After the spawn had ended, the fish were caught and were not used in any other experiment. Forty-eight hours after spawning, the live and dead embryos on each Petri dish were counted. The time between an initial and experimental spawn was 3 weeks.

Experiment 2. Determination of the effect of the inter-spawning period on the spawning results. The issue which was dealt with in the second experiment was what inter-spawning period would be the most beneficial for breeding results. The following indicators of reproduction efficiency were taken as: the number of females which start reproduction and the rate of embryo survival at the eyed-egg stage. Forty reproduction teams, each consisting of one female and two males which had had their first (mass) spawning, were taken for the experiment. After 20, 30, 40 and 60 days, 10 spawner teams were transferred to a separate spawning tank, equipped as in the first experiment. After spawning, the fish were caught and no longer experimented on. Live embryos on each Petri dish were counted 48 hours after spawning in order to determine the survival rate at the eyed-egg stage.

Experiment 3. Determination of the water temperature during the spawning phase on the reproduction results. The effect of three temperatures (20, 23 and 26°C) during the spawning time on the reproduction results were examined in the third experiment. To this end, three groups of fish (10 reproduction teams in each, consisting of one female and two males) were transferred separately to spawning tanks where the temperature was maintained at the levels mentioned above. After spawning, the procedure as in experiment 1 was followed – the spawners were caught and the number of females which started reproduction and the rate of embryos on the Petri dishes was determined. The interval between the initial and experimental spawns was 3 weeks.

Experiment 4. Determination of the effect of water temperature during the period of maintaining spawners before the spawn on the reproduction results. The fourth experiment examined how different water temperatures (20, 23 and 26°C) of maintaining rosy barb spawners for 3 weeks before spawning affected the results of reproduction expressed as the rate of spawning females and the rate of live embryos at the eyed-egg stage. Three fish groups (10 reproduction teams, 1 female and 2 males in each) were used for the purpose. After three weeks of maintenance at those temperatures they were transferred to spawning tanks where the temperature was set at 23°C. The procedure after spawning was the same as in the previous experiments.

Experiment 5. Semi-artificial reproduction of the rosy barb. In the fifth experiment, rosy barb spawners were reproduced by a semi-artificial method. Twenty reproduction teams, one female and two males in each, which had had the first (mass) spawn, were used in the experiment. Ten reproduction teams were used as the control group – the fish reproduced spontaneously. Upon the start of spawning, the females in the study group were caught. After being caught, the fish were given abdominal massage to induce egg shedding onto plastic plates. Subsequently, the males were caught and their abdomens were massaged delicately to get them to shed sperm directly onto the eggs. The spawners were not anaesthetised during those manipulations. Subsequently, water (10 cm^3) from the spawning aquarium was added, gametes were mixed for 30 s and then transferred back to the aquarium. The eggs dropped freely to the bottom, including on the Petri dishes. The procedure followed after spawning was the same as in experiment 1 – spawners were caught after the spawning act, the females which started reproduction and the eyed-egg-stage embryos on the Petri dishes were counted. The interval between the initial and experimental spawn was 3 weeks.

Statistical analysis

The results of the experiments were analysed statistically. The differences in the embryo survival rate between groups in different experiments were subjected to an analysis of variance and Tukey's *post-hoc* test at the significance level of 5%. For the semi-artificial spawning differences were analysed by the Student t-test ($\alpha = 0.05$). Before the statistical analysis, the data expressed in percentage were subjected to arcsine transformation. The relationship between the embryo survival rate and the number of males and the intervals between spawn phases and the temperature of water was examined by means of an analysis of regression.

Results

The parameters studied in the experiment, such as the number of males in a spawning team, and especially the water temperature, affect the percentage of rosy barb females which start reproduction. An analysis of the embryo survival rate has revealed the effect of the number of males in a spawning team has on the reproduction results (Figure 1). The composition of such a team in rosy barb reproduction should be set at a minimal ratio of 1 female: 2 males. It was shown both with such a ratio and when the number of males was greater

(3 or 4) that all the females (100%) started reproduction. Moreover, no statistical differences were found to exist in the rate of embryo survival to the eyed-egg stage. When a female mated with only 1 male, the percentage of females which started reproduction was lower (80%), as was the embryo survival rate.

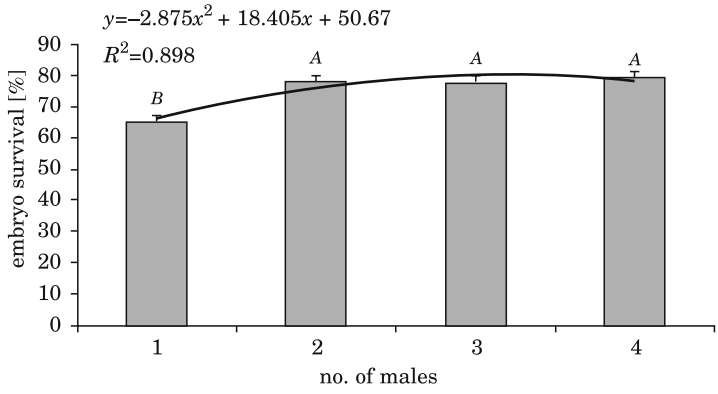

Fig. 1. Survival of rosy barb embryos in relationship of males to females ratio

Examination of the effect of the interval between spawn phases on the reproduction results revealed a reduction of one of the values under examination (embryo survival rate) only when the inter-spawn period was 60 days (Figure 2). This means that the period between reproduction acts in the rosy barb should range from 20 and 40 days. A longer time of maintaining the fish before the next spawn (60 days) resulted in reduction of the embryo survival rate and a reduction of the number of ovulating females to 80%, whereas it ranged from 90% to 100% in the other groups. The temperature was also found to affect the reproduction results. This applies both to the effect of water temperature on spawning (Figure 3) and to the temperature of the water in which the fish were maintained before spawning (Figure 4). Both too low (20°C) and too high (26°C) temperature during the spawning act affected the process of fish reproduction. The percentage of ovulating females was 50% and 40%, respectively, whereas all the females maintained at 23°C started the reproduction process. Moreover, the highest embryo survival rate was also recorded at that temperature (Figure 3). Furthermore, 20°C proved to be the most beneficial temperature for maintaining fish before spawning since 100% of the female fish which started reproduction. Also the highest embryo survival rate were found for this temperature. Raising the temperature of the water in which fish were maintained before spawning resulted in reducing the percentage of ovulating females (23°C – 70%, 26°C – 10%) and the embryo survival

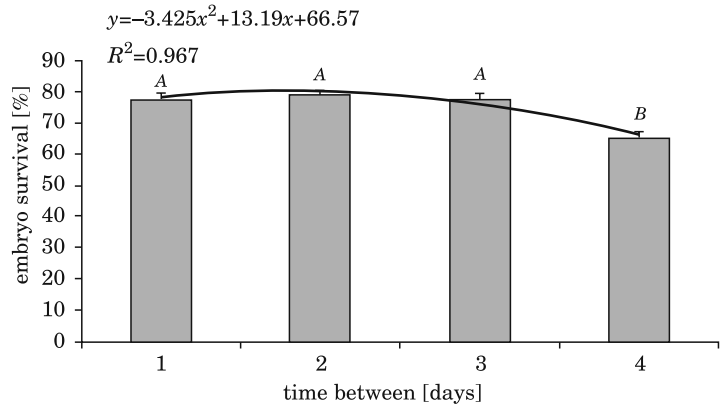

Fig. 2. Survival of rosy barb embryos in relationship to period between following spawns

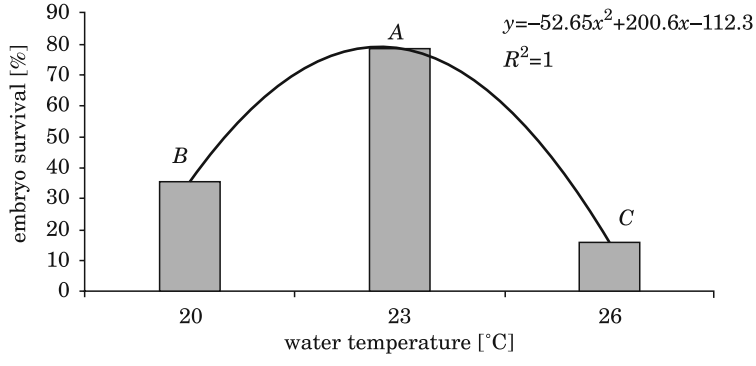

Fig. 3. Survival of rosy barb embryos in relationship to water temperature during spawning

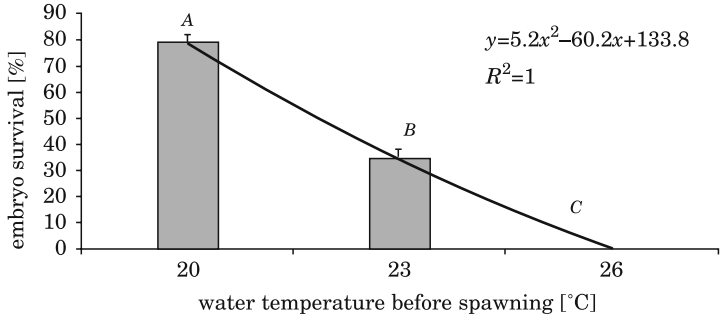

Fig. 4. Survival of rosy barb embryos in relationship to water temperature before spawning

rate (Figure 4). Raising the temperature of water in which the fish were maintained before spawning adversely affected the embryo survival rate to the eyed-egg stage.

