UNIVERSITY OF WARMIA AND MAZURY IN OLSZTYN

# Polish Journal of Natural Sciences

25
(2/2010)

PUBLISHER UWM
OLSZTYN 2010

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

The Journal is also available (from volume 22) in electronic form. The online
edition is hosted by MetaPress (www.metapress.com) in partnership with Versita
(www.versita.com)

PL ISSN 1643-9953



PUBLISHER UWM OLSZTYN

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Ark. wyd. 8, ark. druk. 6,5, nakład egz. 140
Druk – Zakład Poligraficzny UWM w Olsztynie
zam. nr 324

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POLISH JOURNAL OF NATURAL SCIENCES
Abbrev.: Pol. J. Natur. Sc., Vol 25(2): 115–122, Y. 2010

DOI 10.2478/v10020-010-0009-7

# CAPACITY FOR SOMATIC EMBRYOGENESIS IN DIFFERENT PEA CULTIVARS

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K e y w o r d s: pea, somatic embryogenesis, picloram, micropropagation.

A b s t r a c t

Using the method described by GRIGA (1998), embryoids were obtained (through direct or indirect somatic embryogenesis) in cultures of shoot apical meristems of the following pea cultivars: Bankier, Dino, Hazard, Iłówiecki, Konserwowy IHAR, Kosynier, Makler, Oskar, Pegaz, as well as unregistered line HM-6. With cultivars Izolda and Lantra the efforts at somatic embryogenesis (SE) induction remained unsuccessful. The highest responsiveness to SE induction was observed (after 14 days of treatment with a relatively low concentration of picloram – 2.5 µM) in cultivars Oskar, Hazard and line HM-6, in which embryoids were formed with frequencies of 31, 15.9 and 12.5%, respectively. Increasing picloram level to 5 µM and extending period of induction to 28 days, it was possible to obtain SE efficiency above 10% in cultivars Konserwowy IHAR, Dino and Kosynier. Photoperiod affected SE efficiency and the degree and direction of this influence greatly depended on pea cultivar.

# ZDOLNOŚĆ DO SOMATYCZNEJ EMBRIOGENEZY U WYBRANYCH ODMIAN GROCHU

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S ł o w a k l u c z o w e*:* groch, somatyczna embriogeneza, pikloram, mikrorozmnażanie.

A b s t r a k t

Posługując się metodą GRIGI (1998), uzyskano zarodki somatyczne (na drodze embriogenezy bezpośredniej lub pośredniej) w hodowlach wierzchołków pędów następujących odmian grochu: Bankier, Dino, Hazard, Iłówiecki, Konserwowy IHAR, Kosynier, Makler, Oskar, Pegaz. Próby uzyskania somatycznej embriogenezy u odmian Izolda i Lantra się nie powiodły. U odmian Oskar i Hazard, jak również niezarejestrowanej linii HM-6, indukcja somatycznej

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embriogenezy zachodziła najwydajniej – po 14-dniowym pobudzaniu pikloramem o stosunkowo niskim stężeniu (2,5 µM) zarodki tworzyły się z częstością odpowiednio: 31, 15,9 i 12.5%. Podwyższenie stężenia pikloramu do 5 µM i wydłużenie czasu indukcji do 28 dni pozwoliło na uzyskanie częstości SE przekraczającej 10% u odmian Konserwowy IHAR, Dino i Kosynier. Fotoperiod wpływał na wydajność SE, a stopień i kierunek tego oddziaływania silnie zależały od odmiany grochu.

## Introduction

Somatic embryogenesis (SE) raises great interests for both theoretical and practical reasons. It is a very useful system for experiments in plant development and potentially an efficient way of plant vegetative propagation. The responsiveness to SE induction treatments differs markedly across plant species and carrot, cucumber, alfalfa, wheat and maize are among the plants reacting most readily to SE inducers (DUNCAN et al. 2003, HITA et al. 2003, MALINOWSKI et al. 2004, YASUDA et al. 2000, WANG and WEI 2004). Grain legumes (including such important crops as soybean, bean, and pea) are considered rather refractory with respect to SE induction (LAKSHMANAN, TAJI 2000). Pea (*Pisum sativum* L.) is not only an important food legume (FAOSTAT 2007), but it also serves as a favorite object of physiological and molecular research (SCHROEDER et al. 1993). However, somatic embryogenesis in this species is rather time-consuming and the frequencies of embryo formation are often quite low (SCHROEDER et al. 1993, SANAGO et al. 1996). A reliable system for somatic embryogenesis in pea is still missing. In this work we report intervarietal differences in pea competence for somatic embryogenesis.

## Materials and Methods

### Plant material

Pea seeds were surface sterilized in aqueous solution of chloramine B (5%) for 15 minutes (smooth seeds) or 30 minutes (wrinkled seeds), followed by 3 washes with sterile distilled water. After sterilization, seeds were placed in tubes (25 $cm^3$ capacity) on a moist cotton wool. After germination (in darkness at 25-26ºC for 4-7 days) shoot apical meristems were excised and transferred to SE induction medium as primary explants.

Seeds of pea cultivars Bankier, Dino, Hazard, Iłówiecki, Konserwowy IHAR, Izolda, Kosynier, Lantra, Makler, Pegaz were bought from PLANTICO corporation (Poland) and seeds of pea cv. Oskar and line HM-6 were kindly provided by AGRITECH Ltd.(Czech Republic).

### Somatic embryogenesis induction

Two experimental systems were tested:

**System I**. Shoot apices excised from 4-day-old, etiolated, axenically grown seedlings were subjected to 14-days induction on basal medium described by GRIGA (1998), that contained MS salts (MURASHIGE, SKOOG 1962), Gamborg's B5 vitamins (GAMBORG et al. 1968), 3% sucrose and 2,5 μM picloram. During induction, cultures were kept in growing chamber under 16:8 photoperiod (light:darkness) and temperatures of 23–24ºC at day and 19–20ºC at night.

**System II**. It was adopted for those cultivars that did not form embryos under conditions of System I (see Results). Shoot apices excised from 7-days-old, etiolated, axenically grown seedlings were subjected to 28-days induction on basal medium as used in system I, except picloram concentration raised to 5 μM. During the time of induction cultures were kept in growing chamber in darkness at 26–27ºC, or under 16:8 hours photoperiod (light:darkness) at temperatures of 23–24ºC at day and 19–20ºC at night.

### Somatic embryos differentiation

After induction all cultures were transplanted to the differentiation medium (basal medium with no phytohormones) and cultures were kept in growing chamber under 16:8 hours photoperiod with temperatures of 23–24ºC at day and 19–20ºC at night. Physiological state of cultures, their morphogenetic reactions and efficiency of somatic embryogenesis were evaluated 3 weeks after transfer to the differentiation medium. Observations were made every 7–10 days.

The efficiency of somatic embryogenesis was defined as average number of somatic embryos per explant and expressed as per cent.

## Statistical analysis

The effects of cultivars on efficiency of somatic embryogenesis induction were analyzed by one-way analysis of variance (ANOVAs). Means and standard errors (± SE) were calculated for five series of data with four replicates in each series. The means were grouped using Duncan's multiple range test at $P \leq 0.05$.

The Microsoft Excel 2007 and STATISTICA 8.0 computer programs were used.

## Results

At the beginning of culture period explants excised from Konserwowy IHAR and Pegaz seedlings increased their size considerably (to 2–3 mm) without changing their overall shape. Somatic embryogenesis was successfully induced in 10 out of 12 tested cultivars/lines of *Pisum sativum* L. In experiment I direct somatic embryogenesis was observed. Normal somatic embryos with two cotyledons and shoot apex were obtained after 5 weeks of induction culture on explants of Oskar, Bankier, Hazard, Pegaz and HM-6 cultivars. The highest efficiency of somatic embryogenesis was observed in cultures of cv. Oskar and the lowest efficiency of somatic embryogenesis was observed in cultures of cv. Bankier (Table 1). Numerous embryoids in cotyledonary stage were noticed in cultures of line HM-6 (70%), Oskar (55%) or Hazard and Pegaz cultivars (50%). Only one somatic embryo was obtained from all cultures of Lantra cultivar. In cultures of other cultivars no somatic embryos were observed under conditions of experiment I.

Table 1

The efficiency of somatic embryogenesis in 12 pea cultivars under photoperiodic (16:8 h) conditions (mean ± S.E) after 80 days incubation

| Cultivar | Somatic embryogenesis efficiency (%) |
|---|---|
| Makler | $0.0^{a}$ |
| Izolda | $0.0^{a}$ |
| Konserwowy IHAR | $0.0^{a}$ |
| Dino | $0.0^{a}$ |
| Kosynier | $0.0^{a}$ |
| Iłówiecki | $0.0^{a}$ |
| Oskar | $31.0\pm6.8^{d}$ |
| Lantra | $0.0^{a}$ |
| HM-6 | $12.5\pm5.8^{b}$ |
| Bankier | $5.5\pm2.3^{c}$ |
| Hazard | $15.9\pm7.8^{b}$ |
| Pegaz | $7.4\pm3.3^{c}$ |

Values followed by the same superscript are not significantly different at 5% level

In the second experimental system (somatic embryogenesis induction with increased level of picloram at constant darkness), indirect somatic embryogenesis was noticed in cultures of all tested pea cultivars, except Izolda and Iłówiecki (Table 2). The highest efficiency of somatic embryogenesis was observed in cultures of cv. Kosynier, and the lowest efficiency of somatic embryogenesis was observed in cultures of cv. Makler. When somatic embryos induction was carried out under 16-hours

photoperiod conditions the rise of somatic embryogenesis efficiency was observed in cultures of Makler, Iłówiecki and Konserwowy IHAR cultivars. There was no somatic embryogenesis efficiency changes in cultures of Dino cultivar. In cultures of Kosynier cultivar decrease of somatic embryogenesis efficiency (approximately 50%) was noticed. During the first five weeks of culture callus (approximately 2–5 mm wide) developed around the edges of the explants of cultivars Iłówiecki, Izolda and Konserwowy IHAR. Shoot tips isolated from Kosynier, Makler and Dino cultivars produced larger calli, approx. 20 mm wide.

Table 2

The efficiency of somatic embryogenesis in 6 pea cultivars subjected to 28 days induction with 5 μM picloram under constant darkness or photoperiodic conditions (mean ± S.E)

| Cultivar | Somatic embryogenesis efficiency (%) | |
|---|---|---|
| | darkness | photoperiod 16/8h day/night |
| Makler | 1.8±1.8$^{d}$ | 4.4±0.3$^{a}$ |
| Izolda | 0.0$^{c}$ | 0.0$^{c}$ |
| Konserwowy IHAR | 4.0±2.0$^{a}$ | 12.2±1.4$^{b}$ |
| Dino | 11.5±4.8$^{b}$ | 11.9±4.2$^{b}$ |
| Kosynier | 24.8±6.2 | 13.0±5.8$^{b}$ |
| Iłówiecki | 0.0$^{c}$ | 5.8±2.9$^{a}$ |

Values followed by the same superscript are not significantly different at 5% level

The obtained embryos showed many developmental anomalies. Most of them reached only the early torpedo stage. Approximately 15–20% of embryos produced cotyledons, however their further development was disrupted (data not shown). The efficiency of somatic embryogenesis in both experimental systems did not depend on the age of seedlings (4- or 7-day-old) at the time of explant isolation (data not shown). The rate of embryo formation however, i.e. the moment when the first embryos appeared, was strongly affected by duration of the induction culture and picloram concentration. When cultures were exposed for 28 days to higher concentration of picloram (5 μM), callus developed and the induction of embryogenesis was retarded. First somatic embryos were observed on explants after 3 weeks of induction culture of Oskar and HM-6 cultivars (experimental system I) or after 7 weeks culture (3 weeks after transfer to hormone-free medium) of Konserwowy IHAR, Dino, Kosynier i Iłówiecki (experimental system II). Explants of Makler, Konserwowy IHAR, Dino, Kosynier and Iłówiecki cultivars subjected to 28-days induction culture under standard photoperiod conditions generated embryos after 6 weeks of culture (2 weeks after transfer to hormone-free medium).

On some explants multishoots were observed along with somatic embryos. The level of shoot development varied and depended on cultivar. On explants of Kosynier, Hazard and Oskar cultivars shoot clumps clearly

derived from callus tissue. In the other cultures shoots formed from primary apical meristems. Callus formation occurred chiefly on the basal part of the explants.

## Discussion

Many plant species can readily form somatic embryos under *in vitro* culture. It is known, however, that plant species, and even cultivars (PODWYSZYŃSKA 1997) and lines (GAIN et al. 1998) differ widely in responsiveness to somatic embryogenesis induction. Legumes are considered rather tenacious in this respect and the obtained frequencies of somatic embryo formation according to literature range from zero to dozens per cent (NADOLSKA-ORCZYK 1992, BRODA, TORZ 1997, GRIGA 1998, WALKER, PARROT 2001, TAMLIN et al. 2002). The potential for normal germination of somatic embryos is also correlated with cultivar and genotype of explant cells (SALAJOVA et al. 1999, BELMONTE et al. 2007).

In this report significant differences were observed in numbers of somatic embryos across twelve pea cultivars. Somatic embryogenesis efficiency under standard photoperiodic conditions ranged from 0 to 31%. Oskar cultivar seemed most efficient and line HM-6 proved quite good, which corroborates results obtained by GRIGA (1998). Cultivars Hazard, Pegaz and Kosynier also turned out quite responsive to somatic embryogenesis induction. They may thus be added to the list of SE-prone cultivars/lines including, among others, Belman, Brite (LEHMINGER-MERTENS, JACOBSEN 1989), R 4111, PF 5/81, Belinda (KYSELY, JACOBSEN 1990) and Sugar Ann and Patriot (SANAGO et al. 1996).

The efficiency of somatic embryogenesis depends on source of explant, its tissue composition and age of seedling at the time of explant isolation. Fragments of young seedlings or zygotic embryos are usually used as explants in somatic embryos induction cultures of legumes (LAKSHMANAN, TAJI 2000, CHHABRA et al. 2008, JOSHI et al. 2008). Reports by LOISEAU et.al. (1995) and GRIGA (1998) show that shoot apical meristems may also be applicable and our results further support this suggestion.

Type and concentration of plant growth regulators used for induction of somatic embryogenesis have to be adjusted for any specific plant and type of explant. In legumes, with a few exceptions, somatic embryogenesis was induced with the following auxins: 2,4-D; NAA; picloram; IAA and dicamba (LAKHMANAN, TAJI 2000, JOSHI et al. 2008). in pea SE was mainly induced with 2,4-D or NAA at 2–4 μM concentration (KYSELY, JACOBSEN 1990; OZCAN et al. 1993, GRIGA 1998, LAKHMANAN, TAJI 2000). High concentrations of auxins are believed to increase somatic embryogenesis efficiency, however, exceedingly high levels of auxins may also have inhibitory effect on embryo formation (TOMLIN i in. 2002).

## Conclusions

The results show that the choice of cultivar may be critically important for efficient induction of somatic embryogenesis in pea. Among tested cultivars Oskar, Hazard and line HM-6 were most amenable for induction of somatic embryogenesis. With cultivars less prone to form embryoids, the increase of auxin concentration in induction medium and extension of the induction period proved helpful.

Translated by Dariusz Michalczyk Accepted for print 13.04.2010

## References

BELMONTE M.F., TAHIR M., SCHROEDER D., STASOLLA C. 2007. *Overexpression of HBK3, a class IKNOX homeobox gene, improves the development of Norway spruce (Piea abies) somatic embryos.* J. Exp. Bot. 58: 2851–2861.

BRODA Z., TORZ L. 1997. *Zdolności regeneracyjne lucerny mieszańcowej (Medicago media Pers.) poprzez embriogenezę somatyczną. Zeszyty Naukowe AR w Krakowie 318: 235–238.*

CHHABRA G., CHAUDHARY D., VARMA M., SAINGER M., JAIWAL P.K. 2008. *TDZ- induced direct shoot organogenesis and somatic embryogenesis on cotyledonary node explants of lentil (Lens culinaris).* Physiol. Mol. Biol. Plants, 14: 347–353.

DUNCAN D.R, KRIZ A.L., PAIVA R, WIDHOLM J.M. 2003. *Globulin-1 gene expression in regenerable Zea mays (maize) callus.* Plant Cell Rep., 21: 684–689.

FAOSTAT Report 2007. http://faostat.fao.org, acces

GAMBORG O.L, MILLER RA., OJIMA K. 1968. *Nutrient requirements of suspension cultures of soybean root cells.* Exp. Cell Res., 50: 11–158.

GARIN E., ISABEL N, PLOURDE A. 1998. *Screening of large numbers of seed families of Pinus strobes L. for somatic embryogenesis from immature and mature zygotic embryos.* Plant Cell Rep., 18: 37–43.

GRIGA M. 1993. *Some factors affecting somatic embryogenesis efficiency in soybean (Glycine max (L.) Merr.).* Biologia Plantarum, 35: 179–189.

GRIGA M. 1998. *Direct somatic embryogenesis from shoot apical meristems of pea, and thidiazuron--induced high comersion rate of somatic.* Biologia Plantarum, 41: 481–495.

HITA O., GALLEGO P., VILLALOBOS N., LANAS I., BLAZQUEZ A., MARTIN J.P., FERNANDEZ J., MARTIN L., GUERRA H. 2003. *Improvement of somatic embryogenesis in Medicago arborea.* Plant Cell Tiss. Organ Cult. 72: 13–18.

JOSHI M., SUJATHA K, HAZARA S. 2008. *Effect of TDZ and 2,4-D on peanut somatic embryogenesis and in vitro bud deve1opment.* Plant Celi Tiss. Organ Cult., 94: 85–90.

KYSELY W., JACOBSEN H.-J. 1990. *Somatic embryogenesis form pea embryos and shoot apices. Plant* Cell Tiss. Organ Cult., 20: 7–14.

LAKSHMANAN P., TAJI A. 2000. *Somatic embryogenesis in leguminous plants.* Plant Biology, 2: 136–148.

LEHMINGER-MERTENS R., JACOBSEN H.-J. 1989. *Plant regeneration from pea protoplasts via somatic embryogenesis.* Plant Cell Rep., 8: 379–382.

LOISEAU I., MARCHE C., DEUNFF LE 1995. *Effects of auxins, cytokinins, carbohydrates and amino acids on somatic embryogenesis induction from soot apices of pea.* Plant Cell Tiss. Organ Cult., 41: 267–275.

MALINOWSKI R., FILIPECKI M., TAGAASH1RA N., WIŚNIEWSKA A., GAJ P., PLADER W., MALEPSZY S. 2004. *Xyloglucan endotransglucosylase/hydrolase genes in cucumber (Cucumis sativus) – differential expression during somatic embryogenesis.* Physiol. Plant., 120: 678–685.

MCKERSIE B.D., BROWN D.C.W. 1996. *Somatic embryogenesis and artificial seeds in forage legumes.* Seed Sci. Res., 6: 109–126.

MURASHIGE T., SKOOG F. 1962. *A revised medium for rapid growth and bioassays with tobacco tissue culture.* Physiol. Plant., 15. 437–497.

NADOLSKA-ORCZYK A. 1992. *Somatic embryogenesis of agriculturally important lupinus species (Lupinus angustifolius, L. albus, L. mutabilis).* Plant Cell Tiss. Organ Cult., 28: 19–25.

OZCAN S., BARGHCHI M., FIREK S., DRAPER J. 1993, *Efficient adventitions shoot regeneration and somatic embryogenesis in pea.* Plant Cell Tiss. Organ Cult., 34: 271–277.

PODWYSZYŃSKA M., MARASEK A., GABRYSZEWSKA E. 1997. *Indukcja tworzenia pędów przybyszowych i zarodków somatycznych w kulturach in vitro tulipana.* ZESZ. NAUK. AR W KRAKOWIE, 318: 195–199.

SALAJOVA T., SALAJ J., KORMUTAK A. 1999. *Initiation of embryogenic tissues and plantlet regeneration from somatic embryos of Pinus nigra Arn.* Plant Science, 145: 33–40.

SANAGO M.H.M., SHATTUCK VI., STROMMER J. 1996. *Rapid plant regeneration of pea using thidiazuron.* Plant Cell Tiss. Organ Cult., 45: 165–168.

SCHROEDER H.E., SCHOLTZ A.H., WARDLEY-RICHARDSON T., SPENCER D., HIGGINGS T.J.V. 1993. *Transformation and regeneration of two cultivars of pea (Pisum sathmm L.).* Plant Physiol., 101: 751–757.

TOMLIN E.S., BRANCH S.R, CHAMBERLAIN D., GABE H., WRIGHT M.S., STEWARD C.N. 2002. *Screening of soybean, Glycine max (L.) Merrill, lines for somatic embryo induction and maturation capability from immature cotyledons.* In Vitro Cell. Dev. Biol. – Plant, 38: 543–548.

WALKER D. R., PARROT W.A. 2001. *Effect of polyethylene glycol and sugar alcohols on soybean somatic embryo germination and conversion.* Plant Cell Tiss. Organ Cult., 64: 55–62.

WANG C.-T., WEI Z.-M. 2004. *Embryogenesis and regeneration of green plantlets from wheat (Triticum aestivum) leaf base.* Plant Cell Tiss. Organ Cult., 77: 149–156.

YASUDA H., NAKAJIMA M., MASUDA H., OHWADA T. 2000. *Direct formation of heart- shaped embryos for differentiated single carrot cells in culture.* Plant Science, 152: 1–6.

POLISH JOURNAL OF NATURAL SCIENCES
Abbrev.: Pol. J. Natur. Sc., Vol 25(2): 123–131, Y. 2010

DOI 10.2478/v10020-010-0010-1

# THE INFLUENCE OF THE WILD BOAR ON THE BIOLOGICAL AND PERFORMANCE TRAITS OF DOMESTIC PIGS

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K e y w o r d s: wild boar, Lithuanian White, hybrid, prolificacy, bristle color, growth, meatiness, meat quality.

A b s t r a c t

The experiment conducted in a stockyard in the district of Telšiai (Lithuania) involved two groups of animals: I – Lithuanian Whites, mated with the wild boar in an attempt to produce first-generation hybrids ($F_1$) having 50% wild boar blood, and II – female hybrids, mated repeatedly with the wild boar to produce second-generation hybrids ($F_2$) with 75% wild boar blood. An increase in the wild boar gene pool (up to 75%) led to a decrease in the litter size which approximated prolificacy values typical of wild boars. When white homozygous pigs of the Lithuanian White breed were mated with wild boars, white was the dominant color in the first generation ($F_1$), whereas when white, heterozygous female hybrids were crossbred with wild boars, white and striped (torched) second-generation ($F_2$) hybrids were distributed at the ratio of 1.25:1 throughout the population. The aim of this study was to investigate the growth rate, meatiness and meat quality of $F_1$ hybrids. In the control period, (body weight of approximately 30 to 80 kg), the average daily gains of hybrids ($n = 12$) reached 474 g. Boars grew faster (490 g), while gilts were marked by a slower growth rate (457 g). Five boars (approx. 100 kg) were slaughtered for the evaluation of meatiness traits and meat (*musculus longissimus dorsi*) quality, while gilts were left for further breeding. The average half-carcass length of hybrids was 95 cm, backfat thickness at 6–7 and last ribs reached 29 and 16 mm, respectively, and lean meat content was 50.7%. The meat $pH_{48}$ was 5.48, redness was determined at 18.16 ext. u., water holding capacity at 58.15% and cooking loss at 27.35%. Chemical composition composition of meat was as follows: 23.62% protein, 1.54% fat and 1.13% ash. In comparison with Lithuanian White pigs, hybrids grew at a slower rate and their carcasses had lower meatiness traits. The meat of $F_1$ hybrids was characterized by higher redness values, lower cooking loss and satisfactory chemical composition in respect of nutritive value. The meat of male hybrids was also found to deliver a high degree of palatability without a specific, offensive aroma.

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# WPŁYW KRZYŻOWANIA Z DZIKIEM NA CECHY BIOLOGICZNE I UŻYTKOWE ŚWIŃ DOMOWYCH

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Słowa kluczowe: dzik, litewska biała, mieszańce, płodność, kolor szczeciny, wzrost, mięsność, jakość mięsa.

Abstrakt

Eksperyment przeprowadzono w chlewni z wybiegami w okręgu Telšiai (Litwa). Objęto nim dwie grupy zwierząt: I – rasę litewską białą krzyżowaną z dzikiem w celu otrzymania pierwszego pokolenia mieszańców ($F_1$) mających 50% krwi dzika, i II – lochy mieszańce kryte powrotnie dzikiem, aby otrzymać drugie pokolenie mieszańców ($F_2$) z 75% udziałem krwi dzika. Wzrost puli genowej dzika (do 75%) prowadził do obniżenia liczebności miotu, który w przybliżeniu odpowiadał płodności dzika. Gdy lochy czystej rasy litewskiej białej kryto knurami dzika, białe umaszczenie było dominujące w pierwszym pokoleniu mieszańców ($F_1$), natomiast gdy białe heterozygotyczne lochy mieszańce były kryte dzikiem, to białe i kolorowe (z paskami) mieszańce drugiego pokolenia ($F_2$) występowały w proporcji 1,25 : 1. Celem badań było określenie tempa wzrostu, mięsności i jakości mięsa mieszańców $F_1$. W okresie badań (masa ciała ok. 30–80 kg) średnie przyrosty dobowe hybrydów ($n = 12$) wynosiły 474 g. Knurki rosły szybciej (490 g), a loszki cechowały się wolniejszym tempem wzrostu (457g). 5 knurków (o masie ciała 100 kg) ubito w celu określenia mięsności i jakości mięsa (*musculus longissimus dorsi*), loszki zaś pozostały do dalszej hodowli. Średnia długość tuszy hybrydów wynosiła 95 cm, grubość słoniny mierzona na wysokości żeber 6–7 oraz ostatniego wynosiła odpowiednio 29 i 16 mm, a zawartość mięsa – 50,7%. $Ph_{48}$ wyniosło 5,48, udział barwy czerwonej – 18,16, wodochłonność 58,15%, a wyciek termiczny 27,35%. Skład chemiczny mięsa był następujący: 23,62% białka, 1,54% tłuszczu i 1,13% popiołu. W porównaniu ze świniami rasy białej litewskiej mieszańce rosły wolniej, a ich tusze zawierały mniej mięsa. Mięso mieszańców $F_1$ charakteryzowało się wyższym udziałem barwy czerwonej, niższym wyciekiem termicznym i dobrym składem chemicznym w aspekcie wartości odżywczej. Mięso mieszańców samców oceniono jako smaczne, bez specyficznego knurzego zapachu.

## Introduction

As progenitors of pigs (*Sus domesticus*), wild boars (*Sus scrofa* L.) can mate reciprocally to give prolific progeny. There are 26–28 subspecies of wild boars. Lithuania is the habitat of the Central European wild boar (*Sus scrofa scrofa*) (BALEIŠIS et al. 2003). The Lithuanian White breed descends from aboriginal (local) pigs which, in turn, are the successors of the Central European wild boar inhabiting Lithuania (MAKOVECKAS 1986). Owing to centuries of evolution, domestic pigs differ significantly from their wild ancestors. In the process of domestication, man significantly altered the natural environment of wild pigs (wild boars). Due to an abundance of food sources, the pigs' metabolism had changed, their bones became thinner, and changes were also observed in the animals' body covering and bristle color. Domestic animals became short-legged, their productivity was altered and it was followed by changes

in the pigs' external appearance. In wild boars, the front part of body is more developed, while pigs are characterized by more pronounced hinder and middle body parts. The domestication of wild boars also modified the activity of sexual glands. Wild boars rut one or, less frequently, two times a year, whereas the mating regime of pigs has been deprived of its seasonal character. The pregnancy of wild and domestic sows lasts approximately 4 months, but differences in prolificacy are observed. Wild sows deliver an average of 5 piglets (from 1 to 10) (BALEIŠIS et al. 2003), whereas pigs produce 10–14 and more offspring (JANČIENĖ 2005). The prolificacy of domestic pigs is also determined by breed. The average litter size of Lithuanian White sows is 11.2 piglets (DŽIAUGYS et al. 1998, KLIMAS et al. 2006). Domestic pigs mature sexually at the age of 5–6 months (JANČIENĖ 2005), and wild boars – in the second year of life (BALEIŠIS et al. 2003). The aggressive nature of the wild boar has been largely eradicated in the domestication process, pigs are much calmer animals which is why they grow rapidly and accumulate more fat in hypodermic and muscular tissues. Wild boars and pigs differ not only with regard to their appearance but also biological features. The meat of pig and wild boar hybrids is becoming increasingly popular in Great Britain, Poland and other countries (MARCHIORI and FELICIO 2003, *The British wild boar association.* 2005, SZCZEPAŃSKI et al. 2007, SZMANKO et al. 2007). Although in Lithuania boars are prevalent in the wild, attempts are also made to breed those animals in stockyards. Selected farms conduct experiments into hybridizing wild boars with pigs.

The aim of this study was to: 1) investigate the influence of the wild boar on prolificacy of primiparous sows of the Lithuanian White breed and the body covering color of first ($F_1$) and second ($F_2$) generation hybrids; 2) investigate the growth rate, meatiness and meat quality of $F_1$ hybrids.

## Materials and Methods

The experiment was carried out in 2004–2008 in Telšiai district, in the farm of A. Vaitkevičius and a stockyard with the area of 1 ha in the vicinity. Following an examination of the site's suitability for a wild boars stockyard in 2003, the State Food and Veterinary Service of Telšiai district ruled on the site's compliance with the regulations for keeping wild animals in captivity. The Environmental Protection Department of the Šiauliai region issued license No. 14 for stocking a wild boar farm. The experiment involved two groups of animals: I – Lithuanian Whites (*LW*), mated with the wild boar (*WB*) in an attempt to produce first-generation hybrids ($F_1$) having 50% wild boar blood, and II – female hybrids, mated repeatedly with the wild boar to produce second-generation hybrids ($F_2$) with 75% wild boar blood. Wild boars, pigs and hybrids older

than 2 months were fed identical diets (barley, pea and wheat flour), supplemented with minerals and vitamins. Offspring younger than 2 months was fed a special starter compound feed supplied by Kretingos grūdai. Rations were prepared according to the recommended feeding standards. Purebred Lithuanian White gilts were acquired for the experiment from the Berka breeding center (Kelmė district).

The Environmental Protection Department of the Šiauliai region did not extend the license (No. 14) for keeping wild boars and their hybrids, and the experiment was suspended. $F_2$ hybrids grew at a slower rate than $F_1$ hybrids, and when the experiment was brought to an unexpected halt, their average weight upon slaughtering was 50–60 kg. As the result, the growth rate, meatiness and meat quality of $F_2$ hybrids was not analyzed. For this reason, the above performance traits were studied only in $F_1$ hybrids.

**Prolificacy and color of bristles.** The litter size of primiparous sows was determined by the number of hybrid piglets born alive. The distribution of newborn hybrids with white and striped hair covering was analyzed. The number of animals analyzed for the determination of the above attributes is presented in Tables 1–3.

Table 1

The influence of the wild boar on the prolificacy of pigs

| Item | Group | |
|---|---|---|
| | I | II |
| | *LW*x*WB* | ½ *LW* ½ *WB* – $F_1$ x *WB* |
| No. of primiparous sows | 4 | 6 |
| Litter size | 11.25±0.34 | 7.50±0.24*** |

*** $p < 0.001$

Table 2

Distribution of primiparous sows in terms of litter size

| No. of hybrid piglets born alive | Group | | | |
|---|---|---|---|---|
| | I | | II | |
| | primiparous sows | | primiparous sows | |
| | No. | % | No. | % |
| 7 | 0 | 0.0 | 4 | 66.6 |
| 8 | 0 | 0.0 | 1 | 16.7 |
| 9 | 0 | 0.0 | 1 | 16.7 |
| 10 | 1 | 25.0 | 0 | 0.0 |
| 11 | 1 | 25.0 | 0 | 0.0 |
| 12 | 2 | 50.0 | 0 | 0.0 |
| Total | 4 | 100.0 | 6 | 100.0 |

Table 3

Distribution of hybrid piglets in terms of bristle color

| Generation | Genotype | Total number of hybrids | Bristle color | | | |
|---|---|---|---|---|---|---|
| | | | white | | striped | |
| | | | no. | % | no. | % |
| $F_1$ | ½ *LW* ½ *WB* | 45 | 45 | 100.0 | 0 | 0.0 |
| $F_2$ | ¼ *LW* ¾ *WB* | 45 | 25 | 55.6 | 20 | 44.4 |

**Growth rate**. Twelve $F_1$ hybrids (six boars and six gilts) aged 111–116 days were selected for a control fattening test. During the control period (body weight from 30 to 80 kg), all animals were provided with identical housing and feeding conditions in the stockyard – stable. When the hybrids' body weight reached 80 kg, the length of the fattening period, average daily gains and age in days were estimated.

**Control slaughter**. Five boars (approx. 100 kg) were slaughtered in a meat processing plant to evaluate meatiness traits and meat (*musculus longissimus dorsi*) quality, while gilts were preserved for further breeding. The lean meat percentage of fresh carcasses was determined with the use of the Fat-o-meter (FOM) device. Other meatiness traits, including half-carcass length, backfat thickness and loin lean area of cooled left carcasses (stored at 0...+4ºC for 24 h), were determined with the involvement of the approved methodology (SAIKEVIČIUS 2003).

Muscle samples (500 g) for meat quality evaluations were collected from the *musculus longissimus dorsi* at the last rib, 48 hours after slaughter. Meat quality analyses were carried out at the Lithuanian Veterinary Academy using standard methods. The pH of meat was determined in accordance with the *Meat and meat...* ISO 2917:1999 procedure, color – with the Minolta Chroma Meter by measuring lightness ($L$), redness ($a$) and yellowness ($b$), water holding capacity – by the method proposed by Grau and Hamm, as described by OFFER and KNIGHT (1988), cooking loss – by the Warner-Bratzler test, dry matter content – by drying the samples at 105ºC, protein content – according to the Kjeldahl method, intramuscular fat – in accordance with *Meat and meat...* ISO 1443:1973, ash (mineral matter) content – in accordance with *Meat and Meat* ISO 936:1998.

**Statistical analysis**. The results were processed using Statistica for Windows version 6.0 (*StatSoft* 2001) and by observing the analytical principles outlined in the *Basic guide to the statistical analysis of biological data* by TUCKER (2003). Selected hybrid data (Table 4 and Table 5) was compared with the data of purebred Lithuanian White pigs kept in the Berka breeding centre. Differences were regarded as significant at $p < 0.05$.

Table 4

Meatiness traits at 100 kg body weight

| Trait | | $F_1$ hybrids | Lithuanian White pigs | Hybrids/pigs (±) |
|---|---|---|---|---|
| Lean meat, % | | 50.7±0.6 | 54.7±0.3 | –4.0*** |
| Half-carcass length, cm | | 95.0±0.5 | 98.0±0.2 | –3.0** |
| Backfat thickness: | at 6–7 rib, mm | 29.0±0.6 | 22.3±0.3 | +6.7*** |
| | at last rib, mm | 16.0±0.8 | 17.5±0.5 | –1.5* |
| Loin lean area, $cm^2$ | | 36.2±1.2 | 38.3±0.8 | –2.1* |
| Ham weight, kg | | 11.5±0.2 | 11.8±0.1 | –0.3 |

* p<0.05; **p<0.01; ***p<0.001

Table 5

Physicochemical parameters of meat (*musculus longissimus dorsi)* at 100 kg body weight

| Parameter | | $F_1$ hybrids | Lithuanian White pigs | Hybrids/pigs (±) |
|---|---|---|---|---|
| Physical parameters: | | | | |
| $pH_{48}$ | | 5.48±0.02 | 5.48±0.01 | 0 |
| Color ext. u.: | lightness (*L*) | 42.27±1.19 | 53.23±0.09 | –10.96*** |
| | redness (*a*) | 18.16±0.21 | 14.58±0.16 | +3.58** |
| | yellowness (*b*) | 5.56±0.21 | 7.23±0.15 | –1.67* |
| Water holding capacity,% | | 58.15±0.67 | 57.67±0.54 | +0.48 |
| Cooking loss,% | | 27.35±0.99 | 28.70±0.66 | –1.35* |
| Chemical composition: | | | | |
| Dry matter,% | | 26.68±0.36 | 26.05±0.21 | +0.63* |
| Protein,% | | 23.62±0.25 | 23.42±0.14 | +0.20 |
| Fat,% | | 1.54±0.12 | 1.75±0.07 | –0.21 |
| Ash (mineral matter),% | | 1.13±0.01 | 1.17±0.01 | –0.04 |

* $p<0.05$; ** $p<0.01$; *** $p<0.001$

## Results and Discussion

As previously observed by DŽIAUGYS et al. (1998) and KLIMAS et al. (2006), the wild boar had no adverse effect on the prolificacy of purebred pigs. Primiparous sows of group I delivered an average of 11.25 hybrid piglets (Table 1). The prolificacy of female hybrids mated repeatedly with the wild boar was only 7.5, i.e. it was 33.3% lower ($p<0.001$) than in group I primiparous sows. The coefficients of variation relating to the prolificacy rate of group I and group II sows reached 7.37 and 10.18, respectively. A higher degree of variability in litter size was determined in $F_1$ female hybrids paired repeatedly with the wild boar.

The distribution of sows in terms of the number of hybrid piglets born alive was analyzed (Table 2). The majority of group I females (50%) delivered 12 piglets, and group II sows (66.6%) – 7 piglets.

It should be noted that $F_1$ hybrids had white hair covering. In $F_2$ sows, this phenotypic feature was distributed as follows: 55.6% hybrids had white bristles and 44.4% had striped hair covering which is typical of young wild boars (Table 3).

The average daily gains of hybrids reached 474 g (Table 6) in the control fattening period (body weight from 30 to 80 kg). Boars grew at a faster rate (490 g) than gilts (457 g). Hybrids of generation $F_1$ attained the weight of 80 kg in 211 days. The standard body weight for pigs of this age is 100 kg and more (KLIMAS et al. 2006, RIMKEVIČIUS et al. 2008). The results of this study indicate that hybrids have a slower growth rate than pigs (SZCZEPAŃSKI et al. 2007), but they grow at a faster rate than wild boars (*The British Wild Boar Association* 2005).

