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# METHODOLOGICAL REQUIREMENTS FOR IDENTIFYING LODGES COLONIZED BY EUROPEAN BEAVERS *CASTOR FIBER* L. WITH THE USE OF THERMAL IMAGING TECHNOLOGY – PRELIMINARY RESULTS

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Key words: European beaver, *Castor fiber*, thermography, beaver lodges, monitoring.

Abstract

The presence of colonized beaver lodges in a given area may be difficult to confirm during European beaver population surveys. The existing inventory methods do not provide unquestionable results. To avoid survey errors in the future, this study was undertaken to determine whether thermal imaging could be used to confirm the presence of beaver settlements in the analyzed area. The number of beaver colonies estimated using a traditional method that involves the identification of beaver tracks and a method based on an analysis of infrared measurements was different. It was found that thermography could be a useful tool for determining the presence of active beaver colonies, but its effectiveness is dependent upon a number of methodological considerations.

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# METODOLOGICZNE UWARUNKOWANIA WYKORZYSTANIA TERMOWIZJI DO OKREŚLANIA ZASIEDLENIA STANOWISK PRZEZ BOBRA EUROPEJSKIEGO *CASTOR FIBER* L. – WYNIKI WSTĘPNE

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Słowa kluczowe: bóbr europejski, *Castor fiber*, termowizja, stanowiska bobrowe, monitoring.

Abstrakt

Potwierdzenie występowania rodzin bobrowych na danym stanowisku nie jest jednoznaczne, mimo wielu wskazujących na to oznak terenowych. Wyniki dotychczasowych metod inwentaryzacji mogą być zatem obarczone błędem. Podjęto badania, które pomogą uwiarygodnić efekty prac terenowych, mające na celu określenie możliwości wykorzystania termowizji do potwierdzenia zasiedlenia obszaru przez bobra europejskiego.

W eksperymencie stwierdzono, że liczba czynnych stanowisk bobrowych określona metodą tradycyjną, bazującą na oznakach bytowania zwierząt oraz w oparciu o analizę termogramów, była różna. Dowiedziono, że termowizja może być metodą wykorzystaną do określania czynnych stanowisk bobrowych, jednak jej skuteczność jest uzależniona od licznych uwarunkowań metodologicznych określonych podczas prowadzonych badań.

## Introduction

Thermal imaging technology has numerous applications in industry, construction, rescue work, police and medicine (*Infrared thermography*… 2012, McCafferty 2007). It is used in veterinary to diagnose inflammations and diseases affecting locomotive and internal organs (Knizkova et al. 2007), and to determine the insulation values of animal fur coats (Cilulko et al. 2013). Thermographic cameras have also been used to analyze heat energy radiation from different body parts in mammals (Kuhn and Meyer 2009, Lancaster et al. 1997) and birds (McCafferty et al. 1998).

Thermal imaging is a modern research tool for collecting data about the nocturnal activity or presence of animals in selected habitats (Belant and Seamans 2000, Christiansen et al. 2014, Ditchkoff et al. 2005, McCafferty 2007). The European beaver is an important species promoting biodiversity (Janiszewski et al. 2014). The secluded lifestyle of

*Castor fiber* is a frequent cause of errors in beaver population surveys. A beaver family can control more than one site on its territory, and those sites can be separated by considerable distance. Not all parts of the controlled territory are utilized equally by beavers (ŻUROWSKI 1992, WHEATLEY 1997, JANISZEWSKI and HANZAL 2015). The very presence of a beaver family in a given territory can create numerous problems during population surveys. The existing survey methods did not guarantee that the evaluated territory was inhabited by beavers at the time of the inspection. In numerous cases, abandoned lodges, burrows and dens were mistakenly classified as active sites (WHEATLEY 1997).

To avoid survey errors in the future, this study set out to evaluate the applicability of the thermal imaging technology in confirming habitat colonization by the European beaver.

## Materials and Methods

The study was conducted in the Wigry National Park in north-eastern Poland. The park has one of the oldest populations of the European beaver in Poland which dates back to the mid 20$^{th}$ century (ŻUROWSKI and KASPERCZYK 1986). The study was divided into three stages:

– stage I: traditional field survey of beaver sites,

– stage II: measurements performed with a thermographic camera to confirm the colonization status of sites identified in stage I,

– stage III: analysis of thermograms to identify lodges that are and are not inhabited by beavers.

**Stage I.** The information collected during a survey of beaver sites, conducted by the task forces of the Wigry National Park, constituted baseline data. The entire park area was surveyed. A territory colonized by one beaver family was classified as a family site. Active beaver sites were identified based on the presence of:

– winter food stores,

– lodge, burrows and dens,

– diversion dams raising the water table,

– incisions in trees, and shrubs, and scent mounds.

The GPS coordinates of the evaluated sites were marked on a map.

**Stage II.** The presence of beavers in the sites identified in stage I was confirmed with the use of a thermographic camera. The ThermoPro TP8 thermographic camera with an uncooled microbolometer array with 384 × 288 pixels was used. The camera had a thermal sensitivity of 0.08°C to 30°C and temperature range of -20°C to +800°C (down to -40°C and up to

2000°C, optional). The camera had a built-in digital video sensor with a resolution of 1280 × 1024 pixels, a wide-angle 16 mm lens and a 100 mm lens. It featured a touch-screen external display with a resolution of 640 × 480 pixels, which was used to observe changes in the temperature of the analyzed surfaces. Measurements were performed with an accuracy of 1% of the reading.

The temperature on the surface of beaver lodges, burrows and dens classified as inhabited in stage I was measured with the use of the thermographic camera. Thermographic measurements were performed on the day following the completion of stage I to minimize the risk of beavers abandoning the examined sites.

The exact hour of the thermographic measurement and weather conditions were recorded during the survey conducted in stage II of the study:

– air temperature [°C],
– insolation [presence or absence],
– precipitation [presence or absence],
– snow cover [presence or absence],
– wind [m $s^{-1}$].

**Stage III.** Thermograms were analyzed in the Guide IR Analyzer (v. 2010-04-05) program. The maximum and minimum temperature values [°C] in the acquired images were normalized (palette bar). The average temperature across the entire lodge surface was determined. Depending on the shape of the lodge, measurements were performed with the use of elliptical and circular shapes (Figure 1). Maximum and minimum temperatures were marked in every thermogram. Differences in temperature in each evaluated site were interpreted.

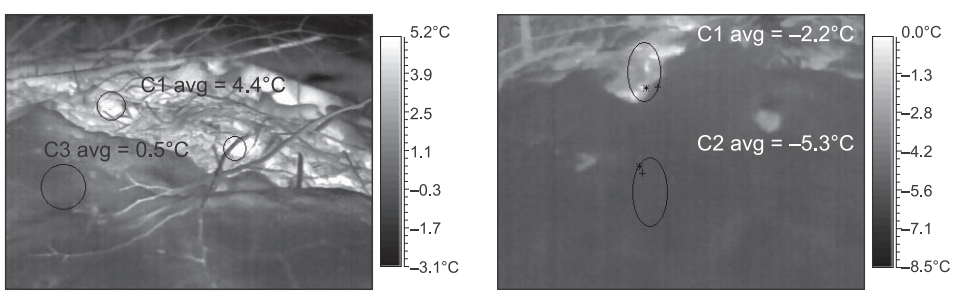


Fig. 1. Thermograms with temperature distribution across the surface of the analyzed lodges which confirm the presence of beavers

Stage III operations were performed by a person who did not participate in stages I or II to eliminate bias in computer analyses of the obtained thermograms.

The study was conducted between 15 November 2014 and 15 February 2015. Beginning and end dates were determined based on previous surveys of active European beaver sites and the relevant research guidelines (JANISZEWSKI et al. 2007, JANISZEWSKI and MISIUKIEWICZ 2012).

## Results and Discussion

### Stage I

A total of 123 signs testifying to the presence of the European beaver in the examined territory were found during a traditional field survey. They included inhabited and abandoned lodges, burrows and dens, as well as the presence of diversion dams raising the water level. The information acquired during stage I was used to select 40 beaver sites for thermographic analysis. Thermographic data revealed that 11 of the selected sites were not inhabited by *Castor fiber* during the study (Table 1).

Table 1

Number of sites classified as inhabited and uninhabited by beavers during each stage of the study

| Stage | I | | II | | III | |
|---|---|---|---|---|---|---|
| Evaluation | inhabited | uninhabited | inhabited | uninhabited | inhabited | uninhabited |
| Number of sites | 29 | 11 | 22 | 18 | 34 | 6 |

The field survey of beaver sites used in stage I of this study had been previously successfully applied by researchers in other regions of Poland. JANISZEWSKI et al. (2007) determined the presence of 1019 active beaver sites in the Province of Mazowsze (Central Poland) based on 4889 beaver tracks identified in the analyzed area. Thus, it can be assumed that the method is effective and provides relatively reliable results.

### Stage II

The results of stage II revealed that the thermographic camera was most effective in measuring the temperature on the surface of beaver lodges. The attempts to determine the presence of beavers inside burrows and dens were ineffective or did not produce conclusive data. The above could be attributed to the structure of burrows and dens, most of which have entrances under water, therefore, their interior temperature cannot be directly determined with a thermographic camera. The layer of soil above

lodge corridors also made it impossible to measure temperature differences. For this reason, thermographic measurements were performed only in lodges in successive analyses.

In stage II, detailed measurements were performed in 40 beaver lodges. The average temperature on the surface of lodges classified as inhabited in stage I was around 3.0–3.5°C higher than ambient temperature (Figure 1). An analysis of thermograms revealed that 18 of the 40 analyzed lodges did not bear any signs indicative of beavers (Table 1).

An analysis of weather conditions during the study demonstrated that thermographic measurements were most effective on overcast winter days when lodges were covered with snow. The most reliable thermographic readouts were produced when ambient temperature was below -5°C. The accuracy with which the presence of beavers was determined inside lodges increased with a drop in ambient temperature due to higher thermal contrast. On cloudless days when the surface of lodges was heated by sunlight, snow-covered ground was characterized by smaller differences in surface temperature. Sun rays heated dark-colored elements of lodge structures, such as tree branches, which led to errors in thermographic readouts and data interpretation problems. These observations indicate that measurements performed on sunny days could falsely imply the presence of beavers in all analyzed lodges, including abandoned structures.

Thermographic data collected on days when wind speed exceeded 4 m $s^{-1}$ and on snowy days were also difficult to interpret. Wind and snow cooled the surveyed surfaces, which produced confusing results. Freshly fallen wet snow which filled crevices in beaver structures and blocked ventilation shafts in lodges was the greatest source of interpretation errors. In these situations, clear temperature stratification was not observed in thermograms, which led to the possibly mistaken conclusion that the analyzed lodges were not inhabited by beavers.

The observations performed in one of the analyzed sites delivered particularly interesting results. Winter food reserves had been accumulated by beavers near the examined lodge approximately three months before the beginning of the study, which suggested the site was colonized. On 21 January 2015, an adult beaver was spotted swimming in the vicinity of the site, and it hid inside the structure. Thermographic measurements on the surface of the lodge were performed immediately after the observation. Measurements were conducted in the morning (9 a.m.), on a snowy and windy day (8 m $s^{-1}$) with an ambient temperature of around 0°C. The animal had been spotted entering the lodge, but the heat emitted by the beaver was not registered by the camera. The lodge was examined repeatedly on 5 February 2015 at around 8 a.m., on a windless (2 m $s^{-1}$) and

clear day with ambient temperature of -3°C. Fresh and clear signs of beavers were found in the site, but the presence of animals was not registered by the thermographic camera. The lodge was examined again at 1 p.m. on the same day, and this time, the thermographic readout confirmed the presence of beavers inside the lodge. These results suggest that thermographic measurements are most effective when conducted in the afternoon, probably because beavers are nocturnal animals that forage for food during the night. Beavers return to lodges in early morning hours when temperature inside the structure is low. Interior temperature rises as beavers remain in the lodge, but this process is relatively slow. For this reason, measurements performed in early morning hours were not reliable because beavers needed more time to generate sufficient amounts of heat and rise the temperature inside the lodge.