A comparison of the semi-artificial spawn did not reveal any statistical differences in the rate of embryo survival to the eyed-egg stage, although the embryo survival rate in the control group was slightly higher than in the study group. The respective values were 82.3 ± 3.2 and 81.1 ± 4.3.

No mortality was observed among the spawners during the experiment, including those which had gametes taken from them manually.

Discussion

Successful reproduction of domestic aquarium and ornamental fish in most cases requires stimulation by environmental conditions, such as temperature, photoperiod and physicochemical parameters of water (YANONG 1996). Such fish include medaka (KOGER et al. 1999, WITTBROAD et al. 2002), zebrafish (KOC et al. 2008, SIMAO et al. 2007, SPENCE et al. 2008, SEGNER 2009), Buenos Aires tetra (KUCHARCZYK et al. 2008a) and rosy barb (CEK et al. 2001, CEK and GOKCE 2005), whose reproduction was examined in this study. The possibility of reproducing fish without the use of any hormonal agents is a very important factor when selecting a species which can be used as a model fish (SPENCE et al. 2008). In rosy barb, raising the water temperature by about 3°C and changing the physicochemical parameters of the water resulted in final gamete maturity achieved after only 12 hours. Similar observations have been made about zebrafish (GERLACH 2006, BALASUBRAMANI and PANDIAN 2008, SPENCE et al. 2008). The time needed for achieving final maturity of gametes in Buenos Aires tetra was longer – it was about 36 hours (KUCHARCZYK et al. 2008a). In some aquarium and ornamental fish it is necessary to apply hormonal injections in order to induce final gamete maturation (YANONG 1996, SEN et al. 2002, DASGUPTA et al. 2009). Reproduction of commercial cyprinids, such as carp, bream, ide, asp, etc. in captivity usually requires hormonal stimulation. It is not possible to obtain gametes without using agents which induce spermation and ovulation (e.g BRZUSKA et al. 2005, KUCHARCZYK et al. 2007, 2008b, TARGOŃSKA et al. 2008, CEJKO et al. 2009). Only in some commercial cyprinids can gametes be obtained by stimulation with environmental conditions: water temperature and photoperiod. This is the case, for example, with the domesticated form of the ide (KREJSZEFF et al. 2009).

The experiment has shown that a spawning team of the rosy barb should consist of a minimum of two males per 1 female. A similar composition of the spawning shoal is recommended in spontaneous reproduction in other species

of aquarium fish (YANONG 1996, KUCHARCZYK et al. 2008a, SPENCE et al. 2008). When the sex ratio was equal to 1:1, a lower rate of rosy barb embryo survival was observed. This may happen for a number of reasons, including a low number of spermatozoa produced by one male, which are insufficient to fertilise an egg, which can be attributed to the relatively high fertility of female rosy barb (VARADI and HORVATH 1993, CEK and GOKCE 2005, TARGOŃSKA 2007). The effect of sperm quality on the embryo survival rate cannot be ruled out, either. The results of studies with other species suggest that the number of spermatozoa per egg should be as high as over 100,000 (RURANGWA et al. 2004). An insufficient number of spermatozoa results in decrease in the percentage of fertilised eggs, which means a reduced percentage of growing embryos. Furthermore, an excessively high number of spermatozoa compared to the minimum number increases the survival rate, and sometimes even compensates for the effect of other adverse factors (RURANGWA et al. 1998, 2004, CASSELMAN et al. 2006). The obtained results suggest, that fish which spawn in stock, like rosy barb need higher proportion of males than females during spawning act. It is probably connected with spawning behaviour of this species and resulted with higher genetic diversity in offspring.

The rosy barb is a fish which spawns in portions. This means that it can reproduce in controlled conditions several times during a short period of time. Based on earlier experiments on the species (TARGOŃSKA 2007, TARGOŃSKA – unpublished data), reproduction trials were made every 20–60 days from the preceding spawn. It has been found that maintaining the fish in water at 20°C for 20–40 days between spawnings gives the best results, understood as the percentage of ovulating females and embryo survival to the eyed-egg stage. The reproduction time between individual spawnings in rosy barb was much longer than that found for the Buenos Aires tetra (*Hemigrammus caudovittatus*) (KUCHARCZYK et al., 2008a) and the neon tetra (KUCHARCZYK et al. 2010). This provides a better opportunity of using the rosy barb as a model fish as compared to the Buenos Aires tetra, because the fish can be reproduced in controlled conditions for several reproduction seasons. Other model fish, such as the medaka or the zebrafish, can be reproduced much more frequently, even every day. However, the obtained fertility rates are often equal to several eggs of spawn per female. It takes a much longer time between spawns to increase fertility (WITTBROTD et al. 2002, SPENCE et al. 2008).

The effect of water temperature on reproduction ability, as well as embryonic and larval development, has been described in a number of fish (BROMAGE et al. 2001, DAVIES and BROMAGE 2002, ANGIUS and CANAVATE 2005), including cyprinids (KUCHARCZYK et al. 1997, 1998, KUPREN et al. 2008b). A similar relationship to the one found in this experiment between the temperature at which the spawners were maintained before reproduction and the embryo

survival rate was observed in the Buenos Aires tetra (KUCHARCZYK et al. 2008a) and in the neon tetra (KUCHARCZYK et al. 2010). Maintaining spawners in excessively warm water before spawning significantly reduces the number of fish which start reproduction and the embryo survival rate. This study has revealed a cumulative effect, both in the possibility of having a spawn and in the proper development of embryos. The effect of environmental factors on the reproduction results has been described, for example, by BROMAGE et al. (2001), DAVIES and BROMAGE (2002), ANGIUS and CANAVATE (2005); studies of cyprinids (nase, *Chondrostoma nasus*, and asp, *Aspius aspius*) in this respect have been conducted by TARGOŃSKA et al. (2008). These studies have shown that the greatest role in the final stage of gametes' maturation is played by temperature. This is similar to embryonic and larval development, when a too high or low temperature reduces the fish survival rate and causes developmental anomalies (KUCHARCZYK 1997, 1998, KUPREN 2008b). Moreover, an excessively short period of maintaining fish in proper thermal conditions before spawning may affect the size of larvae, which has been recorded, for example, in the carp (KUCHARCZYK et al. 2008b).

The possibility of carrying out semi-artificial reproduction and fully transparent spawn provides a perfect opportunity for using rosy barb as a model fish, as is the case with zebrafish (BALASUBRAMANI and PANDIAN 2008, SPENCE et al. 2008). This has been increasingly reflected in studies in different aspects of biotechnology (e.g. AMAZE and IYENGAR 1990, VARADI and HORVATH 1993, ADAM et al. 1995, CEK et al. 2001, KIRANKUMAR et al. 2003, PANDIAN and KIRANKUMAR 2003, CEK and GOKCE 2005, TARGOŃSKA 2007). Considering the fertility of the rosy barb and the possibility of its periodical reproduction for several years, it is a perfect species for model research.

Translated by JOANNA JENSEN

Accepted for print 1.03.2012

References

ADAM M.M., RANA K.J., MCANDREW B.J. 1995. *Effect of cryoprotectants on activity of selected enzymes in fish embryos*. Cryobiology, 32: 92–104.