Table 6

Growth rate of $F_1$ hybrids (body weight of 30 to 80 kg)

| Gender | No. of animals | Initial weight, kg | Final weight, kg | Fattening period, d. | Daily gains, g | Age from birth, d. |
|---|---|---|---|---|---|---|
| Male | 6 | 31.0±1.2 | 79.0±2.0 | 98±0 | 490±15 | 210±1 |
| Female | 6 | 30.2±0.8 | 75.0±2.4 | 98±0 | 457±22 | 212±1 |
| Total | 12 | 30.6±0.7 | 77.0±1.5 | 98±0 | 474±13 | 211±1 |

The backfat of $F_1$ hybrids at 6–7 rib was 6.7 mm thicker ($p < 0.001$), and the lean meat content of the carcass was lower by 4.0% ($p < 0.001$) in comparison with Lithuanian White pigs (Table 5). The lower muscularity of hybrids was determined not only by thicker backfat, but also by a smaller loin lean area (*musculus longissimus dorsi)* (36.2 $cm^2$). The half-carcasses of the investigated animals were 3.0 cm shorter than in pigs ($p < 0.01$).

According to other researchers (SZCZEPAŃSKI et al. 2007), hybrid carcasses are characterized by lower meatiness traits than the carcasses of purebred pigs.

Animal muscle consists of both red and white fibers. The wild boar and the domestic pig are characterized by a nearly reverse ratio of red to white fibers. Wild boar muscles comprise 70% red fibers to 30% white fibers, whereas in the domestic pig, the relevant proportions are 20% red to 80% white fibers. This diversity affects the color, the texture as well as the taste of the two meat types (*The British Wild Boar Association* 2005). As demonstrated by the results of this study (Table 5), wild boars significantly contributed to the quality and physical parameters of hybrid meat. The meat of $F_1$ hybrids was characterized by higher redness values (by 3.58 ext. u., $p < 0.01$) and 1.35% lower cooking loss ($P > 0.05$) in comparison with Lithuanian White pigs. The meat of male hybrids was also marked

by satisfactory chemical composition (Table 5). The dry matter protein content of hybrid meat was 0.20% higher, and intramuscular fat content was 0.21% lower in comparison with the meat of domestic pigs. Similar findings were reported by Polish scientists in a study investigating the quality of meat of domesticated pigs and wild boar hybrids (SZCZEPANSKI et al. 2007, SZMANKO et al. 2007).

The use of male pigs for pork production is limited due to a sensory defect referred to as boar taint. Boar taint, the offensive odor or taste of pork, is caused by the accumulation of androstenone and skatole (BABOL et al. 1999, FONT et al. 1999). The meat of hybrids, on the other hand, is characterized by highly satisfactory taste without the adverse traits of boar taint. It can, therefore, be concluded that wild boars retard the sexual maturation of male hybrids, depriving their meat of an offensive aroma in the first year of life.

## Conclusions

1. An increase in the wild boar gene pool (up to 75%) led to a decrease in the pigs' litter size which approximated prolificacy values typical of wild boars.

2. When white homozygous pigs of the Lithuanian White breed were mated with wild boars, white was the dominant color in the first generation ($F_1$), whereas when white, heterozygous female hybrids were crossbred with wild boars, white and striped (torched) second-generation ($F_2$) hybrids were distributed at the ratio of 1.25:1 throughout the population.

3. In comparison with Lithuanian White pigs, hybrids grew at a slower rate and their carcasses had lower meatiness traits. The meat of $F_1$ hybrids was characterized by higher redness values, lower cooking loss and satisfactory chemical composition in respect of nutritive value. The meat of male hybrids was also found to deliver a high degree of palatability without a specific, offensive aroma. It can be concluded that the biological characteristics of pig and wild boar hybrids enhance the assortment of high-quality pork products.

## Acknowledgements

The authors would like to thank Professor Algimantas Mikelėnas and farmer Antanas Vaitkevičius (Lithuania) for their contribution to this study.

Translated by Authors Accepted for print 19.05.2010

## References


BABOL J., SQUIRES E. J., LUNDSTROM K. 1999. *Relationship between metabolism of androstenone and skatole in intact male pigs.* Journal of Animal Science, 77: 84–92.

BALEIŠIS R., BLUZMA P., BALČIAUSKAS L. 2003. *Lietuvos kanopiniai tvėrys.* Vilnius: 8–21.

DŽIAUGYS V., KLIMAS R., KLIMIENĖ A., RAPKAUSKAS G. 1998. *Lietuvoje veisiamų kiaulių produktyvumas.* Gyvulininkystė, 32: 41–47.

FONT I., FURNOLS M., OLIVER M. A. 1999. *Production and consumption of pork meat with different levels of boar taint. Review.* Food Science and Technology, 5: 367–375.

JANČIENĖ I. 2005. *Kiaulininkystė.* Kaunas, 14–22.

KLIMAS R., KLIMIENĖ A., RIMKEVIČIUS S. 2006. *Kiaulių selekcija ir panaudojimas*. Šiauliai, 63.

MAKOVECKAS R. 1986. *Lietuvos baltosios kiaulės.* Vilnius: 299.

MARCHIORI A. F., FELICIO P. E. 2003. *Quality of wild boar meat and commercial pork*. Science Agricola, 60(1): 1–5.

*Meat and meat products determination of total fat content.* ISO 1443:1973.

*Meat and meat products determination of total ash.* ISO 936:1998.

*Meat and meat products Measurement of pH-Reference method.* ISO 2917:1999.

OFFER G., KNIGHT P. 1988. *The structural basis of water-holding in meat.* Part 1. *General principles and water uptake in meat processing.* In: Lawrie R. Developments in Meat Science – 4. Chapter 3, Elsevier Science, London: 63–171.

RIMKEVIČIUS S., REKŠTYS V., RADIENĖ Z., SĄLYGA G., JUKNA V., SAIKEVIČIENĖ B., RAUDONIKIS A., KERZIENĖ S. 2008. *Kiaulių veislininkystė 2007 metais.* Baisogala, 220.

SAIKEVIČIUS K. J. 2003. *Lietuvos Respublikos gyvulių veislininkystę reglamentuojančių teisės aktų rinkinys. I tomas* Vilnius, 138–157.

*StatSoft, Inc.* 2001. *Statistica for Windows version 6.0. <http://www.statsoft.com>.*

SZCZEPAŃSKI J., SZMANKO T., KORZENIOWSKA M. 2007. *Characteristics of carcass traits and physicochemical properties of meat from pig with wild boar crossbreed*. Animal Science, Proceedings, 1: 134–135.

SZMANKO T., SZCZEPAŃSKI J., KORZENIOWSKA M. 2007. *Culinary and technological usefulness of meat from crossbreed of pig with wild boar.* Animal Science. Proceedings, 1: 136–137.

*The British Wild Boar Association.* 2005. *Wild boar food facts.<http://bwba.co.uk>.*

TUCKER L. A. 2003. *Simplistic statistics. A basic guide to the statistical analysis of biological data.* UK, Welton Lincoln, 65.

POLISH JOURNAL OF NATURAL SCIENCES
Abbrev.: Pol. J. Natur. Sc., Vol 25(2): 132–142, Y. 2010

DOI 10.2478/v10020-010-0011-0

# THE EFFECT OF HERD SIZE ON THE YIELD AND PROXIMATE COMPOSITION OF MILK IN ACTIVE CATTLE POPULATIONS IN THE REGION OF WARMIA AND MAZURY (NE POLAND)*

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Key words: cows, herds size, milk yield, milk composition, fat, protein, inter-calving interval.

Abstract

The milk yield of 24 934 cows from herds monitored by the National Animal Breeding Center, Branch in Olsztyn, was analyzed. The data were collected in the years 1997–2006. The cows were divided into three groups, based on herd size: group I of up to 20 cows, group II of 21–50 cows and group III of more than 50 cows. Additional criteria for the above division were the number of successive 305-day lactations and full lactations as well as the length of inter-calving intervals. The objective of this study was to determine the effect of herd size on the yield and proximate composition of milk in active cattle populations in farms in north-eastern Poland over a ten-year period, taking into account lactations of normal length and full lactations, inter-calving interval (ICI) duration and lifetime cow productivity. The average yield over 305-day lactations was 6579 kg milk (6723 kg FCM), 273 kg fat (4.15%), 213 kg protein (3.24%), 309 kg lactose (4.70%) and 841 kg dry matter (12.78%). Cows in the largest herds (>50 head) were characterized by the highest productivity, and cows in the smallest herds (≤20 head) – by the lowest. The latter produced milk with the highest fat content (4.16%) and the lowest protein content (3.21%). In herds comprising more than 50 animals, cows with the longest ICI (>525 days) were marked by the highest milk production in full lactations (11 010 kg). As regards lifetime productivity, the highest values were noted in cows used for 3.44 years in the smallest herds (19 809 kg milk). In the largest herds cows were used for the shortest period of time (3.31 years), and their lifetime productivity reached 17 185 kg milk.

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* This work was supported by the Ministry of Scientific Research and Information Technology within the framework of grant no. N31105631/0237, funds allocated to research projects in the years 2006–2009.

# WYDAJNOŚĆ I PODSTAWOWY SKŁAD MLEKA KRÓW POPULACJI AKTYWNEJ Z REGIONU WARMIŃSKO-MAZURSKIEGO W ZALEŻNOŚCI OD WIELKOŚCI STADA

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S ł o w a k l u c z o w e: krowy, wielkość stada, wydajność i skład mleka, tłuszcz, białko, okresy międzywycieleniowe.

A b s t r a k t

Do analizy posłużyły wyniki oceny użytkowości mlecznej 24 934 krów pochodzących ze stad kontrolowanych przez KCHZ oddział w Olsztynie w latach 1997–2006. Materiał badawczy podzielono na 3 grupy, uwzględniając wielkość stad: w I grupie (gr. I) było do 20 krów, w II (gr. II): 21–50, w III (gr. III) – ponad 50 krów. Dodatkowymi kryteriami podziału były: kolejne 305-dniowe i pełne laktacje oraz długość okresów międzywycieleniowych. Celem pracy była analiza wpływu wielkości stada na wydajność i podstawowy skład mleka populacji aktywnej krów utrzymywanych w gospodarstwach północno-wschodniej Polski w okresie dziesięciolecia, z uwzględnieniem laktacji standardowych i pełnych, długości okresów międzywycieleniowych (OMW) i wydajności życiowej krów. W badaniach wykazano, że przeciętna wydajność krów w laktacjach 305-dniowych wynosiła: 6579 kg mleka (6723 kg mleka FCM), 273 kg tłuszczu (4,15%), 213 kg białka (3,24%), 309 kg laktozy (4,70%) i 841 kg suchej masy (12,78%). Największa produkcyjność charakteryzowała krowy użytkowane w stadach najliczniejszych (>50 szt.), a najmniejsza była u zwierząt ze stad najmniej licznych (≤ 20 szt.), dających mleko o największej zawartości tłuszczu (4,16%), ale najmniejszej zawartości białka (3,21%). W stadach o liczebności ponad 50 sztuk krowy o najdłuższym OMW (>525 dni) osiągały największą produkcję mleka za laktacje pełne (11 010 kg). Pod względem wydajności życiowej najbardziej efektywne były krowy użytkowane przez 3,44 lata w stadach najmniejszych (19 809 kg mleka). W stadach największych krowy użytkowane były najkrócej (3,31 lat), a ich życiowa wydajność wyniosła 17 185 kg mleka.

## Introduction

Poland's accession to the European Union and the current economic conditions resulted in significant changes in farm structure. According to the data supplied by the *Rocznik statystyczny rolnictwa...* (2008), the total number of agricultural farms decreased by 354 000 (12%), compared with the results of a census of farms carried out five years earlier. The highest drop, at 21%, was noted in the group of farms covering an area of up to 1 ha, while the number of the largest farms, covering an area of 50 ha or more, increased by 7.6%. Changes in cattle herd size were followed by an increase in average milk yield per cow. As a result, commercial milk production is today higher than consumption. Therefore, creating new export opportunities for dairy products outside the EU internal market is an important consideration (OSTOJA-SOLECKI 2002).

Farm fragmentation remains a characteristic feature of Polish agriculture. In 2008, the average size of an individual farm was 10.02 ha (*Ocena i hodowla...* 2009), and over 85% barns housed less than ten cows.

Fragmentation affects also the process of milk cooling and collection from farms (JURCZAK 1999). The development of the dairy sector is dependent upon the presence of large-scale specialized dairy production farms raising cattle with a high proportion of HF genes. Such a trend can be also observed in highly developed countries. In the USA, Holstein-Friesian cattle herds are raised mostly in north- and south-eastern regions (OLEGGINI et al. 2001, ELY et al. 2003). The global HF cattle population continues to increase, and milk yield and composition continue to improve (MELENDEZ, PINEDO 2006), primarily because cows are kept in large herds, are fed well-balanced diets based on concentrated feed and supplemented with protein, minerals and vitamins.

The objective of this study was to determine the effect of herd size on the yield and proximate composition of milk in active cattle populations in farms in north-eastern Poland over a ten-year period (1997–2006), taking into account lactations of normal length and full lactations, inter-calving interval (ICI) duration and lifetime cow productivity.

## Materials and Methods

The milk yield of 24 934 cows from herds monitored by the National Animal Breeding Center, Branch in Olsztyn, was analyzed. The data were collected in the years 1997–2006. The yield of milk, milk fat, milk protein, lactose and dry matter, and the content of milk fat, milk protein, lactose and dry matter were determined for each cow. The actual amount of produced milk was converted into the amount of fat-corrected milk (FCM) and energy-corrected milk (ECM), according to the following formula (ARBEL et al. 2001):

$$\text{FCM [kg]} = 0.4 \cdot \text{milk [kg]} + 15 \cdot \text{fat [kg]}.$$

$$\text{ECM (kg)} = \frac{\text{milk [kg]} \cdot (0.383 \cdot \text{fat (\%)} + 0.242 \cdot \text{protein (\%)} + 0.7832)}{3.14}$$

The cows were divided into three groups, based on herd size: group I of up to 20 cows, group II of 21–50 cows and group III of more than 50 cows. Additional criteria for the above division were the number of successive 305-day lactations and full lactations as well as the length of inter-calving intervals. The effect of herd size on lifetime cow productivity was also determined.

The results were processed statistically using Statistica 7.1 software. Least squares means (LSM) and standard errors (Se) were calculated for the analyzed parameters. The significance of differences between means was estimated by Duncan's test.

## Results and Discussion

Table 1 presents milk yield and composition in 305-day lactations of cows in herds of different size. Average milk yield in the analyzed herds was 6579 kg (6723 kg FCM). The produced milk contained 4.15% fat, 3.24% protein, 4.70% lactose and 12.78% dry matter. Compared with the statistical data collected by the Polish Federation of Cattle Breeders and Dairy Farmers in the Province of Warmia and Mazury in 2008 (*Ocena i hodowla...* 2009), the above milk yield values are similar, while milk fat and protein content values are lower.

Table 1

The effect of herd size on milk yield in 305-day lactations

| Parameter | Statistical measure | Groups of cows | | | |
|---|---|---|---|---|---|
| | | group I ≤ 20 | group II 20.1–50 | group III > 50 | Mean |
| Number of cows | N | 15959 | 4054 | 4921 | 24934 |
| Milk (kg) | LSM | $6105^{A}$ | $6389^{B}$ | $7242^{C}$ | 6579 |
| | Se | 6.453 | 15.502 | 18.382 | 13.443 |
| FCM (kg) | LSM | $6252^{A}$ | $6514^{B}$ | $7405^{C}$ | 6723 |
| | Se | 7.881 | 18.943 | 21.273 | 16.032 |
| ECM (kg) | LSM | $6131^{A}$ | $6412^{B}$ | $7297^{C}$ | 6614 |
| | Se | 7.881 | 18.943 | 21.273 | 16.032 |
| Protein-to-fat ratio | LSM | $0.772^{A}$ | $0.787^{B}$ | $0.788^{B}$ | 0.782 |
| | Se | 0.0005 | 0.0010 | 0.0012 | 0.0009 |
| Fat (kg) | LSM | $254^{A}$ | $264^{A}$ | $301^{B}$ | 273 |
| | Se | 0.314 | 0.738 | 0.745 | 0.559 |
| Protein (kg) | LSM | $196^{A}$ | $208^{B}$ | $237^{C}$ | 213 |
| | Se | 0.220 | 0.531 | 0.634 | 0.462 |
| Lactose (kg) | LSM | $298^{A}$ | $302^{B}$ | $337^{C}$ | 309 |
| | Se | 0.803 | 1.898 | 2.100 | 1.601 |
| Dry matter (kg) | LSM | $781^{A}$ | $816^{B}$ | $926^{C}$ | 841 |
| | Se | 2.173 | 5.088 | 5.256 | 4.172 |
| Fat (%) | LSM | $4.16^{a}$ | $4.13^{b}$ | $4.15^{a}$ | 4.15 |
| | Se | 0.002 | 0.005 | 0.006 | 0.004 |
| Protein (%) | LSM | $3.21^{a}$ | $3.25^{a}$ | $3.27^{b}$ | 3.24 |
| | Se | 0.001 | 0.002 | 0.002 | 0.002 |
| Lactose (%) | LSM | $4.83^{a}$ | $4.75^{a}$ | $4.66^{b}$ | 4.73 |
| | Se | 0.002 | 0.004 | 0.003 | 0.003 |
| Dry matter (%) | LSM | 12.80 | 12.77 | 12.78 | 12.78 |
| | Se | 0.009 | 0.018 | 0.017 | 0.015 |

$A, B, C - p \leq 0.01$; $a, b, c - p \leq 0.05$

Cows in the largest herds (>50 head) were characterized by the highest productivity, manifested in milk yield per cow of 7242 kg in 305-day lactation and the highest protein-to-fat ratio. The lowest values of the above parameters were noted in the smallest herds. The differences between the groups were validated by a statistical analysis ($p \leq 0.01$).

BOJARSZCZUK and KSIĘŻAK (2008) studied diary farms in the Province of Lublin and found that an increase in herd size was followed by an improvement in productivity. Milk yield in the smallest herds (less than 20 head) was by approximately 64% lower than in the largest herds (above 41 head). The cited authors pointed to significant difference in milk production levels between the smallest-scale and largest-scale cattle farms. OLEGGINI et al. (2001), who studied Holstein-Friesian cattle herds in the eastern part of the USA (the Dairy Belt), demonstrated that there were no statistically significant differences in milk yield between herds of 20–49 head and herds of 50–99 head. A significant difference ($p \leq 0.05$) of 1515.63 kg was noted between the smallest and the largest herds (more than 450 head). Similar trends were observed with respect to the yield of milk fat and milk protein – the differences between the smallest and the largest herds were statistically significant ($p \leq 0.05$) and reached 63.27 kg and 54.70 kg respectively.

As regards milk composition, cows in the smallest herds produced milk with the highest fat content (4.16%) and the lowest protein content (3.21%). The values recorded by the Polish Federation of Cattle Breeders and Dairy Farmers in the Province of Warmia and Mazury in 2008 (*Ocena i hodowla...* 2009) were higher, at 4.22% fat and 3.33% protein.

BRZOZOWSKI (1999) reported that cows in the smallest herds produced milk with a higher content of fat and protein, while according to LITWIŃCZUK et al. (1994) herd size had no significant effect on the chemical composition of milk in individual farms in the Province of Lublin. In a study by OLEGGINI et al. (2001), the protein-to-fat ratio in milk was not affected by herd size, but it was substantially higher (0.883) than in the present experiment.

The average lactose content of milk was lowest in the largest herds (4.66%) and highest (4.83%) in the smallest herds. In a study by STANEK et al. (2004), the average lactose content of milk in herds comprising around 30 cows was 4.76%, and it was comparable with the value noted in our study in herds of 21–50 cows (4.75%).

Table 2 shows the effect of herd size on milk yield and composition in successive 305-day lactations. In every lactation, cows in the smallest herds (≤20 head) were characterized by the lowest productivity. Milk production levels increased in consecutive lactations, ranging from 5363 kg in the first lactation to 6869 kg in the fourth and subsequent lactations. Similar values were noted in herds of 21–50 cows, where milk yield ranged from 5935 kg to 7352 kg. Such a tendency was not observed in the largest herds (>50 head). In this group, primiparous cows were marked by the

Table 2

The effect of herd size on milk yield and composition in lactations of normal length

| Parameter | Statistical measure | 1st lactation | | | 2nd lactation | | | 3rd lactation | | | 4th lactation and subsequent lactations | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Groups of cows | | | | | | | | | | | |
| | | group I ≤ 20 | group II 21–50 | group III > 50 | group I ≤ 20 | group II 21–50 | group III > 50 | group I ≤ 20 | group II 21–50 | group III > 50 | group I ≤ 20 | group II 21–50 | group III > 50 |
| Number of cows | N | 15860 | 4171 | 4903 | 12276 | 2805 | 2995 | 7357 | 1439 | 1088 | 6097 | 1058 | 596 |
| Milk (kg) | LSM | $5653^{A}$ | $5935^{B}$ | $6706^{C}$ | $6333^{D}$ | $6768^{C}$ | $7882^{E}$ | $6693^{C}$ | $7179^{F}$ | $7430^{G}$ | $6869^{C}$ | $7352^{F}$ | $7711^{E}$ |
| | Se | 995.52 | 1124.95 | 1523.97 | 1284.57 | 1448.42 | 1994.82 | 1387.71 | 1625.5 | 1670.58 | 1423.73 | 1727.43 | 1782.8 |
| FCM (kg) | LSM | 5729A | 5988B | 6746C | 6523D | 6870C | 7894E | 6834C | 7308F | 7575F | 7096C | 7606F | 7873E |
| | Se | 895.50 | 1223.90 | 1728.07 | 1386.52 | 1848.32 | 2094.12 | 1285.21 | 1329.54 | 1276.50 | 1482.74 | 1925.42 | 1684.81 |
| ECM (kg) | LSM | $5611^{A}$ | $5915^{B}$ | $6662^{C}$ | $6420^{D}$ | $6799^{E}$ | $7808^{F}$ | $6700^{F}$ | $7219^{G}$ | $7457^{H}$ | $6959^{E}$ | $7486^{H}$ | $7731^{F}$ |
| | Se | 1033.72 | 1141.11 | 1469.84 | 1325.73 | 1510.05 | 1864.74 | 1446.72 | 1703.23 | 1712.01 | 1470.8 | 1819.98 | 1733.12 |
| Fat (kg) | LSM | $231^{a}$ | $241^{b}$ | $271^{c}$ | $266^{c}$ | $277^{c}$ | $320^{d}$ | $277^{c}$ | $296^{d}$ | $307^{d}$ | $290^{d}$ | $311^{d}$ | $319^{d}$ |
| | Se | 48.42 | 51.27 | 61.57 | 61.75 | 69.28 | 76.51 | 68.41 | 77.18 | 78.56 | 69.35 | 83.85 | 76.07 |
| Protein (kg) | LSM | $179^{a}$ | $194^{b}$ | $219^{c}$ | $207^{c}$ | $224^{c}$ | $258^{d}$ | $214^{c}$ | $236^{d}$ | $241^{d}$ | $222^{c}$ | $241^{d}$ | $248^{d}$ |
| | Se | 34.32 | 39.22 | 53.85 | 43.21 | 49.5 | 68.25 | 46.27 | 55.17 | 56.6 | 47.5 | 58.77 | 57.9 |
| Lactose (kg) | LSM | $267^{a}$ | $273^{a}$ | $328^{b}$ | $304^{c}$ | $321^{b}$ | $364^{d}$ | $326^{b}$ | $341^{d}$ | $349^{d}$ | $330^{b}$ | $347^{d}$ | $361^{d}$ |
| | Se | 54.6 | 57.72 | 66.09 | 64.48 | 72.63 | 94.03 | 68.12 | 78.01 | 77.01 | 70.22 | 76.24 | 86.57 |
| Dry matter (kg) | LSM | $717^{a}$ | $750^{b}$ | $864^{c}$ | $821^{d}$ | $869^{c}$ | $1003^{e}$ | $861^{c}$ | $922^{c}$ | $950^{c}$ | $886^{c}$ | $942^{c}$ | $988^{c}$ |
| | Se | 144.15 | 149.24 | 170.27 | 169.96 | 190.73 | 230.27 | 184.3 | 210.99 | 215.63 | 189.84 | 206.67 | 225.55 |
| Fat (%) | LSM | $4.09^{a}$ | $4.06^{a}$ | $4.04^{a}$ | $4.20^{b}$ | $4.10^{b}$ | $4.06^{a}$ | $4.14^{b}$ | $4.12^{b}$ | $4.13^{b}$ | $4.22^{b}$ | $4.23^{b}$ | $4.14^{a}$ |
| | Se | 0.46 | 0.48 | 0.56 | 0.49 | 0.52 | 0.58 | 0.5 | 0.48 | 0.54 | 0.49 | 0.47 | 0.522 |
| Protein (%) | LSM | $3.17^{a}$ | $3.27^{b}$ | $3.26^{b}$ | $3.27^{b}$ | $3.31^{b}$ | $3.27^{b}$ | $3.20^{a}$ | $3.29^{b}$ | $3.25^{b}$ | $3.23^{b}$ | $3.28^{b}$ | $3.32^{c}$ |
| | Se | 0.21 | 0.21 | 0.23 | 0.23 | 0.24 | 0.23 | 0.23 | 0.23 | 0.25 | 0.22 | 0.23 | 0.249 |
| Lactose (%) | LSM | $4.72^{a}$ | $4.76^{a}$ | $4.69^{b}$ | $4.80^{b}$ | $4.75^{a}$ | $4.62^{a}$ | $4.83^{b}$ | $4.75^{a}$ | $4.65^{a}$ | $4.80^{b}$ | $4.75^{a}$ | $4.68^{a}$ |
| | Se | 0.14 | 0.12 | 0.12 | 0.017 | 0.14 | 0.14 | 0.15 | 0.014 | 0.16 | 0.017 | 0.15 | 0.145 |
| Dry matter (%) | LSM | $12.68^{a}$ | $12.63^{a}$ | $12.89^{b}$ | $12.97^{c}$ | $12.84^{b}$ | $12.72^{a}$ | $12.87^{b}$ | $12.84^{b}$ | $12.78^{b}$ | $12.90^{b}$ | $12.81^{b}$ | $12.81^{b}$ |
| | Se | 0.66 | 0.65 | 0.75 | 0.75 | 0.73 | 0.78 | 0.71 | 0.74 | 0.86 | 0.72 | 0.66 | 0.723 |

$A\ldots - p \leq 0.01$; $a\ldots - p \leq 0.05$

lowest productivity (6706 kg milk), the highest milk production levels were noted in cows in their second lactation (7882 kg), followed by a drop in milk yield in subsequent lactations. An increase in the yield of milk and milk components from the first to third lactation was also reported by KAMIENIECKI et al. 2001a, MICIŃSKI (2009) and MICIŃSKI et al. (2009).

The above differences became more pronounced when the amount of produced milk was converted to FCM. When the percentage concentrations of fat and protein were taken into account and the amount of produced milk was converted to ECM, statistically significant differences were noted between the largest herds and the remaining groups. The investigated cattle populations were characterized by average concentrations of milk components. Herd size had no influence on the fat content of milk, which exceeded 4% in all groups. The lowest fat concentrations were recorded in primiparous cows (4.04–4.09%) and the highest – in the oldest cows (4.14–4.23%). Smaller differences were noted in the protein content of milk, which ranged from 3.2% to 3.3%. KUCZAJ (2002) studied the effect of breed and lactation on selected performance traits of dairy cows and reported a similar protein content of milk and a higher fat content (over 4.3%). Higher milk production levels in older cows, in comparison with primiparas, were also reported by GULIŃSKI and MŁYNEK (2003).

Cows in their second lactation, kept in the largest herds (>50 head), were characterized by the highest yield of milk, fat, protein, lactose and dry matter. The lowest values of the above parameters were noted in primiparous cows kept in the smallest herds (≤ 20 head). The observed differences were statistically significant ($p \leq 0.01$).

Table 3 illustrates the effect of herd size and inter-calving interval (ICI) duration on milk yield and composition in full lactations. Prolonged ICI resulted in an increase in milk yield in all groups. This is consistent with the findings of BERTILSSON et al. (1997) who observed the highest milk production levels in cows with the longest ICI, and the lowest – in cows with the shortest ICI.

In the present study, the highest milk yield was recorded in the largest herds (> 50 head). Differences between those herds and smaller ones were statistically significant ($p \leq 0.01$). A rise in milk production per cow along with an increase in herd size was also reported by ALLORE et al. (1997, SMITH et al. (2000), KUCZAJ and BLICHARSKI (2005).

The highest milk yield was observed in cows with the longest ICI (over 525 days), kept in the largest herds (> 50 head). The production results of cows in this group were as follows: 11 010 kg milk, 11 225 kg FCM, 11 008 kg ECM, 455 kg fat, 352 kg protein, 517 kg lactose and 1402 kg dry matter. The above results differ significantly ($p \leq 0.01$) from the average values noted in the remaining groups. GULIŃSKI et al. (1996) also found that herd size considerably affected the correlation between ICI length and FCM yield. An increase in herd size, productivity and the age of animals caused a rise in milk yield related to extended ICI.

Table 3

The effect of herd size and inter-calving interval (ICI) duration on milk yield and composition in full lactations

| Parameter | | ICI < 365 days | | | ICI 405–445 days | | | ICI > 525 days | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | Groups of cows | | | | | | | | |
| | | group I (≤ 20) | group II (21–50) | group III (> 50) | group I (≤ 20) | group II (21–50) | group III (> 50) | group I (≤ 20) | group II (21–50) | group III (> 50) |
| Milk (kg) | LSM | $6446^{A}$ | $6668^{B}$ | $7452^{C}$ | $7283^{D}$ | $7501^{C}$ | $8531^{E}$ | $9493^{F}$ | $10182^{G}$ | $11010^{H}$ |
| | Se | 1045.18 | 1207.59 | 1563.03 | 1433.72 | 1569.13 | 2032.8 | 2366.29 | 2765.96 | 3084.72 |
| FCM (kg) | LSM | 6630A | 6778A | 7631B | 7425C | 7659D | 8672E | 9821F | 10503G | 11225H |
| | Se | 1083.05 | 1254.12 | 1473.07 | 1483.57 | 1605.48 | 1931.46 | 2499.08 | 2818.07 | 3056.13 |
| ECM (kg) | LSM | $6492^{A}$ | $6650^{A}$ | $7489^{B}$ | $7338^{C}$ | $7549^{D}$ | $8528^{E}$ | $9614^{F}$ | $10342^{G}$ | $11008^{H}$ |
| | Se | 1083.05 | 1254.12 | 1473.07 | 1483.57 | 1605.48 | 1931.46 | 2499.08 | 2818.07 | 3056.13 |
| Fat (kg) | LSM | $270^{A}$ | $274^{A}$ | $310^{B}$ | $301^{B}$ | $311^{B}$ | $351^{C}$ | $402^{D}$ | $429^{E}$ | $455^{F}$ |
| | Se | 51.18 | 57.76 | 60.81 | 68.58 | 70.3 | 78.48 | 111.57 | 119.83 | 121.88 |
| Protein (kg) | LSM | $206^{A}$ | $213^{A}$ | $240^{B}$ | $240^{B}$ | $245^{B}$ | $276^{C}$ | $305^{D}$ | $334^{E}$ | $352^{E}$ |
| | Se | 36.8 | 41.56 | 54.87 | 50.55 | 54.56 | 70.88 | 87.75 | 98.07 | 111.09 |
| Lactose (kg) | LSM | $304^{A}$ | $307^{A}$ | $364^{B}$ | $350^{B}$ | $356^{B}$ | $394^{C}$ | $462^{D}$ | $484^{E}$ | $517^{F}$ |
| | Se | 330.78 | 352.28 | 421.87 | 465.1 | 393.17 | 472.44 | 409.3 | 444.35 | 490.16 |
| Dry matter (kg) | LSM | $831^{A}$ | $848^{A}$ | $952^{B}$ | $942^{B}$ | $961^{B}$ | $1095^{C}$ | $1228^{D}$ | $1312^{E}$ | $1402^{F}$ |
| | Se | 191.67 | 215.19 | 239.67 | 245.73 | 274.52 | 329.68 | 272.47 | 304.92 | 237.37 |
| Fat (%) | LSM | $4.19^{a}$ | $4.11^{b}$ | $4.16^{c}$ | $4.13^{b}$ | $4.14^{b}$ | $4.11^{b}$ | $4.23^{d}$ | $4.21^{d}$ | $4.13^{b}$ |
| | Se | 0.37 | 0.39 | 0.48 | 0.39 | 0.4 | 0.45 | 0.42 | 0.4 | 0.48 |
| Protein (%) | LSM | $3.20^{a}$ | $3.20^{a}$ | $3.22^{a}$ | $3.30^{b}$ | $3.27^{b}$ | $3.23^{a}$ | $3.21^{a}$ | $3.28^{b}$ | $3.20^{a}$ |
| | Se | 0.19 | 0.20 | 0.21 | 0.2 | 0.19 | 0.2 | 0.22 | 0.2 | 0.21 |
| Lactose (%) | LSM | $4.72^{a}$ | $4.75^{a}$ | $4.89^{b}$ | $4.80^{b}$ | $4.75^{a}$ | $4.62^{a}$ | $4.87^{b}$ | $4.75^{b}$ | $4.70^{a}$ |
| | Se | 0.14 | 0.12 | 0.12 | 0.02 | 0.14 | 0.14 | 0.15 | 0.01 | 0.16 |
| Dry matter (%) | LSM | 12.89 | $12.71^{a}$ | $12.78^{a}$ | $12.93^{b}$ | $12.81^{a}$ | $12.84^{a}$ | $12.94^{b}$ | $12.89^{b}$ | $12.73^{a}$ |
| | Se | 0.73 | 0.71 | 0.76 | 0.73 | 0.63 | 0.76 | 0.71 | 0.68 | 0.86 |

$A, B - p \leq 0.01$; $a, b - p \leq 0.05$

The relationship between herd size and milk yield, as dependent on the number of days from calving to effective insemination, was also investigated by DOMECQ et al. (1997). The cited authors demonstrated that in large herds characterized by high milk production the effectiveness of the first insemination was comparable in primiparous and multiparous cows (47% and 46% respectively), but a tendency towards extended inter-pregnancy intervals was observed in large herds.

The productivity of high-yielding cows was also studied by SAWA et al. (2004) who reported that milk yield related to ICI duration ranged from 9 941 kg to 11 552 kg. According to the above authors, even if the rest period is limited to 60 days, it is impossible to maintain 12-month ICI in cows producing more than 10 000 kg milk over lactation because insemination effectiveness in such cows is considerably reduced, which extends ICI. In a study by STRZAŁKOWSKA et al. (2004), milk yield in 305-day lactation ranged from 8963 kg in cows with 378-day ICI to 9376 kg in cows with 397-day ICI. ICI longer than 411 days resulted in a drop in milk production to 8855 kg.

The effect of herd size on selected performance parameters of cows is presented in Table 4. The largest herds (> 50 head) were characterized by the highest milk production per cow in all lactations (Table 2) and by the lowest lifetime productivity of cows, at 17 185 kg milk. This resulted from

Table 4

The effect of herd size on selected performance parameters of cows

| Parameter | Statistical measure | Groups of cows | | | |
|---|---|---|---|---|---|
| | | group I ≤ 20 | group II 21–50 | group III > 50 | Mean |
| Number of lactations | LSM | $2.67^{A}$ | $2.62^{A}$ | $2.55^{B}$ | 2.61 |
| | Se | 0.092 | 0.093 | 0.092 | 0.092 |
| Herdlife (years) | LSM | $3.44^{A}$ | $3.45^{A}$ | $3.31^{B}$ | 3.40 |
| | Se | 37.49 | 37.73 | 37.50 | 37.57 |
| Lifespan (years) | LSM | $5.67^{A}$ | $5.66^{A}$ | $5.59^{B}$ | 5.64 |
| | Se | 37.70 | 37.93 | 37.70 | 37.78 |
| Milk (kg) | LSM | $19809^{A}$ | $19023^{B}$ | $17185^{C}$ | 18672 |
| | Se | 120.50 | 240.09 | 191.90 | 184.16 |
| FCM (kg) | LSM | $20434^{A}$ | $19624^{B}$ | $17539^{C}$ | 19199 |
| | Se | 141.81 | 285.48 | 227.47 | 218.25 |
| Milk yield (kg) per day of cows' life | LSM | $9.56^{Aa}$ | $9.20^{ab}$ | $8.41^{Ab}$ | 9.06 |
| | Se | 0.22 | 0.22 | 0.22 | 0.22 |
| Milk yield (kg) per day of cows' productive life | LSM | $15.75^{AB}$ | $15.13^{AC}$ | $14.20^{BC}$ | 15.02 |
| | Se | 0.30 | 0.31 | 0.30 | 0.30 |
| Protein-to-fat ratio | LSM | $0.771^{A}$ | $0.794^{B}$ | $0.793^{B}$ | 0.786 |
| | Se | 0.012 | 0.012 | 0.012 | 0.012 |

$A, B, C - p \leq 0.01$; $a, b, c - p \leq 0.05$

the shortest productive life of cows and the lowest number of lactations (2.55). The highest lifetime productivity, reaching 19 809 kg milk and 20 434 kg FCM, was noted in the smallest herds.

KAMIENIECKI et al. (2001b) analyzed the effect of milking method and herd size on the performance parameters of dairy cattle and found that cows kept in the smallest herds had the longest average productive life. SAWA and BOGUCKI (2002) observed an increase in daily milk yield and a decrease in milk quality along with an increase in herd size. In their study, cows in herds comprising 10.1–20.0 head showed the lowest productivity, while the highest milk yield was reported for cows in the largest herds (100.1–200.0 head), and the noted differences were statistically significant.