Situations like those encountered in this study considerably influence the effectiveness of thermographic cameras, the reliability of thermographic readings in detecting the presence of animals, and measurements of temperature distribution across the analyzed surfaces. To account for these drawbacks, the surveys in this study were performed on overcast days, in the afternoon, when the analyzed surfaces were covered by snow. These conditions contributed to clear thermal contrast between the tested surfaces, and they supported accurate measurements.

A negative impact of solar radiation on thermography's accuracy during field surveys of wild animal populations has also been described by other authors (BOONSTRA et al. 1994, GARNER et al. 1995, DITCHKOFF et al. 2005, BUTLER et al. 2006, MCCAFFERTY 2007, HILSBERG-MERZ 2008). Both our findings and the results reported by other researchers show that solar irradiance is the most important potential source of error in thermal imaging, which should be taken into account in field research.

## Stage III

The thermograms obtained in stage II were analyzed with the use of a computer program. The results demonstrated that the presence of high thermal contrast is required to confirm the presence of beavers inside lodges. Data were processed in the Guide IrAnalyzer to reveal that 6 of the 40 lodges examined in stage II were not inhabited by beavers. The presence of beavers was confirmed in the remaining 34 lodges (Table 1).

Due to certain behavioral and environmental factors, it may be difficult to use technical devices for monitoring the distribution and activity of beaver families and individual animals (THOMSEN et al. 2007). The most widely used and recommended method for monitoring the daily and annual

activity patterns of beavers is wildlife radio telemetry (LANCIA et al. 1979, ARJO et al. 2008). JOHN and KOSTKAN (2009) used the Global Positioning System and the Geographic Information System for mapping environmental diversity and beaver activity. All of the described methods support effective monitoring of beaver populations, but they also have certain limitations. BLOOMQUIST and NIELSEN (2009) relied on a remote videography system to characterize the behavior patterns of beavers inside lodges and bank dens. According to the cited authors, the main disadvantage of the applied method was that they could not monitor entire beaver colonies because only animals passing through the field of view were recorded.

## Conclusions

The results obtained in each stage of the study revealed differences in the number of sites that were correctly identified as active beaver sites based on the characteristic signs and thermographic measurements. In the total number of 40 analyzed lodges, 11 were classified as uninhabited in stage I and 18 in stage II. The thermograms analyzed in stage III revealed that only 6 of the 40 tested sites were not colonized by beavers (Table 1). The highest number of lodges classified as uninhabited was determined in stage II, and the lowest number – in stage III.

The classification of beaver lodges as inhabited or uninhabited in stage III was consistent with the results obtained in stages I and II of the study, and it confirmed beyond doubt that 6 lodges were not colonized. A comparison of the results noted in stage I and stage II indicates that only one beaver site was differently classified. The remaining stage I data were partially consistent with stage II findings, which suggests that 4 more lodges were not inhabited by beavers. An analysis of pooled results from stages III and I indicates with full certainty that 10 of the 40 examined lodges were not colonized by beavers.

The effectiveness of thermographic measurements in surveys of active beaver sites is largely determined by the applied research method and the researchers' ability to correctly interpret the results. The use of thermographic cameras can be fraught with problems due to the limitations posed by variable weather conditions and the hour of measurement. For this reason, thermographic cameras do not appear to be highly useful in beaver surveys. However, they can be used in individual cases to confirm the presence of animals in the analyzed sites.

Thermal imaging has not been widely used for field surveys of beaver colonies to date. Therefore, our findings can provide a basis for further research into beaver populations.

The results of this study support the formulation of the following methodological recommendations for surveying active beaver sites with the use of thermal imaging technology:

1. Thermographic measurements should not be performed under the following weather conditions:
   – absence of snow cover,
   – sunny days,
   – falling snow,
   – ambient temperature above -2°C,
   – wind speed higher than 4 m $s^{-1}$.
2. Thermographic measurements produce the most reliable results when performed under the following conditions:
   – ambient temperature below -5°C,
   – beaver lodges are completely covered with snow,
   – measurements are performed not earlier than two days after the last snow fall,
   – fully overcast skies,
   – measurements are conducted in the afternoon,
   – windless weather.

Our findings indicate that thermographic cameras can be used as an auxiliary tool during traditional surveys of active beaver sites, but they should not constitute the only method of measurement during such surveys.

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# SOW LONGEVITY AS AN INDICATOR OF RESISTANCE TO ENVIRONMENTAL STRESSORS

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Key words: sow longevity, reasons for culling, stillborn piglets, piglet mortality, correlations.

Abstract

The longevity and reproductive performance of 190 crossbred Redone sows inseminated with the semen of hybrid Gallia boars were analyzed in this study. The sows were kept on a large commercial farm in optimal environmental conditions, in line with welfare requirements. Sows produced an average of 2.2 litters a year. The total number of litters farrowed was 1380 (7.26 litters per sow on average). The first sows were culled from the herd after the 2nd breeding cycle (5%), whereas the sow characterized by the highest lifetime efficiency and longevity delivered 22 litters (a total of 270 piglets born alive, including 260 weaned piglets). The main reasons for culling were reproductive disorders and lameness, which accounted for 39.7% and 30.7% of all cases, respectively. It should be stressed that sows that delivered at least 7 litters were less often culled from the herd due to high piglet mortality and lameness. The average number of piglets born alive and stillborn piglets per litter was 13 and 0.67, respectively. A positive correlation was found between sow fertility and the size of subsequent litters ($r = 0.17 – p \leq 0.01$), which indicates that sow fertility does not always decreases with age. The group of sows that stayed in the herd for the longest period of time (group 5, at least 12 litters) was characterized by the highest fertility ($p \leq 0.01$) and a similar number of stillbirths in comparison with the remaining groups. Group 5 sows accounted for 17.4% of all sows in the herd, and group 4 sows (at least 6 litters) – for 36.8%. It can be concluded that sows have a high potential for longevity, which can be fully realized only under optimal environmental conditions (adequate nutrition, housing and sanitary conditions, well-trained staff, veterinary care).

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# DŁUGOWIECZNOŚĆ LOCH – WSKAŹNIK ICH ODPORNOŚCI ŚRODOWISKOWEJ

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Słowa kluczowe: długowieczność loch, brakowanie, prosięta martwo urodzone, śmiertelność prosiąt, korelacje.

Abstrakt

Przedstawiono wyniki długości użytkowania oraz użytkowość rozpłodową 190 loch hybrydowych Redone inseminowanych nasieniem knurów linii Galia. Lochy utrzymywano w fermie wielkotowarowej w optymalnych warunkach środowiskowych, zgodnych z wymaganiami dobrostanu. Lochy rodziły średnio 2,2 miotu rocznie. Urodziły łącznie 1380 miotów, średnio 7,26 miotu/lochę. Pierwsze lochy wybrakowano ze stada po drugim cyklu rozpłodowym (5%), natomiast ostatnią, najdłużej użytkowaną, po 22. Locha ta urodziła łącznie 270 prosiąt, a liczba prosiąt odchowanych wyniosła 260 szt. Głównymi przyczynami brakowania loch ze stada były obniżająca się płodność w kolejnych cyklach oraz urazy mechaniczne (odpowiednio 39,7 i 30,7%). Należy zaznaczyć, że lochy, które urodziły 7 i więcej miotów były znacząco rzadziej brakowane z powodu urazów i dużej śmiertelności prosiąt. Średnia płodność loch wynosiła 13,0 prosiąt żywych i 0,67 martwych. Współczynnik korelacji między kolejnym miotem a płodnością był dodatni ($r$ = 017 – $p \leq 0{,}01$), co świadczy o tym, że płodność loch długowiecznych była stale wysoka. Najdłużej użytkowana V grupa loch (12 i więcej urodzonych miotów) charakteryzowała się najwyższą płodnością ($p < 0{,}01$) i podobną liczbą prosiąt martwo urodzonych w porównaniu z pozostałymi podgrupami. Grupa V stanowiła 17,4% udziału wszystkich użytkowanych loch, a grupa loch użytkowanych od 6 do 11 miotu ( IV) – 36,8%.

Podsumowując, lochy mają bardzo duże możliwości długiego użytkowania, które ujawniają się jednak tylko w optymalnych warunkach środowiskowych (żywienie, warunki zoohigieniczne pomieszczeń, fachowa obsługa zootechniczna, profilaktyka weterynaryjna).

## Introduction

Sows usually produce 3 to 4 litters, and annual sow culling rates remain too high (JARCZYK at al. 2014). In 2015, in breeding herds, only 88.9% of Polish Large White (PLW) sows and 78.5% of Polish Landrace (PL) sows delivered 2 litters, whereas the respective values for 4 litters were 71.6% and 59.6% (MUCHA 2016). The above values were lower in 2005 – PLW and PL sows that farrowed 4 litters accounted for 53.3% and 53.4% of sows in the herd, respectively. Even lower values were noted in 1995 at 43.7% and 43.9%, respectively (ORZECHOWSKA and MUCHA 1996, ORZECHOWSKA and MUCHA 2006). This indicates that 30% to 40% of sows

are currently culled after the 4th parity. A positive example could be an English farm where sows produced 10.6 litters on average (ENGLISH et al.1988), which was supervised by the authors of the cited study.

JARCZYK and KONRAD (1995) demonstrated that the heritability index for longevity was relatively high ($h^2 = 0.274$, $p \leq 0.05$) if daughters were born in the first litters. If the daughters of the same mothers were born in later litters, the heritability index was equal to zero. The above resulted from considerable negative prenatal and postnatal environmental effects, including impaired health of multiparous sows, and suboptimal nutrition and management conditions (WU et al. 2006). Therefore, in the past, breeding stock was selected from the 2nd, 3rd and 4th litters, when sows were still in good health. Today, when the welfare of pigs has been considerably improved, efforts are made to improve sow longevity through genetic selection and the implementation of well-designed breeding programs. Some reproductive performance traits (e.g. piglet survival) are associated with a higher level of inherited resistance in the offspring. This was observed by JARCZYK et al. (1999) who investigated sow fertility and parity as maternal effects influencing the quality and number of piglets and weaners reared. In a herd of 200 sows, the highest percentages of weaners were selected for breeding at 84 days of age from litters No. 4, 5 and 6 (66%, 66% and 60%, respectively) compared with litters No. 1 and 2 (37% and 26%, respectively).

Modern sows are expected to produce high numbers of piglets born alive and weaned, both per year and throughout their productive life (REKIEL et al. 2013). In recent years, attempts have been made on Polish farms to improve sow longevity through commercial crossing of large-framed breeds such as Złotnicka Pstra and Duroc. However, reduced fertility was noted in crossbred sows. According to the data of 2015, only 25% of breeding sows delivered more than 4 litters, which points to the ineffectiveness of the proposed breeding strategy (MUCHA 2016).