AMAZE D., IYENGAR A. 1990. *The micropyle: a sperm guidance system in teleost fertilization*. Development, 109: 495–500.

ANGUIS V., CANAVATE J.P. 2005. *Spawning of captive Senegal sole* (*Solea senegalensis*) *under a naturally fluctuating temperature regime*. Aquaculture, 243: 133–145.

BALASUBRAMANI A., PANDIAN T.J. 2008. *Endosulfan suppresses growth and reproduction in zebrafish*. Curr. Sci. India, 94: 883–890.

BANDYOPADHYAY P., SWAIN S.K., MISHRA S. 2005. *Growth and dietary utilisation in goldfish* (*Carassius auratus* Linn.) *fed diets formulated with various local agro-produces*. Bioresource Technol., 96: 731–740.

BHATTACHYARA H., ZHANG S.C., WANG Y.J. 2005. *Embryonic development of the rosy barb Puntius conchonius Hamilton 1822* (Cyprynidae). Trop. l Zool., 18: 25–37.

BHATTACHYARA H., ZHANG S.C., WANG Y.J. XU Y.Y. 2006. *Effects of salinity n embryogenesis and hatching of the rosy barb Puntius conchonius Hamilton 1822* (Cyprynidae). Trop. Zool., 19: 111–118.

BEKASIAK M. 2000. *Brzanka różowa* (*Puntius conchonius*). Nasze Akwarium, 11: 10–13.

BROMAGE N., PORTER M., RANDALL C. 2001. *The environmental regulation of maturation in farmed finfish with special reference to the role of photoperiod and melatonin*. Aquaculture, 197: 63–98.

BRZUSKA E. 2005. *Artificial spawning of carp* (*Cyprinus carpio* L.): *differences between females of Polish strain 6 and Hungarian strain W treated with carp pituitary homogenate*, Ovopel or Dagin. Aquacult. Res., 36: 1015–1025.

CASSELMAN S.J., SCHULTE-HOSTEDDE A.I., MONTGOMERIE R. 2006. *Sperm quality influences male fertilization success in walleye* (*Sander vitreus*). Can. J. Fish. Aquat. Sci., 63: 2119–2125.

CEJKO B.I., KOWALSKI R.K., KUCHARCZYK D., TARGOŃSKA K., KREJSZEFF S., ŻARSKI D., GLOGOWSKI J. 2009. *Influence of the length of time after hormonal stimulation on selected parameters of milt of ide Leuciscus idus* L. Aquacult. Res., 41: 804–813.

CEK S., BROMAGE N., RANDALL C., RANA K. 2001. *Oogenesis, hepatosomatic and gonadosomatic indexes, and sex ratio in rosy barb* (*Puntius conchonius*). Tr. J. F. A. S. 1: 33–41.

CEK S., GOKCE M.A. 2005. *Evaluation of the photocopy method for counting Puntius Conchonius* eggs. Turk. J. Vet. Anim. Sci., 29: 685–689.

CHELAPPA S., CAMARA M.R., VERANI J.R. 2005. *Ovarian development in the Amazonian Red Discus, Symphysodon discus Heckel* (Osteichthyes: Cichlidae). Braz. J. Biol., 65: 609–616.

DASGUPTA S., SARKAR S.K., SARANGI N., BHATTACHARYA S. 2009. *Variation in spawning responses, egg and larval production from induced rohu* (*Labeo rohita*) *during pre-monsoon and monsoon seasons: Relationship with hormonal changes and oocyte responsiveness during final maturation*. Aquaculture, 290: 320–326.

DAVIES B., BROMAGE N. 2002. *The effects of fluctuating seasonal and constant water temperatures on the photoperiodic advancement of reproduction in female rainbow trout*, *Oncorhynchus mykiss*. Aquaculture, 205: 183–200.

GERLACH G. 2006. *Pheromon regulation of reproductive success in female zebrafish: female suppression and male enhancement*. Anim. Behav., 72: 1119–1124.

KIRANKUMAR S., ANATHY V., PANDIAN T.J. 2003. *Hormonal induction of supermale golden rosy barb and isolation of Y-chromosome specific markes*. Gen. Comp. Endocrinol., 134: 62–71.

KOBAYASHI M., SORENSEN P.W., STACEY N.E. 2002. *Hormonal and pheromonal control of spawning behavior in the goldfish*. Fish Physiol. Biochem., 26: 71–84.

KOC N.D., AYTEKIN Y., YUCE R. 2008. *Ovary maturation stages and histological investigation of ovary of the zebrafish* (*Danio rerio*). Braz. Arch. Biol. Tech., 51: 513–552.

KOGER C.S., THE S.J., HINTON D.E. 1999. *Variation of light and temperature regimes and resulting effects on reproductive parameters in medaka* (*Ozyrias latipes*). Biol. Reprod., 61: 1287–1293.

KREJSZEFF S, TARGOŃSKA K., ŻARSKI D., KUCHARCZYK D. 2009. *Domestication affects spawning of the ide* (*Leuciscus idus*) *– preliminary study*. Aquaculture, 295: 145–147.

KUCHARCZYK D., LUCZYNSKI M., KUJAWA R., CZERKIES P. 1997. *Effect of temperature on embryonic and larval development of bream* (*Abramis brama* L.). Aquatic Sci., 59: 214–224.

KUCHARCZYK D., LUCZYNSKI M., KUJAWA R., KAMINSKI R., ULIKOWSKI D., BRZUZAN P. 1998. *Influences of temperature and food on early development of bream* (*Abramis brama* L.). Arch. Hydrobiol., 141: 243–256.

KUCHARCZYK D., KUJAWA R., MAMCARZ A., TARGOŃSKA K., KREJSZEFF S., WYSZOMIRSKA E. 2007. *Artifitial spawning of common tench* (*Tinca tinca* L.) *collected from wild populations*. Pol. J. Nat. Sc., 22(1): 37–45.

KUCHARCZYK D., TARGOŃSKA K., PRUSINSKA M., KREJSZEFF S., KUPREN K., KUJAWA R., MMCARZ A. 2008a. *Reproduction of Buenos Aires tetra* (*Hemigrammus caudovittatus*) *under controlled conditions*. Pol. J. Natur. Sc., 23(4): 858–865.

KUCHARCZYK D., TARGOŃSKA K., HLIWA P., GOMUŁKA P., KWIATKOWSKI M., KREJSZEFF S., PERKOWSKI J. 2008b. *Reproductive parameters of common carp* (*Cyprinus carpio* L.) *spawners during natural season and out-of-season spawning*. Reprod. Biology, 8(3): 285–289.

KUCHARCZYK D., TARGOŃSKA K., ŻARSKI D., KREJSZEFF S., KUPREN K., ŁUCZYŃSKI M.J., SZCZERBOWSKI A. 2010. *The reproduction of neon tetra*, *Paracheirodon innesi* (*Myers*, *1936*), *under controlled conditions*. Pol. J. Natur. Sc., 25(1): 81–92.

KUPREN K., KUCHARCZYK D., PRUSIŃSKA M., KREJSZEFF S., TARGOŃSKA K., MAMCARZ A. 2008a. *The influence of shocking den sity on survival and growth of Buenos Aires tetra* (*Hemigrammus caudovittatus*) *larvae reared under controlled conditions*. Pol. J. Natur. Sc., 23(4): 881–887.

KUPREN K., MAMCARZ A., KUCHARCZYK D., PRUSIŃSKA M., KREJSZEFF S. 2008b. *Influence of water temperature on eggs incubation time and embryonic development of fish from genus Leuciscus*. Pol. J. Natur. Sc., 23(2): 461–481.

LAAN M., RICHMOND H., HE C., CAMPBELL R.K. 2002. *Zebrafish as a model for vertebrate reproduction: characterization of the first functional zebrafish* (*Danio rerio*) *gonadotropin receptor*. Gen. Comp. Endocrinol., 125: 349–364.

PANDIAN T.J., KIRANKUMAR S. 2003. *Androgenesis and conservation of fishes*. Curr. Sci. India, 85: 917–931.

PRUSIŃSKA M., MAMCARZ A., KUPREN K. 2008. *Early ontogeny of Tropheus moorii Boulenger 1898* (*Pisces*, *Cichlidae*, *Lake Tanganyika*) *in laboratory conditions*. Pol. J. Natur. Sc., 23: 888–903.