## Conclusions

The results of this study, which investigated the effect of herd size on milk yield in active cattle populations kept in farms in the region of Warmia and Mazury in the years 1997–2006, show that:

1. The average yield over 305-day lactations was 6579 kg milk (6723 kg FCM), 273 kg fat (4.15%), 213 kg protein (3.24%), 309 kg lactose (4.70%) and 841 kg dry matter (12.78%).

2. Cows in the largest herds (>50 head) were characterized by the highest productivity, and cows in the smallest herds (≤ 20 head) – by the lowest. The latter produced milk with the highest fat content (4.16%) and the lowest protein content (3.21%).

3. In herds comprising more than 50 animals, cows with the longest ICI (>525 days) were marked by the highest milk production in full lactations (11 010 kg).

4. As regards lifetime productivity, the highest values were noted in cows used for 3.44 years in the smallest herds (19 809 kg milk). In the largest herds cows were used for the shortest period of time (3.31 years), and their lifetime productivity reached 17 185 kg milk.

Translated by Aleksandra Poprawska Accepted for print 23.02.2010

## References

ALLORE H.G., OLTENACU P.A., ERB H.N. 1997. *Effects of season, herd size, and geographic region on the composition and quality of milk in the northeast*. Journal Dairy Science, 80: 3040–3049.

ARBEL R., BIGUN Y., EZRA E., SZTURMAN H., HOJMAN D. 2001. *The effect of extended calving intervals in high lactating cows on milk production and profitability*. Journal of Dairy Science, 84: 600–608.

BERTILSSON J., BERGLUND B., RATNAYAKE G., SVENNERSTEN-SJAUNJA K., WIKTORSSON H. 1997. Optimising lactation cycles for the high-yielding dairy cow. A European perspective. Livestock Production Science, 50: 5–13.

Bojarszczuk J., Księżak J. 2008. *Wydajność mleczna krów w zależności od wielkości stada w wybranych rejonach woj. Lubelskiego.* Roczniki Naukowe Stowarzyszenia Ekonomistów Rolnictwa i Agrobiznesu, t. XI(1): 28–32.

Brzozowski P., Ludwiczuk K., Zdziarski K. 1999. *Liczba komórek somatycznych w mleku krów objętych oceną użytkowości mlecznej w Polsce Centralnej*. Zeszyty Naukowe Przeglądu Hodowlanego, 44: 83–89.

Domecq J.J., Skidmore A.L., Lloyd J.W., Kaneene J.B. 1997. *Relationship between body condition scores and conception at first artificial insemination in large dairy herd of high yielding Holstein cows*. Journal Dairy Science, 80: 113–120.

Ely L.O., Smith J.W., Oleggini G.H. 2003. *Regional production differences*. Journal Dairy Sciences (suppl.): E28–E34.

Guliński P., Litwińczuk Z., Młynek K. 1996. *Wpływ wybranych czynników genetycznych i środowiskowych na związek pomiędzy długością okresu międzywycieleniowego a użytkowością mleczną krów*. Roczniki Naukowe Zootechniki, t. 23(4): 9–19.

Guliński P., Młynek K. 2003. *Próba określenia czynników warunkujących produkcję mleka w przebiegu laktacji u krów*. Zeszyty Naukowe Przeglądu Hodowlanego, 68(1): 263–272.

Jurczak M. 1999. *Informacje ogólne o mleczarstwie. Mleko produkcja, badanie, przerób*. Wydawnictwo SGGW, Warszawa.

Kamieniecki H., Czerniawska-Piątkowska E., Sablik P., Wójcik. 2001a. *Badania nad produkcyjnością mleczną i rozrodczą w stadzie krów holsztyńsko-fryzyjskich importowanych z Niemiec jako jałowice cielne*. Zeszyty Naukowe Przeglądu Hodowlanego PTZ, 59: 145–151.

Kamieniecki K., Tietze M., Gnyp J., Pypeć M. 2001b. *Jakość mleka w zależności od metody doju i liczby krów w gospodarstwach sektora drobnotowarowego*. Zeszyty Naukowe PTZ, Warszawa, 59, 159–164.

Kuczaj M. 2002. *Wpływ rasy i laktacji krów mlecznych na wybrane cechy mleka*. Med. Wet., 58(8) 628–631.

Kuczaj M., Blicharski P. 2005. *Porównanie wydajności mlecznej krów rasy czarno- i czerwono-białej utrzymywanych w tych samych warunkach środowiskowych*. Med. Wet., 61(3): 293–296.

Litwińczuk A., Zamielska I., Gnyp. J., Tietze M. 1994. *Jakość mleka dostarczonego do punktu skupu z gospodarstw indywidualnych z uwzględnieniem liczby utrzymywanych krów*. Zeszyty Naukowe Przeglądu Hodowlanego, 14: 175–181.

Melendez P., Pinedo P. 2006. *The association between reproductive performance and milk yield in Chilean Holstein cattle*. Journal of Dairy Science, 90: 184–192.

Miciński J. 2009. *Cechy mleczności i wskaźniki reprodukcji wysoko wydajnych krów w standardowym oraz przedłużonym cyklu produkcyjnym*. Rozprawy i monografie. Wydawnictwo UWM w Olsztynie.

Miciński J., Pogorzelska J., Barański W., Kalicka B. 2009. *Effect of disease incidence on the milk performance of high-yielding cows in successive lactations*. Pol. J. Natur. Sci., 24(2): 102–112.

*Ocena i hodowla bydła mlecznego. Dane za rok 2008*. Polska Federacja Hodowców Bydła i Producentów Mleka, Warszawa.

Oleggini G.H., Ely L.O., Smith J.W. 2001. *Effect of region and herd size on dairy herd performance parameters*. Journal of Dairy Science, 84: 1044–1050.

Ostoja-Solecki J. 2002. *Szacunek możliwości produkcji mleka odpowiadającego unijnym wymogom jakościowym na podstawie liczebności stada i struktury chowu krów w Polsce.* Biuletyn Informacyjny IZ, r. XL. (2): 53–59.

*Rocznik statystyczny rolnictwa i obszarów wiejskich*. 2008. Główny Urząd Statystyczny, Warszawa.

Sawa A., Bogucki M. 2002. *Genetyczne i środowiskowe uwarunkowania wydajności dobowej i jakości mleka*. Acta Sci. Pol., ser. Zootechnika, 1(1–2): 129–138.

Sawa A., Jankowska M., Ziemiński M., Krężel S. 2004. *Okres spoczynku rozrodczego a efektywność użytkowania krów wysoko wydajnych*. Zeszyty Naukowe Przeglądu Hodowlanego, 72(1): 121–128.

Smith J.W., Ely L.O., Chapa A.M. 2000. *Effect of region, herd size, and milk production on reasons cows leave the herd*. Journal of Dairy Science, 83: 2980–2987.

Stanek P., Litwińczuk Z., Teter U., Jankowski P. 2004. *Skład chemiczny i jakość cytologiczna mleka krów czarno-białych utrzymywanych w gospodarstwach farmerskich Lubelszczyzny, z uwzględnieniem pory roku i ich dziennej produkcyjności*. Zeszyty Naukowe Przeglądu Hodowlanego, 72(1): 153–159.

Strzałkowska N., Krzyżewski J., Reklewski Z., Dymnicki E. 2004. *Zależność między wymuszonym wydłużeniem okresu międzyciążowego a wybranymi wskaźnikami reprodukcji i skorygowaną wydajnością mleczną krów*. Med. Wet., 60(12): 1312–1316.

POLISH JOURNAL OF NATURAL SCIENCES
Abbrev.: Pol. J. Natur. Sc., Vol 25(2): 143–153, Y. 2010

DOI 10.2478/v10020-010-0012-z

# HATCHERY WASTE AND HATCHABILITY OF TURKEY EGGS

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Key words: turkeys, age, embryo mortality, hatchability.

A b s t r a c t

The objective of this study was to determine the type and quantity of hatchery waste and egg hatchability during the first laying season of heavy-type turkey hens.

The hatchability of 4536 eggs as well as the weight, type and quantity of waste material produced during nine control incubation cycles were monitored in turkey hens aged from 33 to 57 weeks, at three-week intervals. The day of embryo death was indicated and the percentage of embryos that died on successive days and during three incubation stages was calculated.

The optimum egg weight was recorded in hens aged up to 45 weeks, and in older hens the average egg weight was higher than 95 g. Infertile eggs accounted for 2.0 to 8.3% hatchery waste. Embryo mortality reached 4.6 to 17.2% before puncture, and 1.4 to 5.8% after puncture. Loss due to the delay in hatching ranged from 0.8 to 6.1% fertile eggs. The highest number of newly hatched birds classified as unsuitable for rearing was noted in hens aged 57 weeks (5.2%), while in the remaining weeks of the laying period the number of such poults was substantially lower. The first and second mortality peak accounted for 13.0–28.0% and 13.0–23.0% of the total loss during incubation, respectively. Another embryo mortality peak (13%) was observed in turkey hens aged 42 weeks. The highest hatchability rate, at 93.1%, was reported in week 42. Over the period of best hatchability, embryo mortality reached 43%:15%:42% at three consecutive incubation stages. It was found, based on the type and quantity of hatchery waste and hatch rates, that turkey hens aged between 36 and 48 weeks were characterized by the highest reproductive efficiency. The results of this study may be used as reference values while estimating the total loss during incubation due to hen age in heavy-type turkeys.

# ODPAD INKUBACYJNY I ZDOLNOŚĆ WYLĘGOWA JAJ INDYKÓW

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Słowa kluczowe: indyki, wiek, śmiertelność zarodków, wylęgowość.

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*This study was conducted within the framework of research project no. 0104-225

Abstract

Celem pracy była charakterystyka odpadu inkubacyjnego i ocena zdolności wylęgowej jaj indyków typu ciężkiego w pierwszym sezonie nieśnym. Od 33. do 57. tygodnia życia indyków co 3 tygodnie kontrolowano: masę, rodzaj i wielkość odpadu inkubacyjnego i zdolność wylęgową 4536 jaj w 9 kontrolnych lęgach. W odpadzie inkubacyjnym określono dzień śmierci zarodków, a następnie procent zarodków zamarłych w kolejnych dniach i w 3 fazach lęgu.

Do 45. tygodnia życia niosek masa jaj była optymalna, a po tym okresie średnia ich masa przekroczyła 95 g. Odpad inkubacyjny, którym były jaja niezapłodnione, wynosił od 2,0 do 8,3%. Śmiertelność zarodków przed nakluciem osiągała poziom od 4,6 do 17,2%, a po nakluciu od 1,4 do 5,8%. Straty wynikające z opóźnionego wylęgu wynosiły od 0,8 do 6,1% jaj zapłodnionych. Piskląt nienadających się do chowu w 57. tygodniu życia było 5,2%, a w pozostałych tygodniach znacznie mniej. Wielkość pierwszych szczytów śmiertelności zarodków wynosiła od 13,0 do 28,0%, drugich była mniejsza i wahała się od 13,0 do 23,0%. Dodatkowy szczyt śmiertelności zarodków na poziomie 13% stwierdzono w 42. tygodniu życia indyków. Najwyższą zdolność wylęgową (93,1%) odnotowano w 42. tygodniu życia indyków. W tym okresie śmiertelność zarodków w kolejnych trzech fazach lęgu wynosiła 43% : 15% : 42%.

Rodzaj oraz wielkość odpadu inkubacyjnego i wyniki wylęgu wskazały najlepszy okres do reprodukcji między 36. a 48. tygodniem życia indyków. Uzyskane wyniki mogą być pomocne do oceny strat inkubacyjnych wynikających z wieku indyków typu ciężkiego.

## Introduction

Inadequate hen rearing conditions, unsuitable pre-incubation treatment and errors in the incubation procedure contribute to the production of hatchery waste. The quantity of waste material may be genetically conditioned, and it is higher in heavy-type than in light-type turkeys. The above is due to high egg weight and a high water content of eggs (JANKOWSKI 1989, FARUGA et al. 1996).

The number of infertile eggs is low in turkeys, but it may reach 10% at the beginning and towards the end of the laying season. Incubation loss caused by the death of turkey embryos may exceed 20% of eggs (GRIMES et al. 2004). Live, unhatched poults may account for 0.4–3.9% of fertile eggs (ORŁOWSKA, MRÓZ 2006). In stockbreeding practice, dead embryos and unhatched poults are regarded as the same type of waste which can hinder a correct evaluation of the incubation process (LERNER et al. 1993, FARUGA et al. 1996, MRÓZ, PUDYSZAK 1997). Hatched birds classified as unsuitable for rearing account for around 4% of fertile eggs (BORZEMSKA 1978, ORŁOWSKA, MRÓZ 2006).

It has been found that the quantity of one type of hatchery waste determines the quantity of other types of waste. In a study carried out by MRÓZ and FARUGA (2000), the coefficients of correlation between the percentage of infertile eggs, the percentage of early-dead and late-dead embryos were negative and statistically significant ($-0.150^{xx}$ to $-0.101^{xx}$). They were higher in eggs with morphological defects than in eggs with normal structure. Significant correlation coefficients were observed between the percentage of early-dead and late-dead embryos ($-0.198^{xx}$

to 0.268[xx]). Embryo mortality between the first and the second incubation stage is characterized by high coefficients of correlation also in laying hens at $r = 0.450^{X}$ (KUURMAN et al. 2002). According to a different study, the total quantity of hatchery waste in broilers is determined by the duration of the second mortality peak (JASSIM et al. 1996).

The number of dead embryos was higher in paternal strains than in maternal strains, by 3.31% at the first incubation stage and by 4.28% at the second and third stage (MRÓZ 1998). Under adequate incubation conditions, embryo mortality rates were similar in the first and third incubation stage, while significantly lower mortality rates were noted at stage two (KOSOWSKA 1989, MRÓZ et al. 2002, 2006).

Hatchery waste is evaluated when hatching results are low, usually at the peak or in the middle of the laying season (MALEC et al. 1996., MRÓZ et al. 2002, 2002a, 2006). An in-depth analysis of hatchery waste at selected times of the laying season of Nicholas turkeys was carried out by GRIMES et al. (2004). The study showed an increase in the quantity of hatchery waste after the 19$^{th}$ week of the laying season. At the first incubation stage of Leghorn hens, KOSOWSKA (1989) determined the highest number of dead embryos in week 2, 3 and 34 of the laying season. As regards embryo mortality rates at the first and third incubation stage, the above author reported similar results in the remaining weeks of the laying season.

Radical irregularities in turkey breeding practice and errors in incubation technique increase the quantity of hatchery waste in the third and fourth week of the incubation period (BAGLEY et al. 1990, FRENCH 1994, MALEC et al. 1996). High egg weight and abnormal egg structure disrupt embryo development also in the period of intensive growth (stage two) and in the perihatching period (stage three) (MRÓZ, PUDYSZAK 1997, APPLEGATE, LILBURN 1999, GRIMES et al. 2004, ORŁOWSKA, MRÓZ 2006, MRÓZ et al. 2007). Embryos that died in eggs with abnormal structure are characterized by developmental dysfunctions, problems with protein utilization, renal hyperemia and a wrong position (FRENCH 1994, MRÓZ 1998, MRÓZ et al. 2002, 2002a, 2007, 2007a).

Optimal egg weight not exceeding 95 g is difficult to achieve in turkey farms after week 20 of the laying season (SIOPES 2007, APPLEGATE, LILBURN 1996, LERNER et al. 1993, FARUGA et al. 1996). For better results, turkey eggs weighing in excess of 100 g are incubated at lower temperatures (FRENCH 1994, 1997).

This study was carried out in response to the absence of documented sources presenting the detailed characteristics of hatchery waste in heavy-type turkeys. The objective of this study was to determine the type and quantity of hatchery waste and egg hatchability during the first laying season of heavy-type turkey hens.

## Materials and Methods

The experimental material consisted of eggs of broad-breasted white turkeys. The studied flock comprised 2020 hens and 150 toms kept and fed in line with the recommendations for heavy-type turkeys. The laying season began when turkey hens reached the age of 33 weeks, and it lasted for 24 weeks. Eggs produced by hens aged 33, 36, 39, 42, 45, 48, 51, 54 and 57 weeks were subjected to nine control incubation cycles. Every incubation cycle comprised 504 randomly selected eggs which were weighed before incubation. A batch of 126 eggs was stored over a period of 4, 5, 6 and 7 days for each incubation cycle. Eggs were incubated in a Petersime incubator. Eggs were candled on the $10^{th}$ day and transferred to hatching trays on the $25^{th}$ day of incubation.

After candling and incubation, unhatched eggs were cracked and the number of infertile eggs, eggs with embryos that died before and after puncture, eggs with live, unhatched poults and the number of hatched birds classified as unsuitable for rearing was determined. The gathered data were expressed in percentage terms: the number of infertile eggs in relation to set eggs, the number of hatched poults classified as unsuitable for rearing in relation to hatched poults, the quantity of the remaining hatchery waste in relation to the number of fertile eggs. The day of death of embryos and unhatched poults was determined using the key for determining the age of turkey embryos (DZIACZKOWSKA, FARUGA 1983). The distribution patterns of embryo mortality were determined in the hatchery waste of each incubation cycle on consecutive days of the incubation period (days 1–28) and at three successive incubation stages (first incubation stage – up to day 10, second stage – between day 11 and 24, third stage – from day 25 to day 28). The distribution patterns of embryo mortality are presented in chart form. The days on which mortality rates exceeded 10% were identified as mortality peaks, and the days on which mortality rates ranged from 5% to 10% were defined as days with elevated mortality. Hatchability was evaluated based on fertilization rates and hatch rates of fertile eggs.

Experimental data were processed by one-way analysis of variance, and it was verified by Duncan's multiple range test. Egg weight, hatch rates and the percentage of infertile eggs were presented using means ($\overline{x}$) and coefficients of variation (v). A small number of birds not suited for rearing was reported, and they were not subjected to a statistical analysis. The remaining hatchery waste characterized by high variation was analyzed using the Kruskal–Wallis test (BOCHNO et al. 2001).

## Results and Discussion

The optimum egg weight was recorded in hens aged up to 45 weeks, and it exceeded 95 g in hens aged 48 weeks and older (Table 1). High egg weight and weight variations in hens aged 57 weeks are indicative of a low biological value of eggs. The problem of high egg weight in turkey hens aged 48 weeks and older has also been noted by other authors (APPLEGATE, LILBURN 1996, FARUGA et al. 1996, GRIMES et al. 2004).

Table 1

Turkey egg weight, g ($\bar{x}$, V%)

| Weeks of age | Eggs weight |
|---|---|
| 33 | $81.9^{a}$<br>6.58 |
| 36 | $89.9^{b}$<br>6.74 |
| 39 | $90.3^{b}$<br>7.14 |
| 42 | $92.3^{b}$<br>6.60 |
| 45 | $94.0^{c}$<br>6.79 |
| 48 | $96.0^{cd}$<br>6.42 |
| 51 | $97.4^{d}$<br>6.80 |
| 54 | $97.9^{d}$<br>6.76 |
| 57 | $101.4^{c}$<br>40.25 |
| 33–57 | 93.5<br>16.87 |

Explanation: values in columns followed by abcd differ at $p \leq 0.05$

Eggs were incubated in accordance with the procedure for this bird species. No technological problems were reported. Five types of hatchery waste were identified in control incubation cycles (Table 2). The percentage of infertile eggs was high only in hens aged 33 and 57 weeks, pointing to an impairment in the turkeys' reproductive ability during that period. Low fertilization rates were also reported by GRIMES et al. (2004) at the initial and final stages of the reproductive period, and by LERNER et al. (1993) at the initial stage of the reproductive period. A decrease in fertilization rates in hens older than 45 weeks justifies the need to improve turkey rearing, management and replacement strategies.

Table 2

Hatchery waste characteristics, % (x, V%)

| Weeks of age | Fertile eggs *n* | Infertile eggs (1) | Dead embryos (2) | | | Unhatched poults (2) | Poults classified as unsuitable for rearing (3) |
|---|---|---|---|---|---|---|---|
| | | | unpunctured eggs | punctured eggs | total | | |
| 33 | 472 | $6.4^{ab}$<br>2.16 | 17.2 | 1.5 | 18.7<br>16.35 | 5.8<br>77.20 | – |
| 36 | 492 | $2.4^{b}$<br>1.02 | 9.6 | 2.4 | 12.0<br>40. 12 | 3.3<br>58.85 | – |
| 39 | 493 | $2.2^{b}$<br>0.99 | 6.3 | 1.4 | 7.7<br>46.76 | 3.7<br>28.22 | 1.4 |
| 42 | 494 | $2.0^{b}$<br>1.40 | 4.6 | 1.4 | 6.1<br>41.94 | 0.8<br>1.23 | 0.6 |
| 45 | 486 | $3.6^{b}$<br>2.00 | 7.2 | 1.6 | 8.8<br>34.69 | 2.3<br>56.58 | 1.2 |
| 48 | 483 | $4.0^{ab}$<br>2.91 | 7.1 | 2.3 | 9.5<br>56.86 | 3.1<br>44.52 | 1.2 |
| 51 | 483 | $4.0^{ab}$<br>1.90 | 12.0 | 2.9 | 14.9<br>41.18 | 4.8<br>28.09 | 3.3 |
| 54 | 487 | $3.2^{b}$<br>1.60 | 8.4 | 3.3 | 11.7<br>22.88 | 4.9<br>34.62 | 0.7 |
| 57 | 461 | $8.3^{a}$<br>3.15 | 9.3 | 5.8 | 15.1<br>26.32 | 6.1<br>34.65 | 5.2 |
| 33–57 | 4355 | 4.0<br>2.80 | 9.1 | 2.5 | 11.6<br>45.56 | 3.9<br>62.60 | 1.5 |

Explanation: values in columns followed by ab differ at $p \leq 0.05$
(1) – % in relation to set eggs
(2) – % in relation to fertile eggs
(3) – % in relation to hatched poult

Dead embryos had the highest share of hatchery waste, and embryo mortality before puncture was higher than after puncture (Table 2). Embryo mortality rates after puncture increased in hens aged 48 weeks and older when egg weight exceeded the optimal values. According to the results of statistical tests, age did not affect the percentage of dead embryos. The highest percentage of unhatched birds was noted in turkey hens aged 33 weeks, 51 weeks and older. In this study, the number of poults that did not hatch by the time indicated in the given incubation technology significantly contributed to the quantity of hatchery waste. The percentage of that waste increased when the weight of hatching eggs exceeded 95 g. The share of birds classified as unsuitable for rearing was low (Table 2), and the physiological threshold, which according to BORZEMSKA (1978, 2005) should not be higher than 4%, was exceeded only in hens aged 57 weeks. Weeks 36 to 48 should be regarded as the optimal reproduction age for hens because this period was characterized by low embryo mortality, a small number of live, unhatched birds and disabled

poults (Table 2). The lowest incubation loss in the above rearing period was also reported by other authors (GRIMES et al. 2004).

The highest embryo mortality rates were noted at the first incubation stage, lower mortality rates were reported at the third stage, while embryo mortality was the lowest in the intensive growth phase during the second incubation stage (Figure 1). The highest number of embryos died at the first incubation stage in eggs laid by young hens (aged 33 weeks) and at the end of the rearing period (51–54 weeks). The distribution patterns of embryo mortality at the incubation stage marked by the highest hatchability rates (42 weeks) was 43%:15%:42% at three successive incubation stages (Figure 1). In the period most conducive to reproduction, embryo mortality rates reached 42–43% at the first incubation stage, 40–43% at the third stage and 15–17% at the second stage. The results noted in previous studies suggest that the distribution pattern of embryo mortality fluctuates in the laying season, and it is affected by the age of the flock (KOSOWSKA 1989, TAYLOR 1999, MRÓZ et al. 2002, 2002a, 2006, 2007).

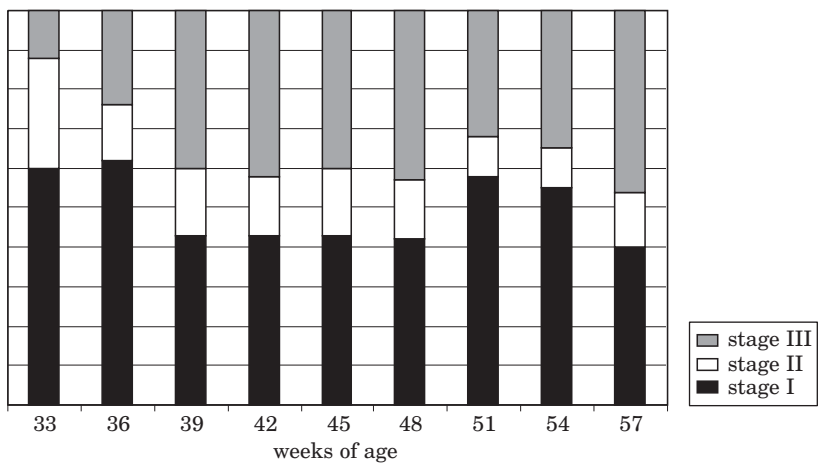


Fig. 1. Distribution patterns of embryo mortality at consecutive incubation stages (%) in relation to all dead embryo

The distribution patterns of embryo mortality on successive incubation days is presented in Figure 2. The first mortality peak was observed up to incubation day 5, and its value ranged from 13% to 28%, subject to hen age. The second, lower mortality peak was noted between incubation day 25 and 27 or on day 28. The mortality peaks reported in this experiment were lower than noted by the authors in their previous studies (MRÓZ et al. 2002). Lower mortality peaks were probably due to advances in incubation technology. An additional mortality peak at 13% dead embryos was reported in hens aged 42 weeks. The laying of eggs without shell pigmentation is intensified in this period, which could explain elevated embryo mortality rates at the second incubation stage (ORŁOWSKA 2007).

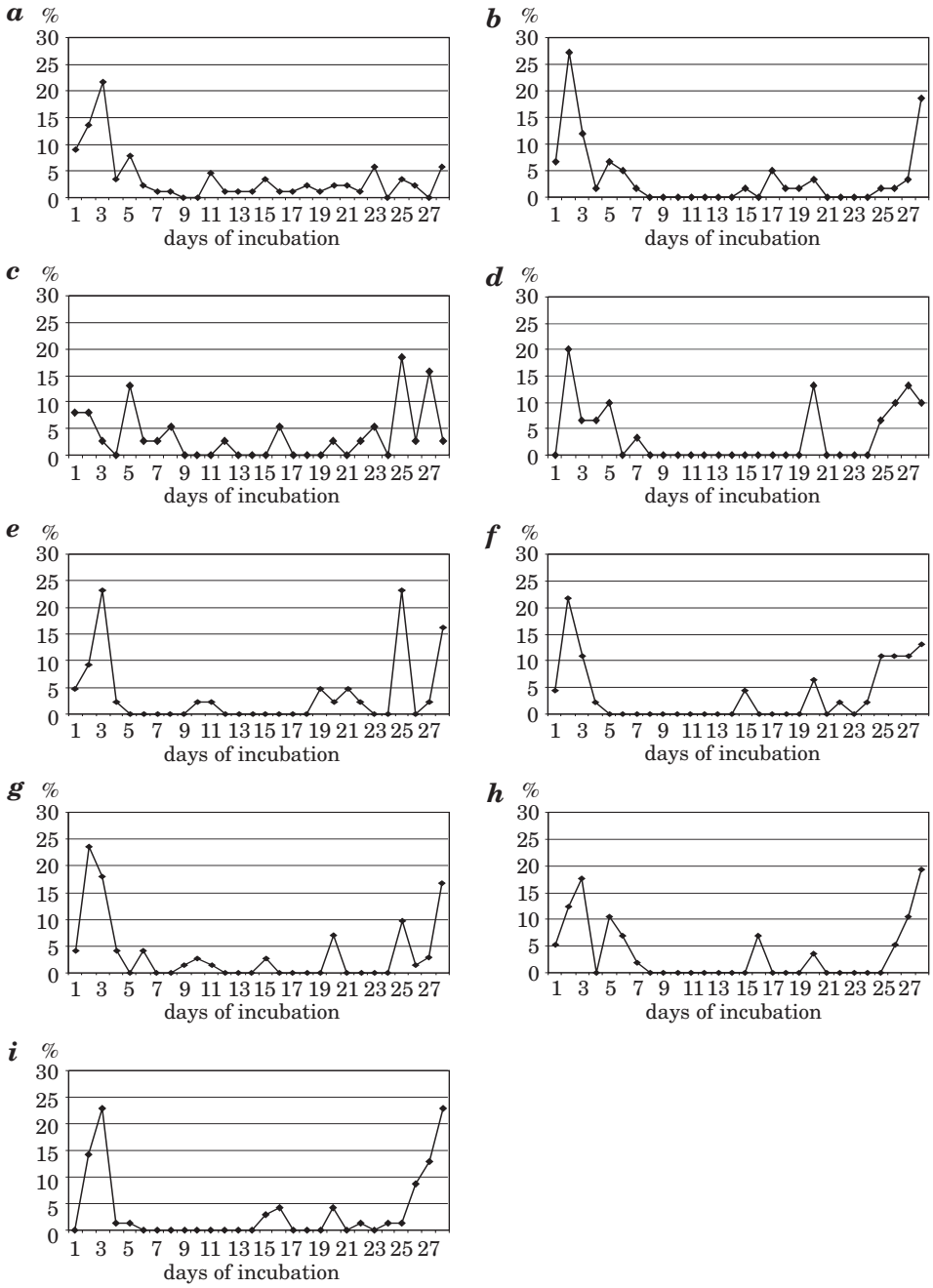


Fig. 2. Distribution patterns of embryo mortality on consecutive incubation days (% in relation to all dead embryos) – weeks of age: a – 33., b – 36., c – 39., d – 42., e – 45., f – 48., g – 51., h – 54., i – 57.

The highest embryo mortality rates were observed during the period of blood island formation, internal and external pipping. An additional mortality peak was noted at the final stage of embryo development on the 20th day of incubation. Low embryo mortality rates persisted for 15 to 21 days during the period of intensive growth (second incubation stage), which is consistent with previous findings (MALEC et al. 1996, MRÓZ 1998, MRÓZ et al. 2002, 2006,2007, GRIMES et al. 2004).

The highest fertilization and hatch rates were reported in hens aged from 36 to 48 weeks (Table 3). The noted values did not differ significantly from the results of a previous study using similar experimental material (ORŁOWSKA, MRÓZ 2006, LERNER et al. 1993). The above authors reported the highest hatch rates in excess of 80% in the second month of the laying season.

Table 3

Selected parameters of egg hatchability,% ($\overline{x}$,V%)

| Weeks of age | Number of set eggs n | Fertilization rates | Hatch rates of fertile eggs |
|---|---|---|---|
| 33 | 504 | $93.7^{ab}$<br>3.85 | $75.5^{a}$<br>7.40 |
| 36 | 504 | $97.6^{b}$<br>0.66 | $84.8^{abc}$<br>7.31 |
| 39 | 504 | $97.8^{b}$<br>1.02 | $88.6^{bc}$<br>3.36 |
| 42 | 504 | $98.0^{b}$<br>0.80 | $93.1^{c}$<br>2.74 |
| 45 | 504 | $96.4^{b}$<br>1.58 | $88.9^{bc}$<br>3.61 |
| 48 | 504 | $96.0^{ab}$<br>2.95 | $87.4^{bc}$<br>5.97 |
| 51 | 504 | $96.0^{ab}$<br>2.02 | $80.3^{abc}$<br>9.20 |
| 54 | 504 | $96.8^{b}$<br>2.00 | $83.4^{abc}$<br>4.83 |
| 57 | 504 | $91.7^{a}$<br>3.21 | $78.8^{ab}$<br>7.32 |
| 33–57 | 4536 | 96.0<br>2.86 | 84.5<br>8.20 |

Explanation: values in columns followed by abcd differ at $p \leq 0.05$

## Conclusions

In this study, hatchery waste consisted of: infertile eggs, eggs with embryos that died before and after puncture, eggs with live, unhatched poults as well as poults classified as unsuitable for rearing which were not determined in young hens.

The type and quantity of hatchery waste and the distribution patterns of embryo mortality at consecutive incubation stages may be used as reference values for estimating the loss during incubation due to hen age in heavy-type turkeys.

The highest hatchability of heavy-type turkeys was noted in hens aged from 36 to 48 weeks.

Translated by Aleksandra Poprawska Accepted for print 2.12.2009

## References

APPLEGATE T.J., LILBURN M.S. 1996. *Independent effects of hen age and egg size on incubation and poult characteristics in commercial turkeys*. Poultry Science, 75: 1210–1216.

APPLEGATE T.J., LILBURN M.S. 1999. *Effect of turkey (Meleagridis gallopavo) breeder hen age and egg size on poult development. 1. Intestinal growth and glucose tolerance of the turkey poult*. Comparative Biochemistry and Physiology, Part B, 124: 371–380.

BAGLEY L.G., CHRISTENSEN V.L., BAGLEY R.A. 1990. *Effect of altering eggshell permeability on the hatchability of turkey eggs incubated at high altitude*. Poultry Science, 69: 451–456.

BOCHNO R., LEWCZUK A., MICHALIK D. 2001. *Biometria stosowana*. UWM Olsztyn.

Borzemska W. 1978. Ważniejsze przyczyny zamierania zarodków indyczych. Med. Wet., 5: 265–267.

BORZEMSKA W.B., 2005. *Patologia lęgów i okresu okołolęgowego*. [W:] Choroby drobiu. Red. M. Mazurkiewicz, 3: 69–119, AR Wrocław.

DZIACZKOWSKA L., FARUGA A. 1983. *Klucz do oznaczania wieku embrionów indyczych*. Maszynopis. COBRD, Poznań.

FARUGA A., PUDYSZAK K., PUCHAJDA H., JANKOWSKI J., KOZŁOWSKI K. 1996. *Charakterystyka jakości jaj w zależności od okresu nieśności i pochodzenia indyków*. Zeszyty Naukowe Przeglądu Hodowlanego, 24: 91–99.

FRENCH N.A.1994. *Effect incubation temperature on the gross pathology of turkey embryos*. British Poultry Science, 35: 363–371.

FRENCH N.A.1997. *Modeling incubation temperature: The effects of incubator design, embryonic development, and egg size*. Poultry Science, 76: 124–133.

GRIMES J.L., NOLL S., BRANNON J., GODWIN J.L., SMITH J.C., ROWLAND R.D. 2004. *Effect of a chelated calcium proteinate dietary supplement on the reproductive performance of Large White Turkey breder hens*. Journal of Applied Poultry Research, 13: 639–649.

JANKOWSKI J.1989. *Reakcja czterech populacji indyków na zastosowane metody hodowlane*. Acta Academiae Agriculturae ac Technicae Olstenensis. Zoot. 31, Supp. F.

JASSIM W.E., GROSSMAN M., KOOPS W.J., LUYKX R.A.J. 1996. *Multiphasic analysis of embryonic mortality in chickens*. Poultry Science, 75: 464–471.

KOSOWSKA G. 1989. *Wzorce strat inkubacyjnych dla kur rasy Leghorn jako kryteria oceny zamarłych zarodków w patologii lęgu*. Acta Agraria et Silvestria, Zoot. 28, 58–70.

KUURMAN W.W., BAILEY B.A., KOOPS W.J., GROSSMAN M. 2002. *Influence of storage days on the distribution for time of embryonic mortality during incubation*. Poultry Science, 81: 1–8.

LERNER S.P., FRENCH N., MCINTYRE D., BAXTER-JONES C. 1993. *Age-related changes in egg production, fertility, embryonic mortality, and hatchability in commercial turkey flocks*. Poultry Science, 72: 1025–1039.

MALEC H., BORZEMSKA W., NIEDZIÓŁKA J. 1996. *Przypadek hipertermii u zarodków indyczych w ostatnim etapie lęgu*. Medycyna Weterynaryjna, 32(10): 645–646.

MRÓZ E., PUDYSZAK K. 1997. *Analiza wyników wylęgowości i jakości jednodniowych indycząt w zależności od wieku indyczek*. Zeszyty Naukowe Przeglądu Hodowlanego, 32: 97–102.

MRÓZ E., 1998. *Studia nad zmiennością cech powierzchni skorupy jaj indyczych i ich związkiem z wylęgowością.* Rozprawy i monografie 4, ART, Olsztyn.

MRÓZ E., FARUGA A. 2000. *Correlations between incubation indices in egggroups with different egg shell and turkey origins*. Natur. Sci., 7: 101–113.

MRÓZ E., PUCHAJDA H., PUDYSZAK K. 2002. *Structure and pigmentation of eggshell and biological value of turkey hatching eggs.* Pol. J. Natur. Sci., 10: 141–152.

MRÓZ E., PUCHAJDA H., MICHALAK K., PUDYSZAK K. 2002a. *Analiza biologiczna wylęgowości jaj indyczych*. Roczniki Naukowe Zootechniki, Sup., 16: 61–66.

MRÓZ E., ORŁOWSKA A., MICHALAK K., REITER K. 2006. *Effect of egg storage time on embryo mortality rates and poult quality*. Pol. J. Natur. Sci., 21: 691–699.

MRÓZ E., MICHALAK K., ORŁOWSKA A. 2007. *Embryo mortality and poult quality depend on the shell structure of turkey hatching eggs*. Animal Science Papers and Reports, 25(3): 161–172.

MRÓZ E., MICHALAK K., ORŁOWSKA A. 2007a. *Hatchability of turkey eggs as dependent on shell ultrastructure*. Pol. J. Natur. Sci., 1(22): 31–42.

ORŁOWSKA A.2007. *Wiek niosek a zdolność wylęgowa jaj i jakość indycząt*. Praca doktorska, UWM. Olsztyn.

ORŁOWSKA A., MRÓZ E. 2006. *Effects of age turkey – hens and egg storage period on embryo mortality and poults quality. XVIII International Poultry Sympsium, Science for poultry practice – poultry practice for science*. PB WPSA, Rogów / Polska, 49–54.

SIOPES T.D. 2007. *Lighting for summer egg production by turkey: day length and light intensity*. Poultry Science, 86: 2413–2419.

TAYLOR G. 1999. *High-yield breeds require special incubation*. World Poultry, 15(3): 27–29.