New hybrid sow lines with different reproductive performance have been developed. Hybrid PenArLan sows were characterized by very high fertility. Studies conducted on three farms revealed also very high fertility of Redone and Naima primiparous sows (from 11.1 to 12.3 piglets) and sows in their 2nd and 3rd parity (from 11.8 to 13.7 piglets). However, some of the sows had an insufficient number of teats to feed piglets – 13.5% of sows had less than 14 teats (JARCZYK et al. 2009), and litter size had to be standardized to match the number of functional teats. Excess piglets often could not be moved to nurse sows due to considerable age differences between litters, which is a commonly encountered problem on small farms. Large litters are also characterized by a relatively high number of low-bir-

th-weight piglets that are at greater risk of death. Thus, it appears that sow longevity and good health status could positively influence piglet survival (AMER et al. 2014, GŁÓD and KACZMARCZYK 1982, GRUDNIEWSKA 1998, JARCZYK et al. 2009, TARRÉS et al. 2006) and be a reliable indicator of welfare (BARNETT et al. 2001, ENGBLOM et al. 2007).

The aim of this study was to analyze the longevity and reproductive performance of sows raised under good environmental conditions, and to determine their culling rates and identify reasons for culling. An attempt was also made to determine whether long-lived sows that produced at least 6 litters had a high number of piglets born alive and weaned, and were characterized by lower culling rates, which could be associated with their increased environmental adaptability and resistance.

## Materials and Methods

The data used in this study come from the records kept on a commercial farm where a breeding herd of 210 sows was maintained. Breeding gilts were purchased from the PenArLan (currently Choice Genetics) company. Redone sows were inseminated with the semen of Gallia boars. The first farrowing took place in 2007, and the last in 2016. Piglets and weaners were raised in the open system, in a 21-day production cycle. Piglets were weaned at 25 to 28 days of age and body weight of 7 to 9 kg. They were transferred to the weaner accommodation where they stayed to approximately 70 days of age.

On the farm, pigs are fed farm-made complete diets formulated so as meet their nutrient requirements at different stages of the reproductive cycle (*Nutrient requirements of swine* 1993). The management conditions are very good. Temperature and humidity in the facilities are continuously monitored. Pig rearing specialists receive information about potential threats via mobile phone. All animals are vaccinated against porcine circovirosis and chlamydia, and sows are administered medicated feed for 3 to 5 days after weaning to protect them against leptospirosis and colibacteriosis.

The reproductive performance of 190 sows that delivered a total of 1380 litters was analyzed. Sows were divided into 5 groups based on the length of their productive life, calculated from the first farrowing and expressed as the number of litters raised:

– group 1 – 2 litters,
– group 2 – 3 to 5 litters,
– group 3 – 6 to 8 litters,

– group 4 – 9 to 11 litters,
– group 5 – 12 litters and more.

The results were analyzed statistically by one-way ANOVA using Statistica PL ver. 12 software (StatSoft, Inc. 2015). The significance of differences was determined by Duncan's test. The coefficients of correlation between sows' age vs. the number of litters farrowed and fertility were calculated. Standard animal handling procedures were not modified because the research protocol was not submitted for approval to the Local Ethics Committee on Animal Experimentation.

## Results

Percentage of sows in successive breeding cycles throughout their productive life is presented in Figure 1. The culling rate reached 5% in the 3rd breeding cycle. In subsequent cycles, the number of sows removed from the herd varied between 10% and 20%. The culling rate exceeded 50% in the 7th cycle, whereas in the 11th cycle, old sows retained in the herd accounted for 25% of the initial population.

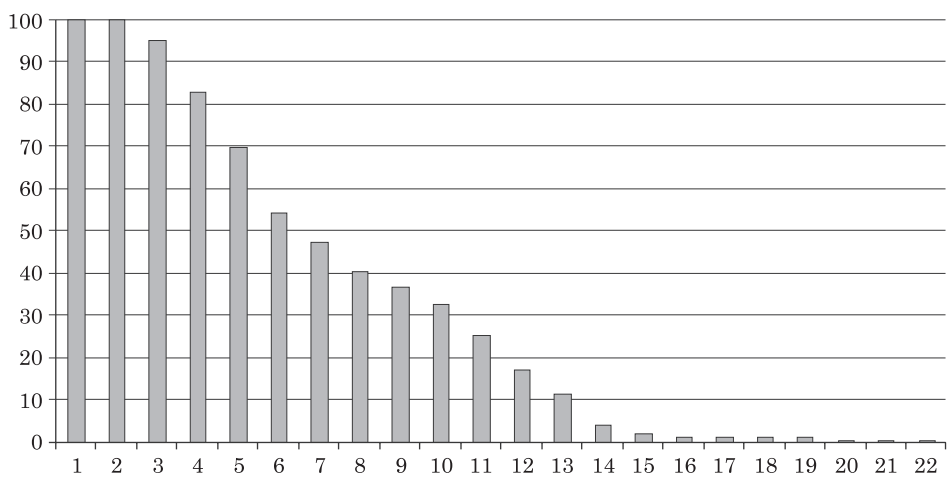


Fig. 1. Percentage of sows in successive breeding cycles

The age of sows at first farrowing and the length of productive life in groups of long-lived sows are shown in Table 1. Gilts characterized by the shortest productive life delivered their first litter at the oldest age. Most probably, they had certain growth and development disorders that prevented effective conception. The average length of productive life from the first farrowing ranged from 215 to 1868 days (7–62 months), and the average

value for the entire population of sows on the farm was 976 days (nearly 32 months). Some sows stayed in the herd for over 5 years, including one sow that remained productive in the breeding herd for 8.5 years (103 months).

Table 1

Age at first farrowing and the length of productive life from the first farrowing

| Group of sows (number of litters) | Number and percentage of sows | Age at first farrowing – days | | Length of productive life – days | |
|---|---|---|---|---|---|
| | | $\bar{x}$ | s | $\bar{x}$ | s |
| 1 (1–2) | 10 (5.3) | 410 | 81.9 | 215 | 125 |
| 2 (3–5) | 77 (40.5) | 375 | 43.5 | 521 | 244 |
| 3 (6–8) | 34 (17.9) | 379 | 46.1 | 915 | 202 |
| 4 (9–11) | 36 (18.9) | 380 | 52.6 | 1376 | 153 |
| 5 (12 and >) | 33 (17.4) | 382 | 43.4 | 1868 | 301 |
| Total | 190 (100) | 378 | 55.2 | 976 | 580 |

The sow characterized by the highest lifetime efficiency and longevity delivered 22 litters: a total of 270 piglets born alive, including 260 weaned and reared piglets. The average length of this sow's breeding cycle was 141 days, and return to estrus after the first insemination was never noted.

Table 2

Number of piglets born alive and stillborn piglets and piglet mortality to 25 days of age

| Group of sows | No. | Piglets born alive | | Stillborn piglets | | Piglet mortality |
|---|---|---|---|---|---|---|
| | | $\bar{x}$ | S | $\bar{x}$ | s | % |
| 1 | 11 | $9.8^{B}$ | 3.92 | 0.63 | 1.18 | 4.1 |
| 2 | 325 | $11.5^{A}$ | 3.46 | 0.61 | 1.48 | 10.8 |
| 3 | 234 | $13.0^{A}$ | 3.45 | 0.73 | 1.62 | 11.5 |
| 4 | 369 | $13.2^{A}$ | 3.05 | 0.70 | 1.99 | 10.6 |
| 5 | 439 | $13.9^{A}$ | 2.91 | 0.65 | 1.08 | 14.4 |
| Total | 1378 | 13.0 | 3.34 | 0.67 | 1.31 | 10.0 |

Explanation: $A$, $B - p \leq 0.01$

Table 2 presents the reproductive performance in 5 groups of long-lived sows. Sows from groups 4 and 5, which were retained in the herd for the longest period of time, were characterized by the highest fertility, and the differences between those groups and group 1 were statistically significant. The number of stillborn piglets per litter was similar and relatively low in all groups. The mortality rate of piglets reared until 25 days of age tended to be higher in group 5 (14.4%), but the number of piglets weaned was similar due to higher fertility of those sows. In group 5, the number of piglets born alive and stillborn piglets remained at a more stable level. In 2015, sows (3–4 generations from the initial population) were still cha-

racterized by high fertility (13.98 piglets on average). Piglet mortality until weaning reached 10.0%. In subsequent litters (13, 14, 15 and 17) average piglet mortality reached approximately 8%, with the exception of litter No. 12 (Figure 2).

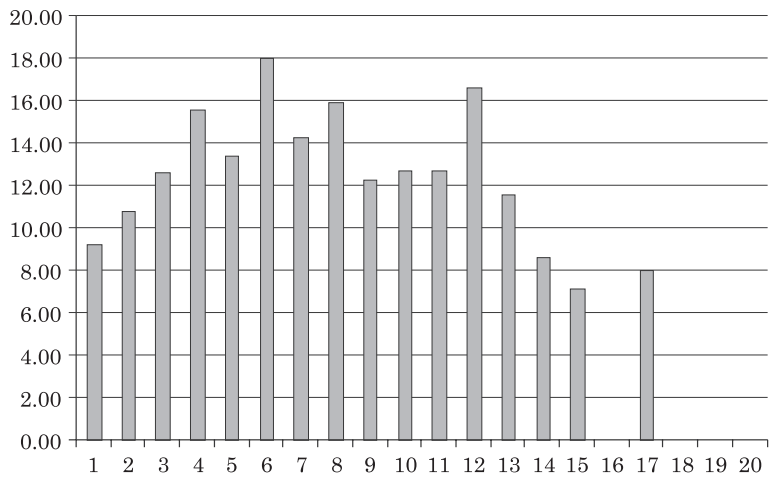


Fig. 2. Piglet mortality in successive litters

Table 3

Reasons for culling sows – total in the herd, before the 6th parity, after the 6th parity

| Item | Culling rate [%] | | |
|---|---|---|---|
| | total in the herd | before the 6th parity | after the 6th parity |
| Conception problems | 39.5 | 39.0 | 40.0 |
| Lameness, leg problems | 30.5 | 38.0 | 22.2 |
| Aging and poor fertility | 16.8 | 7.0 | 27.8 |
| High piglet mortality, stillbirths | 11.1 | 14.0 | 7.8 |
| Death | 2.1 | 2.0 | 2.2 |

The main reasons for culling sows are presented in Table 3. The highest percentage of sows (39.7%) were removed from the herd due to reproductive disorders including lack of estrus and unsuccessful mating, followed by health problems such as lameness and mechanical injuries (30.7%). Sows were also culled for natural reasons, i.e. aging and poor fertility (16.9%). High piglet mortality or even death of all piglets in the litter were responsible for culling 11.1% of sows, whereas 1.6% of sows were culled due to sudden death and sent to slaughter. One sow was culled because of piglet- and stockperson-directed aggression after farrowing.

Table 4
Coefficients of correlation between sow longevity vs. the number of piglets born alive and stillborn piglets

| Item | Piglets born alive | Stillborn piglets |
|---|---|---|
| Subsequent litter | 0.17** | 0.08** |
| Piglets born alive | – | -0.03 |

Explanation: ** – $p \leq 0.01$

The coefficients of correlation between selected reproductive traits of sows are presented in Table 4. A weak positive correlation was observed between successive litters and sow fertility ($r = 0.17$ – $p < 0.01$).

## Discussion

On Polish breeding farms, the culling rates for PLW and PL sows reached 23.84% and 25.14%, respectively, already in the second cycle (MUCHA 2016). The number of multiparous sows was half the number of primiparous sows between litters No. 3 and 4 in both breeds. In the 11$^{th}$ breeding cycle, old PLW and PL sows retained in the herd accounted for only 2% of the initial population, compared with 11% noted in this study. This suggests that their productive lifetime could be considerably increased on both breeding and commercial farms, as confirmed by the results achieved on an English farm where sows delivered an average of 10.6 litters throughout their productive life (ENGLISH 1988).

Poor sow longevity observed on breeding farms, mentioned in the Introduction, indicates that sow welfare standards should be further improved and genetic improvement should be continued. Environmental conditions on farms often exert adverse rather than beneficial effects on animal health and productive lifetime (KONRAD 1997). Culling sows before their 6$^{th}$ parity points to inadequate welfare.