PYRON M. 2003. *Female preferences and male-male interactions in zebrafish* (*Danio rerio*). Can. J. Zool., 81: 122–125.

RURANGWA E., ROELANTS I., HUYSKENS G., EBRAHIMI M., KIME D.E., OLLEVIER F. 1998. *The minimum effective spermatozoa: Egg ratio for artificial insemination and the effects of mercury on sperm motility and fertilisation ability in the African catfish Clarias gariepinus*. J. Fish Biol., 53: 402–413.

RURANGWA E., KIME D.E., OLLEVIER F., NASH J.P. 2004. *The measurement of sperm motility and factors affecting sperm quality in cultured fish*. Aquaculture, 234: 1–28.

SCHOLZ S., ROSLER S., SCHAFFER M. HORNUNG U., SCHARTL M. 2003. *Hormonal Induction and stability of monosex populations in the medaka* (*Ozyrias latipes*): *expression of sex-specific marker genes*. Biol. Reprod., 69: 673–678.

SEGNER H. 2009. *Zebrafish* (*Danio rerio*) *as a model organism for investigating endocrine disruption*. Comp. Biochem. Physiol., part C, 149: 187–195.

SEN U., MUKHERJEE D., BHATTACHARYYA S.P., MUKHERJEE D. 2002. *Seasonal changes in plasma steroid levels in Indian major carp Labeo rohita*: *influence of homologous pituitary extract on steroid production and development of oocyte maturation competence*. Gen. Comp. Endocrinol., 128: 123–134.

SIMAO M.F., PEREZ CAMPS M., GARCIA-XIMENEZ F. 2007. *Short communication. Zebrafish embryo development can be reversibly arrested at the MBT stage by exposure to a water temperature of 16ºC*. Spanish J. Agr. Res., 5: 181–185.

SPENCE R., GERLACH G., LAWRENCE C. SMITH C. 2008. *The behavior and ecology of the zebrafish*, *Danio rerio*. Biol. Rev., 83: 13–34.

STACEY N., FRASER E.J., SORENSEN P., VAN DER KRAAK G. 2001. *Milt production in goldfish: regulation by multiple social stimuli*. Comp. Biochem. Physiol., 130C: 467–476.

TARGOŃSKA K. 2007. *Wykorzystanie larw ochotek z rodzaju Chironomus w hodowli wybranych gatunków ryb*. Praca doktorska, UWM Olsztyn, pp. 140.

TARGOŃSKA K., KUCHARCZYK D. 2011. *The application of hCG, CPH and Ovopel in successful artificial reproduction of Goldfish* (*Carassius auratus auratus*) *under controlled conditions*. Reprod. Dom. Anim., 46: 651–655.

TARGOŃSKA K., ŻARSKI D., KUCHARCZYK D. 2008. *A review of the artificial reproduction of asp*, *Aspius aspius* (L.) *and nase*, *Chondrostoma nasus* (L.). Arch. Pol. Fish, 16(4): 341–354.

TLUSTY M. 2002. *The benefits and risks of aquacultural production for the aquarium trade*. Aquaculture, 205: 203–219.

VARADI L., HORVATH L. 1993. *Propagation System of Rosy Barb*, *Barbus, cocnchonius* (L.) *for production of stripped gametes*. Godollo, University of Agricultural Sciences Institute of Animal Husbandry, Hungary., 1–13.

WHITTINGTON R.J., CHONG R. 2007. *Global trade in ornament al Fish from an Australian perspective. The case for revised import risk analysis and management strategies*. Prev. Vet. Med., 81: 92–116.

WITTBRODT J., SHIMA A., SCHARTL M. 2002. *Medaka – a model organism from Far East*. Nat. Rev. Genet., 3: 53–64.

YANONG R.P.E. 1996. *Reproductive management of freshwater ornamental fish*. Sem. Avian and Exotic Pet Med., 5: 222–235.

YUEN T.T.H., MOK P.Y, CHOW B.K.C. 1997. *Molecular cloning of a cDNA encoding proglucagon from goldfish*, *Carassius auratus.* Fish Physiol. Biochem., 17: 223–230.

POLISH JOURNAL OF NATURAL SCIENCES
Abbrev.: Pol. J. Natur. Sc., Vol 27(2): 229–241, Y. 2012

COMPARISON OF THE QUALITY OF TWO CLASSES OF OLIVE OIL: EXTRA VIRGIN AND REFINED OIL

Marta Ambrosewicz, Małgorzata Tańska, Daniela Rotkiewicz

Chair of Plant Raw Materials Processing and Chemistry
University of Warmia and Mazury in Olsztyn

K e y w o r d s: olive oils, phenolic compounds, tocopherols, squalene, degree of hydrolysis, degree of oxidation, fatty acid composition.

A b s t r a c t

The aim of the study was to perform comparative characterisation of different olive oils with respect to important quality features. Two groups of olive oils, 4 samples of extra virgin oil and 4 samples of refined oils (2 *olive oil* and 2 *pomace*) were used as the study material. The quality features under analysis included: the content of phenolic compounds, tocopherols, squalene, products of hydrolysis and oxidation as well as the fatty acid composition.

The content of phenolic compounds, α-tocopherol and squalene in the oils used in the study varied significantly at the level of significance of 0.05. The *extra virgin* oils contained more phenolic compounds and squalene than the refined oils. On the other hand, the highest amount of α-tocopherol was found in refined *pomace* oils. The degree of hydrolysis and the degree of oxidation of *extra virgin* samples was higher than those of refined oils, which was indicated by a higher acid value and anisidine number for those products. The fatty acid composition in all the oil samples was typical of olive oils; however, the percentage of individual acids varied between the groups of oils under study and within each of them.

PORÓWNANIE JAKOŚCI DWÓCH KLAS OLEJÓW OLIWKOWYCH – EXTRA VIRGIN Z RAFINOWANYMI

Marta Ambrosewicz, Małgorzata Tańska, Daniela Rotkiewicz

Katedra Przetwórstwa i Chemii Surowców Roślinnych
Uniwersytet Warmińsko-Mazurski w Olsztynie

S ł o w a k l u c z o w e: oleje oliwkowe, związki fenolowe, tokoferole, skwalen, stopień hydrolizy, stopień utlenienia, skład kwasów tłuszczowych.

Address: Marta Ambrosewicz, University of Warmia and Mazury, pl. Cieszyński 1, 10-957 Olsztyn, Poland, phone: +48 (089) 523 33 99, e-mail: marta.ambrosewicz@uwm.edu.pl

Abstrakt

Celem badań była charakterystyka porównawcza różnych olejów oliwkowych pod względem ważnych wyróżników jakościowych. Materiał badawczy stanowiły 2 grupy olejów oliwkowych, 4 próbki *extra virgin* oraz 4 próbki olejów rafinowanych (2 *olive oil* oraz 2 *pomace*). Analizowanymi wyróżnikami jakościowymi były: zawartość związków fenolowych, tokoferoli, skwalenu, produktów hydrolizy i utleniania oraz skład kwasów tłuszczowych.

Wykazano, iż zawartość związków fenolowych, α-tokoferolu i skwalenu w badanych oliwach była istotne zróżnicowana na poziomie istotności 0.05. Oliwy *extra virgin* cechowały się wyższą zawartością związków fenolowych oraz skwalenu niż oleje rafinowane. Z kolei najwyższą zawartość α-tokoferolu stwierdzono w rafinowanych oliwach *pomace*. Stopień hydrolizy oraz utlenienia próbek oliwy *extra virgin* był wyższy niż olejów rafinowanych, o czym świadczyły wyższe wartości liczby kwasowej i anizydynowej tych produktów. Skład kwasów tłuszczowych wszystkich próbek olejów był charakterystyczny dla olejów oliwkowych, jednakże między badanymi grupami olejów oliwkowych, jak również w obrębie każdej z nich, stwierdzono zróżnicowanie udziału poszczególnych kwasów tłuszczowych.

Introduction

The European Union is the largest producer of olive oil, with Spain, Italy and Greece being the leading producers, together accounting for 75% of the world's olive oil (*IOOC* 2011). Other important producers include Turkey, Syria, Morocco and Tunisia. According to estimates by the *IOOC* (2011), the global production output and consumption in 2010/2011 amounted to about 3 million tonnes.