POLISH JOURNAL OF NATURAL SCIENCES
Abbrev.: Pol. J. Natur. Sc., Vol 25(2): 154–172, Y. 2010

DOI 10.2478/v10020-010-0013-y

# EVALUATION OF DIVERSITY OF SUBMERGED AND EMERGENT FLORA OF LAKE SZELĄG WIELKI AS THREATENED BY A PESTICIDE TOMB*

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K e y w o r d s: species diversity, macrophyte, pesticide tombs, Lake Szeląg Wielki.

A b s t r a c t

This study was aimed at evaluating the diversity of submerged and emerged flora of Lake Szeląg Wielki and at verifying the hypothesis that a pesticide tomb affects the diversity of submerged and emerged flora of that lake.

Investigations carried out in Lake Szeląg Wielki demonstrated a modifying effect of a pesticide tomb on the floral abundance in phytocoenoses of submerged plants: *Potamettum perfoliati, Ceratophylletum demersi, Ranunculetum circinati*; of emerged plants: *Typhetum angustifoliae, Scirpetum lacustris, Phragmitetum, Glycerietum maximae, Equisetetum fluviatile.* Phytocoenoses of *Scirpetum lacustris* detected in Lake Szeląg Wielki were the most susceptible to the activity of a pesticide tomb.

# OCENA RÓŻNORODNOŚCI GATUNKOWEJ ROŚLINNOŚCI JEZIORA SZELĄG WIELKI POD PRESJĄ MOGILNIKA PESTYCYDOWEGO

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S ł o w a k l u c z o w e: różnorodność gatunkowa, makrofity, mogilnik pestycydowy, jezioro Szeląg Wielki.

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*Research granted from scientific sources as a research project in 2006–2009 MNiSW no. N305 006 31/0326

Abstrakt

Badania miały na celu ocenę różnorodności gatunkowej roślin zanurzonych i wynurzonych, obecnych w jeziorze Szeląg Wielki oraz sprawdzenie hipotezy, że mogilnik pestycydowy wpływa na różnorodność makrofitów występujących w jeziorze.

Wykazano modyfikujący wpływ mogilnika na fitocenozy roślinności wodnej: *Potametum perfoliati, Ceratophylletum demersi, Ranunculetum circinati*; na fitocenozy roślinności szuwarowej: *Typhetum angustifoliae, Scirpetum lacustris, Phragmitetum, Glycerietum maximae, Equisetetum fluviatile*. Na oddziaływanie mogilnika pestycydowego najwrażliwsze były fitocenozy *Scirpetum lacustris*.

## Introduction

Numerous hazardous waste stockpiles, called pesticide tombs, were established in Poland in the 1970s. They were intended for storage of preparations containing chlorinated organic insecticides. They were located without conducting a prior geological survey, at random sites, with the facilities built in a negligent manner and without a proper insulation.

Pesticide tombs are one of the most severe hazards to the natural environment in Poland. In Warlity Wielkie near Ostróda a pesticide tomb was in use until November 3, 2003 – being one of the 16 landfill sites of non-utilized pesticides in the province of Warmia and Mazury in the years 1960–1970. The tomb was used as a landfill of 54 tonnes of toxic substances disposed in 36 silos and 2 unprotected pits. Since 2003, complex analyses of edaphic and aquatic habitats have been carried out on that area (SKIBNIEWSKA et al. 2002, SZAREK et al. 2003, GRZYBOWSKI et al. 2004, GRZYBOWSKI et al. 2005, ZMYSŁOWSKA et al. 2005). A leak of hazardous substances from a pesticide tomb was confirmed by methods of chemical analysis when routes of draining of waters from a dumping ground to a lake was being sought (SKIBNIEWSKA et al. 2005). Due to the poor sorptive ability of soils, the levels of the examined substances were low, except for the samples taken from above the impermeable layer. Chemical analysis of the plants used as indicators by other scholars did not provide any information about the environment pollution. Slightly elevated levels of DDT and its metabolites have been found in the tissues of fish from the ponds adjacent to the pesticide tomb as compared to the fish from other ponds (SKIBNIEWSKA et al. 2004). No effect of the tomb on the bacteria count in the area has been proven to exist, although increased counts of actinomycetales and fungi have been found. A macrophytic assessment of the ecological condition of the Lake Szeląg Wielki has shown a lower value of the lake waters than expected after an analysis of the physicochemical variables of the water (GRZYBOWSKI et al. 2005). The pesticide tomb has also been found to affect the floral diversity of the neighbouring forest (GRZYBOWSKI et al. 2004, GRZYBOWSKI et al. 2007). In addition, histopathological examinations have revealed an negative

effect of the tomb on animals: the number and intensity of morphological changes in the internal organs of the examined animals have been found to decrease with the distance from the source of contamination (SZAREK et al. 2007a, SZAREK et al. 2007b, SZAREK et al. 2007c,). The character and distribution of the morphological changes in the examined animals also indicate the toxic effect of the tomb.

This study was aimed at evaluating the diversity of submerged and emerged flora of Lake Szeląg Wielki and at verifying the hypothesis that a pesticide tomb affects the diversity of submerged and emerged flora of that lake.

## Characteristics of the study area

Lake Szeląg Wielki is located in the Ostróda Commune of the Iławski Lake District, belonging to the province of Warmia and Mazury. It water body is a typical ribbon-lake, narrow and very elongated lake, with the maximum length of 12.5 km. It entries three small watercourses, two of which bring water from the neighboring lakes: Szeląg Mały and Tabórz. Runoff from the lake proceeds south-west to Lake Pauzeńskie and Lake Drwęckie. The surface of the lake accounts for 599 ha, its maximum depth is 35.5 m and its mean depth is 13.5 m.

In the direct basin of Lake Szeląg Wielki the following three villages are located: Warlity Wielkie, Zwierzewo and Kątno (Figure 1). The city of Ostróda is located ca. 4 km south-west of the aquifer (Figure 1). Recreational management of the lake is poor; a recreational centre and a camp site are situated on its south-west shore. On the west shore, it is adjoined by a fish farm.

The lake has a poorly developed shoreline. The zone of littoral and sublittoral is narrow. Lake Szeląg Wielki is stratified. During summer stagnation, epilimnion reaches to a depth of 8 m and constitutes 36% of the lake surface area litoral, additionally it is characterized by good aerobic conditions. In the thermocline stratum, there is a distinct decrease of oxygen concentration that intensifies gradually in the stratum of hypolimnion, which constitutes 44% of lake bottom area (CYDZIK et al. 1995, GRZYBOWSKI et al. 2005). Oxygen depletion at the bottom does not occur and the mean oxygen saturation of lake hypolimnion exceeds 25% (CYDZIK et al. 1995). Results of analyses of physico-chemical variables of Lake Szeląg Wielki waters carried out in 2007 indicated moderate trophy (Table 1).

Indices of the primary production, including the concentration of chlorophyll, dry matter of seston and water transparency, indicate its moderate trophy. Bacteriological analyses of the lake demonstrated its very good sanitary conditions. In addition, concentrations of the pesticides

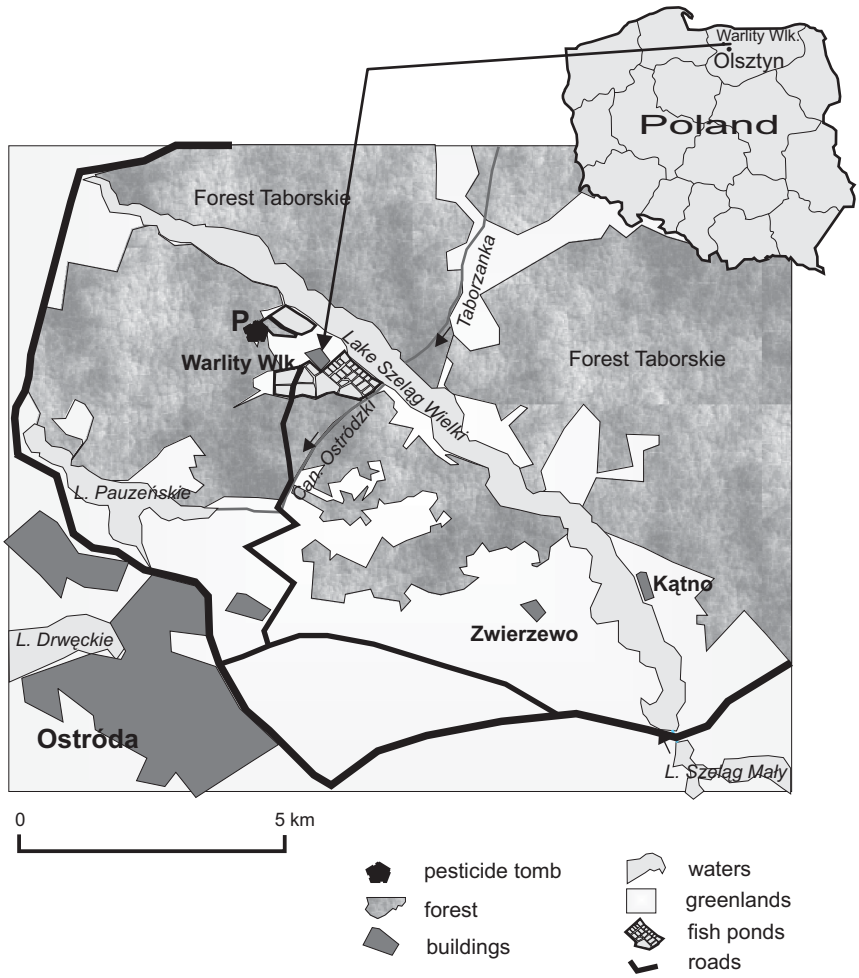


Fig. 1. Location of study area
group 1 – phytocoenosis located at a distance < 2km from pesticide tomb
group 2 – phytocoenosis located at a distance > 2km from pesticide tomb

examined in water were within the binding reference values, yet these were mostly trace amounts (CYDZIK et al. 1995).

Lake Szeląg Wielki is characterized by a high resistance to degradation – I category (CYDZIK et al. 1995). Worthy of notice is the small annual water exchange that prevents the transport of contaminants from the basin area to the lake. The prevalence of forests and relatively low recreational pressure exert an additional beneficial effect on the lake.

Table 1

Physico-chemical variables of Lake Szeląg Wielki

| Physico-chemical variables | Spring | Summer | Autumn |
|---|---|---|---|
| PH | 8.3 | 8.9 | 8.5 |
| Alkalinity, mval $dm^{-3}$ | 2.5 | 2.7 | 2.6 |
| Total hardness, mval $dm^{-3}$ | 2.9 | 2.8 | 3.1 |
| Calcium (Ca), mg $dm^{-3}$ | 44.3 | 41.8 | 45.8 |
| Magnesium (Mg), mg $dm^{-3}$ | 9.03 | 6.2 | 9.50 |
| Chloride (Cl), mg $dm^{-3}$ | 13.4 | 19.6 | 17.5 |
| Oxidizability ($O_2$), mg $dm^{-3}$ | 9.4 | 11.8 | 10.4 |
| Oxygen saturation ($O_2$), % | 121.4 | 115.1 | 146.6 |
| $BOD_5$, mg $dm^{-3}$ | 5.1 | 2.6 | 7.7 |
| Ammonium nitrogen ($N–NH_4$), mg $dm^{-3}$ | 0.86 | 0.05 | 0.02 |
| Nitrite nitrogen ($N–NO_2$), mg $dm^{-3}$ | 0.01 | 0.04 | 0.01 |
| Nitrate nitrogen ($N–NO_3$), mg $dm^{-3}$ | 0.16 | 0.05 | 006 |
| Organic nitrogen ($N_{org.}$), mg $dm^{-3}$ | 0.71 | 1.23 | 1.49 |
| Total nitrogen ($N_{og}$), mg $dm^{-3}$ | 1.74 | 1.37 | 1.58 |
| Phosfphate phosphorus ($P–PO_4$), mg $dm^{-3}$ | 0.07 | 0.04 | 004 |
| Organic phosphorus ($P_{org.}$), mg $dm^{-3}$ | 0.05 | 0.08 | 0.03 |
| Total phosphorus ($P–P_{og.}$), mg $dm^{-3}$ | 0.12 | 0.12 | 0.07 |
| Iron (Fe), mg $dm^{-3}$ | 0.03 | 0.08 | 0.11 |

## Methods

A site survey was conducted in the summer in the years 2006–2007. The ecological characteristics were based on 138 phytosociological relevés taken with the method of BRAUN-BLANQUET (1964).

The nomenclature of vascular plants was adopted after MIREK at al. (2002) and RUTKOWSKI (2005). In the present study, use was made of the division of submerged and emerged plant communities postulated by BRZEG, WOJTERSKA (2001) and MATUSZKIEWICZ (2006).

For a numerical analysis of the phytosociological releves, the quantitative degrees in the BRAUN-BLANQUET (1964) scale were transformed into quantitative degrees on the scale proposed by JANSÉN (1975) following the recommendations of MAAREL VAN DER (1979).

An evaluation of diversity was conducted based on a Shannon-Wiener index (SHANNON, WEAVER 1949). The Shannon-Wiener diversity index ($H$) is defined as a negative sum of a product of probability of subsequent species significance in a set ($p_i$) and a logarithm of that probability.

$$H = -\Sigma p_i \log p_i$$

with 2 adopted as the logarithm base.

The probability of subsequent species significance in the set ($p_i$) is understood as a quotient $n_i/N$, where $n_i$ is a coefficient of significance of a given species, ad $N$ denotes the sum of significance coefficients of all species.

Species distribution in phytocenosis was described by means of a Pielou Evenness index (J) (MAGURRAN 1988). The Pielous Evenness index (J) is defined as a ratio of observed diversity to the maximal diversity at a given number of species $s$:

$$J = \frac{H_{obs}}{H_{\max}}$$

where the maximum diversity $H_{max}$ after transforming the formula into the Shannon-Wiener diversity index:

$$J = \frac{H_{obs}}{\log s}$$

the value of Pielou Evenness reaches maximally 1 when the observed diversity equals the maximum one, namely when all species have equal contribution to the total phytocenosis.

Calculations of diversity indices were carried out using Multi Variate Statistical Package (MVSP) ver. 3.1 software.

To determine statistically significant differences in species abundance, diversity index and the contribution of species to in phytocoenoses between the analyzed communities of submerged and emerged plants, use was made of a parametric Student's t-test (significance level of $\alpha = 0.05$), which assumes a normal distribution of results and differences between results. In the Student's t-test, calculations were performed using Statistica 7.1 software (STATSOFT, Inc. 2005).

The effect of a pesticide tomb on the diversity of submerged and emerged plant communities of Lake Szeląg Wielki was evaluated by comparing the diversity of phytocoenoses noted in the direct proximity of the tomb and phytocenoses of the same communities at least 2 km away from a perpendicular line plotted so as to cut through two points: the closest to the pesticide point of the shoreline of Lake Szeląg Wielki and a point determined by the centre of the tomb. Phytocoenoses of the same plant associations occurring both in the phytolittoral close to the pesticide tomb (< 2 km) and those located far away from it (> 2 km) belonged to the following plant communities noted in Lake Szeląg Wielki: *Ceratophylletum demersi, Potametum perfoliati, Ranunculetum circinati, Equisetetum fluviatile, Glycerietum maximae, Phragmitetum, Scirpetum lacustris, Typhetum angustifoliae.* In those analyses, use was also made of the Student's t-test (significance level of $\alpha = 0.05$) and calculations were made with Statistica 8.0 software (STATSOFT, Inc. 2005).

## Results

The phytosociological analyses carried out in Lake Szeląg Wielki demonstrated the presence of phytocoenoses belonging to: one pleustonic community of the class *Lemnetea minoris* (Table 2), six communities of submerged plants from the class *Potametea* (Table 2), 10 communities of emerged plants from the class *Phragmitetea* (Table 3), and additionally one phytocenosis built of aquatic moss *Fontinalis antipyretica* (Table 2). The maximum depth of the bottom at which the presence of macrophytes was detected reached 3.9 m.

Diversity of phytocoenoses detected in Lake Szeląg Wielki is presented in Table 4.

The Shannon-Wiener diversity index ($H$) was found to be different for submerged and emerged vegetation of Lake Szeląg Wielki ($p = 0.008$), differences ($p < 0.005$) were also observed in the number of species in phytocoenoses (Figure 2). In turn, distribution of species in the communities of submerged and emerged plants, described by means of Pielou Evenness index (J), did not demonstrate important differences ($p = 0.19$) Figure 2.

The Shannon-Wiener diversity index (H) in the community of *Ceratophylletum demersi* was different ($p < 0.003$) for phytocoenoses located in the proximity of the pesticide tomb as compared to those remote from the tomb – being higher in the latter. In addition, an analysis of the number of species in the phytocoenoses examined demonstrated higher value of that index in phytocoenoses located far away from the pesticide tomb ($p < 0.001$) – Figure 3. Differences in species distribution in phytocoenoses of *Ceratophylletum demersi* were not different ($p = 0.18$) – Figure 3.

In the case of the *Potametum perfoliati* community, the Shannon-Wiener diversity index (H) was higher ($p < 0.001$) in the phytocoenoses remote from the tomb in contrast to those located in its vicinity. In addition, the number of species in the analyzed phytocoenoses was higher in those located far away from the tomb ($p = 0.002$) – Figure 4. In species distribution in phytocoenoses of *Potametum perfoliati* ($p = 0.02$) – Figure 4 have not identified any differences.

In the *Ranunculetum circinati* community, the Shannon-Wiener ($H$) diversity index was higher ($p < 0.05$) in phytocoenoses distant from the tomb as compared to phytocoenoses located in its proximity. The number of species in the phytocoenoses examined was higher in those located far away from the tomb ($p < 0.05$) – Figure 5. In species distribution in phytocoenoses of *Ranunculetum circinati* ($p = 0.1$) – Figure 5 have not identified any differences.

In the *Equisetetum fluviatile* community, the Shannon-Wiener ($H$) diversity index differed ($p < 0.05$) in phytocoenoses located in the vicinity of the tomb as compared to those remote from it, being higher in the latter.

Table 2

Phytocoenoses of the class *Lemnetea minoris* R.Tx. 1955, *Potametea* R.Tx. et Prsg 1942, and *Fontinaletea antipyreticae* Hüb. 1957

| Class: *Lemnetea minoris* R.Tx. 1955 | | Class: *Potametea* R.Tx. et Prsg 1942 | | | | | | Class: *Fontinaletea antipyreticae* Hüb.1957 |
|---|---|---|---|---|---|---|---|---|
| community | *Lemno-Spirodeletum* Soó 1927 | *Potametum perfoliati* Koch 1926 | *Potametum natantis* Soó 1927 | *Myriophylletum spicati* Soó 1927 | *Elodeetum canadensis* (Pign. 1953) Pass. 1964 | *Ceratophylletum demersi* Hild 1956 | *Ranunculetum circinati* (Bennema et West. 1943) Segal 1965 | *Fontinaletum antipyreticae* Hüb. 1957 |
| Area, ha | 0.01 | 3.40 | 2.02 | 0.40 | 0.32 | 1.56 | 2.86 | 2.28 |
| Number of relevés | 1 | 12 | 4 | 2 | 2 | 10 | 6 | 5 |
| **Class *Lemnetea*** | | | | | | | | |
| *Lemna minor* L. | 5 | . | . | . | . | . | . | . |
| *Lemna trisulca* L. | . | | . | $1^{+}$ 5 | . | $I^{1}$ 100 | $I^{1}$ 83 | . |
| **Class *Potametea*** | | | | | | | | |
| *Potamogeton perfoliatus* L. | . | $V^{4-5}$ 7917 | | . | | | | . |
| *Potamogeton natans* L. | . | . | . | . | $2^{1}$ 500 | $I^{+}$ 2 | $I^{2}$ 292 | . |
| *Myriophylum spicatum* L. | . | $II^{1-2}$ 229 | $4^{4-5}$ 7500 | . | . | . | . | . |
| *Elodea canadensis* L | . | . | $1^{+}$ 3 | $2^{5}$ 8750 | . | $I^{1}$ 100 | . | . |
| *Cerathopyllum demersum* L | . | $II^{1-3}$ 396 | . | . | $2^{5}$ 8750 | . | $I^{+}$ 2 | $III^{+-1}$ 800 |
| *Ranunculus circinatus* Sibth. | . | $I^{1-2}$ 188 | $1^{+}$ 3 | $1^{+}$ 5 | $1^{+}$ 5 | $V^{4-5}$ 8250 | $V^{+-3}$ 1005 | $I^{+}$ 2 |
| *Nuphar lutea* (L.) Sibth. et sm. | . | $I^{+}$ 1 | . | $2^{+}$ 10 | . | $III^{+-1}$ 800 | $V^{5}$ 8750 | . |
| *Nymphaea alba* L. | . | . | $2^{1-3}$ 1063 | . | . | $I^{1}$ 100 | $I^{2}$ 292 | . |
| *Stratiotes aloides* L. | . | . | . | . | . | . | . | . |
| *Potamogeton obtusifolius* L. | . | . | . | . | . | $II^{2}$ 700 | $I^{1}$ 83 | . |
| **Class *Fontinaletea*** | | | | | | | | |
| *Fontinalis antipyretica* Hedw. | . | . | . | . | . | . | . | $V^{5}$ 8750 |
| **Class *Phragmitetea*** | | | | | $2^{+-2}$ 563 | | | |
| *Phragmites australis* Cav. | + | $I^{+-2}$ 147 | . | . | | | | . |
| *Glyceria maxima* L. | . | $I^{+}$ 1 | $1^{1}$ 125 | . | . | $I^{1}$ 100 | $I^{2}$ 292 | . |
| *Scirpus lacustris* L. | . | . | $1^{2}$ 438 | . | . | $I^{1}$ 100 | . | . |
| *Typha angustifolia* L. | . | . | . | . | . | . | . | . |
| *Acorus calamus* L. | . | . | . | . | . | . | . | . |
| *Typha latifolia* L. | + | . | . | . | . | . | . | . |

| | Class: *Phragmitetea* | | | | |
|---|---|---|---|---|---|
| community | *Scirpetum lacustris* (Allorge 1922) Chouard 1924 | *Phragmitetum australis* (Gams 1927) Schmale 1939 | *Glycerietum maximae* Hueck 1931 | *Equisetetum fluviatilis* Steffen 1931 | *Ttyphaetum latifoliae* Soó 1927 |
| Area, ha | 2.2 | 42.4 | 3.8 | 1.0 | 1.8 |
| Number of relevés | 16 | 13 | 15 | 9 | 6 |
| **Class *Phragnitetea*** | | | | | |
| *Scirpus lacustris* L. | $V^{4-5}$ 7500 | $III^{+-2}$ 252 | $I^{+}$ 1 | $I^{2}$ 194 | $III^{+-2}$ 377 |
| *Phragmites australis* (Cav.) Trin ex Stend | $V^{+-2}$ 518 | $V^{4-5}$ 8558 | $I^{+-1}$ 34 | . | . |
| *Glyceria maxima* L. | $II^{+-3}$ 298 | $I^{1}$ 77 | $V^{4-5}$ 8583 | $IV^{+-3}$ 726 | $III^{+-2}$ 585 |
| *Equisetum fluviatile* L. | $I^{1-2}$ 141 | $I^{+-3}$ 328 | $III^{+-3}$ 718 | $V^{4-5}$ 8472 | . |
| *Typha latifolia* L. | $I^{+}$ 1 | $I^{+}$ 1 | $III^{+-2}$ 385 | $I^{3}$ 417 | $V^{4-5}$ 7500 |
| *Typha angustifolia* L. | $I^{+}$ 1 | $II^{+-2}$ 309 | $I^{2}$ 117 | $II^{+-4}$ 944 | . |
| *Acorus calamus* L. | . | . | $I^{2}$ 117 | $II^{2}$ 389 | . |
| *Sparganium erectum* Huds. | . | . | . | $I^{1}$ 56 | . |
| *Eleochris palustris* (L.) Roem. et Schult | . | . | . | . | . |
| *Sagittaria sagittifolia* L. | . | . | . | . | $I^{+}$ 2 |
| *Carex rostrata* Stokes | . | . | . | . | . |
| *Carex acutiformis* Ehrh. | . | . | . | . | . |
| *Butomus umbellatus* | $I^{+-2}$ 110 | . | . | . | . |
| *Iris pseudacorus* L. | . | . | $I^{+}$ 1 | $I^{1}$ 56 | . |
| *Rumex hydrolapahtum* Hudson | . | . | $I^{+}$ 1 | $I^{1}$ 56 | . |
| *Ranunculus lingua* L. | . | $I^{1}$ 38 | $I^{+}$ 1 | . | . |
| *Mentha aquatica* L. | . | . | $I^{+}$ 1 | $I^{1}$ 56 | . |
| *Lysimachia thyrsiflora* L. | . | . | $I^{+}$ 1 | . | . |
| *Carex verisicaria* Curtis | $I^{+}$ 31 | . | . | . | . |
| *Carex gracilis* Curtis | . | . | . | $I^{+}$ 1 | . |
| *Carex riparia* L. | . | . | . | $I^{1}$ 56 | . |
| *Lysimachia vulgaris* L. | . | . | . | . | . |
| *Cicuta virosa* L. | . | . | . | . | . |
| *Sium latifolium* L. | . | . | . | . | . |
| **Class *Potametea***: | . | . | . | . | . |
| *Nuphar lutea* (L.) Sibth. et Sm. | $III^{+-4}$ 690 | $II^{+-2}$ 212 | $III^{+-1}$ 169 | $III^{+-3}$ 807 | $I^{1}$ 83 |
| *Potamogeton perfoliatus* L. | $I^{1}$ 31 | . | $II^{+-1}$ 35 | $I^{+}$ 1 | $IV^{+-3}$ 1 543 |
| *Ranunculus circinatus* Sibth. | . | . | $I^{2}$ 117 | . | $I^{+}$ 2 |
| *Cerathopyllum demersum* L. | . | . | . | . | . |
| *Myriophylum spicatum* L. | . | . | . | $II^{+-1}$ 57 | . |
| *Potamogeton lucens* L. | $I^{1}$ 31 | $I^{1}$ 38 | . | . | . |
| *Potamogeton natans* L. | $I^{2}$ 109 | . | . | $I^{+}$ 1 | . |
| *Potamogeton pectinatus* L. | . | . | . | . | . |
| *Nymphaea alba* L. | . | $I^{1}$ 38 | . | . | . |
| *Polygonum amphibium* L. | . | | . | | . |
| **Accompanying species**: | | | | | |
| *Lemna minor* L. | . | . | . | . | $I^{+}$ 2 |
| *Salix aurita* L. | $I^{+}$ 1 | . | . | . | . |
| *Poa trivialis* L. | . | . | . | . | . |
| *Fontinalis antipyretica* L. | . | . | . | . | . |
| *Eupatorium cannabinum* L. | . | . | . | . | . |
| *Aegopodium podagraria* L. | . | . | . | . | . |
| *Equisetum pratense* Ehrh. | . | . | . | . | . |
| *Tussilago farfara* L. | . | . | . | . | . |
| *Lycopus europaeus* L. | . | . | . | . | . |
| *Scutellaria galericulata* L. | . | . | . | . | . |
| *Galium palustre* L. | . | . | . | . | . |
| *Lysimachia vulgaris* L. | . | . | . | . | . |

R.Tx. et Prsg. 1942 class

| R.Tx. et Prsg. 1942 | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| *Thyphaetum angustifoliae* (Allorge 1922) Chouard 1924 | *Acoretum calami* Kobendza 1948 | *Sparganietum erecti* Roll. 1938 | *Eleocharitetum palustris* Schennikov 1919 | *Sagittario-Sparganietum emersi* R.Tx. 1953 | *Caricetum rostratae* Ruebel 1912 | *Caricetum acutiformis* Sauer 1937 | *Buttometum umbellati* Konczak 1968 | *Iridetum pseudoacori* Eggler 1933 |
| 4.2 | 1.2 | 0.3 | 0.9 | 0.01 | 0.01 | 0.01 | 0.01 | 0.01 |
| 10 | 5 | 3 | 7 | 3 | 2 | 2 | 2 | 2 |
| $I^{1}$ 63 | . | . | $I^{2}$ 250 | . | . | . | . | . |
| $V^{+-2}$ 1227 | . | $3^{+-1}$ 337 | $I^{2}$ 250 | . | $1^{2}$ 875 | . | . | . |
| . | $III^{+-1}$ 104 | $1^{+}$ 3 | $I^{1}$ 71 | $2^{1}$ 333 | $1^{+}$ 5 | $1^{1}$ 25 | $1^{2}$ 875 | . |
| $I^{2}$ 175 | $IV^{+-1}$ 204 | . | $I^{4}$ 893 | . | $1^{2}$ 875 | | $1^{4}$ 3125 | . |
| $I^{2}$ 175 | . | $1^{+}$ 3 | $I^{2}$ 250 | . | . | | $1^{2}$ 875 | . |
| $V^{4-5}$ 8500 | . | . | . | . | . | | . | . |
| . | $V^{5}$ 8750 | . | . | . | . | $1^{2}$ 875 | . | . |
| . | . | $3^{5}$ 8750 | . | . | . | | . | . |
| . | . | . | $V^{4-5}$ 7321 | . | . | . | . | . |
| . | $I^{+}$ 2 | . | . | $3^{4-5}$ 7917 | . | . | . | . |
| . | . | . | . | . | $2^{5}$ 8750 | . | . | . |
| . | . | . | . | . | . | $2^{5}$ 8750 | . | . |
| . | . | . | . | . | . | . | $2^{5}$ 8750 | . |
| . | . | . | . | . | . | . | . | $2^{5}$ 8750 |
| . | . | . | $I^{2}$ 250 | . | . | . | . | . |
| . | . | . | . | . | $1^{2}$ 875 | . | . | . |
| . | . | . | $I^{1}$ 71 | . | . | . | . | $1^{+}$ 5 |
| . | $I^{+}$ 2 | . | . | . | . | . | . | $1^{+}$ 5 |
| . | . | . | . | . | . | . | . | . |
| . | . | . | . | . | . | . | . | . |
| . | . | . | . | . | . | . | . | . |
| . | . | . | $I^{1}$ 71 | . | . | . | . | . |
| . | . | . | $I^{+}$ 1 | . | . | . | . | . |
| . | . | . | . | . | | . | . | $1^{+}$ 5 |
| . | . | . | . | . | . | . | . | . |
| $I^{+-1}$ 51 | $II^{1}$ 100 | $3^{2-3}$ 2 417 | $II^{2}$ 500 | . | . | $1^{1}$ 25 | $1^{2}$ 875 | . |
| $I^{+}$ 1 | . | . | $II^{+-1}$ 73 | $1^{1}$ 167 | . | . | . | . |
| . | . | $2^{+}$ 7 | $I^{+}$ 1 | $1^{1}$ 167 | . | . | . | . |
| $I^{+}$ 1 | $I^{+}$ 2 | $1^{+}$ 3 | $I^{1}$ 71 | $1^{2}$ 583 | . | . | . | . |
| . | . | . | $III^{+-1}$ 146 | $1^{+}$ 3 | . | . | . | . |
| . | . | . | . | . | . | . | . | . |
| . | . | . | . | . | . | . | . | . |
| . | . | . | . | . | . | . | . | . |
| . | $I^{+}$ 2 | . | . | . | . | . | . | . |
| | . | . | $I^{+}$ 1 | . | . | . | . | . |
| | | | | $1^{+}$ 3 | | | | |
| . | . | . | . | . | . | . | . | . |
| . | . | . | . | . | . | . | . | . |
| . | . | . | $I^{1}$ 71 | . | . | . | . | $1^{+}$ 5 |
| . | . | . | . | $1^{1}$ 167 | . | . | . | . |
| . | . | . | . | . | . | . | . | $1^{2}$ 875 |
| . | . | . | . | . | . | . | . | $2^{+}$ 10 |
| . | . | . | . | . | . | . | . | $1^{1}$ 25 |
| . | . | . | . | . | . | . | . | $1^{+}$ 5 |
| . | . | . | . | . | . | . | . | $1^{+}$ 5 |
| . | . | . | . | . | . | . | . | $1^{+}$ 5 |
| . | . | . | . | . | . | . | . | $1^{+}$ 5 |
| . | . | . | . | . | . | $1^{+}$ 5 | . | . |

Table 4

Biodiversity indices of submerged plant and emerged communities of Lake Szeląg Wielki

| Diversity indices<br>imported data, analysis begun: 21 October 2007 08: 50: 18, Analysing 48 variables x 138 cases, Shannon's method, Log base 2 | | | | | | | |
|---|---|---|---|---|---|---|---|
| **Emerged community** | | | | | | | |
| | sample relčve | index (*H*) | mean | evenness (*J*) | mean | num. spec. | mean |
| *Scirpetum lacustris* | 66*, 73*, 20*, 26*, 3* | 1.366–1.718 | 1.358 | 0.832–0.865 | 0.851 | 3–4 | 3.6 |
| | 71, 75, 59, 115, 10, 14, 70, 25, 29, 125, 98 | 1.939–2.999 | 2.454 | 0.835–0.946 | 0.904 | 5–9 | 6.6 |
| *Phragmitetum australis* | 77*, 27*, 74*, 79*, 34*,90* | 0–1.462 | 0.742 | 0–0.922 | 0.652 | 1–3 | 2 |
| | 11, 95, 12, 31, 49, 62, 128 | 1.366–2.194 | 1.668 | 0.756–0.945 | 0.873 | 3–5 | 3.8 |
| *Glycerietum maximae* | 112*, 45*, 110a*, 126*, 91* | 0–0.94 | 0.462 | 0–0.94 | 0.462 | 1–2 | 1.6 |
| | 132, 37, 47, 94, 50, 58, 5, 30, 41, 39 | 1.297–2.548 | 1.84 | 0.802–0.946 | 0.876 | 3–7 | 4.4 |
| *Equisetetum fluviatile* | 135*, 46*, 102* | 0.684–1.718 | 1.261 | 0.684–0.872 | 0.805 | 2–4 | 3 |
| | 92, 9, 8, 19, 55, 85 | 1.447–2.722 | 2.150 | 0.89–0949 | 0.918 | 3–8 | 5.3 |
| *Typhaetum latifoliae* | 127, 118, 89, 36, 23, 116 | 0.684–2.043 | 1.485 | 0.684–0985 | 0868 | 2–5 | 3.3 |
| *Typhetum angustifoliae* | 78*, 72*, 76*, 113*, 15* | 0–0.94 | 0.487 | 0–0.94 | 0.487 | 1–2 | 1.6 |
| | 57, 82, 69, 84, 13 | 1.287–2.194 | 1.510 | 0.812–0.945 | 0.865 | 3–5 | 3.4 |
| *Acoretum calami* | 42, 134, 7, 106, 40 | 0.684–2.309 | 1.519 | 0.684–0.893 | 0.807 | 2–6 | 3.8 |
| *Sparganietum erecti* | 123, 120, 124 | 1.718–2.064 | 1.943 | 0.859–0.889 | 0.877 | 4–5 | 4.7 |
| *Eleocharitetum palustris* | 111, 104, 109, 96, 97, 60, 64 | 0.998–2.842 | 1.708 | 0.812–0.998 | 0.915 | 2–8 | 4 |
| *Sagittario-Sparganietum* | 6, 131, 1 | 0–2.332 | 1.383 | 0–0.908 | 0.603 | 1–6 | 3.7 |
| *Caricetum rostratae* | 22, 38 | 0.94–2.147 | 1.029 | 0.925–0.94 | 0.622 | 2–5 | 2.3 |
| *Caricetum acutiformis* | 56, 61 | 1.447–1.67 | 1.039 | 0.835–0.913 | 0.583 | 3–4 | 3.5 |
| *Buttometum umbellati* | 138, 140a | 0.997–1.605 | 0.867 | 0.802–0997 | 0.6 | 2–4 | 3 |
| *Iridetum pseudoacori* | 54, 99 | 2.22–2.722 | 1.647 | 0.859–0.907 | 0.589 | 6–8 | 7 |
| **Submerged community** | | | | | | | |
| *Lemno-Spirodeletum* | 136 | 1.198 | | 0.756 | | 3 | |
| *Potametum perfoliati* | 101*, 117*, 67*, 68*, 100* | 0–0.961 | 0.356 | 0–0.961 | 0.356 | 1–2 | 1.1 |
| | 107, 133, 52, 81, 80, 105, 44 | 0.845–1.873 | 1.418 | 0.79–0.937 | 0.871 | 2–4 | 3.1 |
| *Potametum natantis* | 21, 119, 114, 53 | 1.252–2.295 | 1.41 | 0.79–0.919 | 0.686 | 3–6 | 3.4 |
| *Myriophylletum spicati* | 93, 35 | 1.198 | 1.198 | 0.756 | 0.756 | 3 | 3 |
| *Elodeetum canadensis* | 130, 129 | 1.287–1.78 | 1.022 | 0.812–0.89 | 0.567 | 3–4 | 3.5 |
| *Ceratophylletum demersi* | 88*, 33*, 83*, 145* | 0.684–1.447 | 0.944 | 0.684–0.913 | 0.808 | 2–3 | 2.8 |
| | 86, 18, 83a, 144, 146, 147 | 1.605–2.164 | 1.904 | 0.802–0.938 | 0.881 | 4–5 | 4.5 |
| *Ranunculetum circinati* | 2*, 108*, 121* | 0.684–1.382 | 0.959 | 0.684–0.872 | 0.789 | 2–3 | 2.3 |
| | 65, 17, 110 | 1.447–1.833 | 1.687 | 0.89–0.916 | 0.906 | 3–4 | 3.7 |
| *Fontinaletum antipyreticae* | 139, 140, 141, 142, 143 | 0–1.366 | 0.554 | 0–0.862 | 0.454 | 1–3 | 1.8 |

66 * – phytocoenosis located at a distance <2 km from pesticide tomb
71 – phytocoenosis located at a distance >2 km from pesticide tomb

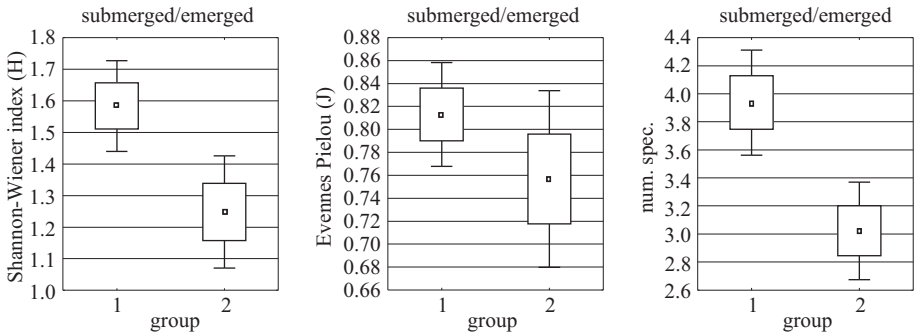


Fig. 2. Statistical analysis of diversity indices for phytocoenoses of submerged and emerged plants: group 1 – phytocoenosis located at a distance < 2 km from pesticide tomb; group 2 – phytocoenosis located at a distance > 2 km from pesticide tomb; student's t-test; grouping var.: stadium (sheet 1) group 1: submerged plants: 1; group 2: emerged plants 2

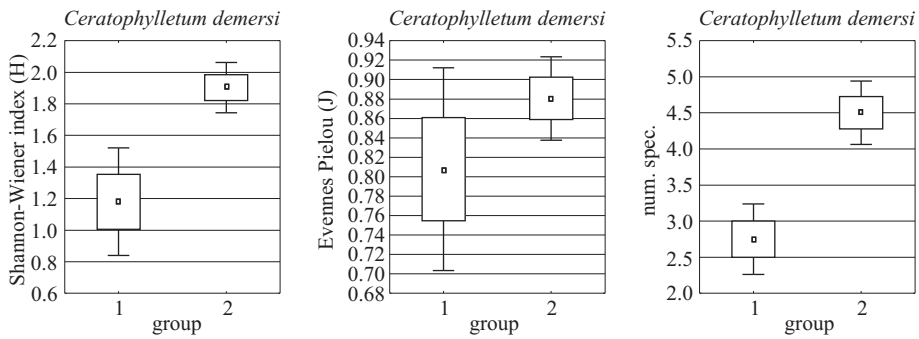


Fig. 3. Statistical analysis of diversity indices of *Ceratophylletum demersi* phytocoenoses: group 1 – phytocoenosis located at a distance < 2 km from pesticide tomb; group 2 – phytocoenosis located at a distance > 2 km from pesticide tomb; student's t-test; grouping var.: stadium (sheet 1) group 1: relévе 101*, 117*, 67*, 68*, 100*: 1; group 2: relévе 107, 133, 52, 81, 80, 105, 44: 2

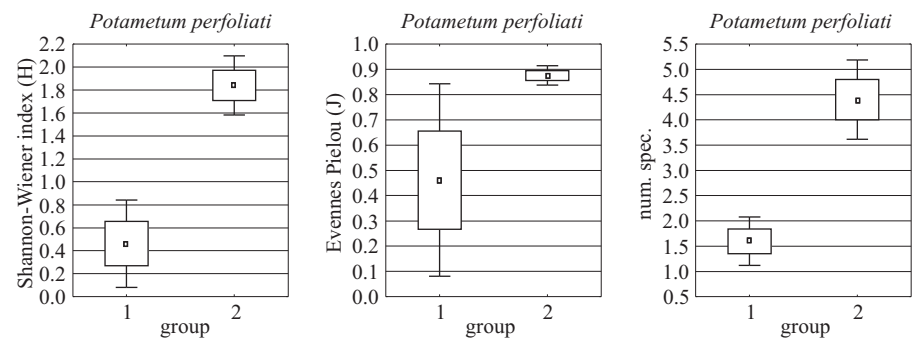


Fig. 4. Statistical analysis of diversity indices of *Potametum perfoliati* phytocoenoses: group 1 – phytocoenosis located at a distance < 2 km from pesticide tomb; group 2 – phytocoenosis located at a distance > 2 km from pesticide tomb; student's t-test; grouping var.: stadium (sheet 1) group 1: relévе 2*, 108*, 121*: 1; group 2: relévе 65, 17, 110: 2

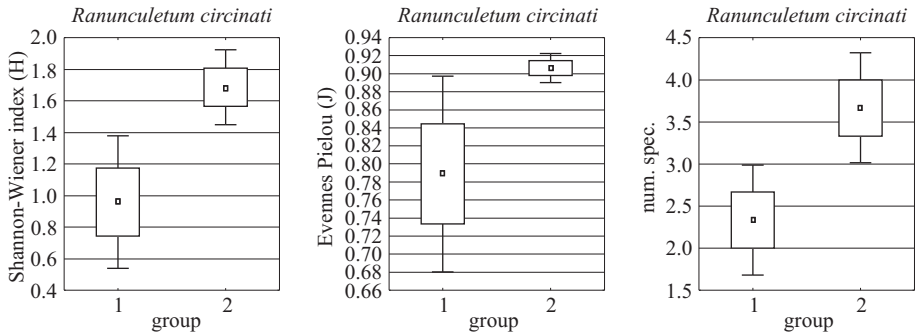


Fig. 5. Statistical analysis of diversity indices of *Ranunculetum circinati* phytocoenoses: group 1 – phytocoenosis located at a distance < 2 km from pesticide tomb; group 2 – phytocoenosis located at a distance > 2 km from pesticide tomb; student's t-test; grouping var.: stadium (sheet 1) group 1: relévе 135*, 46*, 102*: 1; group 2: relévе 92, 9, 8, 19, 55, 85: 2

In addition, species distribution in communities of *Equisetetum fluviatile* between the phytocoenoses examined ($p = 0.05$) – Figure 6. No differences were found between the number of species in the phytocenoses close to the dumping ground and those situated some distance from it ($p < 0.09$) – Figure 6.