Our results of the reproductive performance of long-lived sows correspond to the findings of KNECHT et al. (2011) and the values noted on selected (10%) top German farms raising Naima and Gallia sows (REKIEL et al. 2009). Differences in the results point to a significant impact of environmental conditions, in particular adequate nutrition and veterinary care. On the analyzed farm, attention was paid to ensuring sufficient dietary intake of fiber (dried pomace) to stimulate peristalsis and inhibit the growth of *E. coli* bacteria. Satiated sows are calmer, which reduces piglet mortality.

In the present study, average piglet mortality until weaning reached 10.0% (Table 2). In the study SCHWARZ et al. (2009) the number of piglets that died before weaning increased with increasing parity number (from

0.75 piglets in litter 1 to 1.60 piglets in litter 9). Probably the reason was the large size and weight of sows. In 2015, the mortality rate of piglets produced by PLW and PL sows, usually kept in smaller herds, ranged from 6% to 8% until 21 days of age (MUCHA 2016). Taking into account very high fertility of sows, piglet mortality can be considered low. In groups 3 and 4, the low mortality rates of piglets could be associated with their greater environmental adaptability inherited from long-lived mothers. Table 2 data show that piglet mortality was lower in group 4 (10.6%) than in groups 2 and 3, whereas sows from groups 2 and 3 were characterized by lower fertility than group 4 sows. Such relationships were also observed in subsequent litters (13, 14, 15 and 17) where average piglet mortality reached approximately 8%, with the exception of litter No. 12 (Figure 2). It should be noted that the above piglet mortality rates were lower than that recorded in piglets delivered by sows of the conservative breed Złotnicka Pstra, raised on an organic farm (straw-bedded pens), which reached 21.1% on average, at sow fertility of 9.42 piglets per litter (SZULC 2012).

In the current study, the main reasons for culling sows were reproductive disorders accounted for 39,5% . It should be noted that a high percentage of sows were culled due to mechanical injuries (30.7%). This indicates that selection for improved constitution, i.e. inherited and acquired physical and mental traits typical of a given animal in a given environment. Therefore, in evaluations of exterior traits of pigs particular attention should be paid to constitutional and conformation defects such as dipped back, shoulder instability, crooked spine behind the shoulder and weak pasterns. This is confirmed by Table 3 data showing the reasons for culling sows before and after the $6^{th}$ parity. Sows in the latter group, which stayed in the herd for the longest period of time, were less frequently (by 15,8%) culled due to leg problems and high piglet mortality (7.8% vs. 14%) than sows in the former group. In other studies, similarly to the analyzed farm, poor reproductive performance was the most common reason (approx. 30%) for culling sows (KOZERA et al. 2015, KULISIEWICZ et al. 2010, MASAKA et al. 2014, SCHWARZ et al. 2007, ULGUIM et al. 2014). In a study conducted in China (ZHAO at al. 2015), the total percentage of culled sows was similar to that noted in the present experiment; in the cited study, 35.3% and 22.5% of sows were culled due to reproductive disorders and lameness, respectively. In a study by DAGORN and AUMAITRE (1979), the highest percentage of sows were culled due to fertility problems (31%) and decreasing productivity (27%). Mechanical leg injuries accounted for 8.8% of all cases, and as many as 6.5% of sows were culled because of sudden death. In a study comparing data from Dutch farms (KROES and VAN MALE 1979), the profitability of sow production was two-fold lower at an annual repla-

cement rate of 40% compared with 15%. Despite substantial differences in culling rates between the analyzed farms, the above findings demonstrate that long-lived sows are less susceptible to leg injuries, and are less frequently culled due to poor results of piglet rearing.

Positive correlation between successive litters and sow fertility ($r = 0.17 – p < 0.01$) was observed in the present study. This indicates that sows were removed from the herd due to poor fertility in two consecutive parities and when repeat breeding was required (maximum two services). An insignificant negative correlation was found between sow fertility and the number of stillborn piglets, which shows that the percentage of stillbirths was lower in sows characterized by high fertility. This is an important consideration, especially that the opposite relationship was noted in other studies (BORGES et al. 2005, JARCZYK et al. 2011), i.e. older sows had a higher tendency to deliver stillborn piglets. In our study, as shown in Table 2, piglets inherited greater environmental adaptability and resistance from long-lived sows, which was reflected in higher piglet survival rates.

In can be concluded that adequate nutrition, continuous electronic monitoring of housing and sanitary conditions on the farm (temperature, humidity), accompanied by veterinary care and supervision, contributed to achieving optimal reproductive performance with regard to sow longevity. The results noted in our study, i.e. the average number of litters farrowed per sow – 7.21, the average number of piglets born alive – 13.0, and piglet mortality rate – 10.0%, could be proposed as target values for genetic improvement, breeding and veterinary programs in the nearest future.

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# EFFECTS OF QUALITATIVE AND QUANTITATIVE FEED RESTRICTION ON CARCASS YIELD AND PORK QUALITY

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Key words: Feed restriction, *ad libitum feeding*, cut parts, meat quality.

Abstract

A 3 x 3 factorial experiment was carried out to investigate the carcass yield and meat quality characteristics of growing pigs subjected to qualitative and quantitative feeding strategies. Eighty one (81) Large White growing pigs (54 males and 27 females) with initial weight of 6.7 ± 0.39 kg were assigned to nine treatments of three replicates of three pigs per replicate. Treatments consist of three levels of qualitative (20, 18 and 16% crude protein) and quantitative (*ad libitum*, 90% and 80% *ad libitum*) feed restriction. Fasted weight values (31.75, 26.77, 23.98 kg) of growing pigs significantly ($p < 0.05$) decreased with decreasing levels of dietary crude protein. The same trends were observed in other carcass yield parameters of growing pigs studied except left carcass weight which was not significantly affected by qualitative restriction. Dietary crude protein levels had significant ($p < 0.05$) effect on percentage cooking loss with the values obtained increasing with decreasing levels of dietary crude protein content. Quantitative feed restriction influenced ($p < 0.05$) head, shoulder, fore-leg, hind-leg, liver, kidney, spleen and stomach weights but had no influence on meat quality parameters of growing pigs. Interaction between qualitative and quantitative restriction had significant effect on all carcass yield parameters but with no effect on meat quality indices considered in this study. It can be concluded from this study that qualitative and quantitative restriction can be used as management tool to alter the carcass yield, cut-parts and visceral organs of growing pigs; the cooking loss of pork likewise was impacted positively by the quality of ration offered.

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# WPŁYW OGRANICZONEGO ILOŚCIOWO I JAKOŚCIOWO ŻYWIENIA NA WYDAJNOŚĆ TUSZ I JAKOŚĆ MIĘSA WIEPRZOWEGO

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Słowa kluczowe: żywienie restrykcyjne, żywienie *ad libitum*, wyręby tuszy, jakość mięsa.

Abstrakt

Doświadczenie w układzie 3 x 3 przeprowadzono w celu określenia wydajności rzeźnej i jakości mięsa rosnących świń żywionych restrykcyjnie pod względem ilościowym i jakościowym. 81 świń rasy wielkiej białej (54 wieprzki i 27 loszek) o początkowej masie ciała 6,7 ± 0,39 kg podzielono na 9 grup doświadczalnych, w trzech powtórzeniach po 3 osobniki w każdym. Czynnikami doświadczalnymi były trzy różne poziomy żywienia jakościowego (20, 18 lub 16% białka ogólnego w paszy) i ilościowego (żywienie ad *libitum,* 90% lub 80% poziomu *ad libitum*). Końcowa masa ciała świń w grupach żywionych z ograniczeniami jakościowymi statystycznie istotnie ($p < 0,05$) zmniejszała się wraz z malejącym poziomem białka ogólnego w mieszankach. Takie same zależności obserwowano w odniesieniu do cech jakości tusz, z wyjątkiem masy lewej półtuszy, która nie była zróżnicowana statystycznie istotnie. Zawartość białka ogólnego w stosowanych mieszankach miała statystycznie istotny ($p < 0,05$) wpływ na straty w procesie gotowania, które rosły wraz z obniżaniem się zawartości białka. Żywienie restrykcyjne w zakresie ilości podawanej paszy spowodowało statystycznie istotne ($p < 0,05$) zróżnicowanie masy poszczególnych wyrębów tuszy i narządów wewnętrznych: głowy, łopatki, nogi przedniej, nogi tylnej, wątroby, nerek, śledziony i żołądka. Jednocześnie zróżnicowanie ilościowe żywienia nie miało wpływu na cechy jakości pozyskanego mięsa.

Stwierdzono statystycznie istotne interakcje między żywieniem restrykcyjnym ilościowym i jakościowym w zakresie wszystkich badanych cech wydajności tuszy, jednocześnie nie stwierdzono ich w zakresie badanych cech jakości mięsa.

Można stwierdzić, że zastosowanie żywieniowych ograniczeń jakościowych i ilościowych może być metodą kształtowania wydajności rzeźnej, masy wyrębów tuszy i narządów wewnętrznych rosnących świń. Podobnie jakość podawanej paszy może wpływać na mniejsze straty podczas obróbki cieplnej wieprzowiny.

## Introduction

Pig production still rank highest as one of the most veritable sources of animal protein to the growing populations of the world especially the resource-deficient countries. Nutritional requirement of pigs is a major concern to the pig farmers. Most of them are unaware of the nutrient requirements of pigs and how feeding strategies can be manipulated in order to attain production goals of the pig enterprise. One of the main obstacles to livestock improvement in developing countries of the world is

that of inadequate and unbalanced ration (NJOKU et al. 2015a). This has immensely affected the level of production of domestic animal especially pig. Pig farmers feed their animals with unwholesome feed ingredients without recourse to the carcass characteristics and pork quality from such pigs. However, the consumers have become far more health conscious and are often aware of the relationship between meat quality and food safety (DUCLOS et al. 2007). The quality of the meat is mainly influenced by genotype of animals and its environment, especially either nutrients or stress undergone during growing period or before slaughter, taking into account the degree of fat and muscle tissue.

Potential benefits of feeding strategy include production of animals with maximum lean body mass, better feed efficiency, premium meat quality, reduced feed cost per unit gain, lower inputs for feed and manure handling and possibly less feed wastages (WOOD et al. 2004, TERLOUW et al. 2005, NJOKU et al. 2013). *Ad libitum* feeding has been reported to make farm animals to be obese and thus predispose to thermal discomfort, high incidence of lameness and high morbidity and mortality due to skeletal disorders and heart failure (NJOKU et al. 2017). Excessive body weight is also associated with reduced disease resistance (KORNIEWICZ et al. 2012) and increased incidence of multiple ovulations in female animals, resulting to low productivity. Feed restriction could be a useful management tool for improving the biological and economic performance; and meat quality characteristics of domestic animal through prevention of excessive accumulation of body and abdominal fats (LEBRET et al. 2001, MEHMOOD et al. 2007). Only a limited number of studies have addressed the effect of dietary protein content or levels of lysine on the carcass composition and meat quality (PUGLIESE et al. 2013). Likewise, the limit of feed restriction as to not impair carcass yield and meat quality has not yet been established (YANEZE et al. 2007). To this effect, this study was carried out to evaluate the carcass yield and meat quality characteristics of growing pigs reared in a hot humid environment to qualitative and quantitative restricted feeding strategy.