Oil quality depends on many factors, including olive variety, maturity of fruit and the harvesting method, the time between harvest and processing, processing technology (method of extraction, clarification, refinement), type of packaging and storage conditions (exposure to oxygen and light, change of the material and oil temperature). Agriclimatic factors, associated with the cultivation area, are also important (JELNICKA et al. 2008, KWIATKOWSKA 2007, PTASZNIK 2006). It is supposed that the quality features of olive oil may be affected by flavour additives, such as herbs (basil, thyme, estragon) and the addition of other vegetable oils (AMBROSEWICZ et al. 2011).

Growing interest in olive oil stems mainly from its beneficial effect on human health. Although oil contains small amounts of polyunsaturated fatty acids, it has a strong protective effect on the circulatory system and a beneficial effect on the function of the heart by reducing blood viscosity, coagulability and inhibits atherosclerosis (WATERMAN and LOCKWOOD 2007). A high content of oleic acid and antioxidants in oil, i.e. phenolic compounds (hydroxytyrosol, tyrosol and oleuropein), sterols and squalene, prevents formation of free radicals and peroxides, which slows down the ageing processes and explains its anticancer properties (WATERMAN and LOCKWOOD 2007, BENDINI et al. 2006, FLACZYK et al. 2004, PROCYK 2001, KOLANOWSKI 1998). The presence of those

compounds is also important for inhibition of oil oxidation, extending its shelf life period as compared to other oils. It has also been found that hydroxytyrosol, tyrosol and oleuropein have antibacterial properties with regard to strains which cause infections of the intestines and the respiratory system (WATERMAN and LOCKWOOD 2007).

Due to an increasing number of olive oil producers and the multitude of products on the market, the aim of the product was to compare the quality of two classes of olive oil, *extra virgin* with refined *olive oil* and *pomace*, available on the Olsztyn market.

Materials and Methods

Study material

Four *extra virgin* oil samples and 4 samples of refined oil, including 2 of *olive oil* and 2 of *pomace* oil were used as the study material (Table 1). They differed in shelf life period and type of packaging. All the oils were purchased at retail outlets in June 2010.

Table 1

Characterisation of the study material

Oils sample	Oil type	Packaging type	Usability period at the time of analyses (months)
O_1	*extra virgin* – oil extracted from fresh olives at the temperatures < 25°C within 24 h of harvest, with no chemical processes applied. Required acidity <0.8%	clear, light glass bottle	1
O_2	*extra virgin* – as above	clear, light glass bottle	15
O_3	*extra virgin* – as above	clear, light glass bottle	3
O_4	*extra virgin* – as above	clear, light glass bottle	17
O_5	*olive oil* – oil which contains refined oil and extra virgin oil	clear bottle made of emerald glass	1
O_6	*olive oil* – as above	clear bottle made of emerald glass	15
O_7	*pomace* – refined oil extracted from olive pomace	clear, light glass bottle	1
O_8	*pomace* – as above	clear, light glass bottle	11

Methods

The study involved determination of antioxidants (phenolic compounds, tocopherols, squalene), degree of hydrolysis (acid value), degree of oxidation (peroxide number, anisidine number, content of coupled diene and triene fatty acids and fatty acid composition.

The phenolic compound content was determined by the spectrophotometric method described by RIBEREAU-GAYON (1972). Samples for analysis were prepared by the method described by KANIA et al. (2004). Absorbance was measured by a UNICAM UV/Vis UV2 spectrophotometer. The total phenolic compound content was calculated from the standard curve prepared for different concentrations of D-catechol.

Tocopherols were determined by RP-HPLC using the method described by GIMENO et al. (2000). The compounds were separated with a liquid chromatography analyser (manufactured by Agilent Technologies, series 1200) and a fluorescence detector (a LiChrospher Si 60,5 μm column [250 mm × 4 mm] by Merck) and 0.7% solution of isopropanol in hexane as the mobile phase. Tocopherol isomers were identified by their retention times determined for the standards of those compounds, supplied by Merck.

Squalene content was determined by the method described by CZAPLICKI et al. (2009). The analysis was performed with a liquid chromatography analyser manufactured by Agilent Technologies, series 1200, and a photodiode detector and a LiChrospher RP-18, 5 μm column (250 × 4.6 mm). A mixture of isopropanol, acetonitrile, hexane was used as the mobile phase. Squalene detection was conducted at the wavelength of 218 nm. The quantitative analysis was based on the standard curve plotted for a standard supplied by Sigma-Aldrich.

The degree of hydrolysis of the olive oils was found by determination of the acid values in accordance with the standard PN-ISO 660:1998 (*Oleje i tłuszcze*. PN-ISO 660:1998).

The degree of oxidation of the olive oils was determined by determination of the peroxide number (*Oleje i tłuszcze*. PN-ISO 3960:1996), anisidine number (*Tłuszcze*. PN-93/A-86926) and the content of coupled diene and triene acids (*Ultraviolet*. AOCS Standard. Official method Cd 7-58:1973).

Fatty acid composition in olive oils was determined in accordance with PN-EN ISO-5508:96, by preparing methyl esters in accordance with *Oleje i tłuszcze.* PN-EN ISO-5509:2001.

Statistical analysis

Obtained results of researches were statistically analyzed using the Statictica 9.0 PL (StatSoft Poland) program. In order to indicate significance of differences between oils of peanut samples unvaried analysis of variance (ANOVA) with Tukey's test of $p \leq 0.05$ significance level was used. Moreover, there were determined Pearson correlation coefficients (r) between individual quality factors.

Results and discussion

Antioxidants

The content of antioxidants, i.e. phenolic compounds, tocopherols and squalene, varied significantly in the olive oils under study (Table 2).

Table 2

The content of phenolic compounds, α-tocopherol and squalene in olive oils

Specification	Olive oils							
	extra virgin				oilve oil		pomace	
	O_1	O_2	O_3	O_4	O_5	O_6	O_7	O_8
Phenolic compounds [mg kg^{-1}]	$19.4^{b} \pm 2.92$	$52.6^{d} \pm 4.90$	$30.0^{bc} \pm 0.95$	$40.1^{c} \pm 3.25$	$1.8^{a} \pm 0.66$	$3.1^{a} \pm 0.69$	$4.2^{a} \pm 0.59$	$4.7^{a} \pm 0.08$
α-tocopherols [mg 100 g^{-1}]	$35.9^{e} \pm 0.28$	$32.4^{a} \pm 0.58$	$32.1^{a} \pm 0.34$	$23.0^{b} \pm 0.79$	$27.2^{d} \pm 0.30$	$24.1^{c} \pm 0.21$	$73.5^{g} \pm 0.28$	$71.1^{f} \pm 0.30$
Squalene [mg 100 g^{-1}]	$458.6^{h} \pm 1.23$	$406.8^{g} \pm 2.20$	$248.9^{f} \pm 0.45$	$212.8^{e} \pm 0.53$	$118.9^{d} \pm 2.63$	$66.3^{a} \pm 0.42$	$109.3^{c} \pm 2.61$	$96.7^{b} \pm 0.65$

The content of phenolic compounds varied within a broad range from 1.8 to 52.6 mg kg^{-1} (Table 2). The highest and the most varied content of those compounds was found in *extra virgin* oils (O_1–O_4, 19.4–52.6 mg kg^{-1}), while the lowest, 2.4 mg kg^{-1} on average, in *olive oil* O_5 and O_6. The content of phenolic compounds in samples of *pomace* oil O_7 and O_8 was equal to 4.2 and 4.7 mg kg^{-1}, respectively. OWEN and TUCK and HAYBALL (cit. WATERMAN and LOCKWOOD 2007) report that, in general, *extra virgin* oils contain more phenolic compounds than refined oils. GÓMEZ-CARAVACA et al. (2007) and TSIMIDOUR et al. (2005) examined the effect of filtration of olive oil on phenolic compounds content and their oxidative stability and found non-filtered oils to contain more such compounds and are oxidised more slowly than filtered oils.

Phenolic compounds present in oil are responsible for its stability, smell and bitterish taste. They inhibit oxidation processes through various mechanisms, for example, by free radical sweeping, transfer of a hydrogen atom and by chelating metal ions (BENDINI et al. 2007). OWEN et al. (cit. WATERMAN and LOCKWOOD 2007) report that phenolic compounds present in oil can remove free radicals formed in faeces matrix, which prevents development of colorectal cancer.