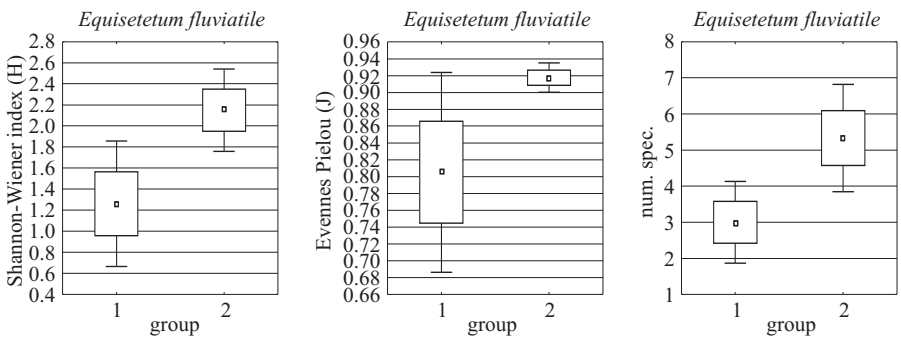


Fig. 6. Statistical analysis of diversity indices of *Equisetetum fluviatile* phytocoenoses: group 1 – phytocoenosis located at a distance < 2 km from pesticide tomb; group 2 – phytocoenosis located at a distance > 2 km from pesticide tomb; student's t-test; grouping var.: stadium (sheet 1) group 1: relévе 112*, 45*, 110a*, 126*, 91*: 1; group 2: relévе 132, 37, 47, 94, 50, 58, 5, 30, 41, 39: 2

The diversity index ($H$) determined in the community *Glycerietum maximae* was higher ($p < 0.0001$) in phytocoenoses remote from the pesticide tomb as compared to those located in its proximity. In the phytocoenoses analyzed, the number of species was higher in those remote from the tomb ($p < 0.009$) – Figure 7. Differences have been found in the distribution of species in the phytocenoses of *Glycerietum maximae* ($p < 0.009$) – Figure 7.

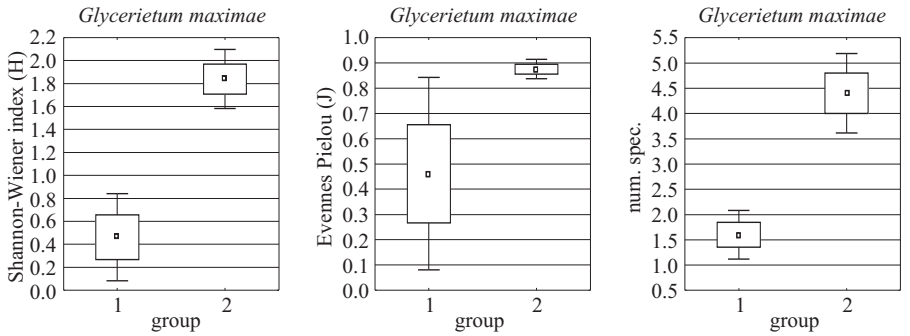


Fig. 7. Statistical analysis of diversity indices of *Glycerietum maximae* phytocoenoses: group 1 – phytocoenosis located at a distance < 2 km from pesticide tomb; group 2 – phytocoenosis located at a distance > 2 km from pesticide tomb; student's t-test; grouping var.: stadium (sheet 1) group 1: relève 112*, 45*, 110a*, 126*, 91*:1; group 2: relève 132, 37, 47, 94, 50, 58, 5, 30, 41, 39:2

In the *Phragmitetum* community, the Shannon-Wiener diversity index ($H$) was statistically significantly higher ($p < 0.003$) in phytocoenoses located far away from the tomb in contrast to those located in its vicinity (Figure 8). The number of species in the phytocoenoses examined was higher in those remote from the tomb as compared to those located at a distance of over 2 km ($p < 0.004$) – Figure 8. No differences were found in the distribution of species in the phytocenoses of *Phragmitetum* ($p = 0.11$) – Figure 8.

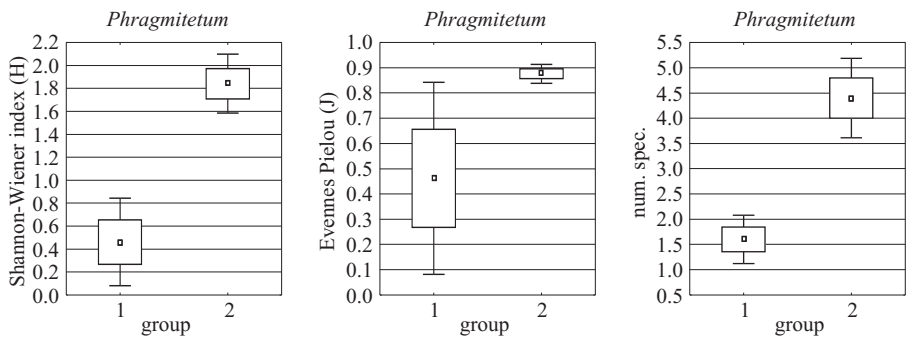


Fig. 8. Statistical analysis of diversity indices of *Phragmitetum australis* phytocoenoses: group 1 – phytocoenosis located at a distance < 2 km from pesticide tomb; group 2 – phytocoenosis located at a distance > 2 km from pesticide tomb; student's t-test: grouping var.: stadium (sheet 1) group 1: relève 77*, 27*, 74*, 79*, 34*, 90*:1; group 2: relève 11, 95, 12, 31, 49, 62, 128:2

All diversity indices appeared to be higher in phytocoenoses of *Scirpetum lacustris* remote from the tomb as compared to those located in its vicinity ($p < 0.002$) – Figure 9.

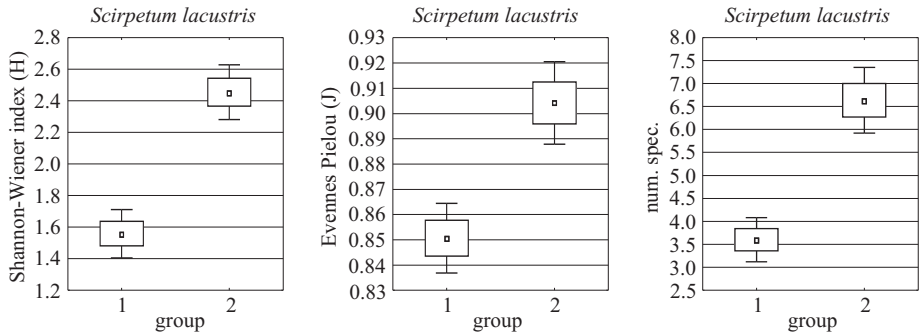


Fig. 9. Statistical analysis of diversity indices of *Scirpetum lacustris* phytocoenoses: group 1 – phytocoenosis located at a distance < 2 km from pesticide tomb; group 2 – phytocoenosis located at a distance > 2 km from pesticide tomb; student's t-test; grouping var.: stadium (sheet 1) group 1: reléve 66*, 73*, 20*, 26*, 3*:1; group 2: reléve 71, 75, 59, 115, 10, 14, 70, 25, 29, 125, 98:2

The Shannon-Wiener diversity indices ($H$) in the community of *Typhetum angustifoliae* were higher ($p < 0.005$) in phytocoenoses remote from the tomb as compared to those in its proximity (Figure 10). The number of species in the phytocoenoses analyzed was also higher in phytocoenoses located far away from the pesticide tomb ($p < 0.005$) – Figure 10. No differences were found in the distribution of species in the phytocoenoses of *Typhetum angustifoliae* ($p = 0.1$) – Figure 10).

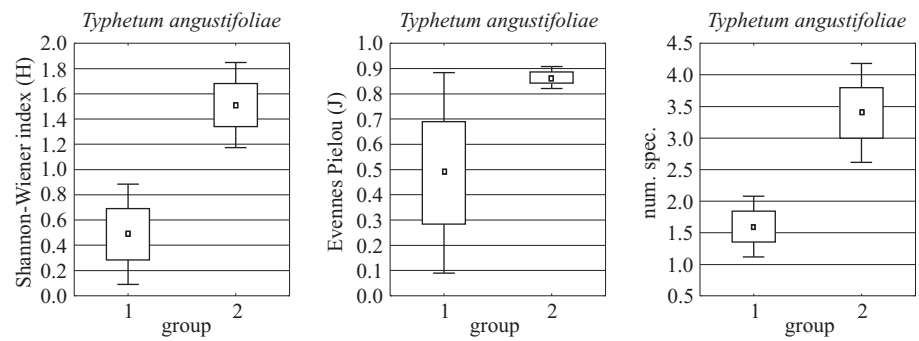


Fig. 10. Statistical analysis of diversity indices of *Typhetum angustifoliae* phytocoenoses: group 1 – phytocoenosis located at a distance < 2 km from pesticide tomb; group 2 – phytocoenosis located at a distance > 2 km from pesticide tomb; student's t-test; grouping var.: stadium (sheet 1) group 1: reléve 78*, 72*, 76*, 113*, 15*:1; group 2: reléve 57, 82, 69, 84, 13:2

## Discussion

Submerged and emerged vegetation are of key significance to the proper functioning of the entire lacustrine ecosystem and demonstrate high susceptibility to changes in the aquatic ecosystem (Baattrup-Peder-

SEN et al. 2001, SMOLDERS et al. 2001, CIECIERSKA, 2004, SCHAUMBURG et al., 2004a,b). The greater the taxonomic and syntaxonomic diversity of phytolittoral, the higher the ecological status of a lake (ENDLER et al. 1999, GRZYBOWSKI, ENDLER 2003, CIECIERSKA et al. 2006). The mean value of the Shannon-Wiener diversity index (H) for submerged and emerged plants of Lake Szeląg Wielki (Table 4), that of the Evenness index (J) – Table 4, and the mean number of species (Table 4) recorded in the lake under study are typical of eutrophic lakes (ENDLER et al. 1999).

The number of plant communities in a lake results, among others, from diversified habitat conditions of littoral (JENSÉN 1977, ROONEY, KALFF 2000). The size and length of a lake as well as the development of its shoreline affect the number of microhabitats for macrophytes. Lake Szeląg Wielki is characterized by good habitat conditions. The average number of phytocoenoses amongst 153 reference lakes originate from a database of lakes selected for the purpose of lake monitoring in Poland in compliance with Directive 2000/60/EC (*Directive*... 2000) ranges from 21 to 23 depending on the type of lake (CIECIERSKA et al. 2006). Analyses carried out in Lake Szeląg Wielki demonstrated the presence of 24 phytocoenoses, which indicates the high phytocenotic diversity of its phytolittoral.

The analysis of reference lake stations from countries of Central Europe showed that the maximum depth of bottom colonization with plants reached 4.6 m in deep lakes, including Lake Szeląg Wielki, and that for the same group of lakes the value of boundary between very good and good states accounted for 3.6 m (PHILLIPS 2006, *Milestone 6 Report*... 2006). This indicates that Lake Szeląg Wielki, in which the maximum range of submerged plants reaches 3.9 m and the mean one exceeds 3 m, is at a boundary between good and very good ecological state.

An important indicator of high macrophytic evaluation of the ecological state of lakes are communities of green stoneworts (*Charophyceae*) (FORSBERG 1964, KRAUSE 1981), yet, they were not detected in Lake Szeląg Wielki. In contrast some phytocoenoses were observed being negative indicators of the ecological state (CIECIERSKA et al. 2006) of Lake Szeląg Wielki, namely: *Ceratophylletum demersi*, *Elodeetum canadansis, Potametum pectinati*. The presence of these phytocoenoses and the lack of stoneworts algae point to the existence of human impact (CIECIERSKA et al. 2006).

Phytosociological examinations carried out in 2006 in Lake Szeląg Wielki demonstrated differences compared to the results of assays conducted in 2002 (GRZYBOWSKI et al. 2005). In 2002, the following phytocoenoses: *Fontinaletum antipyreticae, Lemno-Spirodelletum, Caricetum rostratae, Caricetum acutiformis, Iridetum pseudacori*, were not detected in the investigated lake. The analysis of biodiversity indices in communities of: *Potametum perfoliati, Ceratophylletum demersi, Ranunculetum circinati, Typhetum angustifoliae, Scirpetum lacustris, Phragmitetum, Glycerietum maximae,* and *Equisetetum fluviatile*, consisting of a comparison of diversity

indices: Shannon-Wiener index (H), Pielou Evenness index (J), and the mean number of species in phytocoenoses remote from the pesticide tomb as compared to phytocoenoses located in its vicinity demonstrates the differences between the examined phytocoenoses. Statistically significantly lower values of the Shannon-Wiener (H) were recorded in phytocoenoses located closest to the tomb. A similar statistically-confirmed correlation was found for the number of species in the phytocoenoses under scrutiny.

The changes in the Pielou Evenness index (J) were not statistically significant, except for phytocoenoses of *Scirpetum lacustris,* which indicates a marginal effect of the pesticide tomb on species distribution in the analyzed communities. This corresponds with the results of investigations on biodiversity obtained from anthropogenic forest phytocoenoses located in the proximity of the pesticide tomb in Warlity Wielkie (GRZYBOWSKI et al. 2007).

Investigations carried out in Lake Szeląg Wielki demonstrated:

– a modifying effect of a pesticide tomb on the floral abundance in phytocoenoses of submerged plants: *Potamogetonetum perfoliati, Ceratophylletum demersi, Ranunculetum circinati*,
– a modifying effect of pesticide tomb on the floral abundance of phytocoenoses of emerged plants: *Typhetum angustifoliae, Scirpetum lacustris, Phragmitetum, Glycerietum maximae, Equisetetum fluviatile*,
– phytocoenoses of *Scirpetum lacustris* detected in Lake Szeląg Wielki to be the most susceptible to the activity of a pesticide tomb,
– the Pielou index of species evenness (J) changed to the least extent in the phytocoenoses examined, which indicates a negligible impact of the pesticide tomb on species distribution in the community.

Translated by: Joanna Jensen Acctepted for print: 23.12.2009

## References

BAATTRUP-PEDERSEN A., ANDERSSON B., BRANDRUD T. E., KARTTUNEN K., RIIS T., TOIVONEN H. 2001. Macrophytes. [In:] *Biological monitoring in Nordic rivers and lakes.* Ed. J. SKRIVER. National Environmental Research Institute, Denmark. Temanord. 513: 53–60.

BRAUN-BLANQUET J. 1964. *Pflanzensoziologie. Grundzuge der Vegetationskunde*. 3 Aufl. Springer., Wien-New York.

BRZEG A., WOJTERSKA M. 2001. *Zespoły roślinne Wielkopolski, ich stan poznania i zagrożenie.* [In: ] *Szata roślinna Wielkopolski i Pojezierza Południowopomorskiego. Przewodnik sesji terenowych 52. Zjazdu PTB*. Ed. M. WOJTERSKA. 24–28 września 2001, Poznań, 39–110.

CIECIERSKA H. 2004. *Phytocoenotic diversity of littoral in lakes of the Masurian Landscape Park – current state and changes. Teka Komitetu Ochrony Kształtowania Środowiska Przyrodniczego*, Lublin, 1: 32–38.

CIECIERSKA H., KOLADA A., SOSZKA H., GOŁUB M. 2006. *Opracowanie podstaw metodycznych dla monitoringu biologicznego wód powierzchniowych w zakresie makrofitów i pilotowe ich zastosowanie dla części wód reprezentujących wybrane kategorie i typy.* T. II Jeziora. IOŚ, UWM. Warszawa-Poznań-Olsztyn.

CYDZIK D., KUDELSKA D., SOSZKA H. 1995. *Atlas stanu czystości jezior Polski badanych w latach 1989–1993.* Biblioteka Monitoringu Środowiska PIOŚ, Warszawa.

DIRECTIVE 2000/60/EC of the European Parliament and of the Council of the European Union 23 October, 2000. Off. J. Eur. Communities, 22: 1–72.

ENDLER Z., GRZYBOWSKI M., JUŚKIEWICZ B. 1999. *Przegląd fitocenoz wodnych i szuwarowych Pojezierza Mazurskiego.* Zeszyty WSP, Prace Biologiczne, 2: 35–43.

FORSBERG C. 1964. *Phosphorus, a maximum factor in the growth of Characeae*. Nature, 201: 517–518.

GRZYBOWSKI M., ENDLER Z. 2003. *Zmiany zbiorowisk makrofitów podmiejskiego Jeziora Trackiego pod wpływem antropopresji.* Acta Sci. Pol., Formatio Circumiectus, 2(2): 149–156.

GRZYBOWSKI M., SZAREK J., SKIBNIEWSKA K., GUZIUR J., SAWICKA-KAPUSTA K., ZAKRZEWSKA M. 2007. *Evaluation of point diversity of an anthropogenic forest threatened by pesticide tomb* (in press).

GRZYBOWSKI M., SZAREK J., SKIBNIEWSKA K., SAWICKA-KAPUSTA K., GUZIUR J., ENDLER Z. 2005. *The characteristics of plants in the littoral zone of lake Szeląg Wielki in the Iława Lake District threatened by pesticide tomb.* Fresenius Environ. Bull., 14(5): 357–362.

GRZYBOWSKI M., SZAREK J., ZMYSŁOWSKA I., GUZIUR J., ENDLER Z., SKIBNIEWSKA K. 2004. *Ecological characteristic of pesticide tomb in the Warmia region on the basis of index numbers of vascular plants.* Polish J. Environ. Stud., 13(6): 683–692.

JANSÉN J. 1975. *A simple clustering procedure for preliminary classification of very large sets of phytosociological relevés.* Vegetatio, 30: 67–71.

JENSÉN S. 1977. *An objective method for sampling the macrophyte vegetation in lakes.* Vegetatio, 33: 107–118.

KRAUSE W. 1981. *Characeen als Bioindikatoren für den Gewässerzustand.* Limnologica. 13(2): 399–418.

MAAREL E. VAN DER 1979. *Transformation of cover-abundance values in phytosociology and its effects on community similarity.* Vegetatio, 39–2: 97–114.

MAGURRAN A. 1988. *Ecological diversity and its measurement*. Princeton, New Jersey: Princeton University Press.

MATUSZKIEWICZ W. 2006. *Przewodnik do oznaczania zbiorowisk roślinnych Polski.* Ser. Vademecum Geobotanicum. PWN, Warszawa.

*Milestone 6 Report* – Lakes GIGs 2006 JRC: JRC-EEWAI Intercalibration.

MIREK Z., PIĘKOŚ-MIRKOWA H., ZAJĄC A., ZAJĄC M. 2002. *Flowering plants and pteridophytes of Poland. A checklist. Biodiversity of Poland. 1.* W. Szafer Institute of Botany, Polish Academy of Sciences, Kraków.

PHILLIPS G. 2006. *Derivation of chlorophyll boundaries based on changes to maximum depth distribution of submerged macrophytes,* http: //forum.europa.eu.int/Members/jrc/jrc/jrc_eewai/library.

ROONEY N., KALFF J. 2000. *Inter-annual variation in submerged macrophyte community biomass and distribution: the influence of temperature and lake morphometry.* Aquat. Bot., 68: 321–335.

RUTKOWSKI L. 2005. *Klucz do oznaczania roślin naczyniowych Polski niżowej.* PWN, Warszawa.

SHANNON C.E., WEAVER W. 1949. *The mathematical theory of communication.* Urbana, University of Illinois Press.

SCHAUMBURG J., SCHRANZ CH., HOFMANN G., STELZER D., SCHNEIDER S., SCHMEDTJE U. 2004a. *Handlungsanweisung für die ökologische Bewertung von Seen żur Umsetzung der EU-Wasserrahmenrichtlinie. Makrophyten und Phytobenthos.* Bayerisches Landesamt für Wasserwirtschaft, München.

SCHAUMBURG J., SCHRANZ CH., HOFMANN G., STELZER D., SCHNEIDER S., SCHMEDTJE U. 2004b. *Macrophytes and phytobenthos as indicators of ecological status in German lakes – a contribution of the implementation of the Water Framework Directive.* Limnologica, 34: 302–314.

SKIBNIEWSKA K., SZAREK J., RÓŻAŃSKI S., GUZIUR J. 2002. *Pesticide tombs in Warmia and Mazury voivodship as environmental hazard.* Abstracts of the 7th Regional Meeting of the Central and Eastern European Section, Brno, Czech Republik, 243–245.

Skibniewska K, J. Guziur, J. Szarek. 2004. *DDT and its metabolites in fat of fish sampled in reservoirs influenced by pesticide tomb.* Bull VUHR Vodnany, (4): 164–170.

Skibniewska K, Szarek J., Sawicka-Kapusta K., Guziur J., Zmysłowska I., Grzybowski M., Zakrzewska M., Różański S., Andrzejewska A. 2005. *Analytical problems in searching for hidden wastes in the environment*. [In:] *On Analytical and Environmental Problems.* Ed. Z. Gallbacs, 54–57.

Smolders A.J.P., Lamers L.M.P., Roelofs J.G.M. 2001. *Aquatic macrophytes in assessment and monitoring of ecological status of aquatic environments.* TemaNord 2001, 563: 23–31.

StatSoft, Inc. 2005. STATISTICA (data analysis software system), version 7.1. www.statsoft.com.

Szarek J., Lipinska J., Guziur J., Andrzejewska A., Skibniewska K., Babińska I. 2003. *Pathomorphological pattern of the internal organs in carp (Cyprinus carpio) from a pond near a pesticide tomb*. 21" Annual Meeting of the European Society of Veterinary Pathology, Dublin, Irleand.

Szarek J., Skibniewska K., Grzybowski M., Guziur J., Sawicka-Kapusta K., Zakrzewska M., Andrzejewska A., Zmyslowska I., Felsmann M. 2007a. *Pathomorphology as a tool in searching for sources of environmental pollution. Proceedings of the International Conference on Environmental Management, Engineering, Planning and Economics.* Skiathos, GRAFIMA Publ., Thessaloniki, Greece, 7–12.

Szarek J., Skibniewska K., Wojtacka J., Guziur J., Babińska I., Andrzejewska A., Sawicka--Kapusta K., Grzybowski M., Zakrzewska M., Zmysłowska I., Felsmann M. 2007b. *Effect of the pesticide tomb in Ilawskie Lake District on the morphological pattern of the liver and kidneys of the black-striped field mouse (Apodemus agrarius, Pallas 1771) in the year 2004 and 2006.* Pol. J. Pathol., 58(2): 147.

Szarek J., Skibniewska K., Guziur J., Wojtacka J., Grzybowski M., Babinska I., Andrzejewska A., Sawicka-Kapusta K., Felsmann M., Zakrzewska M., Zmyslowska I. 2007c. *Pathomorphological pattern of the hepatopancreas and kidneys in carp (Cyprinus carpio Linnaeus, 1758) bred in pond nearby pesticide tomb in Ilawskie Lake District in the years 2004 and 2006.* Proceedings of the III International Conference "Fishery", Ideja Print, Loznica, Republic of Serbia, 198–206.

Tomaszewicz H. 1979. *Roślinność wodna i szuwarowa Polski.* Wyd. UW. Warszawa.

Zmysłowska I., Grzybowski M., Guziur J., Skibniewska K., Szarek J., Golaś I., Andrzejewska A., Sawicka-Kapusta K., Zakrzewska M. 2005. *Mikrobiologiczne badania gleby, stawów rybnych i jeziora w pobliżu mogilnika pestycydowego.* Zesz. Probl. Post. Nauk Roln., 505: 531–537.

POLISH JOURNAL OF NATURAL SCIENCES
Abbrev.: Pol. J. Natur. Sc., Vol 25(2): 173–181, Y. 2010

DOI 10.2478/v10020-010-0014-x

# APPLICATION OF MICROSATELLITE DNA VARIATION IN RUSSIAN STURGEON (*ACIPENSER GUELDENSTAEDTI*) AND STERLET *ACIPENSER RUTHENUS* CULTURED IN A POLISH FISH FARM

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Key words: genetic diversity, genetic monitoring, microsatellite DNA, Russian sturgeon, sterlet.

Abstract

Six microsatellite loci were used to examine the genetic variability of Russian sturgeon and sterlet cultured in a Polish fish farm. Within 47 individuals of Russian sturgeon and 35 individuals of sterlet, a total of 113 alleles were detected in six polymorphic microsatellite loci. The number of alleles per locus ranged from 9 to 23 in Russian sturgeon and 3–8 in sterlet. The genetic diversity of six microsatellite loci varied from 0.404 to 0.880 in Russian sturgeon and from 0.515 to 0.971 in sterlet. Microsatellite analysis has a great potential for aquaculture of sturgeon fishes, especially when levels of genetic variation could be monitored and inbreeding controlled in commercial breeding programs.

## ZASTOSOWANIE MIKROSATELITARNEGO DNA W GENETYCZNYM MONITORINGU HODOWLI JESIOTRA ROSYJSKIEGO (*ACIPENSER GUELDENSTAEDTI*) I STERLETA (*ACIPENSER RUTHENUS*)

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Słowa kluczowe: zmienność genetyczna, monitoring genetyczny, mikrosatelitarny DNA, jesiotr rosyjski, sterlet.

Abstrakt

W opracowaniu przeprowadzono analizę polimorfizmu mikrosatelitarnego DNA w celu określenia zmienności genetycznej jesiotra rosyjskiego *Acipenser gueldenstaedti* i sterleta *Acipenser ruthenus* pochodzących z akwakultury. W badaniach zastosowano 6 par starterów mikrosatelitarnego DNA do analizy genetycznej wymienionych gatunków ryb. Analizom mo-

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lekularnym poddano 47 osobników jesiotra rosyjskiego oraz 35 osobników sterleta, u których zidentyfikowano 113 allele w badanych loci mikrosatelitarnego DNA. W analizowanych loci obserwowano od 9 do 23 alleli u jesiotra rosyjskiego oraz od 3 do 8 alleli u sterleta. Zmienność genetyczna badanych prób wynosiła od 0,404 do 0,880 u jesiotra rosyjskiego oraz od 0,515 do 0,971 u sterleta. Badania ukazują możliwość zastosowania analizy polimorfizmu mikrosatelitarnego DNA w akwakulturze ryb jesiotrowatych, szczególnie w przypadku monitorowania zmienności genetycznej stad tarłowych podczas prowadzenia programów hodowlanych.

## Introduction

Today, aquaculture is gaining importance on the food market and aquaculture production relies on commercial fisheries. Sturgeon aquaculture is a common industry branch in Poland, with commercial production of sturgeons in Poland increasing every year. This corresponds to an increasing demand for sturgeon meat and caviar. The tree main species of sturgeon cultured in Polish fish farms are Siberian sturgeon (*Acipenser baeri*), Russian sturgeon (*Acipenser gueldenstaedti*), sterlet (*Acipenser ruthenus*) and sturgeon hybrids. The relatively abundant broodstocks of sturgeons in Europe create a true opportunity for increasing production of valuable sturgeon products, although natural populations of this fish have been declining.

Sturgeons reared under controlled conditions show rapid growth and reach sexual maturity faster than in nature (CHEBANOV, BILLARD, 2001). Proper exploitation of broodstock together with innovative technologies should increase farming production in private sturgeon farms in Poland. Fish farms and breeding stations involved in sturgeon broodstock production shoud protect the gene diversity of these fish. Our knowledge of the genetic structure of broodstock should help to preserve the genetic diversity among cultured sturgeon species. Regular monitoring of genetic diversity of spawning populations is very important as well as prevention of any loss of the current polymorphism due to inbreeding and outbreeding problems. The most reliable method for studying gene diversity in broodstock is an application of microsatellite DNA markers (microsatellites). The microsatellite DNA is a powerful genetic marker, useful in many areas of fish genetics and breeding (MCCONNEL et al. 1995, NIELSEN et al. 1996, NORRIS et al. 1999, CHISTIACOV et al. 2005, CLIFFORD et al. 1998, FOPP-BAYAT 2004, FOPP-BAYAT 2009). Polymorphic microsatellite loci have been frequently applied to the analysis of genetic diversity in fish population, species identification, parentage identification, breeding programmes of food fish and monitoring of gene diversity in cultured fishes (MCCONNEL et al. 1995, NIELSEN et al. 1996, NORRIS et al. 1999, CHISTIACOV et al. 2005, CLIFFORD et al. 1998, FOPP-BAYAT 2009).

The purpose of the present study has been to apply microsatellite DNA fragments to the estimation of gene diversity in Russian sturgeon and sterlet farmed in a Polish fish farm. The results will be applied to testing

the purity of hatchery broodstocks in Polish fish farms and will be helpful in increasing the efficiency of selective breeding and performance testing programs of the examined sturgeon species.

## Material and Methods

Fin clips were sampled from 47 specimens of Russian sturgeon and 35 specimens of sterlet reared at Wasosze Fish Farm near Konin, Poland. Genomic DNA for amplification of six microsatellite loci: *Afu-19*, *Afu-39, Afu-68, AfuB-68,* (MAY et al., 1997), *Spl-163, Spl-168* (McQuown *et al*., 2000) was extracted using Chelex 100 method (WALSH et al., 1991) – Table 1.

Table 1

Repeat motif, primer sequences, annealing temperature and references of studied micxrosatellite loci in Russian sturgeon (*Acipenser gueldenstaedti*) and sterlet (*Acipenser ruthenus*) specimens

| Lp | Micro-satellite locus | Repeat motif | Primer sequence | T°C | References |
|---|---|---|---|---|---|
| 1 | *Afu-19* | $(TTG)_9$ | F..CATCTTAGCCGTCTGTGGTAC<br>R..CAGGTCCCTAATACAATGGC | 53 | MAY et al. 1997 |
| 2 | *Afu-39* | $(GTT)_{10}$ | F..TCCTGAAGTTCACACATTG<br>R..ATGGAGCATTATTGGAAGG | 57 | MAY et al. 1997 |
| 3 | *Afu-68* | $(GATA)_{13}$ | F..TTATTGCATGGTGTAGCTAAAC<br>R..AGCCCAACACAGACAATATC | 55 | MAY et al. 1997 |
| 4 | *AfuB-68* | $(GATA)_{28}$ | F..AACAATATGCAACTCAGCATAA<br>R..AGCCCAACACAGACAATATC | 55 | WELSH et al. 2003 |
| 5 | *Spl-163* | $(GATA)_{17}$ | F..TGCTTGTAAACTGCCCCACT<br>R..CCACATGCAGTTTGAGCTGC | 57 | MCQUOWN et al. 2000 |
| 6 | *Spl-168* | $(TATC)_{18}$ | F..CACTGATTCGCTACAACCGT<br>R..AGAAGGACTTGCAGTCCGAA | 57 | MCQUOWN et al. 2000 |

All microsatellite loci were amplified using the Polimerase Chain Reaction procedure (PCR). The primer sequences, annealing temperature and references of studied microsatellite loci in Russian sturgeon (*Acipenser gueldenstaedti*) and sterlet (*Acipenser ruthenus*) specimens were described in Table 1. Reaction mixes and amplification procedures of microsatellite DNA fragments were described by FOPP-BAYAT (2009). Amplification was conducted with a Mastercycler gradient thermocycler (Eppendorf, Germany). Aliquots containing PCR products and reaction buffer were electrophoresed using 6% polyacrylamidae gel, and DNA bands were visualized by the silver staining method (Tegelström, 1986). Electrophoresis was conducted on a Bio-Rad SequiGen Sequencing Cell-system, and the gel size was 38x30cm. Amplified fragments were sized by comparing migration with two DNA standards: ϕX 174 DNA/*Hinf* I DNA

Step Ladder (Promega, Madison, WI, USA) and 25bp DNA Step Ladder (Promega, Madison, WI, USA). The size and intensity of alleles in the studied microsatellite loci was estimated using Densitometer GS-800 with Quantity One software (Bio-Rad).

Allele frequencies, observed heterozygosity ($H_o$), expected heterozygosity ($H_e$) and polymorphism information content (PIC) values for each locus were computed using the microsatellite toolkit macro for MICROSOFT EXCEL$^{TM}$ (Park 2001). The gene diversity was calculated based on the heterozygosity value ($H$).

$$\overline{H} = \frac{2N \cdot (1 - \sum q_i^2)}{2N - 1}$$

H – heterozygosity value at locus
$q_i$ – frequency of i-th allele at locus
$N$ – number of specimens

For calculation of more loci, the formula was:

$$\overline{H} = \frac{\sum_{r=1}^{r} \frac{2N \cdot (1 - \sum q_i^2)}{2N - 1}}{r}$$

H – heterozygosity value at locus
$q_i$ – frequency of i-th allele at locus
$N$ – number of specimens
$r$ – number of studied loci

The Polymorphism Information Content (PIC value) was calculated for each locus.

$$PIC = 1 - \left( \sum_{i=1}^{n} p_i^2 \right) - \sum_{i=1}^{n-1} \sum_{j=i+1}^{n} 2 p_i^2 p_j^2$$

$p_i$ – frequency of i-th alleli in population
$p_j$ – frequency of j-th alleli in population.