## Materials and Methods

The experimental protocols involving the use of animals were in compliance with the animal welfare requirements for care and management of experimental animal and was in line with the guidelines of the Animal Welfare Committee of the Federal University of Agriculture, Abeokuta, Nigeria (FUNAAB 2013)

The experiment was carried out at the Piggery Unit of the Teaching and Research Farms Directorate (TREFAD) and Meat Processing Laboratory of Department of Animal Production and Health, Federal University of Agriculture, Abeokuta, Ogun State, Nigeria. The farm lies within latitude 7°10'N, longitude 3°2'E and altitude 76 mm. It is located in the derived savannah zone of South-Western Nigeria. It has a humid climate with mean annual rainfall of about 1037 mm and temperature of about 34.7°C. The relative humidity ranges from 63 to 96% in the rainy season (late March to October) and from 55 to 82% in the dry season (November to early March) with an annual average of 82%. The seasonal distribution of annual rainfall is approximately 44.96 mm in the late dry season (January–March); 212.4 mm in the early wet season (April–June); 259.3 mm in the late wet season (July–September) and 48.1 mm in the early dry season (October–December) as documented by Federal University of Agriculture, Abeokuta Meteorological Station.

A total of eighty one (81) Large White breed of pigs weighing 6.72 ± 0.38 kg were bought from the established drift in the Teaching and Research Farm of the Federal University of Agriculture, Abeokuta, Nigeria. The pigs were grouped on weight equalisation into (9) treatments with 3 replicates of 3 pigs per replicate. Sex was balanced across the treatment groups. The floor of the housing unit was washed with detergent and disinfectant, the pen was repaired and put into good working conditions before the arrival of the experimental animals. On arrival the pigs were given water and feed containing anti-stress and multi-vitamins. The pigs were injected with ivomec® (ivermectine) against endo and ecto-parasites and allowed to acclimatize for one week (7 days) before the commencement of the experiment. The pen has a floor area of 3 m by 2 m, equipped with concrete feeding and watering trough. The pens are half-walled of about 1.4–1.6 m high and the rest were open-sided for proper ventilation. Daily routine management practices were carried out on daily basis, fresh water was supplied *ad libitum* throughout the 150 days experimental period.

The experimental design was a 3 × 3 factorial arrangement with factor A consisting of three (3) levels of qualitative feed restriction (20%, 18% and 16% crude protein) and factor B involving quantitative feed restriction (*ad libitum,* 90% and 80% of *ad-libitum* feeding). Treatment 1 consists of pigs fed *ad libitum* with daily ration containing 20% crude protein, group 2 was fed *ad libitum* with daily ration containing 18% crude protein, group 3 was fed *ad libitum* with daily ration containing 16% crude protein, group 4 consists of pigs on 90% of *ad-libitum* feeding with 20% crude protein ration, group 5 was fed 90% of *ad-libitum* feeding with 18% crude protein ration, group 6 was fed with 90% of *ad-libitum* feeding with 16% crude protein

ration, group 7 consists of pigs on 80% of *ad-libitum* feeding with 20% crude protein ration, group 8 was fed 80% of *ad-libitum* feeding with 18% crude protein ration, and group 9 was fed 80% of *ad-libitum* feeding with 16% crude protein ration.

Table 1

Percentage composition of experimental diets on as-fed basis

| Ingredients | Diet 1 | Diet 2 | Diet 3 |
|---|---|---|---|
| Maize | 29.00 | 40.00 | 45.50 |
| Groundnut cake | 18.00 | 13.00 | 8.00 |
| Wheat offal | 30.00 | 20.00 | 19.05 |
| Palm kernel cake | 19.65 | 23.65 | 24.10 |
| Bone meal | 2.60 | 2.60 | 2.60 |
| Salt | 0.35 | 0.35 | 0.35 |
| L-Lysine | 0.05 | 0.05 | 0.05 |
| DL-Methionine | 0.05 | 0.05 | 0.05 |
| Premix* | 0.30 | 0.30 | 0.30 |
| Total | 100.00 | 100.00 | 100.00 |
| Calculated Nutrients [%] | | | |
| Crude protein | 20.30 | 18.00 | 16.16 |
| Fat | 6.81 | 7.34 | 7.26 |
| Crude fibre | 5.09 | 4.47 | 4.25 |
| Calcium | 0.77 | 0.79 | 0.84 |
| M.E [MJ/kg feed] | 11.19 | 12.00 | 12.26 |
| Calcium | 1.00 | 1.00 | 1.00 |
| Phosphorus | 0.50 | 0.50 | 0.40 |

* To supply the following per kg diet; vit. A – 12600 IU; vit. $D_3$ – 2800 IU; vit. E – 49 IU; vit. $K_3$ – 2.8 mg; vit. $B_1$ – 1.4 mg; vit. $B_2$ – 5.6 mg; vit. B6 – 1.4mg; vit $B_{12}$ – 0.014 mcg; nacin – 21 mg; pantothenic acid – 14 mg; folic acid – 1.4 mg; biotin – 0.028 mcg; choline chloride – 70 mg; manganese – 70 mg; zinc – 140 mg; iron – 140 mg; copper – 140 mg; iodine – 1.4 mg; selenium – 0.28 mg; cobalt – 0.7 mg; antioxidant – 168 mg.

Three experimental diets were formulated to meet the body requirements of growing pigs as shown in Table 1. The ration contained 20.30%, 18.00% or 16.16% crude protein and metabolisable energy of 11.19, 12.00 or 12.26 MJ $kg^{-1}$ feed (Auto Feed Formulator, NRC 1998) for diets 1, 2 and 3 respectively. Feed was offered to the experimental pigs at 08:00 hour daily throughout the experimental period. *Ad libitum* feeding level had been previously determined as standard used on the Teaching and Research Farm of the Federal University of Agriculture for different categories of pigs and adjusted on weekly basis throughout the study period while 80 and 90% feeding levels were calculated based on the weekly value obtained for *ad libitum* fed group.

Fifty four (54) pigs consisting of 6 pigs whose live weight were close to the mean treatment weight were selected from each treatment, slaughtered and analysed for carcass yield at the end of the experiment. The pigs were weighed and fasted for 16 hours, and the fasted weight of each pig meant for slaughtering were taken before they are stunned by percussion method and bled by incision using a sharp knife cutting through the jugular vein between the skull and the atlas. Complete bleeding and dehairing were done.

The stomach of the pigs was opened along the greater curvature and emptied. After the removal of the visceral organs, the remaining part were measured as carcass weight and later expressed as percentage of the live weight to get the dressing percentage. The carcass was divided longitudinally. The left half of the carcass was dissected as described by BARCA et al. (2006). Ham was separated by locating the division between the 2nd and 3rd sacral vertebrae and saw perpendicularly along axis of the ham. Shoulder of the pig was separated from the loin and belly by a straight cut between the second and third ribs and a straight cut 2.5 cm ventral to the ventral edge of the scapula. The cut parts was weighed and recorded.

Water Holding Capacity (WHC): The water holding capacity of meat was determined by the press method as modified BY KAUFFMAN et al. (1992). Approximately one gram (1 g) of meat sample was cut from the ham of the slaughtered pigs in each treatment. Each sample was collected, hygienically placed into two plexi glasses at about 34.6 kg $cm^{-3}$ absolute pressure between two Whatman filter papers for 1 minute, using a bench vice. Thereafter, the meat samples were transferred to a hot air oven and dried for 1 hour, then weighed and returned back to the oven for another 30 minutes and weighed again to determine the moisture content. Thereafter, the pressed sample is placed on a tracing sheet and the circumference of the water loss and the sample weight were traced and measured for all using a graph sheet of 1 cm. The Water holding capacity of the meat was then calculated as:

$$\text{W.H.C.} = 100 - \frac{(A_r - A_m) \cdot 9.47}{(W_o \cdot W_m)}$$

where:
$A_r$ – area of water in $cm^2$
$A_m$ – area of meat in $cm^2$
$W_o$ – weight of water loss (initial weight – final weight)
$W_m$ – weight of meat
9.47 – constant

Cooking loss: This was measured using the procedure of OMOJOLA (2007) with some modifications. It was determined by collecting about 50 g meat samples from the ham of each treatment, hygienically wrapped in air-tight polythene bag, labelled according and immediately cooked in water bath at about $70^0$C for 15 minutes. The temperature was checked using a skewer thermometer. Thereafter, the meat samples were allowed to cool at a room temperature and weighed again using a sensitive scale. It was expressed as:

Cooking loss [g] = weight of raw meat – weight of cooked meat

$$\text{Cooking loss [\%]} = \frac{\text{weight of raw meat} - \text{weight of cooked meat}}{\text{weight of raw meat}} \cdot 100$$

Refrigeration loss: This was determined by collecting about 50 g meat samples from the ham of each treatment, hygienically wrapped in air-tight polythene bag, labelled according and placed in refrigeration for 24 hours. Thereafter, the weight of the meat was determined after refrigeration using a sensitive scale.

Refrigeration loss [g] = weight of raw meat – weight of refrigeration meat

$$\text{Refrigeration loss [\%]} = \frac{\text{weight of raw meat} - \text{weight of refrigeration meat}}{\text{weight of raw meat}} \cdot 100$$

The experimental layout was a 3 x 3 factorial arrangement. Data generated were subjected to a one-way Analysis of Variance using SAS (2000). Significantly ($P < 0.05$) different means among the variables were separated using New Duncan Multiple Range Test as contained in the same statistical package. The statistical model is as described below:

$$Y_{ijk} = \mu + A_i + B_j + AB_{ij} + \sum_{ijk}$$

where:
$Y_{ijk}$ – observed values of dependent variable
$\mu$ – population mean
$A_i$ – effect of $i^{th}$ qualitative feed restriction (16, 18, 20% crude protein)
$B_j$ – effect of $j^{th}$ quantitative feed restriction (*ad libitum*, 90%, 80% of *ad libitum*)
$(AB)_{ij}$ – interaction between qualitative and quantitative feed restriction
$\sum_{ijk}$ – random residual error.

## Results

Table 2 shows the effects of qualitative and quantitative feed restriction on carcass yield of growing pigs. Significant differences were observed across all the parameters considered except left carcass weight, head, fore--leg, hind-leg, kidney, and heart weights. The values of live weight (33.50, 27.58, 24.39 kg), fasted weight (31.75, 26.77, 23.98 kg), bled weight (27.81, 22.89, 19.93 kg) and eviscerated weight (21.33, 17.67, 15.00 kg) decreased significantly with decreasing levels of dietary crude protein. Comparable means values were obtained for ham (2.68, 2.28%) and liver (0.63, 0.60%)