An analysis of tocopherol content revealed only the presence of α-tocopherol. The isomer content in the oils under study ranged from 23.0 mg kg^{-1} (*extra virgin* oil O_4) to 73.5 mg kg^{-1} (*pomace* oil O_7) (Table 2). The differences in α-tocopherol content were also found to exist both between two classes of olive oil and within samples of the same class. E*xtra virgin* oils (O_1–O_4) differed by about 13.0 mg content of the compound per kg of oil. O*live oils* contained three times less α-tocopherol than pomace oils. GLISZCZYŃSKA-ŚWIGŁO et al. (2007) analysed tocopherol content, e.g. in *extra virgin* oil, and found a much higher value for the α form (163.0 mg kg^{-1}) as well as the presence of other isomers, i.e. β, γ and δ. PSOMIADOU et al. (2000) analysed tocopherol content in 25 samples of Greek oil and found 15 of them to contain more than 200 mg of tocopherol per kg. BASUNY et al. (2008) showed that the tocopherol content in extra virgin oil depended on the olive variety and on whether olives were stoned prior to oil extraction. The authors found olive oils from unstoned olives to contain less tocopherol than oils from stoned fruit. ANTONOPOULOS et al. (2006) found the decrease in tocopherol content in refined oils to be a result of using steam, high temperature and pressure in oil deodorisation.

Tocopherols are natural antioxidants which protect oils from free radicals, reactive oxygen species and peroxides (STUCHLÍK and ŽÁK 2002). The main physiological functions of tocopherols include preventing peroxidation of lipids during the seeds' resting period, their germination and early development of seedlings (SATTLER et al. 2004). According to AZZI and STOCKER (cit. GLISZCZYŃSKA-ŚWIGŁO et al. 2007) they may have a beneficial effect on human health by prevention of such diseases as atherosclerosis, cataract, cancers and neural tube defects.

Squalene content in the oils under study ranged from 66.28 to 458.59 mg/100 g (Table 2). The highest, and at the same time the most varied, content of the compound was found in the four extra virgin oil samples, with O_1 and O_2 samples containing 406.8–458.6 mg/100 g, while O_3 and O_4 contained nearly twice less of it (212.8 and 248.9 mg/100 g, respectively). Squalene content in *olive oil* O_5 and O_6 samples was equal to 66.28 and 118.94 mg/100 g, whereas in the *pomace* oil O_7 and O_8 samples – about 103 mg/100 g. OWEN et al. (cit. WATERMAN and LOCKWOOD 2007) report that *extra virgin* oils contain only a little more of the compound as compared to refined oils.

Squalene is a three-pentene hydrocarbon and the main mediator in the synthesis of plant and animal steroids. According to NEWMARK (1997), the content of the compound in olive oil is about 0.7%, whereas it ranges from 0.002 to 0.03% in other animal and plant oils (LIU et al. 1976). NEWMARK (1997) also reported that food control units use the compound assay as an indicator of commercial purity of olive oil. Squalene has antioxidant properties and owing to its structure it is more effective in sweeping singlet oxygen species than hydroxyl radicals (WATERMAN and LOCKWOOD 2007). Its highest concentration in the human body was found in the skin (12%), whereas its content in the adipose tissue ranges from 0.001 to 0.04%. There is a high probability of the presence of a reactive singlet oxygen species in skin exposed to high levels of UV radiation, but a high concentration of the squalene in it may produce a chemopreventive effect (NEWMARK 1997). OWEN et al. (cit. WATERMAN and LOCKWOOD 2007) report that epidemiological studies conducted on a population using a Mediterranean diet with a high squalene content have confirmed that the compound reduces the risk of skin cancer occurrence.

Degree of hydrolysis

The acid values for the oils under study ranged from 0.14 to 1.03 mg KOH g^{-1} (Table 3). The highest values were found for the *extra virgin* oils (O_1–O_4), but they did not exceed the highest acceptable value for extra virgin edible oils, i.e. 4 mg KOH g^{-1} (*Tłuszcze*. ZN-94/SGO-01). Samples of *olive oil* (O_5 and O_6) and *pomace* oil (O_8) had low values of the degree of hydrolysis, indicated by the acid values, which did not exceed the highest acceptable value for refined vegetable oil – 0.3 mg KOH g^{-1} (*Oleje i tłuszcze*... PN-A-86908:2000). Only a sample of oil O_7 did not meet the criteria set out in the standard, which can be attributed to the fact that the oil was at the end of its shelf life (1 month) and to probable failure to meet the required storage conditions by the trade entities, i.e. wholesalers and retailers. The lower acid values of refined olive oil were probably caused by the removal of free fatty acids in the refinement process, i.e. in dehydration. TAŃSKA and ROTKIEWICZ (2003) analysed two commercial *extra virgin* oils and found higher acid values of 1.68 and 1.75 mg KOH g^{-1}. TYNEK and SZUKALSKA (2006) analysed, *inter alia*, *extra virgin* and *pomace* oils and found the values of the quality feature similar to those determined in this study.

Table 3

Discriminate of olive oils technological value

Gualitative discriminants	Olive oils							
	extra virgin				oilve oil		pomace	
	O_1	O_2	O_3	O_4	O_5	O_6	O_7	O_8
Acid value [mg KOH g^{-1}]	$0.67^{e} \pm 0.001$	$0.53^{d} \pm 0.040$	$1.03^{g} \pm 0.041$	$0.83^{f} \pm 0.001$	$0.17^{a} \pm 0.000$	$0.14^{a} \pm 0.040$	$0.44^{c} \pm 0.003$	$0.22^{d} \pm 0.001$
Peroxide value [mEq O_2 kg^{-1}]	$0.62^{a} \pm 0.045$	$0.51^{b} \pm 0.001$	$0.58^{a} \pm 0.001$	$0.57^{ab} \pm 0.003$	$1.60^{f} \pm 0.033$	$1.51^{e} \pm 0.070$	$1.31^{d} \pm 0.018$	$1.01^{c} \pm 0.010$
Anisidine value	$9.18^{e} \pm 0.27$	$5.39^{b} \pm 0.15$	$7.04^{c} \pm 0.05$	$7.06^{c} \pm 0.83$	$6.27^{d} \pm 0.35$	$3.43^{a} \pm 0.07$	$5.42^{b} \pm 0.02$	$3.62^{a} \pm 0.07$
Conjugated compounds [%]								
Diene	$0.12^{bc} \pm 0.003$	$0.12^{bc} \pm 0.004$	$0.21^{ad} \pm 0.004$	$0.11^{b} \pm 0.047$	$0.16^{cd} \pm 0.012$	$0.22^{a} \pm 0.000$	$0.22^{a} \pm 0.041$	$0.23^{a} \pm 0.014$
Triene	$0.001^{e} \pm 0.000$	$0.000^{a} \pm 0.000$	$0.014^{d} \pm 0.000$	$0.000^{a} \pm 0.000$	$0.000^{a} \pm 0.000$	$0.000^{a} \pm 0.000$	$0.009^{b} \pm 0.000$	$0.012^{c} \pm 0.001$

Degree of oxidation

The peroxide number ranged from 0.51–1.60 mEq O_2 kg^{-1}, which indicates a very low degree of the oils oxidation (Table 3). Samples of *extra virgin* oils had similar values of the feature, ranging from 0.51 to 0.62 mEq O_2 kg^{-1}. The highest acceptable peroxide number for extra virgin oils is 10 mEq O_2 kg^{-1} (*Tłuszcze...* ZN-94/SGO-01). The peroxide number for refined olive oils did not exceed the highest acceptable value of 5 mEq O_2 kg^{-1} (*Oleje i tłuszcze...* PN-A-86908:2000) (Table 3).

The values of anisidine number, which describes the content of secondary oxidation products (JERZEWSKA 1991), were statistically different for individual oils and ranged from 3.43 to 9.18 (Table 3). MAKAREVICIENE and JANULIS report that the maximum value of the anisidine number for extra virgin oils should not exceed 3 units. The samples of extra virgin oils analysed in this study had much higher values of the feature (5.39–9.18) – Table 3. The high degree of oxidation of the oils was probably caused by the use by the producer of packages which did not protect the oils against light and the final part of the shelf life of two samples: O_1 and O_3 (1 and 3 months). The anisidine numbers for refined olive oils did not exceed 8, which is the highest value accepted by the standard for refined oils. TYNEK and SZUKALSKA (2006) analysed extra virgin oils and pomace oils and found the feature to have a lower value for extra virgin oils. TAŃSKA and ROTKIEWICZ (2003) found much higher values of the anisidine number for *extra virgin* (about 13).