## Results and Discussion

In the present study, all six microsatellite loci have been successfully amplified in Russian sturgeon and five loci in sterlet. Locus *Spl-168* was not evaluated in sterlet because it did not amplify. The gels obtained with each of the six microsatellite loci in the sturgeon species were analyzed

with respect to the length of alleles. Table 2 describes the allele frequency at the studied microsatellite DNA loci in Russian sturgeon and sterlet. The size of the alleles at individual loci varied between 84 base pairs (bp) and 272 bp (Table 2). In total, 78 alleles were detected in Russian sturgeon and 27 in the studied strain of sterlet. The number of allele per locus ranged from 3 for loci *Afu-19* and *Afu-39* in sterlet to 23 for locus *AfuB-68* in Russian sturgeon (Table 2). The observed number of alleles in Russian sturgeon is larger than in sterlet because of different ploidy status of the studied two sturgeon species. Russian sturgeon is tetraploid (~240 chromosomes) while sterlet is diploid (~120 chromosomes) (FONTANA 1994). The three of our analyzed microsatellite loci (*Afu-19*, *Afu-39* and *Afu-68*) were studied in Russian sturgeon and sterlet by LUDWIG et al. (2001) during studies of genome duplication events and functional reduction of ploidy levels. In this study at locus *Afu-19* ten alleles were observed in Russian sturgeon and four in sterlet. Locus *Afu-39* were represented by 16 alleles in Russian sturgeon and four in sterlet while at locus *Afu-68* 28 and 15 alleles were observed in Russian sturgeon and sterlet respectively (LUDWIG et al. 2001). The high number of allels per locus observed by LUDWIG et al. (2001) was probably connected to numerous sample of studied fish. The same three microsatellite DNA loci: *Afu-39*, *Afu-68* and *Spl-168* were applied in Chinese sturgeon. All the three primer pairs revealed polymorphic loci in Chinese sturgeon (ZHU et al. 2002). At locus *Afu-39* four alleles were observed ranging from 110–180 base pairs (bp); locus *Afu-68* was represented by 12 alleles 110–180 bp in length, while *Spl-168* contained 31 alleles 147–238 bp in length. With these highly

Table 2

Allele frequency at studied microsatellite loci in Russian sturgeon and sterlet

| Locus/Allele | | Species | | Locus/Allele | | Species | |
|---|---|---|---|---|---|---|---|
| 1 | | 2 | 3 | 4 | | 5 | 6 |
| *Afu-19* | | Russian sturgeon | sterlet | *Afu-39* | | Russian sturgeon | sterlet |
| *N* | bp | frequency | | *N* | bp | frequency | |
| 1 | 124 | 0.093 | 0.000 | 1 | 124 | 0.109 | 0.000 |
| 2 | 133 | 0.065 | 0.000 | 2 | 127 | 0.185 | 0.417 |
| 3 | 136 | 0.087 | 0.014 | 3 | 130 | 0.092 | 0.383 |
| 4 | 139 | 0.239 | 0.572 | 5 | 133 | 0.299 | 0.200 |
| 5 | 142 | 0.277 | 0.414 | 6 | 139 | 0.022 | 0.000 |
| 6 | 145 | 0.016 | 0.000 | 7 | 145 | 0.081 | 0.000 |
| 7 | 148 | 0.109 | 0.000 | 8 | 148 | 0.098 | 0.000 |
| 8 | 154 | 0.011 | 0.000 | 9 | 151 | 0.022 | 0.000 |
| 9 | 157 | 0.103 | 0.000 | 10 | 160 | 0.033 | 0.000 |
| | | | | 11 | 163 | 0.054 | 0.000 |
| | | | | 12 | 166 | 0.005 | 0.000 |

cont. Table 2

| 1 | | 2 | 3 | 4 | | 5 | 6 |
|---|---|---|---|---|---|---|---|
| *Afu-68* | | Russian sturgeon | sterlet | *AfuB-68* | | Russian sturgeon | sterlet |
| *N* | bp | frequency | | *N* | bp | frequency | |
| 1 | 128 | 0.100 | 0.000 | 1 | 84 | 0.046 | 0.000 |
| 2 | 132 | 0.095 | 0.000 | 2 | 88 | 0.024 | 0.000 |
| 3 | 136 | 0.244 | 0.000 | 3 | 96 | 0.003 | 0.000 |
| 4 | 140 | 0.083 | 0.000 | 4 | 100 | 0.008 | 0.000 |
| 5 | 144 | 0.006 | 0.000 | 5 | 104 | 0.046 | 0.000 |
| 6 | 148 | 0.133 | 0.000 | 6 | 108 | 0.027 | 0.000 |
| 7 | 184 | 0.039 | 0.091 | 7 | 112 | 0.053 | 0.278 |
| 8 | 192 | 0.022 | 0.000 | 8 | 116 | 0.136 | 0.000 |
| 9 | 204 | 0.017 | 0.136 | 9 | 120 | 0.125 | 0.143 |
| 10 | 208 | 0.000 | 0.439 | 10 | 124 | 0.136 | 0.000 |
| 11 | 212 | 0.033 | 0.046 | 11 | 128 | 0.077 | 0.000 |
| 12 | 216 | 0.000 | 0.106 | 12 | 132 | 0.077 | 0.000 |
| 13 | 224 | 0.011 | 0.000 | 13 | 136 | 0.035 | 0.000 |
| 14 | 228 | 0.000 | 0.182 | 14 | 140 | 0.021 | 0.000 |
| 15 | 240 | 0.111 | 0.000 | 15 | 164 | 0.019 | 0.064 |
| 16 | 244 | 0.095 | 0.000 | 16 | 168 | 0.013 | 0.000 |
| 17 | 248 | 0.011 | 0.000 | 17 | 180 | 0.008 | 0.000 |
| | | | | 18 | 184 | 0.000 | 0.064 |
| | | | | 19 | 188 | 0.000 | 0.286 |
| | | | | 20 | 192 | 0.027 | 0.029 |
| | | | | 21 | 196 | 0.000 | 0.043 |
| | | | | 22 | 204 | 0.008 | 0.093 |
| | | | | 23 | 216 | 0.005 | 0.000 |
| | | | | 24 | 220 | 0.053 | 0.000 |
| | | | | 25 | 224 | 0.045 | 0.000 |
| | | | | 26 | 228 | 0.008 | 0.000 |
| *Spl-163* | | Russian sturgeon | sterlet | *Spl-168* | | Russian sturgeon | sterlet |
| *N* | bp | frequency | | *N* | bp | frequency | |
| 1 | 100 | 0.144 | 0.000 | 1 | 212 | 0.021 | 0.000 |
| 2 | 168 | 0.048 | 0.071 | 2 | 224 | 0.107 | 0.000 |
| 3 | 172 | 0.175 | 0.000 | 3 | 232 | 0.043 | 0.000 |
| 4 | 176 | 0.027 | 0.000 | 4 | 236 | 0.021 | 0.000 |
| 5 | 180 | 0.058 | 0.000 | 5 | 248 | 0.261 | 0.000 |
| 6 | 188 | 0.000 | 0.257 | 6 | 252 | 0.032 | 0.000 |
| 7 | 192 | 0.011 | 0.000 | 7 | 260 | 0.021 | 0.000 |
| 8 | 196 | 0.037 | 0.115 | 8 | 264 | 0.037 | 0.000 |
| 9 | 200 | 0.096 | 0.000 | 9 | 272 | 0.037 | 0.000 |
| 10 | 204 | 0.106 | 0.200 | | | | |
| 11 | 208 | 0.144 | 0.029 | | | | |
| 12 | 212 | 0.154 | 0.271 | | | | |

polymorphic genetic markers, ZHU et al. (2002) distinguished artificially and naturally propagated individuals among juvenile samples of Chinese sturgeon in the estuary of the Yangtze River.

Table 3 presents expected heterozygosity, observed heterozygosity, number of alleles and PIC value at studied microsatellite loci in Russian sturgeon and sterlet. The studied groups of fish were characterized by various gene diversity in the analyzed microsatellite loci (Table 3). The level of gene diversity (based on observed heterozygosity) in Russian sturgeon was high (0.814–0.880; Table 3) except locus *Spl-168* (0.404, Table 3). The genetic diversity in sterlet approximated from 0.971 at locus *Spl-163* to 0.515 at locus *Afu-68* (Table 3). Locus *AfuB-68* was the most polymorphic one in Russian sturgeon (PIC-value 0.796, Table 3), analogously to locus *Spl-163* in sterlet (PIC-value 0.769; Table 3).

Recently, microsatellite DNA analyses have been applied in the management of cultured sturgeon species. ZHU et al. (2002) used microsatellite DNA markers in their analysis of genetic variation in Chinese sturgeon (*Acipenser sinensis*) and demonstrated high levels of polymorphism. Parentage analysis based on microsatellite DNA analysis described by ZHU et al. (2002) revealed a detectable proportion (5–10%) of artificially propagated individuals in a natural population of juveniles. RODZEN et al. (2004) applied microsatellites for parentage and relatedness analysis

Table 3

Expected heterozygosity ($H_{exp}$), observed heterozygosity ($H_{obs}$), number of alleles ($N_a$) and polymorphism information content (PIC) at studied microsatellite loci in Russian sturgeon and sterlet

| Lp. | Locus | Gatunek | $N_o$ | $H_{exp}$ | SD | $H_{obs}$ | SD | $N_a$ | *PIC*-value |
|---|---|---|---|---|---|---|---|---|---|
| 1 | *Afu-19* | Russian sturgeon | 46 | 0.774 | 0.004 | 0.880 | 0.034 | 9 | 0.732 |
| | | sterlet | 35 | 0.509 | 0.000 | 0.629 | 0.081 | 3 | 0.389 |
| 2 | *Afu-39* | Russian sturgeon | 46 | 0.785 | 0.046 | 0.880 | 0.034 | 11 | 0.743 |
| | | sterlet | 30 | 0.650 | 0.000 | 0.767 | 0.077 | 3 | 0.563 |
| 3 | *Afu-68* | Russian sturgeon | 45 | 0.776 | 0.066 | 0.833 | 0.039 | 14 | 0.735 |
| | | sterlet | 33 | 0.745 | 0.000 | 0.515 | 0.087 | 6 | 0.702 |
| 4 | *AfuB-68* | Russian sturgeon | 47 | 0.828 | 0.026 | 0.814 | 0.028 | 23 | 0.796 |
| | | sterlet | 35 | 0.723 | 0.062 | 0.586 | 0.059 | 8 | 0.676 |
| 5 | *Spl-163* | Russian sturgeon | 47 | 0.799 | 0.00 | 0.840 | 0.037 | 11 | 0.763 |
| | | sterlet | 35 | 0.809 | 0.000 | 0.971 | 0.028 | 7 | 0.769 |
| 6 | *Spl-168* | Russian sturgeon | 47 | 0.735 | 0.018 | 0.404 | 0.051 | 10 | 0.695 |
| | | sterlet | 35 | – | – | – | – | – | – |

in white sturgeon (*Acipenser transmontanus*), while Fopp-Bayat (2009) used microsatellite DNA fragments for identification of mixed groups of farmed Siberian sturgeon (*Acipenser baeri*). In sturgeons, the inheritance of microsatellite DNA loci has been studied, for example in lake sturgeon (*Acipenser fulvescens*) (PYATSKOWIT et al. 2001) and in Siberian sturgeon (FOPP-BAYAT 2008).

As the infrastructure for sturgeon farming in Poland is expanding, genetic management of broodstock in aquaculture is becoming very important for protection of sturgeon diversity. Microsatellite DNA can be applied in many studies on the management of sturgeon fishes, for example the use of microsatellite information in polyploid species, for parental localization, genetic tagging and for estimation of relatedness between possible future breeders.

## Conclusions

The present study has clearly demonstrated a potential of genetic assignment of Russian sturgeon and sterlet in aquaculture, providing farmers with a tool to monitor various aspects of production and management. The results of the present study could be applied to testing the purity (or hybrids identification) of hatchery broodstocks in Polish fish farms and increasing the efficiency of selective breeding. The results of the research will provide baseline data for the development of scientific management of farmed Russian sturgeon and sterlet in Poland.

## Acknowledgments

The study was supported by UWM grant number 0804.0206. We would like to thank Ms Elżbieta Fopp and Mr Andrzej Fopp from Wasosze Fish Farm for kindly providing fish for the study. We are also grateful to Paweł Woźnicki for laboratory assistance.

Translated by Dorota Fopp-Bayat Accepted for print 9.03.2010

## References

CHEBANOV M., BILLARD R. 2001. *The culture of sturgeons in Russia: production of juveniles for stocking and meat for human consumption*. Aquatic Living Resources, 14: 375–381.

CHISTIACOV D.A., HELLEMANS B., HALEY CH.S., LAW A.S., TSIGENOPOULUS. KOTULAS G., BERTOTTO D., Libertini A., Voleckaert F.A.M. 2005. *A microsatellite linkage map of the European Sea Bass Dicentrarchus labrax L*. Genetics, 170: 1821–1826.

CLIFFORD S.L., MCGINNITY P., FERGUSON A. 1998. *Genetic changes in Atlantic salmon population resulting from escaped juvenile farm salmon.* Journal of Fish Biology, 52: 118–127.

FONTANA F. 1994. *Chromosomal nucleolar organizer regions in four sturgeon species as markers of karyotype evolution in Acipenseriformes (Pisces).* Genome, 37: 888–892.

FOPP-BAYAT D. 2004. *Genetic identification of sturgeons and sturgeon-derived food products: meat and caviar.* Archives of Polish Fisheries, 12: 231–241.

FOPP-BAYAT D. 2008. *Inheritance of microsatellite loci in polyploid Siberian sturgeon (Acipenser baeri Brandt) based on uniparental haploids*. Aquaculture Research, 39: 1787–1792.

FOPP-BAYAT D. 2009**.** *Application of DNA fingerprint analysis for identification of mixed groups of Siberian sturgeon (Acipenser baeri Brandt).* Pol. J. Natur. Sci., 24: 169–176.

LUDWIG A., BELFIORE N.M., PITRACH Ch., SIVIRSKY V., JENNECKENS I. 2001. *Genome duplication events and functional reduction of ploidy levels in sturgeon (Acipenser, Huso, Scaphirhynchus).* Genetics, 158: 1203–1215.

MAY B., KRUEGER C.C., KINCAID H.L. 1997. *Genetic variation at microsatellite loci in sturgeon: primer sequence homology in Acipenser and Scaphirhynchus.* Canadian Journal of Fisheries and Aquatic Sciences, 54: 1542–1547.

MCCONNEL S.K.J., O'REILLY P., HAMILTON L., WRIGHT J.N., BENTZEN P. 1995. *Polymorphic microsatellite loci from Atlantic salmon (Salmo salar) – genetic differentiation of North-American and European populations.* Canadian Journal of Fisheries and Aquatic Sciences, 52: 1863–1872.

MCQUOWN E.C., SLOSS B.L., SHEEHAN R.J., RODZEN J., TRANAH G.J., MAY B. 2000. *Microsatellite analysis of genetic variation in sturgeon: new primer sequences for Scaphirhynchus and Acipenser*. Transactions of Amamerican Fish Society, 129: 1380–1388.

NIELSEN E.E., HANSEN M.M., LOESCHCKE V. 1996. *Genetic structure of European populations of Salmo salar L. (Atlantic salmon) inferred from mitochondrial-DNA*. Heredity, 77: 351–358.

NORRIS A.T. BRADLEY D.G. CUNNINGHAM E.P. 1999. *Microsatellite genetic variation between and within farmed and wild Atlantic salmon (Salmo salar) populations*. Aquaculture, 180: 247–264.

PARK S.D.E. 2001. *Trypanotolerance in West African cattle and the population genetic effects of selection.* Dissertation, University of Dublin.

PYATSKOWIT J.D., KRUEGER C.C., KINCAID H.L., MAY B. 2001. *Inheritance of microsatellite loci in the polyploid lake sturgeon (Acipenser fulvescens).* Genome, 44:185–191

RODZEN A.A., THOMAS R. FAMULA, BERNIE MAY. 2004. *Estimation of parentage and relatedness in the polyploid white sturgeon (Acipenser transmontanus) using a dominant marker approach for duplicated microsatellite loci.* Aquaculture, 232: 165–187.

TEGELSTRÖM, H. 1986. *Mitochondrial DNA in natural populations: an improved routine for the screening of genetic variation based on sensitive silver staining*. Electrophoresis, 7: 226–229.

WALSH P.S., METZGER D.A., HIGUCHI R. 1991. *Chelex 100 as a medium for simple extraction of DNA for PCR-based typing from forensic material*. Biotechniques, 10: 506–513.

WELSH A.B., BLUMBERG M., MAY B. 2003. *Identification of microsatellite loci in lake sturgeon, Acipenser fulvescens, and their variability in green sturgeon, A. medirostris*. Molecular Ecology Notes, 3: 47–55.

ZHU B., ZHOU F., CAO H., SHAO Z, ZHAO N., MAY B., CHANG, J. 2002. *Analysis of genetic variation in the Chinese sturgeon, Acipenser sinensis: estimating the contribution of artificially produced larvae in a wild population.* Journal of Applied Ichthyology, 18: 301–306.

POLISH JOURNAL OF NATURAL SCIENCES
Abbrev.: Pol. J. Natur. Sc., Vol 25(2): 182–189, Y. 2010

DOI 10.2478/v10020-010-0015-9

# HETEROGENEITY IN DEVELOPMENT OF THE OVARIES OF BLEAK, *ALBURNUS ALBURNUS* (L.) IN LAKE KORTOWSKIE IN NORTH-EASTERN POLAND*

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K e y w o r d s: *Alburnus alburnus*, development of ovaries, heterogeneity.

A b s t r a c t

Bleak ovaries during the period preceding the reproduction and during reproduction in Lake Kortowskie were studied as concerns reproductive diversity applying standard histological methods. Among 90 females, 88 represented batch spawning while 2 females the total spawning. Presence of female total spawners suggests that sexual maturity can occur in case of the crossbreeds of bleak with one of the total spawner species crossing with bleak.

# NIEJEDNORODNOŚĆ W ROZWOJU JAJNIKÓW UKLEI, *ALBURNUS ALBURNUS* (L.) W JEZIORZE KORTOWSKIM W PÓŁNOCNO-WSCHODNIEJ POLSCE

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Słowa kluczowe: *Alburnus alburnus*, rozwój jajników, niejednorodność.

A b s t r a k t

Zbadano, stosując standardowe metody histologiczne, jajniki 90 samic uklei w okresie poprzedzającym rozród i rozrodczym w Jeziorze Kortowskim. U 88 samic stwierdzono cechy porcyjnego tarła, a u 2 całkowitego. Występowanie samic o całkowitym tarle sugeruje, że może dochodzić do osiągania dojrzałości płciowej przez mieszańce uklei z którymś spośród czterech krzyżujących się z ukleją gatunków ryb (płoć, kleń, jelec, leszcz) o całkowitym tarle.

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* The study was financed from grant no. 0208.805, University of Warmia and Mazury in Olsztyn.

## Introduction

Among the species of polycyclic Teleostei fish there are species that are total spawners and species that are batch spawners. On the ovaries of the total spawner species, during the period preceding the reproductive season, vitellogenetic oocytes are present while there are no oocytes in the stage of *cortical alveoli*. The vitellogenetic oocytes, after reaching the stage of full maturity are excreted during spawning and their numbers determine the actual year fertility of those species. This is the determined fertility (HUNTER et al. 1992). In the ovaries of batch spawner females, during the period preceding the reproductive season, vitellogenetic oocytes that form the first batch of spawn and oocytes in the stage of *cortical alveoli*, of which consecutive batch of the spawn will form during the reproductive season are present (SAKUN, BUCKAÂ 1968). The year potential fertility is not determined before commencement of spawning and the species with that organization of ovaries development are referred to as species with undetermined fertility (HUNTER et al. 1992). Both the organization of the development of vitellogenetic oocytes in ovaries of female fish and the spawning type are characteristics of individual species (MURUA, SABORIDO-REY 2003). However, in rudd and silver bream – the batch spawning species, females that are total spawners are encountered (TRÂPICYNA 1975, KOPIEJEWSKA 1996, KOPIEJEWSKA 1997, KOPIEJEWSKA, KOZŁOWSKI 2006, 2007). Both species belong to the Leuciscinae subfamily characterized by a relatively large number of combinations of natural crossbreeds (KOTTELAT 1997, ÂKOVLEV et al. 2000) and both species cross with total spawner species: rudd with roach and bream, silver bream with bream (KUDERSKIJ 1956, WHEELER 1969, NIKOLÛKIN 1972, SCHWARTZ 1972, 1981, KENNEDY, FITZMAURICE 1973, BRASSINGTON, FERGUSON 1976, KUTUZOV 1983, ECONOMIDS, WHEELER 1989). The crossbreeds of those species reach sexual maturity (KUDERSKIJ 1956, WHEELER 1969, NIKOLÛKIN 1972, KUTUZOV 1983, KOPIEJEWSKA et al. 2004). Sexually mature females of rudd and bream crossbreeds (as indicated by experimental research) can be total as well as batch spawners (KOPIEJEWSKA et al. 2007). It can be assumed, as a consequence, that total spawner females in the populations of the rudd and silver bream can be the crossbreeds of rudd and silver bream with total spawner species. Bleak, *Alburnus alburnus,* is also a species belonging to the Leuciscinae subfamily (KOTTELAT 1997). It is characterized by batch spawning and it crossbreeds with total spawner species such as roach, chub, dace and bream (KUTUZOV 1983, BIAŁOKOZ, MŁYNIEC 2000). So far, sexually mature crossbreeds of bleak with those species are not known. The purpose of the presented study was to determine whether among the female bleaks there are total spawner females.

## Materials and Methods

During the years 2004 and 2005, during the period preceding reproduction and during reproduction season 90 female bleaks were collected from Lake Kortowskie (north-eastern Poland) – Table 1.

The central cuts of the right and left part of the ovaries of those females were fixed in buffer formalin, dehydrated and next placed in paraffin. Slices 7 µm thick were stained with haematoxylin and eosin (ZAWISTOWSKI 1986).

Table 1

Body length and weight of bleak females in Lake Kortowskie

| Date | n | Body length (Sl cm) | Body weight (g) |
|---|---|---|---|
| 02.06.2004 | 16 | 8.9–12.7 | 8.7–27.7 |
| 10.06.2004 | 2 | 9.0–9.8 | 9.2–9.9 |
| 25.06.2004 | 10 | 8.7–12.6 | 11.0–27.9 |
| 28.06.2004 | 5 | 10.5–12.7 | 15.0–30.0 |
| 14.07.2004 | 7 | 9.6–11.7 | 10.4–23.5 |
| 24.05.2005 | 22 | 9.3–12.2 | 11.7–24.8 |
| 01.06.2005 | 10 | 9.2–12.3 | 9.0–24.0 |
| 15.06.2005 | 9 | 9.6–12.3 | 11.5–26.0 |
| 24.06.2005 | 9 | 10.1–12.1 | 10.6–20.7 |

The oocytes development stages were determined according to SAKUN and BUCKAÂ (1968) as well as WALLACE and SELMAN (1981), TYLER and SUMPTEM (1996), MURUA and SABORIDO-REY (2003). In the ovaries the following stages were identified: primary growth, *cortical alveoli*, vitellogenesis and maturation. The percentage shares of oocytes in the stages of *cortical alveoli* and vitellogenesis were determined according to the formula by ABERCROMBIE after MARRABLE (1962):

$$N = \frac{n\mathrm{T}}{(T + D)}$$

where $N$ – numbers of oocytes, $n$ – number of cross sections of oocytes in a given maturity stage in three serial slices of the left and right part of the ovaries, T – slice thickness, D – arithmetic average of the diameters of 20 oocytes at a given stage of maturity. The Abercrombie formula in studies of that type can be applied which was proven in the work by KOPIEJEWSKA (2003).

## Results

Among 40 female bleaks collected for studies in 2004, 38 had the ovaries possessing characteristics of batch spawner and 2 with the characteristics of total spawner (Figure 1A, 1B, 1C, Table 2). In the ovaries of females showing the characteristics of batch spawner simultaneously two groups of oocytes: in the stage of vitellogenesis / maturing and oocytes in the stage of *cortical alveoli* were present. In the ovaries of one of the females possessing the characteristics of total spawner there were oocytes in the stage of maturing only and there were no oocytes in the stage of *cortical alveoli*. In the ovaries of the second female there were follicles

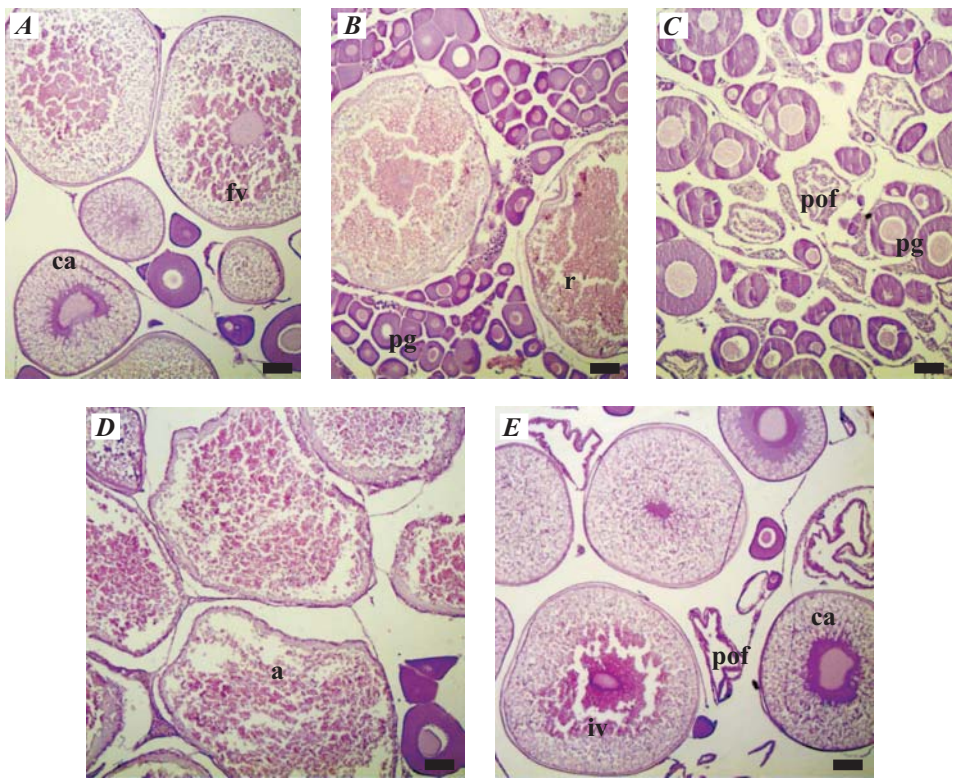


Fig. 1. Cross sections of ovaries of bleak from Lake Kortowskie: A – oocytes in the final stage of vitellogenesis (fv) and oocytes in the stage of *cortical alveoli* (ca) in ovaries of bleak during the period preceding spawning, B – oocytes that are ripe (r) and oocytes in the stage of primary growth (pg); absence of oocytes in the stage of *cortical alveoli* in the ovaries of the bleak 11.7 cm in length and 23.5 g in weight collected for studies on 14.07.2004, C – post-ovulatory follicles (pof) and oocytes at the stage of primary growth (pg); absence of oocytes in the stage of *cortical alveoli* in the ovaries of the bleak 10.3 cm in length and 12.1 g in weight collected for studies on 14.07.2004, D – atrophy of oocytes of the oldest generation (a) in the ovaries of the bleak collected for studies on 14.07.2004, E – post-ovulatory follicles (pof), oocytes in the initial stage of vitellogenesis (iv), oocytes in the stage of *cortical alveoli* (ca) in ovaries of the bleak after the first spawning. Scale = 100 µm

from ovulation of oocytes and there were no oocytes in the stage of *cortical alveoli*. During the period of June and the first half of July, only in the above mentioned female with characteristics of a total spawner the post-ovulatory follicles were observed while in case of the other females the oocytes of the eldest generations did not ovulate. At the end of June and in July the oocytes of those generations were subject to atrophy (Figure 1D).

All the females collected for the studies in 2005 had ovaries with characteristics of batch spawners. Oocytes in the stage of *cortical alveoli* were present in the ovaries simultaneously with the oocytes in the stage of vitellogenesis or maturing during the period preceding the reproduction season (Table 2) as well as after the first spawning simultaneously with the post-ovulatory follicles. Following the first spawning, in the eldest generations of oocytes in that stage, vitellogenesis started (Figure 1E).

Table 2

Share of oocytes in the stages of: vitellogenesis/maturing and *cortical alveoli* in the ovaries of bleak from Lake Kortowskie during the period preceding the reproductive season of the years 2004–2005

| Body length (cm) | Body weight (g) | Final stage of vitellogenesis/mature | | | Final stage of *cortical alveoli* | | | Initial stage of *cortical alveoli* | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | % | Ø µm | SD | % | Ø µm | SD | % | Ø µm | SD |
| Year 2004 | | | | | | | | | | |
| 9.4 | 9.7 | 52.3 | 736 | 35.83 | 39.5 | 481 | 51.52 | 8.2 | 350 | 23.45 |
| 10.3 | 13.2 | 50.2 | 967 | 79.73 | 38.3* | 504 | 105.53 | 11.5 | 317 | 30.55 |
| 10.8 | 16.2 | 38.0 | 914 | 59.41 | 50.1* | 500 | 77.20 | 11.9 | 300 | 7.07 |
| 12.0 | 27.9 | 51.2 | 962 | 38.83 | 33.2 | 533 | 50.82 | 15.6 | 287 | 43.10 |
| 12.5 | 27.3 | 41.2 | 862 | 62.24 | 44.3 | 467 | 69.65 | 14.5 | 292 | 40.40 |
| Year 2005 | | | | | | | | | | |
| 9.2 | 9.0 | 51.6 | 725 | 34.89 | 36.5* | 422 | 58.13 | 11.9 | 263 | 23.87 |
| 10.0 | 12.3 | 54.9 | 921 | 45.09 | 27.5* | 486 | 83.63 | 17.6 | 277 | 47.35 |
| 10.9 | 18.9 | 51.4 | 925 | 69.12 | 33.1 | 496 | 50.92 | 15.5 | 284 | 48.53 |
| 11.4 | 21.0 | 56.1 | 832 | 73.83 | 38.8* | 534 | 82.54 | 5.1 | 318 | 69.69 |
| 12.3 | 24.0 | 55.2 | 906 | 73.71 | 33.0* | 519 | 54.46 | 11.8 | 271 | 25.94 |

* oocytes at final and middle *cortical alveoli* stages

The first spawning of the bleak took place during the first half of the third decade of June. All the females examined, during that period, had post-ovulatory follicles in the ovaries. However, during the earlier period (May 24, June 1 and 15) in each sample of the females there was one female that has completed the spawning. In the ovaries of those females post-ovulatory follicles and oocytes at the initial stage of vitellogenesis were present.

## Discussion

The results obtained confirm that bleak is a batch spawner with undetermined fertility (HUNTER et al. 1992). The results showed that among batch spawner females, females that are total spawners with defined fertility can be found. Batch spawning, according to BURT et al. (1988), FORDHAM and TRIPPEL (1999) is linked to small body size and relatively small size of the ovaries. The bleak females that were total spawners in this study did not stand out by the body size and were within the range of body length and weight of batch spawner females. Similarly, in the silver bream populations, total spawner females were average or among the larger ones in the group of females that were batch spawners (KOPIEJEWSKA 1997, KOPIEJEWSKA, KOZŁOWSKI 2007). In the population of rudd, the female that was the total spawner was the smallest among the batch spawner females (KOPIEJEWSKA, KOZŁOWSKI 2006).

In the populations of *Cottus gobio* and *Noemacheilus barbatulus* (batch spawners) populations are found in which females spawn once a year. The changes in the number of the batches of oocytes are linked to the growth rate, environment productivity and geographic latitude at which the population is present (MANN et al. 1984). In the population of rudd in the Volga River delta, total spawning in some of the females was found three times during the periods of studies. In the ovaries of the females after spawning the oocytes in the stage of primary growth, *cortical alveoli* and numerous resorbed empty follicles were found (TRÂPICYNA 1975). Experimental studies on sexual maturing of crossbreeds of rudd and bream indicated that crossbreeding of fish species with different types of spawning results in different types of spawning in case of those crossbreeds (KOPIEJEWSKA et al. 2007). It can be assumed that total spawner rudds in the delta of the Volga as well as the total spawner bleaks from the presented studies could be the sexually mature crossbreeds with total spawner species. As a consequence, bleak was another species of Leuciscinae, the crossbreeds of which with Leuciscinae species that are total spawners with which bleak can crossbreed (KUTUZOV 1983) that achieves sexual maturity. The issue requires conducting studies on crossbreeds of bleak with species crossbreeding with it that are total spawners under experimental conditions.

## Conclusions

The studies showed that reproductive heterogeneity of females can be found in populations of bleak, which manifests in presence of females that are total spawners among the batch spawners. Presence of total spawner females suggests that sexual maturity can be reached by crossbreeds of bleak with one of the species crossbreeding with bleak (roach, silver bream, dace, bream) that is a total spawner.

## Acknowledgements

The photographs were prepared by Mr A. Koryzno, University of Warmia and Mazury in Olsztyn.

Translated by Jerzy Gozdek Accepted for print 13.04.2010

## References

Âkovlev V.N., Slyn'ko Û.V., Grečanov I.G., Krysanov E.Û. 2000. *Problema otdalennoj gibridizacii u ryb*. Vopr. Ikhtiol., 40(3): 312–326.

Białokoz W., Młyniec B. 2000. *Ukleja Alburnus alburnus.* [W:] *Ryby słodkowodne Polski*. Red. M. Brylińska, PWN, Warszawa, ss. 328–332.

Brassington R.A., Ferguson A. 1976. *Electrophoretic identification of roach* (*Rutilus rutilus* L.), *rudd* (*Scardinius erythrophthalmus* L.), *bream* (*Abramis brama* L.) *and their natural hybrids*. J. Fish Biol., 9: 471–477.

Burt A., Kramer D.L., Nakatsura K., Spry C. 1988. *The tempo of reproduction in Hyphessobrycon pulchripinnis (Characidae), with a discussion on the biology of multiple spawning in fishes*. Environmental Biology of Fishes, 22: 15–27.

Economidis P.S., Wheeler A. 1989. *Hybrids of Abramis brama* with *Scardinius erythrophthalmus* and *Rutilus rutilus from lake Volvi, Macedonia, Greece*. J. Fish Biol., 35: 295–299.

Fordham S.E., Trippel E.A. 1999. *Feeding behaviour of cod* (*Gadus morhua*) *in relation to spawning*. J. Appl. Ichthyol., 15: 1–9.

Hunter J.R., Macewicz B.J., Lo N.C.H., Kimbrell C.A. 1992. *Fecundity, spawning and maturity of female Dover sole*, *Microstomus pacificus*, *with an evaluation of assumptions and precision*. Fish. Bull. U.S., 90: 101–128.

Kennedy M., Fitzmaurice P. 1973. *Occurrence of cyprinid hybrids in Ireland*. Ir. Nat. J., 17: 349–351.

Kopiejewska W. 1996. *Germ line of white bream Blicca bjoerkna* (L.) *females in the Włocławek Dam Reservoir*. Acta Ichthyol. Piscat., 26(2): 35–47.

Kopiejewska W. 1997. *Oogeneza w aspekcie liczebnego dojrzewania oocytów u wybranych gatunków ryb kostnoszkieletowych Osteichthyes*. Acta Acad. Agric. Tech. Olst. Prot. Aqua. Piscat., B 22: 1–53.

Kopiejewska W. 2003. *Determination of frequency distribution of oocytes at different maturity phases in the ovaries of the roach Rutilus rutilus* (L.). Acta Ichthyol. Piscat., 33(1): 47–53.

Kopiejewska W., Terlecki J., Chybowski Ł. 2004. *Attainment of sexual maturity by hybrids of rudd*, *Scardinius erythrophthalmus* (L.) *and carp bream*, *Abramis brama* (L.) *under experimental conditions*. Acta Ichthyol. Piscat., 34(1): 43–50.

Kopiejewska W., Kozłowski J. 2006. *Reproductive biology of rudd Scardinius erythrophthalmus* (L.) *females in Lake Kortowskie in North-Eastern Poland*. Zool. Pol., 51(1–4): 21–32.

Kopiejewska W., Kozłowski J. 2007. *Development structure of ovaries in female white bream*, *Abramis bjoerkna* (L.) *from Lake Kortowskie in North-Eastern Poland*. Folia Zool., 56(1): 1–8.

Kopiejewska W., Terlecki J., Chybowski Ł. 2007. *Development of vitellogenic oocytes in ovaries of female reciprocal hybrids of rudd, Scardinius erythrophthalmus* (L.) *and carp bream*, *Abramis brama* (L.) *under experimental conditions.* Acta Sci. Pol. Piscaria, 6(3–4): 33–45.

Kottelat M. 1997. *European freshwater fishes*. Biology (s. zool.). Acad. Press Slovak. Bratislava, 52(5): 1–271.

Kuderskij L.A. 1956. *Pomes' leŝa i gustery v Kotkozere (ûžnaâ Kareliâ).* Trudy Karel'skogo Filiala AN SSSR 5: 152–153.

Kutuzov A.M. 1983. *Estestvennye gibridy gustery Blicca bjoerkna (L.) s uklejkoj Alburnus alburnus* (L.*) i leŝom Abramis brama (L.) (Cyprinidae) v Sviâžskom zaliže Kujbyševskogo vodohraniliŝa.* Vopr. Ikhtiol., 23(3): 373–381.

MANN R.H.K., MILLS C.A., CRISP D.T. 1984. *Geographical variation in the life – history tactics of some species of freshwater fish*. [W:] *Fish Reproduction: Strategies and Tactics*. Red. G.W. Potts, R.J. Wootton, Academic Press, New York, ss. 171–186.