Table 2

Effects of qualitative and quantitative restriction on carcass yield of growing pigs

| Parameters/Treatments | Qualitative restriction | | | | Quantitative restriction | | | |
|---|---|---|---|---|---|---|---|---|
| | 20% C.P. | 18% C.P. | 16% C.P. | S.E.M | *Ad libitum* | 90% of *ad libitum* | 80% of *ad libitum* | S.E.M |
| Initial weight [kg/pig] | 6.78 | 6.83 | 6.56 | ±0.38 | 6.44 | 7.00 | 6.72 | ±0.38 |
| Live weight [kg/pig] | $33.50^{a}$ | $27.58^{b}$ | $24.39^{c}$ | ±0.48 | $28.58^{ab}$ | $29.58^{a}$ | $27.31^{b}$ | ±0.48 |
| Fasted weight [kg/pig] | $31.75^{a}$ | $26.77^{b}$ | $23.98^{c}$ | ±0.37 | $28.30^{a}$ | $28.32^{a}$ | $25.88^{b}$ | ±0.37 |
| Bled weight [kg] | $27.81^{a}$ | $22.89^{b}$ | $19.93^{c}$ | ±0.43 | $26.51^{a}$ | $23.33^{b}$ | $20.78^{c}$ | ±0.43 |
| Eviscerated weight [kg] | $21.33^{a}$ | $17.67^{b}$ | $15.00^{c}$ | ±0.58 | $19.00^{a}$ | $18.00^{ab}$ | $17.00^{b}$ | ±0.58 |
| Left carcass weight [kg] **Cut parts [% live weight]** | 7.15 | 6.67 | 5.33 | ±0.57 | 5.33 | 6.81 | 7.00 | ±0.57 |
| Head weight | 2.89 | 2.71 | 2.54 | ±0.17 | $2.55^{b}$ | $3.11^{a}$ | $2.49^{b}$ | ±0.17 |
| Ham weight | $3.62^{a}$ | $2.68^{b}$ | $2.28^{b}$ | ±0.16 | 3.02 | 2.84 | 2.73 | ±0.16 |
| Shoulder weight | $3.40^{a}$ | $3.18^{a}$ | $2.75^{b}$ | ±0.13 | $3.35^{a}$ | $3.25^{a}$ | $2.74^{b}$ | ±0.13 |
| Fore-leg weight | 0.39 | 0.38 | 0.37 | ±0.02 | $0.36^{b}$ | $0.47^{a}$ | $0.31^{b}$ | ±0.02 |
| Hind-leg weight **Offals weight [% live weight]** | 0.45 | 0.47 | 0.40 | ±0.03 | $0.48^{a}$ | $0.52^{a}$ | $0.32^{b}$ | ±0.03 |
| Liver weight | $1.05^{a}$ | $0.63^{b}$ | $0.60^{b}$ | ±0.03 | $0.86^{a}$ | $0.78^{a}$ | $0.64^{b}$ | ±0.03 |
| Lung weight | $0.44^{a}$ | $0.33^{b}$ | $0.26^{c}$ | ±0.02 | 0.36 | 0.34 | 0.33 | ±0.02 |
| Kidney weight | 0.18 | 0.15 | 0.12 | ±0.02 | $0.20^{a}$ | $0.15^{ab}$ | $0.09^{b}$ | ±0.02 |
| Heart weight | 0.19 | 0.26 | 0.27 | ±0.12 | 0.17 | 0.14 | 0.41 | ±0.12 |
| Spleen weight | $0.34^{a}$ | $0.16^{b}$ | $0.13^{b}$ | ±0.03 | $0.28^{a}$ | $0.22^{ab}$ | $0.14^{b}$ | ±0.03 |
| Full G.I.T weight | $5.27^{a}$ | $4.25^{b}$ | $3.58^{b}$ | ±0.28 | 4.65 | 4.68 | 3.77 | ±0.28 |
| Full stomach weight | $0.86^{a}$ | $0.44^{b}$ | $0.34^{b}$ | ±0.05 | $0.39^{b}$ | $0.49^{b}$ | $0.77^{a}$ | ±0.05 |
| Empty stomach weight | $0.34^{a}$ | $0.28^{b}$ | $0.19^{c}$ | ±0.01 | $0.23^{b}$ | $0.28^{a}$ | $0.30^{a}$ | ±0.01 |
| Full intestine weight | $2.54^{a}$ | $2.30^{b}$ | $2.07^{b}$ | ±0.07 | 2.34 | 2.39 | 2.19 | ±0.07 |

$^{abc}$ – values with different superscripts within row are significantly different ($P < 0.05$)
S.E.M – standard error mean; C.P – crude protein

weights of pigs on 18% and 16% crude protein diets which differed significantly ($p < 0.05$) from the values (3.62 and 1.05%, respectively) gotten for pigs fed 20% dietary protein. Shoulder weights (3.40, 3.18%) of pigs on dietary protein levels of 20 and 18% were statistically similar but differed ($p < 0.05$) from 2.75% recorded by those on 16% crude protein diets.

Lung weight (0.44, 0.33, 0.26%) and empty stomach weight (0.34, 0.28 and 0.19%) decreased significantly ($p < 0.05$) with decreasing levels of dietary crude protein levels. Pigs fed 20% dietary crude protein had the highest ($p < 0.05$) values in spleen, full gastrointestinal tract, full stomach and full intestinal compared to their counterparts on 16% crude protein diet that recorded the least values.

Significant ($p < 0.05$) differences were observed across all parameters considered except for left carcass, ham, lung, heart, full gastrointestinal tracts and full intestine weights. Pigs on 90% *ad libitum* feed offered had the highest ($p < 0.05$) live weight value of 29.58 kg while those fed 80% of *ad libitum* recorded the least value of 27.31 kg. Comparable mean values (28.30, 28.32 kg) were obtained for fasted weight of pigs on *ad libitum* feed offered and 90% of *ad libitum* which differed significantly ($p < 0.05$) from 25.88 kg gotten for pigs on 80% of *ad libitum* feed offered. Values of bled (26.51, 23.33, 20.78 kg) and eviscerated (19.00, 18.00, 17.00 kg) weights of pigs decreased ($p < 0.05$) with decreasing levels of feed offered. Comparable means values (2.55, 2.49%) were obtained for head weight of pigs fed *ad libitum* and 80% *ad libitum* feed offered these were significantly different from 3.11% noted for pigs on 90% *ad libitum* feed offered. The shoulder weight (3.35%) and hind-leg weight (0.48%) values of pigs fed *ad libitum* are comparable to those (3.25 and 0.52%, respectively) documented for pigs fed 90% *ad libitum* and these values were significantly higher than 2.73% and 0.32% respectively documented for pigs on 80% *ad libitum* feeding. Quantitative restriction significantly influenced the fore-leg weight of pigs with the range values from 0.31% (pigs fed 90% *ad libitum*) to 0.47% (pigs on 90% *ad libitum* feeding). Liver (0.86, 0.78, 0.64%), kidney (0.20, 0.15, 0.09%) and spleen (0.28, 0.22, 0.14%) weights decreased ($p < 0.05$) with increasing levels of feed restriction. Full stomach increased from 0.39% (*ad libitum* fed pigs) to 0.77% (80% *ad libitum* fed pigs). Empty stomach followed the same trend with full stomach.

Table 3 shows the interaction between qualitative and quantitative feed restriction on carcass yield of a growing pigs. Significant ($p < 0.05$) differences were observed across all parameters considered. Pigs fed 20% dietary protein at *ad libitum* recorded highest value in live weight (35.50 kg), fasted weight (34.00 kg), bled weight (30.95 kg) and eviscerated weight (22.00 kg) while those fed 18% crude protein diet at 80% *ad libitum* feed

Table 3

Interaction between qualitative and quantitative feed restriction on carcass yield of a growing pigs

| Parameters/Treatments | 20% *ad libitum* | 18% *ad libitum* | 16% *ad libitum* | 20% 90% *ad libitum* | 18% 90% *ad libitum* | 16% 90% *ad libitum* | 20% 80% *ad libitum* | 18% 80% *ad libitum* | 16% 80% *ad libitum* | S.E.M |
|---|---|---|---|---|---|---|---|---|---|---|
| Initial weight [kg] | 6.67 | 6.67 | 7.00 | 6.67 | 7.00 | 6.83 | 6.00 | 7.33 | 6.33 | ±0.66 |
| Live weight [kg] | $35.50^{a}$ | $27.50^{de}$ | $25.50^{e}$ | $32.50^{b}$ | $28.50^{d}$ | $27.50^{de}$ | $31.50^{bc}$ | $29.50^{cd}$ | $21.50^{f}$ | ±0.89 |
| Fasted weight [kg] | $34.0^{a}$ | $26.00^{de}$ | $24.00^{e}$ | $31.00^{b}$ | $27.00^{d}$ | $26.00^{de}$ | $30.00^{bc}$ | $28.00^{cd}$ | $20.00^{f}$ | ±0.89 |
| Bled weight [kg] | $30.95^{a}$ | $25.25^{bc}$ | $22.85^{de}$ | $26.00^{b}$ | $22.00^{ef}$ | $21.00^{f}$ | $24.00^{cd}$ | $22.50^{def}$ | $16.00^{g}$ | ±0.50 |
| Eviscerated weight [kg] | $22.00^{a}$ | $17.00^{d}$ | $15.00^{f}$ | $21.00^{b}$ | $16.00^{e}$ | $14.00^{g}$ | $18.00^{c}$ | $17.50^{cd}$ | $13.00^{h}$ | ±0.06 |
| Left carcass weight [kg] **Cut parts [% live weight]** | $8.00^{a}$ | $6.00^{c}$ | $5.00^{d}$ | $8.35^{a}$ | $8.00^{a}$ | $7.00^{b}$ | $8.00^{a}$ | $8.50^{a}$ | $7.00^{a}$ | ±0.06 |
| Head weight | $2.80^{c}$ | $3.10^{bc}$ | $2.65^{cd}$ | $3.80^{a}$ | $3.10^{bc}$ | $3.10^{bc}$ | $3.50^{ab}$ | $2.95^{bc}$ | $2.10^{d}$ | ±0.07 |
| Ham weight | $3.85^{a}$ | $2.65^{d}$ | $2.55^{e}$ | $3.35^{b}$ | $2.35^{f}$ | $2.30^{f}$ | $3.15^{c}$ | $3.08^{c}$ | $2.00^{g}$ | ±0.00 |
| Shoulder weight | $4.10^{a}$ | $3.20^{c}$ | $3.05^{cd}$ | $3.60^{b}$ | $3.00^{cd}$ | $2.90^{d}$ | $2.55^{ef}$ | $2.80^{de}$ | $2.35^{f}$ | ±0.01 |
| Fore-leg weight | $0.35^{b}$ | $0.25^{d}$ | $0.35^{b}$ | $0.30^{c}$ | $0.30^{c}$ | $0.30^{c}$ | $0.45^{a}$ | $0.48^{a}$ | $0.35^{b}$ | ±0.00 |
| Hind-leg weight **Offals weight [% live weight]** | $0.50^{a}$ | $0.50^{a}$ | $0.50^{a}$ | $0.45^{a}$ | $0.50^{a}$ | $0.50^{a}$ | $0.23^{b}$ | $0.29^{b}$ | $0.25^{b}$ | ±0.00 |
| Liver weight | $1.15^{a}$ | $0.75^{a}$ | $0.60^{cd}$ | $1.00^{ab}$ | $0.60^{cd}$ | $0.65^{c}$ | $1.00^{ab}$ | $0.75^{bc}$ | $0.35^{d}$ | ±0.01 |
| Lung weight | $0.45^{a}$ | $0.40^{a}$ | $0.30^{b}$ | $0.45^{a}$ | $0.30^{b}$ | $0.30^{b}$ | $0.45^{a}$ | $0.40^{a}$ | $0.20^{c}$ | ±0.00 |
| Kidney weight | $0.20^{a}$ | $0.15^{b}$ | $0.10^{c}$ | $0.10^{c}$ | $0.10^{c}$ | $0.10^{c}$ | $0.10^{c}$ | $0.09^{c}$ | $0.05^{d}$ | ±0.00 |
| Heart weight | $0.20^{a}$ | $0.15^{b}$ | $0.10^{c}$ | $0.15^{b}$ | $0.10^{c}$ | $0.15^{b}$ | $0.15^{b}$ | $0.13^{bc}$ | $0.10^{c}$ | ±0.00 |
| Spleen weight | $0.30^{a}$ | $0.20^{abc}$ | $0.10^{cd}$ | $0.30^{a}$ | $0.10^{cd}$ | $0.10^{cd}$ | $0.25^{ab}$ | $0.15^{bc}$ | $0.03^{d}$ | ±0.00 |
| Full G.I.T weight | $5.40^{a}$ | $4.40^{cd}$ | $4.30^{d}$ | $5.00^{ab}$ | $4.20^{d}$ | $4.05^{d}$ | $4.80^{bc}$ | $4.40^{cd}$ | $2.85^{e}$ | ±0.04 |
| Full stomach weight | $0.55^{bc}$ | $0.40^{bc}$ | $0.35^{c}$ | $0.55^{bc}$ | $0.40^{bc}$ | $0.25^{c}$ | $1.20^{a}$ | $0.83^{ab}$ | $0.35^{c}$ | ±0.03 |
| Empty stomach weight | $0.35^{a}$ | $0.30^{b}$ | $0.20^{d}$ | $0.25^{c}$ | $0.25^{c}$ | $0.20^{d}$ | $0.35^{a}$ | $0.33^{ab}$ | $0.20^{d}$ | ±0.00 |
| Full intestine weight | $2.70^{a}$ | $2.25^{cd}$ | $2.10^{d}$ | $2.20^{cd}$ | $2.35^{bcd}$ | $2.25^{cd}$ | $2.65^{a}$ | $2.43^{abc}$ | $1.60^{e}$ | ±0.01 |