The content of coupled diene acids in oils was low and ranged from 0.11 to 0.23% (Table 3). A generally higher content of such compounds was found in refined *olive oil* and *pomace oil*. TAŃSKA and ROTKIEWICZ (2003) analysed two *extra virgin* olive oils and found coupled diene acids at similar content.

The content of coupled triene acids in oils was low and it did not exceed 0.014% (Table 3). Of the eight analysed olive oils, those compounds were not found in four of them (O_2, O_4, O_5 and O_6). Olive oils analysed by TAŃSKA and ROTKIEWICZ (2003) also contained coupled triene acids at low concentrations.

Fatty acids' composition

The analysis results revealed significant differentiation of the fatty acids; composition in olive oils (Table 4).

Table 4

The share [%] of fatty acid of olive oils [%]

Olive oils		Fatty acids						
		$C_{16:0}$	$C_{16:1}$	$C_{18:0}$	$C_{18:1}$	$C_{18:2}$	$C_{18:3}$	others
Extra virgin	O_1	$11.19^{b} \pm 0.02$	$0.53^{a} \pm 0.06$	$3.23^{c} \pm 0.07$	$80.42^{h} \pm 1.00$	$4.23^{c} \pm 0.34$	$0.34^{bc} \pm 0.00$	$0.06^{a} \pm 0.01$
	O_2	$14.13^{e} \pm 0.04$	$0.81^{c} \pm 0.07$	$2.78^{ab} \pm 0.06$	$76.52^{d} \pm 5.41$	$5.41^{e} \pm 0.06$	$0.33^{b} \pm 0.04$	$0.00^{a} \pm 0.00$
	O_3	$14.81^{g} \pm 0.071$	$0.93^{b} \pm 0.04$	$2.86^{b} \pm 0.04$	$77.57^{f} \pm 0.07$	$3.83^{b} \pm 0.00$	$0.00^{a} \pm 0.00$	$0.00^{a} \pm 0.00$
	O_4	$14.70^{f} \pm 0.01$	$0.97^{b} \pm 0.00$	$2.44^{d} \pm 0.01$	$74.84^{b} \pm 0.07$	$7.04^{a} \pm 0.08$	$0.00^{a} \pm 0.00$	$0.00^{a} \pm 0.00$
Olive oil	O_5	$12.60^{d} \pm 0.07$	$0.61^{a} \pm 0.06$	$3.13^{c} \pm 0.03$	$79.25^{g} \pm 0.06$	$4.41^{d} \pm 0.04$	$0.00^{a} \pm 0.00$	$0.00^{a} \pm 0.00$
	O_6	$17.16^{h} \pm 0.09$	$1.66^{d} \pm 0.01$	$2.69^{a} \pm 0.09$	$67.56^{a} \pm 0.13$	$10.52^{g} \pm 0.00$	$0.40^{c} \pm 0.00$	$0.00^{a} \pm 0.00$
Pomace	O_7	$12.41^{c} \pm 0.06$	$0.59^{a} \pm 0.04$	$2.81^{ab} \pm 0.11$	$76.84^{e} \pm 0.03$	$7.04^{a} \pm 0.00$	$0.00^{a} \pm 0.00$	$0.31^{b} \pm 0.09$
	O_8	$10.42^{a} \pm 0.03$	$0.54^{a} \pm 0.06$	$3.24^{c} \pm 0.03$	$75.33^{c} \pm 0.04$	$9.94^{f} \pm 0.06$	$0.52^{d} \pm 0.04$	$0.01^{a} \pm 0.00$

Oleic acid, which accounts for 67.56 (O_6) to 80.42% (O_1) of the total (Table 4), is the dominant fatty acid in the oils under study. Its highest average content, 74.84 to 80.42%, was found in *extra virgin* oils O_1–O_4. The lowest and the most varied content of oleic acid was found in *olive oils* O_5 and O_6 (79.25 and 67.56%). This acid accounted for 76.84 and 75.33% of total acids in *pomace* oils O_7 and O_8. The results in this study were similar to the findings of studies by TYNEK and SZUKALSKA (2006) as well as by TAŃSKA and ROTKIEWICZ (2003).

A molecule of oleic acid contains one double bond, so it is regarded as less susceptible to oxidation than polyunsaturated fatty acids (WATERMAN and

LOCKWOOD 2007). According to literature data, the oxidation rate of the acid is 10–40 times lower than linoleic acid (DROZDOWSKI 2002, FREGA et al. 1999). Owing to a high content of oleic acid, olive oil is regarded as an oil with high oxidative stability, which gives it a long shelf life (BENDINI et al. 2006). Monounsaturated fatty acids are thought to reduce the risk of cardiovascular diseases by lowering the level of triacylglycerols, total cholesterol and its LDL fraction (GILL et al. 2003, KRIS-ETHERTON et al. 1999). Literature reports contain some information about the anticancer properties of oleic acid, but the data are inconclusive (WATERMAN and LOCKWOOD 2007).

Polyunsaturated fatty acids in the oils under study were represented by linoleic acid, which accounted for 3.83–10.52% of the total fatty acids (Table 4). The most varied content of the acid was found in samples of *olive oil* (O_5 and O_6), where the difference was as high as about 6.0 percentage points. Linoleic acid accounted for 3.83–7.04% of total acids in *extra virgin* oils and for 7.04 and 9.94% in *pomace* oils O_7 and O_8.

Of the eight oil samples, the presence of h-linolenic acid was not found in four of them (O_3–O_5 and O_7). In the other samples, the acid accounted for 0.33–0.52% of the total acids.

In total, saturated fatty acids, palmitic and stearic, accounted for 13.66 (O_8) to 19.85% (O_6) of all the acids, with palmitic acid dominating and accounting for >76% of total saturated acids (Table 4). The average content of saturated fatty acids in *extra virgin* O_1–O_4 was about 16.5%, whereas in *pomace* oils O_7 and O_8 it was 14.4%. The most varied in this regard were *olive oils* O_5 and O_6, in which the percentage of the acids was 15.22 and 13.66%, respectively. Both TYNEK and SZUKALSKA (2006) and TAŃSKA and ROTKIEWICZ (2003) found a lower content of saturated fatty acids in the oils they analysed.

Correlations

An analysis of correlation coefficients between the features which describe the degree of hydrolysis and the degree of oxidation of olive oils and the content of antioxidants revealed the existence of significant relationships only in several cases (Table 5). Acid value was negatively correlated with the content of phenolic compounds and squalene, for both: r = -0.86. The peroxide number increased with a decrease in squalene content (r = -0.78). The content of coupled diene acids was negatively correlated with the content of phenolic compounds and squalene.

Tabela 5
Significant correlation between individual quality factors of olive oils (Pearson correlation coefficients – *r*)

Discriminants	Phenolic compounds [mg kg^{-1}]	Tocopherols [mg kg^{-1}]	Squalene [mg/100 g]	Oleic acid [%]	Linoleic acid [%]
Acid value [mg KOH g^{-1}]	-0.86	–	-0.86	–	–
Peroxide value [mEq O_2 kg^{-1}]	–	–	-0.78	–	–
Anisidine value	–	–	–	0.73	-0.82
Conjugated diene fatty acids [%]	-0.71	–	-0.75	–	–
Conjugated triene fatty acids [%]	–	–	–	–	–

Conclusion

Extra virgin oils have considerably higher nutritional value than refined oils (*olive oil*, *pomace oil*). Higher nutritional value of *extra virgin* oils is a result of much higher content of phenolic compounds and squalene, which protects oil from excessive hydrolysis and oxidation, thereby protecting a consumer from consuming toxic oxidation products. This is indicated by the negative correlation coefficients between those compounds and the numbers which describe the degree of hydrolysis and degree of oxidation. A lower α-tocopherol content in *extra virgin* oils seems to be of lesser importance because no significant correlation was found to exist between the compound content and the characteristic numbers for the oils. Growing consumer awareness of the wholesomeness of olive oil, which results from a high content of antioxidants and oleic acid, has led to an increase in interest in the product. Producers want to increase the competitiveness of olive oils by producing refined oils with additives, i.e. spices and flavours, to persuade consumers to buy their product. However, one should think about whether such oils meet consumers' expectations not only with respect to their flavour and taste, but also to their nutritional value and durability.