MARRABLE A.W. 1962. *The counting of cells and nuclei in microtome sections*. Quarterly J. Microsc. Sci., 103: 331–347.

MURUA H., SABORIDO-REY F. 2003. *Female reproductive strategies of marine fish species of the North Atlantic*. J. Northwest Atlantic Fish. Sci., 33: 23–31.

NIKOLÛKIN N.I. 1972. *Otdalennaâ gibridizaciâ osetrovyh i kostistyh ryb*. Piŝevaâ Promyšlennost, Moscow.

SAKUN O.F., BUCKAÂ N.A. 1968. *Opredelenie stadii zrelosti i izučenie polovyh ciklov ryb*. Ministerstvo Rybnogo Hozâjstva SSSR, Polârnyj naučno-issledovatel'skij i proektnyj institut morskogo rybnogo hozâjstva i okeanografii im. Knipoviča, Murmansk.

SCHWARTZ F.J. 1972. *World literature on fish hybrids, with an analysis by family, species, and hybrid*. Publ. Gulf Coast Res. Lab. Mus., 32: 1–328.

SCHWARTZ F.J. 1981. *World literature on fish hybrids with an analysis by family, species, and hybrid*. Supplement 1. NOAA Technical Report NMFS SSRF (750), 1–507.

TRÂPICYNA L.N. 1975. *Ekologiâ krasnoperki i gustery delty Volgi v usloviâh Zaregulirovanego stoka*. Nauka, Moskva.

TYLER C.R., SUMPTER J.P. 1996. *Oocyte growth and development in teleosts*. Rev. Fish Biol. Fish., 6: 287–318.

WALLACE R.A., SELMAN K. 1981. *Cellular and dynamic aspects of oocyte growth in teleosts*. Am. Zool., 21: 325–343.

WHEELER A. 1969. *The fishes of the British Isles and North-West Europe*. Macmillan, London.

ZAWISTOWSKI S. 1986. *Technika histologiczna, histologia oraz podstawy histopatologii*. PZWL, Warszawa.

POLISH JOURNAL OF NATURAL SCIENCES
Abbrev.: Pol. J. Natur. Sc., Vol 25(2): 190–199, Y. 2010

DOI 10.2478/v10020-010-0016-8

# AN EXPERIMENTAL DEVICE FOR EGGS INCUBATION AND FISH LARVAE REARING UNDER LABORATORY CONDITIONS

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Key words: larviculture, eggs incubation, rearing device, experimental system, laboratory research.

Abstract

This paper presents and describes a practical application of an experimental system for spawn incubation and fish larvae rearing under laboratory conditions in a closed water circulation. The experiment determined the effectiveness of water filtration in the system during the rearing of larvae of selected ornamental fish and native cyprinids. The results have shown full usability and diversity of application of the system for experimental purposes. The results obtained during experimental rearings with the system can be successfully used in practice.

# EKSPERYMENTALNE URZĄDZENIE DO INKUBACJI IKRY ORAZ PODCHOWU LARW RYB W WARUNKACH LABORATORYJNYCH

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Słowa kluczowe: larwikultura, inkubacja ikry, urządzenie podchowowe, system eksperymentalny, badania laboratoryjne.

Abstrakt

W artykule przedstawiono praktyczne zastosowanie eksperymentalnego układu do inkubacji ikry oraz podchowu larw ryb w warunkach laboratoryjnych, pracujące w zamkniętym obiegu wody. Określono efektywność filtracji wody w układzie w trakcie podchowu larw wybranych gatunków ryb ozdobnych oraz rodzimych ryb karpiowatych. Uzyskane efekty wskazują na pełną przydatność oraz wszechstronność zastosowania opisywanego systemu do celów eksperymentalnych. Wyniki badań mogą być z powodzeniem zastosowane w praktyce.

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## Introduction

Currently, aquaculture plays two very important roles. It is a source of high quality healthy food but it is also a tool used to protect endangered fish populations or species (PHILIPPART 1995, BABIAK et al. 1998). Technology transfer, applied in the industrial production of aquatic organisms, aimed at the application of sustainable aquaculture, requires accurate and precise data on the procedures of reproduction biotechniques and rearing stocking material of various species. Of particular importance are data on techniques of inducing final gamete maturation, determination of their quality and methods of storage and handling (GLOGOWSKI et al. 1999, ŁUCZYŃSKI et al. 1997, KOWALSKI et al. 2003, SZCZERBOWSKI et al. 2009, ŻARSKI et al. 2009b, CEJKO et al. 2010, KUCHARCZYK et al. 2010). Data related to fish reproduction biotechniques and larvae rearing are obtained by experimental methods in scientific centres around the world. The process involves designing various devices which make it possible to examine several biotechnical indexes, such as stocking density, type of feed given, feeding frequency and temperature of eggs incubation and larvae rearing, in the optimum way (KUJAWA et al. 1999, 2000, HAMÁČKOVÁ et al. 2009). Devices which enable eggs incubation (KUCHARCZYK et al. 1996a, 2008a, KUJAWA et al. 1997) or rearing larvae, whose sensitivity requires special environmental conditions (SZCZERBOWSKI et al. 1997, KUJAWA 2004, WOLNICKI 2005, ŻARSKI et al. 2009a), are among the most frequently designed and constructed. Relatively good results have been achieved in rearing fry in illuminated cages (MAMCARZ et al. 1998, SKRZYPCZAK et al. 1998).

The effectiveness of production associated with sustainable aquaculture is affected by numerous factors. One of them is controlled reproduction which, for some species, is not possible without previous hormonal stimulation (PHILIPPART 1995, KREJSZEFF et al. 2008, 2009a, SZCZERBOWSKI et al. 2009, ŻARSKI et al. 2009b). The type of hormonal preparation and the way other manipulations are performed directly affect the quality of gametes and, consequently, the quality of the stocking material (KUCHARCZYK et al. 1998a, KREJSZEFF et al. 2008, YARON et al. 2009, CEJKO et al. 2010). The effectiveness of protocols associated with controlled reproduction and with genome manipulation or gamete cryopreservation is determined by various parameters; the percentage of live embryos in the eyed-egg stage or hatching stage are the most frequently used ones. To determine it, a small amount of eggs is incubated on Petri dishes or in small tanks (BABIAK et al. 1998, KUCHARCZYK et al. 1996b, 1997, GLOGOWSKI et al. 1999, ŁUCZYŃSKI et al. 1997, KREJSZEFF et al. 2008, 2009a, SZCZERBOWSKI et al. 2009, ŻARSKI et al. 2009b).

The effectiveness of larvae rearing depends on such factors as: temperature, photoperiod, stocking density, type and amount of feed given, feeding frequency, as well as physicochemical parameters of water (SZCZERBOWSKI et al. 1997, KUCHARCZYK et al. 1998b, KUJAWA 2004, WOLNICKI

2005, ŻARSKI et al. 2008b). However, the effect of each factor is species-dependent and should be considered separately (KUJAWA 2004, WOLNICKI 2005). Determination of the effect of one such factor is possible only if the others are maintained at the same level.

The aim of the study is to describe a device for spawn incubation and fish larvae rearing under laboratory conditions and its practical application for experimental purposes based on the published data.

## Materials and Methods

### Description of the rearing system

The rearing system consists of 18 small glass tanks with a total volume of 1.3 $dm^3$ (10×10×13 cm) each, which have one wall partially replaced with a net with a mesh size of 200 μm. This prevents larvae from escaping outside the tank, keeps the feed inside and enables free exchange of water

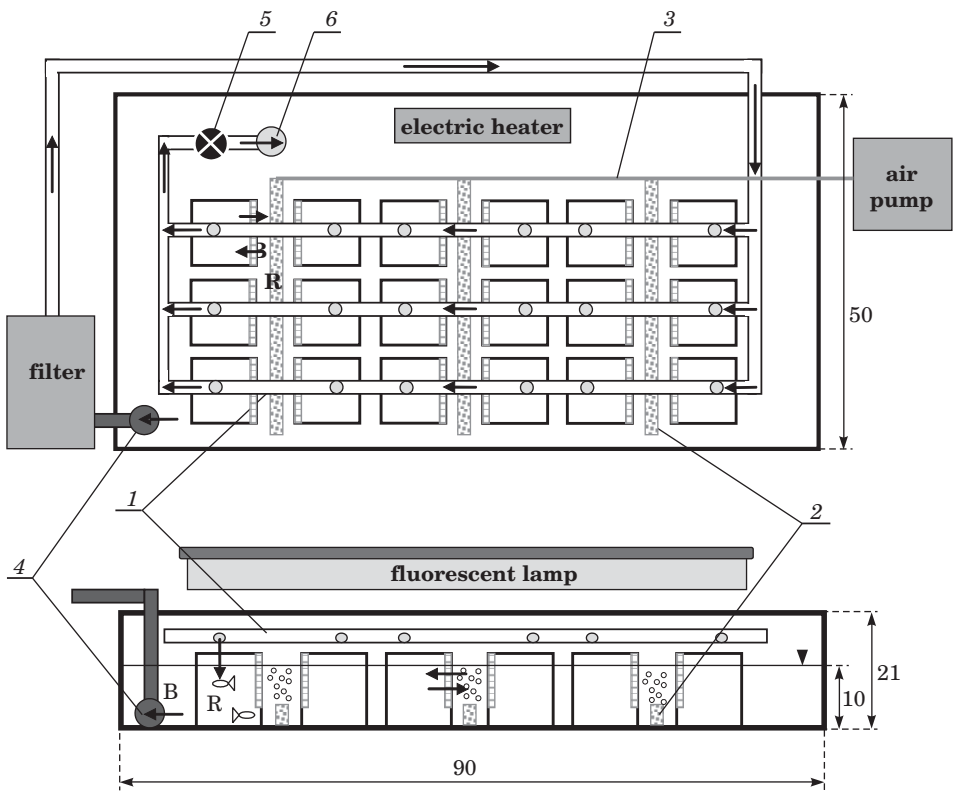


Fig. 1. Scheme of experimental rearing unit. R – rearing tank; B – bath tank; 1 – top-inlet (inlet opening – ○); 2 – aerating stone; 3 – air duct; 4 – outlet (●); 5 – valve regulating water pressure in top-inlet pipes; 6 – overflow. Arrows are showing direction of water flow and/or free circulation between R and B. Dimensions are presented in centimeters

between a rearing tank and a larger glass tank which plays the role of water bath (Figure 1). The bath size (working capacity 50 $dm^3$) enables free positioning of smaller tanks, placing a heater inside (power – 100 W), longitudinal aerating stones and devices draining water for filtration. The water in the bath is dammed up to such a height (10 cm), that it makes the working capacity of small tanks equal to 1 $dm^3$. Water temperature is regulated by a thermoregulator (±0.1ºC), and the entire system is lit by fluorescent light (30 W) with adjustable lighting time. The lamp is hung 30 cm above the water surface. Circulating water is filtered mechanically and biologically by an external Fluval 405 filter with a capacity of 8.5 $dm^3$, equipped with a pump, whose throughput is equal to 1300 $dm^3\ h^{-1}$. Water from the filter may be channelled directly to the water bath or, with a specially designed sprinkler, made of PVC pipes, supplying water separately to each rearing tank. The sprinkler has 18 holes with the diameter of about 1 mm and a water surplus overflow system with a valve to adjust water pressure in the sprinkler; increasing the valve lumen lowers the pressure, thereby reducing the amount of water flowing into the rearing tanks. It is possible to adjust water flow through the rearing tanks from 5 to 25 $dm^3\ h^{-1}$.

## Application of the system to eggs incubation

The device was used for incubation of eggs of selected fish species. To this end, eggs of cyprinids was obtained following hormonal stimulation by the method described by ŻARSKI et al. (2009b), eggs from ornamental fish – by following the procedures described by KUPREN et al. (2008a) and KREJSZEFF (2008) and that of perch – by reproduction following hormonal stimulation according to the procedure described by SZCZERBOWSKI et al. (2009). Depending on the species, fertilised eggs samples were placed in rearing tanks freely or with substrate (ornamental species), on Petri dishes (cyprinids) or fragmented ribbon (perch). The embryo survivability was determined at the eyed-egg stage. The hatching and deformation percentages were also determined.

## Application of the system to larvae rearing

A number of rearing operations were performed in the system under examination with a wide range of densities of larvae of selected species of ornamental fish and cyprinids. The content of ammonium nitrogen and nitrites was determined during the operations with the use of LF 205 photometer (Slandi, Poland). The content of dissolved oxygen was measured with an oxygen probe HI 91410 (Hanna Instrument, Italy). The larvae were measured at the beginning and at the end of each rearing. 30 individual fish were taken for measurement and put down

Table 1

Main characteristic of rearing procedures and results obtained during rearing of chosen fish species larvae (mean value ± SD)

| Species | Temperature (°C) | Rearing period (days) | Water flow ($dm^3\ h^{-1}$) | Maximum stocking density (ind. $dm^{-3}$) | Ammonia concentration (mg $dm^{-3}$) | Nitrites concentration (mg $dm^{-3}$) | Dissolved oxygen (%) | Survival (%) | Total length of larvae (mm) | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | | | Initial | Final |
| Buenos aires tetra (*Hemigrammus caudovittatus*) | 26 ± 0.1 | 25 | *D** | 200 | 0.01–0.05 | < 0.05 | 65–82 | 89.0 ± 7.56 | 4.25 ± 0.19 | 18.67 ± 2.07 |
| Siamese fighting fish (*Betta splendens*) | 27 ± 0.1 | 17 | *D** | 150 | 0.01–0.05 | < 0.05 | 61–80 | 89.5 ± 6.3 | 4.16 ± 0.14 | 13.23 ± 1.71 |
| Paradise fish (*Macropodus opercularis*) | 27 ± 0.1 | 17 | *D** | 150 | 0.01–0.05 | < 0.05 | 61–80 | 56.7 ± 2.0 | 3.46 ± 0.11 | 13.77 ± 1.37 |
| Dwarf gourami (*Colisa lalia*) | 27 ± 0.1 | 17 | *D** | 150 | 0.01–0.05 | < 0.05 | 61–80 | 44.0 ± 8.2 | 3.09 ± 0.06 | 13.50 ± 2.17 |
| Zebrafish (*Danio rerio*) | 27 ± 0.1 | 20 | *D** | 120 | 0.01–0.05 | < 0.05 | 62–85 | 51.1 ± 12.0 | 4.34 ± 0.08 | 15.89 ± 1.55 |
| Ide (*Leuciscus idus*) | 25 ± 0.1 | 21 | 6–10 | 400 | 0.01–0.1 | < 0.05 | 75–85 | 93.6 ± 1.2 | 8.57 ± 0.23 | 22.34 ± 2.27 |
| Asp (*Aspius aspius*) | 25 ± 0.1 | 21 | 6–10 | 400 | 0.01–0.1 | < 0.05 | 75–85 | 94.3 ± 0.7 | 9.6 ± 0.35 | 24.07 ± 1.86 |
| Chub (*Leuciscus cephalus*) | 25 ± 0.1 | 21 | 6–10 | 400 | 0.01–0.1 | < 0.05 | 75–85 | 85.0 ± 2.7 | 7.76 ± 0.21 | 20.79 ± 2.98 |
| Dace (*Leuciscus leuciscus*) | 25 ± 0.1 | 21 | 6–10 | 400 | 0.01–0.1 | < 0.05 | 75–85 | 89.4 ± 1.4 | 8.83 ± 0.65 | 20.33 ± 2.43 |
| Crucian carp (*Carassius carassius*) | 25 ± 0.1 | 21 | 6–10 | 600 | 0.01–0.1 | < 0.05 | 70–85 | 89.0 ± 3.2 | 5.49 ± 0.03 | 17.77 ± 0.38 |

* without top water inflow (*free circulation of water*)

in 2-fenoxyethanol solution (Sigma-Aldrich, Niemcy) (0.4 $cm^3$ $dm^{-3}$). The results were documented and analysed by ProgRes® Capture Pro 2.5 software (Jenoptik, Germany) – Table 1.

The same procedure was followed in each rearing operation, regardless of the species, fish density or size. Throughout the rearing the larvae were fed *ad libitum* with freshly hatched Artemia nauplii. The water in the rearing tanks was replaced in two ways. Due to their small initial sizes, ornamental species were reared without the use of a sprinkler and the water was replaced freely, through the net. The process was aided by water movement in the bath, caused by aeration. A sprinkler was used in the rearing of cyprinids. The photoperiod was set at 12 h (12L : 12D). Feed remnants and faeces were removed from the rearing tanks daily before the first feeding.

## Results and Discussion

Larvae rearing can be carried out in open, semi-closed or closed systems. It is much more beneficial economically to use a semi-closed or closed system than an open one. The benefits also apply to the fish health and the possibility of controlling the environmental conditions (Barak, Rijn van 2000, Żarski et al. 2008a). However, large amounts of nitrogen and phosphorus compounds are accumulated during the system use (Barak, Rijn van 2000, Żarski et al. 2008a). Such compounds, especially ammonium and nitrites, have a negative effect on fish (Randall, Tsui 2002). Also, being biogenic compounds, they increase primary productivity in open water bodies, thereby intensifying their eutrophication (Oliva-Teles et al. 1998, Barak, Rijn van 2000, Read, Fernandes 2003, Crab et al. 2007). Therefore, research into the development of recirculation systems and the dynamic nature of by-products has been going on for years (Rijn van 1996, Rijn van et al. 2006, Żarski et al. 2008a).

The system under investigation was tested during incubation of spawn of ornamental fish, cyprinids and perch in the course of experimental determination of the effectiveness of reproduction procedures and was comparable with the findings of other authors (Kupren et al. 2008a, Krejszeff 2009b, Targońska et al. – unpublished, Kupren et al. – unpublished, Szczerbowski et al. – unpublished). Owing to the small dimensions of tanks the frequent water replacement in each of them (if a sprinkler is used), it is possible to maintain the proper water parameters during incubation of species with even the greatest requirements. This especially concerns eggs incubation on Petri dishes, which has been successfully applied in reproduction biotechnology experiments (Targońska-Dietrich et al. 2004, Mamcarz et al. 2006, Krejszeff et al. 2008, 2009a, Żarski et al. 2009b). Moreover, embryos after hatching stay in the tank, where they can be observed and reared.

The system presented in this paper has a small total volume, but relatively high volume of the biological filter (17% of the entire system) as compared to the system described by ŻARSKI et al. (2008a) (6% of the entire system). The filter construction enables concurrent mechanical filtration, but using nets with such a small mesh prevented the feed from getting out of the rearing tank. As a result, it effectively prevented potential suspensions from entering the filter. Contamination of water only with dissolved substances (e.g. nitrogen compounds) significantly affected the nitrification effectiveness. The data obtained during pilot-scale rearing operations show that the effectiveness of biological water filtration is very high compared to the findings of other authors (e.g. SINGH et al. 1999, ŻARSKI et al 2008a), who recorded ammonia concentrations of 0.1 to 0.93 mg $dm^{-3}$. Intensive aeration (which enriched water with oxygen) enabled successful larvae rearing, as the results of mean larvae length measured in the rearing operations did not differ from data obtained by other authors in other rearing systems (e.g. KUJAWA 2004, WOLNICKI 2005, KWIATKOWSKI et al. 2008, ŻARSKI et al. 2008b). This also concerns the most important indicator – larvae survivability. Another important, although intangible, advantage of the system is its ease of operation by one person. Owing to the small numbers of the rearing tanks it is possible to maintain very good sanitary conditions, which may directly affect the experiment results.

The cost of the operation, which depends on the scale of the enterprise, is also a very important aspect (KUPREN et al. 2008b, TURKOWSKI et al. 2008, HAKUĆ-BŁAŻOWSKA et al. 2009). In experimental rearing, a small number of larvae can be used. This is especially important with valuable fish species and ornamental fish which produce small number of offspring (KREJSZEFF 2008, KUCHARCZYK et al. 2008b, 2010). The small amounts of water necessary to carry out the experiment is also important; if reconstituted water has to be used (a mixture of water obtained by reversed osmosis or distilled water or other necessary solutions and substances which modify water parameters, such as pH, hardness or salinity) (e.g. KANE et al. 1990) it can significantly reduce the cost of rearing. This also concerns the live feed used in the rearing, whose small amounts used are reflected in the scale of the necessary culture of feed organisms.

Translated by Jerzy Gozdek Accepted for print 16.03.2010

## References

BABIAK I., GŁOGOWSKI J., KUJAWA R., KUCHARCZYK D., MAMCARZ A. 1998. *Cryopreservation of sperm from asp Aspius aspius*. Progr. Fish-Cult., 60: 146–148.

BARAK Y., RIJN VAN J. 2000. *Biological phosphate removal in a prototype recirculating aquaculture treatment system*. Aquacult. Eng., 22: 121–136.

CEJKO B.I., KOWALSKI R.K., KUCHARCZYK D., TARGOŃSKA K., KREJSZEFF S., ŻARSKI D., GŁOGOWSKI J. 2010. *Influence of the length of time after hormonal stimulation on selected parameters of milt of ide Leuciscus idus L.* Aquacult. Res., 41: 804–813.

CRAB R., AVNIMELECH Y., DEFOIRDT T., BOSSIER P., VERSTRAETE W. 2007. *Nitrogen removal techniques in aquaculture for a sustainable production*. Aquaculture, 270: 1–14.

GŁOGOWSKI J., BABIAK I., KUCHARCZYK D., ŁUCZYŃSKI M., PIROS B. 1999. *Some properties of bream Abramis brama L. sperm and its cryopreservation*. Aquacult. Res., 30: 765–772.

HAKUĆ-BŁAŻOWSKA A., KUPREN K., TURKOWSKI K., TARGOŃSKA K., JAMRÓZ M., KREJSZEFF S., KWIATKOWSKI M., ŻARSKI D., KUCHARCZYK D. 2009. *Comparison of economic effectiveness of applying different hormonal preparations for reophile cyprinid fish reproduction stimulation based on the example of asp Aspius aspius (L.) and ide Leuciscus idus (L.)*. Pol. J. Natur. Sc., 24: 224–234.

HAMÁČKOVÁ J., PROKEŠ M., KOZÁK P., PEŇÁZ M., STANNY L.A., POLICAR T., BARUŠ V. 2009. *Growth and development of vimba bream (Vimba vimba) larvae in relation to feeding duration with live and/or dry starter feed*. Aquaculture, 287: 158–162.

KANE A.S., BENNET R.O., MAY E.B. 1990. *Effect of hardness and salinity on survival of striped bass larvae*. North Amer. J. Fish. Manag., 10: 67–71.

KOWALSKI R., GŁOGOWSKI J., KUCHARCZYK D., GORYCZKO K., DOBOSZ S., CIERESZKO A. 2003. *Proteolytic activity and electrophoretic profiles of proteases from seminal plasma of teleosts*. J. Fish Biol., 63: 1008–1019.

KREJSZEFF S. 2008. *Early development of the selected species of labyrinth fishes*. PhD Thesis. University of Warmia and Mazury, Olsztyn, Poland, 90p.

KREJSZEFF S., KUCHARCZYK D., KUPREN K., TARGOŃSKA K., MAMCARZ A., KUJAWA R., KACZKOWSKI Z., RATAJSKI S. 2008. *Reproduction of chub, Leuciscus cephalus L., under controlled conditions*. Aquacult. Res., 39: 907–912.

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

KREJSZEFF S., MAMCARZ A., KUCHARCZYK D., KUJAWA R., KUPREN K. 2009b. *Comparative rearing of three larval labyrinth fishes (Betta splendens, colisa lalia, and Macropodus opercularis) under controlled conditions*. Europ. Aquacult. Soc. Spec. Publ., 38: 213–216.

KUCHARCZYK D., KUJAWA R., MAMCARZ A. 1996a. *New experimental incubation unit for eggs of the perch Perca fluviatilis*. Progr. Fish-Cult., 58: 281–283.

KUCHARCZYK D., ŁUCZYŃSKI M.J., JANKUN M., ŁUCZYŃSKI M. 1996b. *Preliminary observations on the induction of triploidy in Abramis brama by cold shock*. Cytobios, 354: 153–160.

KUCHARCZYK D., ŁUCZYŃSKI M.J., ŁUCZYŃSKI M., GŁOGOWSKI J., BABIAK I., JANKUN M. 1997. *Triploid and gynogenetic bream (Abramis brama (L.)) produced by heat shock*. Pol. Arch. Hydrobiol., 44: 33–38.

KUCHARCZYK D., KUJAWA R., MAMCARZ A., SKRZYPCZAK A., WYSZOMIRSKA E. 1998a. *Induced spawning in perch, Perca fluviatilis L., FSH + LH with pimozide or metoclopramide*. Aquacult. Res., 29:131–136.

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

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

KUCHARCZYK D., TARGOŃSKA K., PRUSIŃSKA M., KREJSZEFF S., KUPREN K., KUJAWA R., MAMCARZ A. 2008b. *Reproduction of buenos aires tetra (Hemigrammus caudovittatus) under controlled conditions*. Pol. J. Natur. Sc., 23: 858–865.

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: 81–92.

KUJAWA R.J. 2004. *Biologiczne podstawy podchowu larw reofilnych ryb karpiowatych w warunkach kontrolowanych*. Rozprawy i monografie, 88. Wyd. UWM, Olsztyn.

KUJAWA R., KUCHARCZYK D., MAMCARZ A. 1997. *Effect of temperature on embryonic development of asp (Aspius aspius L.)*. Pol. Arch. Hydrobiol., 44: 139–143.

KUJAWA R., KUCHARCZYK D., MAMCARZ A. 1999. *A model system for keeping spawners of wild and domestic fish before artificial spawning*. Aquacult. Eng., 20:85–89.

KUJAWA R., MAMCARZ A., KUCHARCZYK D., SKRZYPCZAK A. 2000. *An experimental unit for rearing of larval freshwater fish*. Folia Univ. Agric. Stetin., Piscaria, 205: 103–108.

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

KUPREN K., TURKOWSKI K., KUCHARCZYK D., KREJSZFF S., ŻARSKI D., HAKUĆ-BŁAŻOWSKA A., TARGOŃSKA K., KWIATKOWSKI M., JAMRÓZ M., CZARKOWSKI T. 2008b. *Economic aspects of rearing larval asp, Aspius aspius (L.), and ide, Leuciscus idus (L.), in closed recirculating systems*. Arch. Pol. Fish., 16: 413–420.

KUPREN K., ŻARSKI D., KREJSZEFF S., KUCHARCZYK D., TARGOŃSKA K., MAMCARZ A. 2009. *The influence of stocking density on survival and growth of dace Leuciscus leuciscus (L.) larvae reared under laboratory conditions*. Europ. Aquacult. Soc. Spec. Publ., 38, 217–220.

KWIATKOWSKI M., ŻARSKI D., KUCHARCZYK D., KUPREN K., JAMRÓZ M., TARGOŃSKA K., KREJSZEFF S., HAKUĆ-BŁAŻOWSKA A., KUJAWA R., MAMCARZ A. 2008. *Influence of feeding natural and formulated diets on chosen rheophilic cyprinid larvae*. Arch. Pol. Fish., 16: 383–396.

ŁUCZYŃSKI M.J., GLOGOWSKI J., KUCHARCZYK D., ŁUCZYŃSKI M., DEMSKA-ZAKĘŚ K. 1997. *Gynogenesis in northern pike (Esox lucius L.) induced by heat shock – Preliminary data*. Pol. Arch. Hydrobiol., 44: 25–32.

MAMCARZ A., KUCHARCZYK D., KUJAWA R., SKRZYPCZAK A., FURGALA-SELEZNIOW G. 1998. *Ontogeny of feeding habits in northern pike, Esox lucius (Esocidae), larvae reared in illuminated cages*. Ital. J. Zool., 65: 251–253.

OLIVA-TELES A., PEREIRA J.P., GOUVEIA A., GOMES, E. 1998. *Utilisation of diets supplemented with microbial phytase by seabass (Dicentrarchus labrax) juveniles*. Aquat. Liv. Res., 11: 255–259.

PHILIPPART J.C. 1995. *Is captive breeding an effective solution for the preservation of endemic species?* Biol. Cons., 72: 281–295.

RANDALL D.J., TSUI T.K.N. 2002. *Ammonia toxicity in fish*. Mar. Poll. Bull., 45: 17–23.

READ P., FERNANDES T. 2003. *Management of environmental impacts of marine aquaculture in Europe*. Aquaculture, 226: 139–163.

RIJN VAN J. 1996. *The potential for integrated biological treatment systems in recirculating fish culture. A review*. Aquaculture, 139: 181–201.

RIJN VAN J., TAL Y., SHREIER H.J. 2006. *Denitrification in recirculating systems, Theory and applications*. Aquacult. Eng., 34: 364–376.

SINGH S., EBELING J., WHEATON F. 1999. *Water quality trials in four recirculating aquacultural system configurations*. Aquacultural Engineering, 20: 75–84.

SKRZYPCZAK A., MAMCARZ A., KUJAWA R., KUCHARCZYK D., FURGALA-SELEZNIOV G. 1998. *Feeding habits of larval Eurasian perch, Perca fluviatilis (Percidae)*. Ital. J. Zool., 65: 243–245.

SZCZERBOWSKI A., ZAKĘŚ Z., ŁUCZYŃSKI M.J., SZKUDLAREK M. 1997. *Maturation and growth of a stunted form of crucian carp (Carassius carassius L.) in natural and controlled conditions*. Pol. Arch. Hydrob., 44: 171–180.

SZCZERBOWSKI A., KUCHARCZYK D., MAMCARZ A., ŁUCZYŃSKI M.J., TARGOŃSKA K., KUJAWA R. 2009. *Artificial off-season spawning of Eurasian perch Perca fluviatilis L.*. Arch. Pol. Fish., 17: 95–98.

TARGOŃSKA-DIETRICH K., ZIELAZNY T., KUCHARCZYK D., MAMCARZ A., KUJAWA R. 2004. *Out-of-season spawning of cultured ide (Leuciscus idus L.) under controlled conditions*. EJPAU 7 (2): #02, www.ejpau.media.pl.

TURKOWSKI K., KUCHARCZYK D., KUPREN K., HAKUĆ-BŁAŻOWSKA A., TARGOŃSKA K., ŻARSKI D., KWIATKOWSKI M. 2008. *Economic aspects of the experimental rearing of asp, Aspius aspius (L.), ide, Leuciscus idus (L.), and dace, Leuciscus leuciscus (L.), under controlled conditions*. Arch. Pol. Fish., 16: 397–411.

WOLNICKI J. 2005. *Intensywny podchów wczesnych stadiów ryb karpiowatych w warunkach kontrolowanych*. Arch. Pol. Fish., 13: 5–87.

YARON Z., BOGOMOLNAYA A., DRORI S., BITON I., AIZEN J., KULIKOVSKY Z., LEVAVI-SIVAN B. 2009. *Spawning Induction in the Carp: Past Experience and Future Prospects – A Review*. Israeli J. Aquacult. – Bamidgeh, 61: 5–26.

ŻARSKI D., KUCHARCZYK D., TARGOŃSKA K., CHYŁA B., DOBROŁOWICZ A. 2008a. *Dynamics of changes in nitrogen and phosphorus compounds during intensive culture of ide Leuciscus idus (L.) in a recirculating system*. Arch. Pol. Fish., 16: 459–467.

ŻARSKI D., KUCHARCZYK D., KWIATKOWSKI M., TARGOŃSKA K., KUPREN K., KREJSZEFF S., JAMRÓZ M., HAKUĆ-BŁAŻOWSKA A., KUJAWA R., MAMCARZ A. 2008b. *The effect of stocking density on the growth and survival of larval asp Aspius aspius (L.) and European chub Leuciscus cephalus (L.) during rearing under controlled conditions*. Arch. Pol. Fish., 16: 371–381.

ŻARSKI D., SASINOWSKI W., KUCHARCZYK D., KWIATKOWSKI M., KREJSZEFF S., TARGOŃSKA K. 2009a. *Mass initial rearing of burbot Lota lota (L.) larvae under controlled conditions*. Pol. J. Natur. Sci., 24: 76–84.

ŻARSKI D., KUCHARCZYK D., TARGOŃSKA K., JAMRÓZ M., KREJSZEFF S., MAMCARZ A. 2009b. *Application of ovopel and ovaprim and their combinations in controlled reproduction of two reophilic cyprinid fish species*. Pol. J. Natur. Sc., 24: 235–244.

POLISH JOURNAL OF NATURAL SCIENCES
Abbrev.: Pol. J. Natur. Sc., Vol 25(2): 200–208, Y. 2010

DOI 10.2478/v10020-010-0017-7

# THE EFFECT OF PREPARED ACTIVATING LIQUID ON THE SURVIVABILITY OF IDE *LEUCISCUS IDUS* (L.) EMBRYOS

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K e y  w o r d s: gamete activation, fertilisation, activating liquid, ide, *Leuciscus idus*.

A b s t r a c t

A preliminary examination was carried out into the effect that various activating liquids have on the survivability of ide *Leuciscus idus* (L.) embryos. It was found to be similar (54–59%) in four study groups; the embryo survivability was found to have decreased (44%) only in the group where non-sterilised tap water was used for gamete activation. A negative correlation was found between water conductivity, salinity and sperm motility. A positive correlation was found between water conductivity, salinity and embryo survivability. A negative correlation between the sperm motility and the survivability of ide embryos at the eyed-egg stage shows that the fertilisation result is more affected by the duration of sperm movement than by the percentage of motile sperm.

# WPŁYW RODZAJU PRZYGOTOWANEGO PŁYNU AKTYWUJĄCEGO NA PRZEŻYWALNOŚĆ EMBRIONÓW JAZIA *LEUCISCUS IDUS* (L.)

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S ł o w a  k l u c z o w e: aktywacja gamet, zapłodnienie, płyn aktywujący, jaź, *Leuciscus idus*.

A b s t r a k t

Przeprowadzono wstępne badania wpływu różnych płynów aktywujących na przeżywalność embrionów jazia *Leuciscus idus* (L.). Stwierdzono, że w czterech grupach badawczych nie różniła się ona między sobą (54–59%). Jedynie w grupie, w której do aktywacji gamet użyto niesterylizowanej wody kranowej, odnotowano obniżenie przeżywalności embrionów

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(44%). Wykazano ujemną korelację między przewodnością wody oraz jej zasoleniem a ruchliwością plemników. Stwierdzono także dodatnią korelację między przewodnością wody i jej zasoleniem a przeżywalnością embrionów. Ujemna korelacja między ruchliwością plemników a przeżywalnością embrionów jazia w stadium zaoczkowania świadczy o większym wpływie na wyniki zapłodnienia czasu ruchu plemników niż odsetka ruchliwych plemników.

## Introduction

The stocking material production result is affected by many factors. The most important of them include gamete quality, percentage of fertilised eggs and water temperature during incubation (KUCHARCZYK et al. 2008b, TARGOŃSKA et al. 2008b, SZCZERBOWSKI et al. 2009). The significance of gamete quality (especially sperm in cyprinids) in controlled reproduction was examined, for example, by GLOGOWSKI et al. (1999), BABIAK et al. (1998), KOWALSKI et al. (2003) and CEJKO et al. (2010). Their findings show that the following factors affect fertilisation: percentage of motile sperm and their duration of movement and concentration. This has practical implications when it is necessary to apply liquids used to store sperm or to evaluate their motility, which can significantly affect the duration of sperm movement and motility. For example, 0.5% solution of NaCl positively affects those parameters, while the same saline at the concentration of 0.9% NaCl blocks sperm movement (KUCHARCZYK 1999, GLOGOWSKI et al. 1999).

A significant effect on the survivability of fertilised embryos is exerted by external biotic factors, mainly bacteria and parasitic moulds from the genera *Achlya* and *Saprolegnia* (CZECZUGA, MUSZYŃSKA 1999, CZECZUGA et al. 1999). Bacterial infections can be transmitted vertically, e.g. *Renibacterium salmoninarum* (BULLOCK et al. 1978, BRUNO, MUNRO 1986, EVELYN et al. 1986), or horizontally, e.g. *Aeromonas* sp. (BULLOCK, STUCKEY 1987). In the first case, infection takes place before ovulation or soon after it, through ovarian fluid in a female body cavity (EVELYN et al. 1984, LEE, EVELYN 1989). In horizontal infections, the source is usually water or the activating liquid used in egg fertilisation. The most sensitive place which is susceptible to infection is the open micropyle or pores in the egg shell. Bacteria cells can enter to oocytes as they are small enough to pass through micropyle. Although fish eggs contain substances which protect them against microorganism growth (YOUSIF et al. 1994), bacteria infiltration inside an egg may cause it to die. Dead eggs become food for moulds and a source of infection of further grains of eggs. Due to the risk of eggs infection, activating liquids used for fertilisation should be sterilised. Therefore, many methods of sterilisation are applied in aquacultural practice and many liquids are used, which are subjectively regarded as sterile. However, the actual properties and the effect of the liquids on spawning effectiveness, expressed as embryo survivability, has

not been corroborated by proper research. In hatchery practice, water available at the hatchery units is used as the gamete activating liquid.

The aim of the study was to determine the effect of sterilisation of activating liquid on fertilisation results, evaluated in the study as the survivability of embryos at the eyed-egg stage.

## Materials and Methods

Ide, *Leuciscus leuciscus* (L.), spawners were obtained from Lake Mosąg near Olsztyn. Following their delivery to the hatchery, they were divided into two groups according to sex and put into 1000 $dm^3$ tanks equipped with devices which enable controlling environmental conditions (KUJAWA et al. 1999). Fish reproduction was carried out according to the methodology described by KREJSZEFF et al. (2009). In order to induce final gamete maturity, the fish were administered Ovopel (Unic-Trade, Hungary) in the first injection and Ovaprim (Syndel, Canada) in the second, according to the methodology provided by ŻARSKI et al. (2009). Gametes were obtained from 12 females and 8 males. After eggs taken from all the females were mixed, small samples of eggs (100–200 grains) were placed on Petri dishes. 0.05 ml of sperm mixture was added to each sample, followed by specially prepared water to activate the gametes (Table 1). Five different liquids for fertilisation were prepared:

– water circulating in the hatchery system (total volume 1 $m^3$), which was chemically disinfected (cw group) 12 hours before the experiment (simulation of routine disinfection of hatchery devices prior to eggs incubation);
– water boiled (bw group) 12 hours before the experiment;
– tap water, disinfected (UVw group) with ultraviolet radiation (UV) in a mini-circulation with the total volume of 7 $dm^3$ with a UV lamp (JBL – AquaCristal UV-C 11W). The disinfection time – 12 hours;
– water obtained by reversed osmosis (ow group) 12 hours before the experiment;
– tap water (tw) put aside 12 hours before the experiment, non-sterilised.