*abcdefgh* – values with different superscripts within rows are significantly different ($P < 0.05$)

S.E.M – standard error mean

offered had the least mean values of 21.50, 20.00, 16.00 and 13.00 respectively. Pigs on 18% dietary crude protein at 80% *ad libitum* feed offered had the highest left carcass weight (8.50 kg) while their counterparts fed *ad libitum* on 16% crude protein diet recorded the least value (5.00 kg). The ham, shoulder and hind-leg weights of pigs fed *ad libitum* with diet containing 20% crude protein had the highest ($p < 0.05$) mean values while those fed *ad libitum* on ration containing 16% crude protein recorded the least values in all these measurements. The head weight values ranged from 2.10% (pigs on 16% crude protein diet at *ad libitum* feed offered) to 3.80% (pigs on 20% dietary crude protein at 90% feed offered). Fore-leg weight of pigs on 18% crude protein diet fed at 80% *ad libitum* feed offered was the highest while those offered 18% crude protein diet at *ad libitum* had the least value. Liver, kidney, heart, full gastrointestinal tract and empty stomach weights had the highest mean values documented for pigs fed *ad libitum* with diet containing 20% crude protein. Full stomach values ranged from 0.25% noted for pigs on 16% dietary crude protein ration at 90% *ad libitum* feed offered to 1.20% recorded for pigs fed diet containing 20% crude protein at 80% *ad libitum* feeding. Pigs on 16% crude protein diet at 80% feeding level had the least full intestine weight while their counterparts fed ad libitum with 20% crude protein diet recorded the highest mean value.

Table 4

Effects of qualitative and quantitative feed restriction on meat quality of growing pigs

| Parameters/Treatments | Qualitative restriction | | | | Quantitative restriction | | | |
|---|---|---|---|---|---|---|---|---|
| | 20% C.P. | 18% C.P. | 16% C.P. | S.E.M | *ad libitum* | 90% of *ad libitum* | 80% of *ad libitum* | S.E.M |
| Water holding capacity | 27.72 | 31.29 | 45.69 | ±8.55 | 37.19 | 28.57 | 38.95 | ±14.8 |
| Cooking loss [g] | $8.95^{b}$ | $10.51^{ab}$ | $11.87^{a}$ | ±0.73 | 10.66 | 10.34 | 10.33 | ±1.26 |
| Cooking loss [%] | $17.89^{b}$ | $20.88^{ab}$ | $23.74^{a}$ | ±1.49 | 21.32 | 20.53 | 20.66 | ±2.59 |
| Refrigeration loss [g] | 0.41 | 0.41 | 0.63 | ±0.09 | 0.55 | 0.34 | 0.56 | ±0.16 |
| Refrigeration loss [%] | 0.82 | 1.26 | 0.82 | ±0.19 | 1.09 | 0.68 | 1.12 | ±0.32 |
| pH | 5.7 | 6.0 | 6.1 | ±0.21 | 5.7 | 6.0 | 6.1 | ±0.37 |

$^{ab}$ – values with different superscripts within row are significantly different ($p < 0.05$)
S.E.M – standard error mean

Table 4 shows the effects of qualitative and quantitative feed restriction on meat quality characteristics of growing pigs. The change in the level of dietary crude protein offered to the growing pigs had significant ($p < 0.05$) effect on cooking losses and percentage cooking losses. Percentage cooking loss had the highest value of 23.74% documented for growing pigs fed 16% dietary crude protein while the least

value of 17.89% was recorded for pigs given ration containing 20% crude protein. Dietary protein level had no significant ($p > 0.05$) effect on water holding capacity, refrigeration loss, percentage refrigeration loss and pH values of the meat. The water holding capacity and pH values decreased numerically ($P > 0.05$) with increasing level of dietary protein.

There were no significant ($p > 0.05$) effect on water holding capacity, cooking loss, percentage cooking loss, refrigeration loss, percentage refrigeration loss. Water holding capacity mean values ranged from 28.57 to 38.95%, the highest value was noted for the animals placed on 80% *ad libitum* and the least value was recorded for animal fed 90% *ad libitum*. Percentage cooking loss mean values ranged from 20.53 in pigs fed 90% *ad libitum* feeding to 21.32% documented for pigs offered feed at *ad libitum*. Refrigeration loss mean values ranged from 0.34 to 0.56 g, the highest numeric value was noted for the pigs placed on 80% *ad libitum* and the least value was recorded for those fed 90% *ad libitum*. Percentage refrigeration loss ranged from 0.68 to 1.12%, the highest value was noted on the animals placed on 80% *ad libitum*, and the least value was recorded on animal fed 90% *ad libitum*. pH mean values ranged from 5.7 to 6.1 with the highest value noted for the animals placed on 80% *ad libitum* while the least value was recorded for animal fed *ad libitum*.

The interaction between quantitative and qualitative feed restriction had no significant ($p > 0.05$) effect on cooking loss, percentage cooking loss, refrigeration loss, percentage refrigeration loss, water holding capacity and pH of meat (Table 5). The mean values of cooking loss ranged from 8.13 to 12.21 g, the highest value was documented for pigs offered 18% dietary crude protein ration at 90% *ad libitum*, while the least value was recorded for pigs fed *ad libitum* with 18% crude protein diet. Percentage cooking loss mean values ranged from 16.3 to 24.4%, the highest value was noted for pigs fed ration containing 18% crude protein at 90% *ad libitum*, while the least value was recorded for pigs offered *ad libitum* with diet containing 18% crude protein.

Table 5

Interaction between qualitative and quantitative feed restriction on meat quality of growing pigs

| Parameters/Treatments | 20% *ad libitum* | 18% *ad libitum* | 16% *ad libitum* | 20% 90% *ad libitum* | 18% 90% *ad libitum* | 16% 90% *ad libitum* | 20% 80% *ad libitum* | 18% 80% *ad libitum* | 16% 80% *ad libitum* | S.E.M |
|---|---|---|---|---|---|---|---|---|---|---|
| Water holding capacity | 37.1 | 40 | 34.6 | 21.0 | 44.1 | 20.6 | 35.8 | 53.1 | 28.0 | ±3.52 |
| Cooking loss [g] | 9.93 | 10.5 | 11.6 | 8.13 | 10.69 | 12.21 | 8.79 | 10.4 | 11.84 | ±0.45 |
| Cooking loss [%] | 19.9 | 21.0 | 23.1 | 16.3 | 20.90 | 24.40 | 17.6 | 20.7 | 23.7 | ±0.90 |
| Refrigeration loss [g] | 0.42 | 0.65 | 0.57 | 0.14 | 0.61 | 0.27 | 0.67 | 0.64 | 0.39 | ±0.06 |
| Refrigeration loss [%] | 0.84 | 1.3 | 1.14 | 0.28 | 1.21 | 0.54 | 1.33 | 1.27 | 0.77 | ±0.13 |
| pH | 5.7 | 5.5 | 5.9 | 6.2 | 6.0 | 5.9 | 6.5 | 5.7 | 6.3 | ±0.11 |

S.E.M – standard error mean

## Discussion

Intake limitation strategy modifies the body composition of domestic animal. Based on tissue allometry deposition, feed restriction leads to changes in differential growth of internal organs and body tissues during the realimentation period (CHIBA et al. 2002, FABIAN et al 2004). The live weight of pigs in this study increased with increasing levels of dietary crude protein. Two and four percent increase in dietary crude protein levels resulted to 13.08 and 37.35% increase in live weight, respectively. Several authors have reported increase in live weight with increasing levels of dietary crude protein content (RUUSUNEN et al. 2007, PEINADO et al. 2009). The differences in live weight could be attributed to the higher lysine content in the high protein diets which has been established to correlate with the rate of protein synthesis (RUUSUNEN et al. 2007). The significant difference in fasted, bled and eviscerated weights were attributable to the differences in live weight of the pigs which increased significantly with increasing levels of dietary protein. PEINADO et al. (2009) noted high correlation between live weight and hot carcass weight of Chato Murciano pigs on high and low protein diets, whereas, WOOD et al. (2004) observed similarity in hot carcass yield values of pigs on varied levels of crude protein diets. Dietary protein levels showed significant effect on ham and shoulder weights. The results are in agreement with the observation of PEINADO et al. (2009) who reported a significant heavier ham of pigs on high protein with low fibre diet compared to the value obtained for pigs on low protein with high fibre diet. Several factors (body conformation, restriction and environmental temperature) have been reported to influence the organ weights of slaughtered pigs (RUUSUNEN et al. 2007). Liver weight has been reported to increase with increasing dietary protein content or with an increasing lysine-to-digestible energy ratio (SZABO et al. 2001). This is in line with the result of this study that buttress the fact that dietary crude protein levels significantly influenced the liver, lung and spleen weights of growing pigs. The liver and spleen hypertrophy associated with intake of the high protein ration was probably related to the higher amount of nitrogenous compounds processed by these organs (ANUGWA et al. 1989). The elevated values of stomach (both full and empty) and full intestinal weights with increasing levels of dietary crude protein might be due to increase in apparent maintenance requirement caused by repartitioning of nutrients from growth of the edible carcass to the visceral organs, leading to the increase in the mass of these organs. KOONG et al. (1983) asserts that pigs on higher plane of nutrition exhibited significantly heavier weights for metabolically active organs (stomach,

intestines, pancreas, liver and kidneys) than their counterparts on low plane of nutrition.

The significant difference noted in the live weight of pigs with variation in feed accessibility is an indication that nutrient requirements for maintenance and growth of the pigs were compromised by quantitative feed restriction. This observation is consistent with the reports of previous researchers (LEBRET et al. 2001, NJOKU et al. 2013) that found lower growth rate in feed restricted animals in comparison with their full-fed counterparts. LEBRET et al. (2001) observed that 25% reduction in feed offered to pig decreases the weight gain by 27%. WATANABE et al. (2010) stated that restricted feeding had effect on feed consumption which negatively influenced the growth rate of the animal as the severity of restriction increased. The higher live weight recorded by pigs on 10% decrease in feeding allowance could be attributed to a reduction in metabolic heat production, leading to a better feed utilization (LOVATTO et al. 2006) and improvement in the welfare of pigs during the realimentation period which must have translated to the superior final weight. The results of this present study are in line with the observation of SHEIKH et al. (2014) who reported that replacement of maize with paddy during finishing phase of pigs resulted to significant differences in bled and eviscerated weights. The observed differences are reflection of the differences in live weight of the pigs. On the contrary, WATANABE et al. (2010) noted no significant difference in the slaughtered weights of pig subjected to different inclusion levels of citrus pulp. Carcass parameters are important factors to be well thought-out since increase in weight of the cut-parts will result to higher profitability (AGUNBIADE 2009). The significant means observed in the head, shoulder, fore-leg and hind-leg weights were in line with the reports of LATORRE et al. (2003) and NJOKU et al. (2015b) who reported a significantly higher proportion of head, ham, shoulder and feet weights in *ad libitum* fed pigs compared to their restricted fed counterparts. Pigs on 90% *ad libitum* feed offered had higher head, fore-leg and hind-leg weights which could be attributed to the variation in the live weight values of the pigs. However, these observations were in variance with the report of LO FIEGO et al. (2005) who noted that primal cut proportion decreased with increasing body weight because the growth rate of the primal cuts correlate more with age than the growth rate of the whole body. Kidney and liver play important roles in the detoxification and excretion of most toxic materials from body. The decrease in liver and kidney weights with decreasing level of feed offered can be attributed to low proliferation of nephrons of these organs as the liver is main location of energetic metabolism (LEHNINGER et al.1995), where most of lipids and lipoprotein syn-

thesis occurs. DE LANGE et al. (2003) enthused that higher level feed intake stimulates visceral organ growth and alters the distribution of body amino acid. The weights of full and empty stomach were significantly increased with increasing levels of restriction. The hyperphagia shown by intermittently starved animals was associated by earlier workers with a marked hypertrophy of the gastric mucosa and musculature, which in turn increased the weight of the gastrointestinal tract (LOW 1989, PLUSKE et al. 1998).