Translated by JOANNA JENSEN

Accepted for print 24.11.2011

References

ANTONOPOULOS K., VALET N., SPIRATOS D., SIRAGAKIS G. 2006. *Olive oil and pomace olive oil processing*. Grasas y aceites, 57(1): 56–67.

AMBROSEWICZ M., ROTKIEWICZ D., TAŃSKA M. 2012. *Evaluation method of labellimg of vegetable oils on the Olsztyn mosket*. Pol. J. Natur. Sc., 27(1): 67–79

Analiza estrów metylowych kwasów tłuszczowych metodą chromatografii gazowej. PN-EN ISO 5508.

BASUNY A.M.M., DALIA A.M.S., MOSTFA M.M. 2008. *Virgin olive oil quality: relationship between bioactive components and organoleptic evaluation*. Alex. J. Fd. Sci. & Tchnol., *special volume conference*, 21–29.

BENDINI A., CERRETANI L., CARRASCO-PANCORBO A., GÓMEZ-CARAVACA A., SEGURA-CARRETERO A., FERNÁNDEZ-GUTIÉRREZ A., LECKER G. 2007. *Phenolic molecules in virgin olive oils: a survey of their sensory properties, health effects, antioxidant activity and analytical methods. An overview of the last decade*. Molecules, 12: 1679–1719.

BENDINI A., CERRETANI L., VECCHI S., CARRASCO-PANCORBO A., LERCKER G. 2006. *Protective effects of extra virgin olive oil phenolics on oxidative stability in the presence or absence of copper ions*. J. Agric. Food Chem., 54: 4880–4887.

CZAPLICKI S., ZADERNOWSKI R., OGRODOWSKA D. 2009. *Triacylglycerols from viper bugloss (Echium vulgare L.) seed bio-oil*. Eur. J. Lipid Sci. Technol., 111: 1266–1269.

DROZDOWSKI B. 2002. Lipidy. Rozdział 7. [In:] W.E. Sikorski, *Chemia żywności*. Warszawa, WNT.

FLACZYK E., RUDZIŃSKA M., GÓRECKA D., SZCZEPANIAK B., KLIMCZAK S., KORCZAK J. 2004. *Ocena wybranych wskaźników jakościowych przechowywanej oliwy „extra virgin"*. Rośliny Oleiste, 25(1): 213–224.

FREGA N., MOZZON M., LERCKER G. 1999. *Effect of free fatty acids on oxidative stability of vegetable oil*. J. Amer. Oil Chem. Soc., 76(3): 325–329.

GILL J., BROWN J., CASLAKE M., WRIGHT D., COONEL J., BEDFORD D., HUGHES D.A., STANLEY J.C., PACKARD CH.J. 2003. *Effects of dietary monounsaturated fatty acids on lipoprotein concentrations, compositions, and subfraction distributions and on lipoprotein concentrations, compositions, and subfraction distributions and on VLDL apolipoprotein B kinetics*. Am. J. Clin. Nutr., 78(1): 47–56.

GIMENO E., CASTELLOTE A., LAMUELA-RAVENTOS R., DE LA TORRE M. 2000. *Rapid determinatoin of vitamin E in vegetable oils by reversed-phase high-performance chromatography*. J. Chromatogr. A., 881: 251–254.

GLISZCZYŃSKA-ŚWIGŁO A., SIKORSKA E., KHMELINS I., SIKORSKI M. 2007. *Tocopherol content in edible plant oils*. Pol. J. Food Nutr. Sci., 4(A): 157–161.

GÓMEZ-CARAVACA A., CERRETANI L., BENDINI A., SEGURA-CARRETERO A., FERNÁNDEZ-GUTIÉRREZ A., LERCKER G. 2007. *Effect of filtration systems on the phenolic content in virgin olive oil by HPLC-DAD-MSD*. Am. J. Food Technol., 2(7): 671–678.

IOOC. 2011. International Olive Oil Council. www.internationaloliveoil.org.

JELNICKA K., PTASZNIK S. 2008. *Ocena sensoryczna wybranych gatunków oliwy z oliwek extra virgin*. Tłuszcze Jadalne, 43(3–4), 77–88.

JERZEWSKA M. 1991. *Wprowadzenie metody oznaczenia liczby anizydynowej i współczynnika Totox w olejach roślinnych do krajowej praktyki laboratoryjnej*. Rocz. Inst. Przem. Mięs. Tłuszcz., 28: 108–117.

KANIA M., MICHALAK M., GOGOLEWSKI M., HOFFMANN A. 2004. *Antioxidative potential of substances contained in cold Pressem soybean oil and after each chase of refining process*. Acta Sci. Pol., Technol. Aliment., 3(1): 113–121.

KOLANOWSKI W. 1998. *Oliwa czy olej oliwkowy – podstawa diety śródziemnomorskiej. Charakterystyka, klasyfikacja, znaczenie zdrowotne*. Żywność, Żywienie a Zdrowie, 3: 285–290.

KRIS-ETHERTON P., PEARSON T., WAN Y., HARGROVE R., MORIARTY K., FISHELL V., ETHERTON T.D. 1999. *High – monounsaturated fatty acids diets lower both plasma cholesterol and triacyloglicerol concentration*. Am. J. Clin. Nutr., 70(6): 1009–1015.

KWIATKOWSKA E. 2007. *Właściwości zdrowotne oliwy z oliwek*. Postępy Fitoterapii, 3: 168–171.

LIU C., AHRENS E., SCHREIBMAN H., CROUSE R. 1976. *Measurement of squalene in human tissues and plasma: validation and application*. J. Lipid Res., 17: 38–45.

MAKAREVICIENE V., JANULIS P. *Analiza jakości olejów jadalnych oraz obowiązkowe wymagania*. Tłuszcze Jadalne, 34(1–2): 15–32.

NEWMARK H. 1997. *Squalene, olive oil, and cancer risk: a review and hypothesis*. Cancer Epidemiol. Biomarkers Prev., 6: 1101–1103.

Oleje i tłuszcze roślinne oraz zwierzęce. Oznaczanie liczby kwasowej i kwasowości. PN-ISO 660:1998.

Oleje i tłuszcze roślinne oraz zwierzęce. Oznaczanie liczny nadtlenkowej. PN-ISO 3960:1996.

Oleje i tłuszcze roślinne oraz zwierzęce. Przygotowanie estrów metylowych kwasów tłuszczowych. PN-EN ISO-5509:2001.

Oleje i tłuszcze roślinne oraz zwierzęce. Rafinowane oleje roślinne. PN-A-86908:2000.

PROCYK A. 2001. *Oliwa europejska.* Wiadomości Zielaskie, 43(5): 3–16.

PSOMIADOU E., TSIMIDOU M., BOSOKU D. 2000. *Alpha-tocopherol content of Greek virgin olive oils.* J. Agric. Food Chem., 48(5): 1770–1775.

PTASZNIK S. 2006. *Oliwa z oliwek – historia a rzeczywistość, skład chemiczny i aspekty jakościowe*. Tłuszcze Jadalne, 41(1–2): 3–16.

SATTLER S., GILLILAND L., MAGALLANES-LUNDBACK M., POLLARD M., DELLAPENN D. 2004. *Vitamin E is essential for seed longevity and for preventing lipid peroxidation during germination*. The Plant Cell, 16: 1419–1432.

STUCHLÍK M., ŽÁK S. 2002. *Vegetable lipids as components of functional foods*. Biomed. Papers, 146(2): 3–10.

TAŃSKA M., ROTKIEWICZ D. 2003. *Stopień przemian lipidów wybranych olejów roślinnych i konsumpcyjnych nasion oleistych.* Tłusz. Jad., 38(3/4): 147–155.

Tłuszcze roślinne jadalne. Oleje tłoczone na zimno. ZN-94/SGO-01.

Tłuszcze roślinne jadalne. Oznaczanie liczby anizydynowej oraz obliczanie wskaźnika oksydacji tłuszczu Totox. PN-93/A-86926.