Each type of water was prepared 12 hours before the planned spawning to make the experiment conditions uniform because some parameters (e.g. pH or hardness) change over time. Immediately before fertilisation, the temperature of each type of water was unified by placing the beakers with each liquid in a water bath at the temperature in which eggs were later incubated. The physicochemical parameters of water (electric conductivity, salinity, redox potential, content of dissolved oxygen, pH and temperature) were measured by a multiparametric device HI 9828 (Hanna Instruments, Italy) – Table 1.

Table 1

A listing of the examined parameters of the water used for gamete activation and sperm motility in the water. The data on the sperm motility marked with the same letter index are not statistically different

| Group | Conductivity µS cm$^{-1}$ | Salinity ‰ | ORP | $O_2$ | | pH | Temperature °C | Sperm motility % | Survival of embryos (%) |
|---|---|---|---|---|---|---|---|---|---|
| | | | | % | ppm | | | | |
| cw | 578 | 0.28 | 102 | 68 | 6.6 | 8.4 | 14.8 | $70^b$ | $55^a$ |
| bw | 652 | 0.32 | 126 | 93.9 | 9.2 | 8.25 | 14.6 | $45^c$ | $59^a$ |
| UVw | 625 | 0.31 | 31 | 83.4 | 8.4 | 8.71 | 15.1 | $85^a$ | $54^a$ |
| ow | 342 | 0.17 | 94 | 74.5 | 7.3 | 7.21 | 14.9 | $90^a$ | $57^a$ |
| tw | 14 | 0.01 | 77 | 79.8 | 7.1 | 6.02 | 14.9 | $90^a$ | $44^b$ |

Five minutes after gamete activation, the water used in fertilisation was poured off. Subsequently, Petri dishes with eggs stuck to their bottoms were poured over with water taken from the tank where incubation would take place. Incubation was then carried out in a closed water circulation. The water temperature during the activation and fertilisation processes was 14–15°C, which is the optimum value for ide embryo growth (KUCHARCZYK et al. 2008b, KUPREN et al. 2008). Sperm motility was assessed subjectively under a microscope (magnification 500X) (GLOGOWSKI et al. 1999). Embryo survivability was determined at the eyed-egg stage. The experiment was carried out in three replications.

Embryo survivability and sperm motility data for different groups was subjected to arcsine transformation, which was followed by an analysis of variance (ANOVA) and *post-hoc* Tukey's test ($\alpha = 0.05$). The relationships between the parameters in question and the percentage of motile sperms and embryo survivability at the eyed-egg stage was examined using linear correlation.

## Results

Sperm motility in different types of water prepared for gamete activation is shown in Table 1. Sperm motility decreased from 85–90% to 70% in group cw, and to 45% in group bw. The type of water used in fertilisation affected ide embryo survivability at the eyed-egg stage. Survivability in the group in which non-sterilised tap water (tw) was used for gamete activation was statistically lower (44%) compared to the other groups (54–59%), while no significant differences were found between the latter. The highest survivability was recorded in the group in which boiled water was used for fertilisation.

An correlation analysis between the parameters under consideration revealed a negative correlation between sperm motility and water

conductivity and salinity (Figure 1 and Figure 2). An analysis of the two latter parameters and embryo survivability to the eyed-egg stage revealed a significant relationship. An increase in salinity to 0.32‰ positively affected embryo survivability (Figure 3). Moreover, the results indicate a positive correlation between water conductivity and embryo survivability (Figure 4). A negative correlation was also found between sperm motility and embryo survivability (Figure 5).

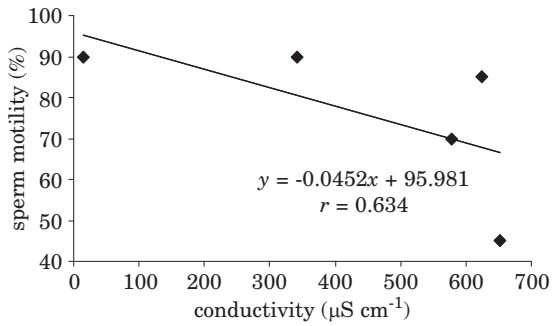


Fig. 1. The relationship between conductivity of water used for gamete activation and sperm motility in it

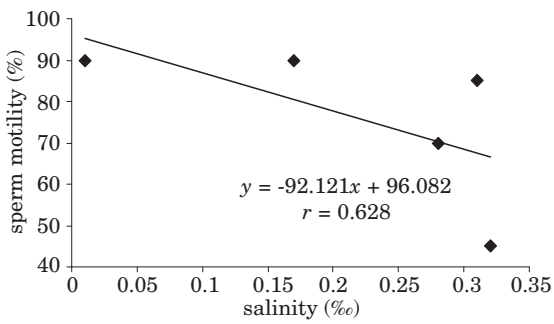


Fig. 2. The relationship between salinity of water used for gamete activation and sperm motility in it

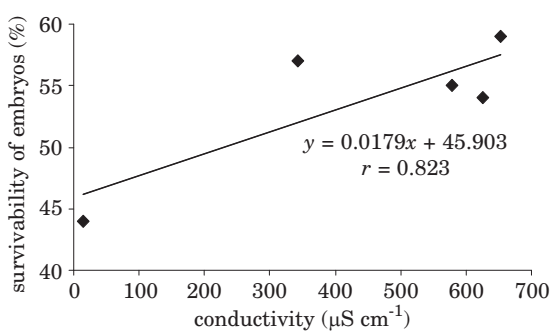


Fig. 3. The relationship between conductivity of water used for gamete activation and survivability of embryos to the eyed-egg stage

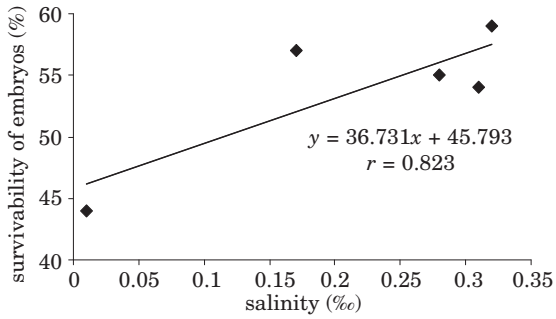


Fig. 4. The relationship between salinity of water used for gamete activation and survivability of embryos to the eyed-egg stage

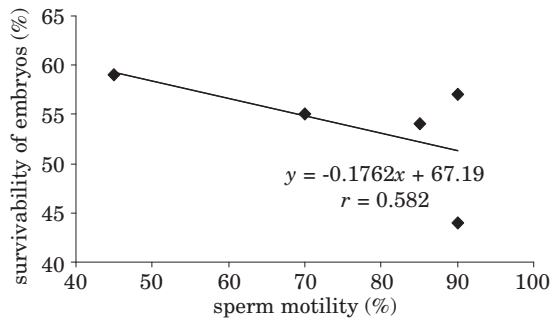


Fig. 5. The relationship between motility of sperm used for spawn fertilisation and survivability of embryos to the eyed-egg stage

## Discussion

Great progress has been observed in recent years in the controlled reproduction of cyprinids, including the ide (KUCHARCZYK et al. 1997a, 1997b, 1997c, 2008b, TARGOŃSKA et al. 2008a, KREJSZEFF et al. 2009, ŻARSKI et al. 2009). However, there is still a problem of variable survivability of embryos achieved in controlled reproduction. According to KUCHARCZYK et al. (2008b), ide embryo survivability, recorded at the eyed-egg or hatching stage, usually fluctuates within the range of ±50% and is lower than the values recorded for other cyprinids, such as the chub *Leuciscus cephalus* (L.), the asp *Aspius aspius* (L.) or the common nase *Chondrostoma nasus* (L.) (TARGOŃSKA et al. 2008b). One of the methods of seeking improvement of ide embryo survivability was the use of new hormone agents in controlled reproduction. Improvement of embryo survivability was achieved following the application of Ovaprim, as compared to Ovopel – the most commonly used agent (ŻARSKI et al. 2009). It is noteworthy that the final effectiveness of breeding measures is affected by indirect factors, such as incubation temperature – in the case of the burbot *Lota lota* L. (ŻARSKI et al. 2010), the asp (KUJAWA et al. 1997) or the bream

*Abramis brama* (L.) (KUCHARCZYK et al. 1997d, 1998), as well as – recorded for characids – the number of reproduction cycle repetitions (KUCHARCZYK et al. 2008c, 2010).

One of the factors which affects fertilisation and, consequently, embryo survivability is the quality of oocytes and semen and the number of sperm per egg (RURANGWA et al. 1998, 2004, KUCHARCZYK 2008a, 2008b). The quality of semen is expressed by percentage of motile sperm, the duration of their movement, plasma composition, sperm damage, etc. (GLOGOWSKI et al. 1999, BABIAK et al. 1998, RURANGWA et al. 1998, 2004, LAHNSTEINER et al. 2003, KOWALSKI et al. 2003, CEJKO et al. 2010, TARGOŃSKA et al. 2008a). It is much more difficult to determine the biological quality of oocytes, whose most frequent determinant is embryo survivability at the eyed-egg or hatching stage, which requires both proper conditions of fertilisation and incubation (KUCHARCZYK et al. 2005, KREJSZEFF et al. 2008, ŻARSKI et al. 2008, 2009).

The type of liquid used for gamete activation also affects egg fertilisation (ŁUCZYŃSKI et al. 1997, RODINA et al. 2004, KUJAWA et al. 2009), by reducing or extending the duration of sperm movement and egg activation time to micropyle closing (AMANZE, IYENGAR 1990, COSSON et al, 2008). It has been found in this study that the way an activating liquid is prepared, including the way it is sterilised, is important in affecting sperm motility. A statistically significant negative correlation has been found between water conductivity, salinity and sperm motility (Figure 1, Figure 2). However, a comparison of the two parameters of water with embryo survivability at the eyed-egg stage reveals an opposite relationship. An increase in salinity and conductivity of water used in fertilisation was accompanied by an increase in the percentage of live embryos (Figure 3, Figure 4). Interestingly, a negative correlation was found between sperm motility and embryo survivability (Figure 5). According to the findings of a study by TARGOŃSKA et al. (2008a), ide embryo survivability is more affected by duration of sperm movement than by motility. The results seem to corroborate the claim. An absence of a positive correlation between sperm motility and embryo survivability must result not only from different duration of sperm movement but also from different time of micropyle closing (AMANZE and IYENGAR 1990, COSSON et al. 2008).

The findings of this study show an important problem for fishery and aquaculture economy. The manner of preparation of water used for gamete activation directly affects the effectiveness and, consequently, profitability of controlled reproduction of the ide. Therefore, the same methods of controlled reproduction in various fish farms with different water characteristics may yield different results as the differences found in this study corroborate a significant relationship between embryo survivability and the liquid activating gametes used. Apparently small differences in salinity or conductivity may result in a considerable increase in the percentage of live embryos.

Translated by Jerzy Gozdek Accepted for print: 16.03.2010

## Reference


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

BABIAK, I., GLOGOWSKI, J., KUJAWA, R., KUCHARCZYK, D., MAMCARZ, A. 1998. *Cryopreservation of asp, Aspius aspius (L.) sperm*. Progr. Fish-Cult., 60: 146–148.

BRUNO D.W., MUNRO A.L.S. 1986. *Observations on Renibacterium salmoninarum and the salmonid egg*. Dis. Aquat. Org., 1: 83–87.

BULLOCK G.L., STUCKEY H.M., MULCAHY D. 1978. *Coryaebacterial kidney disease: Egg transmission following iodophore disinfection*. Fish Health News, 7: 51–52.

BULLOCK G.L., STUCKEY H.M. 1987. *Studies of vertical transmission of Aeromonas salmonicida*. Prog. Fish Cult., 49: 302–302.

CEJKO B.I., KOWALSKI R.K., KUCHARCZYK D., TARGOŃSKA K., KREJSZEFF S., ŻARSKI D., GLOGOWSKI J. 2010. *Influence of the length of time after hormonal stimulation on selected parameters of milt of ide Leuciscus idus L*. Aquacult. Res., 41: 804–813.

COSSON J., GROISON A.L., SUQUET M., FAUVEL C., DREANNO C., BILLARD R. 2008. *Marine fish spermatozoa: racing ephemeral swimmers*. Reproduction, 136: 277–294.

CZECZUGA B., MUSZYŃSKA E. 1999. *Aquatic fungi growing on percid fish eggs (Percidae) in Poland*. Pol. J. Environ. Stud., 8(1): 31–34.

CZECZUGA B., MUSZYŃSKA E., WOSSUGHI G., KAMALY A., KIZIEWICZ B. 1999. *Aquatic fungi growing on the eggs of several species acipenserid fishes*. Acta Ichthyol. Et Piscat., 25(2): 71–79.

EVELYN T.P.T., KETCHESON J.E., PROSPERI-PORTA L. 1984. *Further evidence for the presence of Renibacterium salmoninamm in salmonid eggs and the failure of providoneiodine to reduce the intra-ovum infection rate in waterhardened eggs*. J. Fish Dis., 7: 173–182.

EVELYN T.P.T., PROSPERI-PORTA L., KETCHESON J.E. 1986. *Experimental intra-ovum infection of salmonid eggs with Renibacterium salmoninarum and vertical transmission of the pathogen with such eggs despite their treatment with erythromycin*. Dis. Aquat. Org., 1: 197–202.

GLOGOWSKI J., BABIAK I., KUCHARCZYK D., LUCZYNSKI M., PIROS B. 1999. *Some properties of bream Abramis brama L. sperm and its cryopreservation*. Aquacult. Res., 30: 765–772.

KOWALSKI R., GLOGOWSKI J., KUCHARCZYK D., GORYCZKO K., DOBOSZ S., CIERESZKO A. 2003. *Proteolytic activity and electrophoretic profiles of proteases from seminal plasma of teleost*. J. Fish Biol., 63: 1008–1019.

KREJSZEFF S., KUCHARCZYK D., KUPREN K., TARGOŃSKA K., MAMCARZ A., KUJAWA R., KACZKOWSKI Z., RATAJSKI S. 2008. *Reproduction of chub, Leuciscus cephalus L., under controlled conditions*. Aquacult. Res., 39: 907–912.

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. 1999. *Genetic inactivation of ide (Leuciscus idus L.) sperm using UV irradiation*. Cytobios, 392: 149–158.

KUCHARCZYK D., KUJAWA R., ŁUCZYŃSKI M., GLOGOWSKI J., BABIAK I., WYSZOMIRSKA E. 1997a. *Induced spawning in bream, Abramis brama (L.), using carp and bream pituitary extract and hCG*. Aquacult. Res., 28: 139–144.

KUCHARCZYK D., KUJAWA R., MAMCARZ A., WYSZOMIRSKA E. 1997b. *Artificial spawning in bream (Abramis brama L.)*. Pol. Arch. Hydrobiol., 44: 203–207.

KUCHARCZYK D., KUJAWA R., MAMCARZ A., WYSZOMIRSKA E. 1997c. *Induced spawning in rudd (Scardinius erythrophthalmus L.)*. Pol. Arch. Hydrobiol., 44: 207–211.

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

KUCHARCZYK D., ŁUCZYŃSKI M., KUJAWA R., KAMIŃSKI 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-DIETRICH K., WYSZOMIRSKA E., GLOGOWSKI J., BABIAK I., SZABO T. 2005. *Induced spawning in bream (Abramis brama L.) using pellets containing GnRH*. Czech J. Anim. Sci., 50(3): 89–95.

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

KUCHARCZYK D., TARGOŃSKA K., ŻARSKI D., KUJAWA R., MAMCARZ A. 2008b. *A review of the reproduction biotechnology for fish from the genus Leuciscus*. Arch. Pol. Fish., 16(4): 319–340.

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

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

KUJAWA R., MAMCARZ A., KUCHARCZYK D. 1997. *Effect of temperature on embryonic development of asp (Aspius aspius L.)*. Pol. Arch. Hydrobiol., 44: 139–143.

KUJAWA R., KUCHARCZYK D., MAMCARZ A. 1999. *A model system for keeping spawners of wild and domestic fish before artificial spawning*. Aquacult. Eng., 20: 85–89.

KUJAWA R., KUCHARCZYK D., MAMCARZ A. 2009. *The effect of tannin concentration and egg unsticking time on the hatching success of tench Tinca tinca (L.) larvae*. Rev. Fish Biol. Fish., (in press).

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

LAHNSTEINER F., BERGER B., WEISMANN T. 2003. *Effect of media, fertilization technique, extender, straw volume and sperm to egg ratio on hatchability of cyprinid embryos, using cryopreserved semen*. Theriogenology, 60: 829–841.

LEE E.G.H., EVELYN T.P.T. 1989. *Effect of Renibacterium salmoninarum levels in the ovarian fluid of spawning Chinook salmon on the prevalence of the pathogen in their eggs and progeny*. Dis.Aquat. Org., 7: 179–184.

ŁUCZYŃSKI M.J., GLOGOWSKI J., KUCHARCZYK D., ŁUCZYŃSKI M., DEMSKA-ZAKĘŚ K. 1997. *Gynogenesis in northern pike (Esox lucius L.) induced by heat shock – Preliminary data*. Pol. Arch. Hydrobiol., 44: 25–32.

RODINA M., COSSON J., GELA D. AND LINHART O. 2004. *Kurokura solution as immobilization solution for spermatozoa of tench (Tinca tinca L.)*. Aquacult. Int., 12: 119–131.

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 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.

SZCZERBOWSKI A., KUCHARCZYK D., MAMCARZ A., ŁUCZYŃSKI M.J., TARGOŃSKA K., KUJAWA R. 2009. *Artificial off-season spawning of Eurasian perch Perca fluviatilis L.*. Arch. Pol. Fish., 17: 95–98.

TARGOŃSKA K., KUCHARCZYK D., MAMCARZ A., GLOGOWSKI J., KREJSZEFF S., PRUSIŃSKA M., KUPREN K. 2008a. *Influence of individual variability in the percentage of motile spermatozoa and motility time on the survival of embryos of chosen fish species*. Pol. J. Natur. Sc., 23(1): 178–187.

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

YOUSIF A.N., ALBRIGHT L.J., EVELYN T.P.T. 1994. *In vitro evidence for the antibacterial role of lysozyme in salmonid eggs*. Dis. Aquat. Org., 19: 15–19.

ŻARSKI D., TARGOŃSKA K., RATAJSKI S., KACZKOWSKI Z., KUCHARCZYK D. 2008. *Reproduction of nase, Chondrostoma nasus (L.) under controlled conditions*. Arch. Pol. Fish., 16(4): 355–362.

ŻARSKI D., KUCHARCZYK D., TARGOŃSKA K., JAMRÓZ M., KREJSZEFF S., MAMCARZ A. 2009. *Application of Ovopel, Ovaprim and their combination in artificial reproduction of two rheophilic cyprinid fishes*. Pol. J. Natur. Sc., 24(4): 235–244.

ŻARSKI D., KUCHARCZYK D., SASINOWSKI W., TARGOŃSKA K., MAMCARZ A. 2010. *The influence of temperature on successful reproduction of burbot Lota lota L. under hatchery conditions*. Pol. J. Nat. Sc., 25: 93–105.

POLISH JOURNAL OF NATURAL SCIENCES
Abbrev.: Pol. J. Natur. Sc., Vol 25(2): 209–214, Y. 2010

DOI 10.2478/v10020-010-0018-6

# CRANBERRY AND STRAWBERRY JUICES – INFLUENCE OF METHOD PRODUCTION ON ANTIOXIDANTS CONTENT AND ANTIOXIDATIVE CAPACITY

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Key words: juices, cranberry, strawberry, antioxidants, DPPH• radical scavenging.

Abstract

The objective of this study was to analyze fresh and pasteurized cranberry and strawberry juices, based on the content of polyphenols, anthocyanins and ascorbic acid, as well as DPPH• radical scavenging capacity. Significant differences were found between the investigated juices, dependent on both fruit species and the technological process. Cranberry juices were characterized by higher concentrations of the tested compounds, except for anthocyanins, and by greater DPPH• radical scavenging capacity. Pasteurization was found to exert a significant, destructive effect on the properties of the examined juices, particularly on the anthocyanin content of strawberry juice.

# SOKI Z ŻURAWINY I TRUSKAWKI – WPŁYW SPOSOBU OTRZYMYWANIA NA ZAWARTOŚĆ ZWIĄZKÓW PRZECIWUTLENIAJĄCYCH I POJEMNOŚĆ PRZECIWUTLENIAJĄCĄ

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Słowa kluczowe: soki, żurawina, truskawka, przeciwutleniacze, zmiatanie rodników DPPH•.

Abstrakt

W pracy oceniano soki świeże oraz utrwalone przez pasteryzację z owoców żurawiny błotnej i truskawki pod względem zawartości polifenoli, antocyjanów, kwasu askorbinowego oraz zdolności zmiatania rodników DPPH•. Wykazano istotne różnice zależne zarówno od gatunku owoców, jak i od procesu technologicznego. Większą zawartością analizowanych związków, poza antocyjanami, oraz większą zdolnością zmiatania rodników DPPH• charakteryzowały się soki z żurawiny. Stwierdzono znaczący, destrukcyjny wpływ pasteryzacji, zwłaszcza na antocyjany soku z truskawki.

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## Introduction

Cranberries and strawberries are grown in temperate climates. Cranberry and strawberry fruits are excellent raw materials for juice production, as they contain numerous antioxidants including phenolic compounds, vitamin C, minerals and many other. Their health-promoting properties include antioxidative activity (HANNUM 2004, SZAJDEK, BOROWSKA 2008, BOROWSKA et al. 2009a). Apart from the genetic characters of raw materials, also the conditions of the technological process exert a significant effect on the concentrations of antioxidants in juices and on their final properties (DIETRICH et al. 2004, LANDBO, MEYER 2004, BOROWSKA et al. 2009b). The release of antioxidants into the juice is considerably affected by the parameters of unit operations during processing, such as fruit crushing and mash heating, as well as by the type of enzymatic preparation used for mash maceration, and juice pressing conditions (OSZMIAŃSKI, SOŻYŃSKI 1989, PŁOCHARSKI, MARKOWSKI 2003, LANDBO, MEYER 2004, BAGGER-JØRGENSEN, MEYER 2004, BUCHERT et al. 2005, SZAJDEK et al., 2009). Of particular note is fruit mash maceration prior to juice pressing. Under industrial conditions, fruit mash is subjected to thermal processing or is treated with highly specific enzymes that act upon cell wall polysaccharides (HELBIG 2001, HILZ et al. 2005, URLAUB 2005). The application of enzymatic preparations facilitates juice extraction and improves the extractability of phenolic compounds. However, enzymes often contribute to the destruction of antioxidants, followed by a decline in antioxidant capacity and undesirable changes in color and flavor (KADER et al. 1999, SKREDE et al. 2000).

The objective of this study was to analyze fresh and pasteurized cranberry and strawberry juices, based on the content of polyphenols, anthocyanins and ascorbic acid, as well as DPPH$^{\bullet}$ radical scavenging capacity. The analyzed juices differed with regard to the method of fruit mash treatment prior to pressing. It should be stressed that enzymatic preparations were not used for mash maceration in this experiment, which means that juices of that type can be produced directly at eating places and in agritourism farms.

## Materials and Methods

Cranberries were picked up from their natural habitat near Olsztyn, and strawberries cv. Senga Sengana were purchased on a plantation near Olsztyn. The juices used in the study were produced under laboratory conditions. Fruits were crushed in a laboratory food processor (type ZM Mesko), and the mash was divided into two parts. One part was subjected to juice pressing with a laboratory hydraulic press (ZPBB Bydgoszcz), to

obtain fresh control juice (I, I'). Half volume of the juice was pasteurized in jars with twist-off caps at a temperature of 100ºC for 10 min., to obtain pasteurized juice (II, II'). The other part of fruit mash was heated at a temperature 85ºC for 5 min. before juice pressing. The further procedure was the same as above. Non-pasteurized juice after mash treatment (III, III') and pasteurized juice after mash treatment (IV, IV') were obtained.

Juice samples were assayed for the content of: total phenolic compounds (as gallic acid equivalent) – as described by SINGLETON and ROSSI (1965), anthocyanins (as cyanidin-3-glucoside) – by the method proposed by WROLSTAD (1976), ascorbic acid – according to the Polish Standard (Przetwory owocowe... PN-90/A-75101/11) and DPPH$^{\bullet}$ radical scavenging capacity (as μmol trolox/ml juice) – as described by BRAND-WILLIAMS et al. (1995). All analyses were performed in triplicate.

The results were verified statistically by a one-factor analysis of variance and Duncan's test, at a significance level of $P < 0.05$, using STATISTICA 8.0 software.

## Results and Discussion

Significant differences were found between cranberry and strawberry juices, regarding the concentrations of the analyzed antioxidants and DPPH$^{\bullet}$ radical scavenging capacity (Table 1). Cranberry juices, produced

Table 1

Antioxidants and DPPH$^{\bullet}$ radical scavenging of cranberry and strawberry juices

| Type of juice | Total phenols (mg/l) | Anthocyanins (mg/l) | Ascorbic acid (mg/100 g) | DPPH$^{\bullet}$ radical scavenging (μmol trolox/ml) |
|---|---|---|---|---|
| Cranberry juices | | | | |
| Fresh – control (I) | $1272^{a} \pm 13$ | $134.6^{c} \pm 1.1$ | $17.5^{b} \pm 0.2$ | $10.66^{b} \pm 0.13$ |
| Pasteurized (II) | $1256^{a} \pm 14$ | $86.3^{a} \pm 0.8$ | $9.6^{a} \pm 0.2$ | $10.34^{a} \pm 0.10$ |
| Non pasteurized – after mash treatment (III) | $1883^{c} \pm 29$ | $170.2^{d} \pm 1.3$ | $25.6^{d} \pm 0.6$ | $14.92^{d} \pm 0.18$ |
| Pasteurized – after mash treatment (IV) | $1542^{b} \pm 17$ | $116.4^{b} \pm 1.9$ | $20.7^{c} \pm 0.3$ | $11.94^{c} \pm 0.09$ |
| Strawberry juices | | | | |
| Fresh – control (I') | $640.2^{B} \pm 8$ | $320.8^{C} \pm 1.7$ | $12.6^{D} \pm 0.3$ | $4.05^{B} \pm 0.03$ |
| Pasteurized (II') | $541.2^{A} \pm 12.4$ | $103.0^{A} \pm 2.1$ | $4.6^{A} \pm 0.2$ | $3.94^{A} \pm 0.01$ |
| Non pasteurized – after mash treatment (III') | $801.7^{C} \pm 13$ | $235.2^{B} \pm 1.0$ | $10.5^{C} \pm 0.3$ | $4.35^{D} \pm 0.02$ |
| Pasteurized – after mash treatment (IV') | $632.9^{B} \pm 6.3$ | $121.8^{A} \pm 1.7$ | $5.1^{B} \pm 0.2$ | $4.23^{C} \pm 0.03$ |

Mean values in the same column having different small letters for cranberry juices ($a$, $b$, $c$…) and different large letters for strawberry juices ($A$, $B$, $C$...) are significantly different at $P < 0.05$

under identical technological conditions as strawberry juices, were characterized by a significantly higher content of polyphenols and ascorbic acid, a lower anthocyanin content and greater DPPH• radical scavenging capacity. These differences resulted primarily from a different qualitative and quantitative composition of materials used for juice production (HANNUM 2004, BOROWSKA, SZAJDEK 2005, SZAJDEK et al. 2008). Compared with other berry fruit species, cranberries have an average polyphenols content and a low anthocyanins content. Proanthocyanidins and phenolic acids are known for their strong antioxidative properties (VATTEM et al. 2005, BOROWSKA et al. 2009a). Strawberry fruits contain less total phenolics and more anthocyanins than cranberry fruits (SZAJDEK et al. 2008).

The heat processing of fruit mash, preceding juice pressing, leads to partial degradation of polysaccharides (pectins, cellulose) and decreases the system's viscosity. This, in turn, improves the extractability of antioxidants (HILZ et al. 2005). The heat treatment of cranberry mash caused a statistically significant ($P < 0.05$) increase in the concentrations of all tested compounds and in the DPPH• radical scavenging capacity of juice (III), in comparison with control juice (I). Strawberry juice (III') made from heat-treated mash was marked by a significantly ($P < 0.05$) lower content of anthocyanins and ascorbic acid than fresh control juice (I') – Table 1. The results of our previous experiments with juices from other berry fruit species (BOROWSKA et al. 2009b, SZAJDEK et al. 2009) and the findings of other authors (LANDBO, MEYER 2004, BUCHERT et al. 2005) show that both juice yield and the extractability of compounds may be enhanced by thermal and enzymatic maceration of fruit mash. On the other hand, research results pointed to the low thermal stability of anthocyanins in strawberry fruits during processing. The degradation of strawberry anthocyanins resulting from fruit crushing and mash treatment was reported, among others, by SKREDE et al. (1992). According to VERSARI et al. (1997), anthocyanins may be converted into aglycones which may then form brown-colored polymers.

In the present study, the thermal processing of cranberry and strawberry juices had a destructive influence on the evaluated properties (Table 1, Figure 1). As a result of juice pasteurization, the greatest losses were noted with respect to anthocyanins and ascorbic acid. In strawberry juices, the content of ascorbic acid and anthocyanins decreased by 41–53% and 60–70% respectively. In cranberry juices, total anthocyanin loss did not exceed 40%. Less pronounced changes were observed in total phenolic compounds, thus pointing to higher stability of polyphenol components other than anthocyanins. As indicated by numerous studies conducted on berry fruit juices, changes in composition during the technological process are largely determined by fruit species (BOYLES, WROLSTAD 1993, LANDBO, MEYER 2004, BUCHERT et al. 2005, BOROWSKA et al. 2009b).

Despite a high decrease in the concentrations of studied compounds during pasteurization, changes in the DPPH• radical scavenging capacity

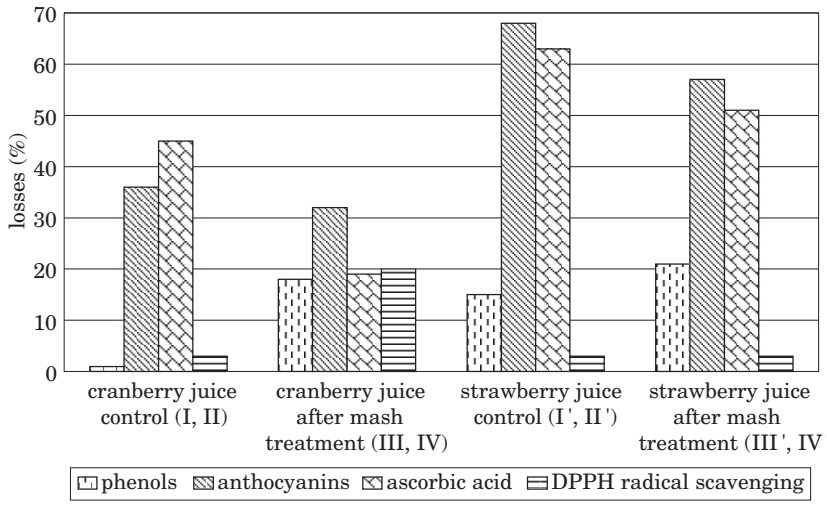


Fig. 1. The influence of pasteurization on antioxidants and DPPH• radical scavenging of juices: I, I' – fresh juice control; II, II' – pasteurized juice; III, III' – after mash treatment juice; IV, IV' – pasteurized – after mash treatment juice

of juices were relatively small (Figure 1). This may suggest that the compounds formed as a result of processing also exhibit antioxidant activity (OSZMIAŃSKI, SOŻYŃSKI 1989, GRAJEK 2003).

## Conclusions

An analysis of cranberry and strawberry juices revealed that they differed significantly with regard to composition and antioxidant properties. Particular attention should be paid to the approximately twofold higher DPPH• radical scavenging capacity of cranberry juices. Heat treatment of cranberry mash, prior to juice pressing, contributed to higher concentrations of the studied components in the final product. As regards strawberry juices, both the heat treatment of fruit mash before pressing and juice pasteurization had a destructive effect on the levels of anthocyanins and ascorbic acid.

It should be stressed that juices produced without the use of commercial enzymatic preparations for fruit mash maceration can be offered as fresh, non-pasteurized products. According to market research reports, increased consumer awareness has contributed to growing demand for unprocessed products, including fresh juices characterized by a high content of antioxidants and organoleptic properties similar to those of raw materials.

Translated by Aleksandra Poprawska Accepted for print 13.03.2010

## References


BAGGER-JØRGENSEN R., MEYER A.S. 2004. *Effects of different enzymatic pre-press maceration treatments on release of phenols into blackcurrant juice.* Eur. Food Res. Technol., 219: 620–629.

BOROWSKA E.J., MAZUR B., GADZAŁA-KOPCIUCH R., BUSZEWSKI B. 2009a. *Polyphenol, anthocyanin and resveratrol mass fractions and antioxidant properties of cranberry cultivars.* Food Technol. Biotechnol., 47: 56–61.

BOROWSKA E.J., SZAJDEK A. 2009b. *Effect of heat and enzyme treatment on yield, phenolic content and antioxidant capacity of juices from chokeberry mash.* Ital. J. Food Sci., 21: 197–209.

BOYLES M.J., WROLSTAD R.E. 1993. *Anthocyanin composition of red raspberry juice: influences of cultivar, processing and environmental factors.* J. Food Sci., 58: 1135–1141.

BUCHERT J., KOPONEN J.M., SUUTARINEN M., MUSTRANTA A., LILLE M., TÖRRÖNEN R., POUTANEN K. 2005. *Effect of enzyme-aided pressing on anthocyanin yield and profiles in bilberry and blackcurrant juices.* J. Sci. Food Agric., 85: 2548–2556.

BRAND-WILLIAMS W., CUVELIER M.E., BERSET C. 1995. *Use of free radical method to evaluate antioxidant activity.* LWT-Food Sci. Technol., 28: 25–30.

DIETRICH H., RECHNER A., PATZ C.D. 2004. *Bioactive compounds in fruit and juice.* Fruit Process., 14: 50–55.

GRAJEK W. 2003. *Zmiany potencjału przeciwutleniającego surowców roślinnych w procesach przetwórczych i w czasie trawienia.* Żywność, 4: 26–35.

HANNUM S.M. 2004. *Potential impact of strawberries on human health: a review of the science.* Critic. Rev. Food Sci. Nutr., 44: 1–17.

HELBIG J. 2001. *Production of colour-intensive and colour-stable coloured juices.* Fruit Process., 9: 342–347.

HILZ H., BAKX J., SCHOLS H.A., VORAGEN A.G.J. 2005. *Cell wall polysaccharides in black currants and bilberry – characterization in berries juice and press cake.* Carbohydrate Polymers, 59: 477–488.

KADER F., NICOLAS J.P., METCHE M. 1999. *Degradation of pelargonidin 3-glucoside in the presence of chlorogenic acid and blueberry polyphenol oxidase.* J. Sci. Food Agric., 79: 517–522.

LANDBO A.K., MEYER A.S. 2004. *Effects of different enzymatic maceration treatments on enhancement of anthocyanins and other phenolics in black currant juice.* Innov. Food Sci. Emerg. Technol., 5: 503–513.

OSZMIAŃSKI J., SOŻYŃSKI J. 1989. *Wpływ warunków otrzymywania oraz przechowywania soku z aronii na związki fenolowe i barwę.* Zesz. Nauk., AR Wrocław, 184: 90–96.

PŁOCHARSKI W., MARKOWSKI J. 2003. *Ocena uzysku soku i jego parametrów jakościowych przy obróbce miazgi owoców czarnej porzeczki preparatami enzymatycznymi nowej generacji firmy Novozymes Switzerland.* Przem. Ferm., 6: 24–26.

*Przetwory owocowe i warzywne. Oznaczanie witaminy C.* PN-90/A-75101/11.

SINGLETON V.L., ROSSI A. 1965. *Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents.* Am. J. Enol. Viticult., 16: 144–158.

SKREDE G., WROLSTAD R.E., LEA P., ENERSEN G. 1992. *Color stability of strawberry and blackcurrant syrups.* J. Food Sci., 57: 172–177.

SKREDE G., WROLSTAD R.E., DURST R.W. 2000. *Changes in anthocyanins and polyphenolics during juice processing of highbush blueberries (Vaccinium corymbosum L.).* J. Food Sci., 65: 357–364.

SZAJDEK A., BOROWSKA E.J. 2008. *Bioactive compounds and health-promoting properties of berry fruits: A review.* Plant Foods Hum. Nutr., 63: 147–156.

SZAJDEK A., BOROWSKA E.J., CZAPLICKI S. 2009. *Effect of bilberry mash treatment on the content of some biologically active compounds and the antioxidant activity of juices.* Acta Aliment., 58: 281–292.

URLAUB R. 2002. *Modern use of enzymes in fruit processing.* Fruit Process., 8: 158–163.

VATTEM D.A., GHAEDIAN R., SHETTY K. 2005. *Enhancing health benefits of berries through phenolic antioxidant enrichment: focus on cranberry.* Asia Pac. J. Clin. Nutr., 14: 120–130.

VERSARI A., BIESENBRUCH S., BARBANTI D., FARNELL P.J., GALASSI S. 1997. *Effects of pectolytic enzymes on selected phenolic compounds in strawberry and raspberry juices*. Food Res. Int., 30: 811–817.

WROLSTAD R.E. 1976. *Color and pigment analyses in fruits products.* Agricultural Experiment Station Bulletin, 624, Oregon State University, Corvallis, OR, USA.