Previous reports indicate that dietary protein levels do not have a remarkable effect on the meat quality parameters (TEYE et al. 2006). From this study, the water holding capacity, refrigeration loss and muscle pH were not influenced by the levels of dietary protein. The non significant treatment effect on water holding characteristic, refrigeration loss and muscle pH is an indication that the pigs were in good pre-slaughter condition. This observation is consistent with the report of TEYE et al. (2006) that declared that a decrease in dietary protein had no impact on drip loss and muscle pH value. Dietary crude protein content did not modify the water holding capacity of pork. Although, increasing levels of dietary crude protein resulted to numeric decrease in water holding capacity of the meat. The water holding capacity is inversely related to protein content of the meat (OUHAYOUN and DALLE-ZOTTE 1996), and the loss of water is influenced by the rate of rigor shrinkage and cell membrane permeability of water as well as the pace at which protein denatures which is driven by decreasing pH and calcium values during rigor development (OFFER and TRINICK 1983). LASKAR and NATH (1998) enthused that ultimate pH of meat is positively correlated to the water holding capacity of the meat. The pH values (5.7–6.1) in this present study are within the range values (5.5–6.3) previous reported for meat from pigs of different genotypes, with different regimes of exercise, nutritional levels, stunning methods etc (CORREA et al. 2006). The non significant difference in the pH could be attributed to the similarities in the meat acidity which has been associated with numerous other meat quality characteristics such as meat colour, water holding capacity, juiciness, tenderness and microbial stability (ANDERESEN et al. 2005). This observation is consistent with other studies that reported no effect of decreased dietary protein on drip loss and muscle pH value (SEE and ODLE 2000, WITTE et al. 2000, TEYE et al. 2006). The significant increased in cooking loss with decreasing dietary protein content is in line with the studies of SIRTORI et al. (2014) and PUGLIESE et al. (2013) who observed that lower protein diets offered to Cinta Senese pigs resulted to increase in carcass fat and high IMF, cooking loss, lightness and redness of meat.

Feeding levels had no significant effect on water holding capacity, cooking loss, and refrigeration loss and muscle pH values. The non significant effect observed in this present study indicates that compensatory growth must have occurred during the realimentation period which must have led to homogeneous growth rate and developmental pattern. This must have positioned all the pigs in good pre-slaughter condition irrespective of feeding strategies practiced during the rearing period that led to normal development of pH and water holding capacity which are major factors that influences other physical attributes of pork. The present observation affirms the report of LEHESKA et al. (2002) who found no significant effect on pork quality traits upon feeding low digestible carbohydrates and high protein diet during the last two weeks prior to slaughter. However, HEYER and LEBRET (2007) observed small difference in quality parameters of pork after realimentation period. The authors declared that restrictive-compensatory feeding strategy improved meat quality of pig. However, BEE et al. (2006) stated that restrictive feeding had detrimental impact on meat quality, with lower water holding capacity and tenderness. The differences in the result of these studies could be attributed to the variations in the level of feed restriction and breeds of pig used by different researcher.

## Conclusion

Carcass yield, retail cut parts and visceral organs of growing pigs were influenced by limited feeding strategies. Qualitative feed restriction positively influenced the cooking loss of meat while Quantitative restriction had no impact on meat quality characteristics of growing pigs, hence, moderate feed restriction both qualitatively and quantitatively can be used to improve the carcass yield and meat quality traits of growing pigs.

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# SELECTED MORPHOMETRIC PARAMETERS AND MINERAL DENSITY OF TIBIOTARSAL BONES IN GREEN-LEGGED PARTRIDGE COCKERELS AND CAPONS

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Key words: caponization, bones, bone mineral density, testosterone.

Abstract

The aim of this study was to determine the effect of caponization on selected morphometric parameters and mineral density of tibiotarsal bones in Green-legged Partridge cockerels. The experiment was conducted on 200 cockerels. At 8 wks of age, 100 birds were surgically castrated. At 12 and 24 wks of age, blood samples were collected from 10 intact cockerels and 10 capons, and the birds were slaughtered. Tibiotarsal bones were dissected from individual birds. Age had a significant effect on tibia weight in both cockerels and capons ($P \leq 0.01$), whereas the effect of castration on this parameter was noted in older birds, at 24 wks of age (age x sex category interaction, $P \leq 0.01$). Tibiotarsal bones were longer in cockerels than in capons ($P \leq 0.01$). The tibiotarsal bones of capons had higher BMD values than the bones of cockerels ($P < 0.01$).

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# WYBRANE CECHY MORFOMETRYCZNE ORAZ GĘSTOŚĆ MINERALNA KOŚCI PISZCZELOWO-STĘPOWEJ KOGUTÓW I KAPŁONÓW ZIELONONÓŻKI KUROPATWIANEJ

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Słowa kluczowe: kapłonienie, kości, gęstość mineralna kości, testosteron.

Abstrakt

Celem badań było określenie wpływu kastracji i wieku na wybrane cechy morfometryczne oraz gęstość mineralną kości piszczelowo-stępowej kogutów zielononóżki kuropatwianej. Materiał do badań stanowiło 200 kogutów, z których 100 poddano kastracji chirurgicznej w wieku 8. tygodni. W wieku 12. i 24. tyg. od 10 losowo wybranych kogutów i 10 kapłonów pobrano próby krwi, a następnie po uboju wypreparowano kość piszczelowo-stępową.

Stwierdzono, że wiek ptaków wpłynął istotnie na masę kości piszczelowo-stępowej kogutów i kapłonów ($P \leq 0.01$), natomiast wpływ kastracji ujawnił się u ptaków starszych, w wieku 24. tyg. (interakcja wiek × kategoria płciowa, $P \leq 0.01$). Kość piszczelowo-stępowa była dłuższa u kogutów, w porównaniu z kośćmi kapłonów, zarówno w 12., jak i w 24. tyg. ($P \leq 0.01$). Kości kapłonów wykazywały wyższe wartości BMD ($P < 0.01$).

## Introduction

In Europe, the meat of capons (surgically castrated male chickens) is appreciated by consumers for its tenderness and flavor, and it is more expensive than meat from broiler chickens and organic chickens (MURIEL DURÁN 2004, FRANCO et al. 2016). Caponized chickens of native breeds are most popular. Recent years have witnessed a growing interest in native chicken breeds which are well adapted to extensive egg and meat production systems (PADHI 2016). Male layer-type chicks of native breeds, which are considered "waste products" in the egg industry, are often used for capon production. The Green-legged Partridge is one of such breeds.

Castration is a hormonal intervention which permanently influences metabolic processes in birds (RIKIMARU et al. 2011, ADAMSKI et al. 2016).

Due to reduced synthesis of sex steroids, caponization leads to increased fat deposition in the carcass, which was confirmed by post-mortem analyses, chemical analyses of muscles and histological analyses in castrated birds (DÍAZ et al. 2010, GESEK et al., 2017). Age and sex exert significant effects on bone tissue parameters during postnatal development (CHARUTA et al. 2013). Androgens promote bone formation, Ca and P retention (JOHNSON and RENDANO 1984), whereas androgen deficiency is a risk factor for low bone mineral density (BMD) (FINKELSTEIN et al. 1987). In poultry, adverse changes are most frequently observed in the tibia. Research shows that the volumetric mineral density of tibiotarsal bones in males of various poultry species decreases at different ages, e.g. at 4 wks of age in broiler chickens (CHARUTA et al. 2013) and at 9 wks of age in turkeys (CHARUTA et al. 2012).

Some studies (LIN and HSU 2003, CHEN et al., 2014) have revealed that castration affects the weight, length and histological parameters of bones. MUSZYŃSKI et al. (2017) reported lower breaking strength of tibiotarsal bones in castrated males, which could be a risk factor in capon production. According to MANOLAGAS et al. (2002), blood Ca is the substrate for bone ossification and mineralization, whereas decreased androgen concentrations contribute to osteoclast formation, bone erosion, and Ca and P release from bones. In contrast, MAHMUD et al. (2014) found that caponization had no significant osteometric effects on almost all long bones. The results of studies investigating the effects of castration on the qualitative and quantitative parameters of bones are inconclusive, most likely due to differences in the origin and age of caponized birds. Testosterone levels also vary across breeds, which is another important consideration (GRYZIŃSKA et al. 2011, MURAWSKA et al. 2015).

The objective of this study was to determine the effect of caponization and age on selected morphometric parameters and mineral density of tibiotarsal bones in Green-legged Partridge cockerels.

## Materials and Methods

The experiment was conducted on 200 Green-legged Partridge cockerels. Day-old birds were weighed, marked with wing tags, and randomly distributed to 10 pens in the experimental center of the Department of Commodity Science and Animal Improvement of the University of Warmia and Mazury in Olsztyn, Poland. The birds were raised to 24 wks of age, and were fed commercial diets *ad libitum* (Table 1). At 8 wks of age, 100 birds were surgically castrated by a qualified veterinarian in accor-

dance with Commission Regulation (EC) No. 543/2008. The procedure was approved by the Local Ethics Committee in Olsztyn, Poland. The birds were divided into two sex categories (with 5 replications per group and 20 birds per replication).

Table 1

Composition of experimental diets. Composition of diets 1 and 2: ground cereal grain (cornmeal, ground wheat), soybean meal (line 40-3-2), calcium carbonate, monocalcium phosphate, sodium chloride

| Nutritional value of diets | Diet 1 (weeks 1–8) | Diet 2 (weeks 9–24) |
|---|---|---|
| Metabolizable Energy [MJ $kg^{-1}$]* | 11.64 | 11.94 |
| Crude protein [%] | 18.77 | 20.88 |
| Crude fat [%] | 2.81 | 3.64 |
| Crude ash [%] | 5.22 | 5.47 |
| Crude fiber [%] | 2.51 | 2.55 |
| Lysine [%] | 0.94 | 1.08 |
| Met + Cyst [%] | 0.72 | 0.75 |
| Threonine [%] | – | 0.80 |
| Tryptophan [%] | – | 0.24 |
| Methionine [%] | – | 0.52 |
| Calcium [%] | 0.77 | 0.87 |
| Total phosphorus [%] | 0.59 | 0.59 |
| Sodium [%] | 0.18 | 0.14 |
| Mineral-vitamin premix [%] | 1.0 | 1.0 |

* Metabolizable energy content estimated based on the percentages of selected ingredients determined analytically

Provided per kg of diet: microelements: Cu – 8.0 mg, Fe – 116.0 mg, Mn – 80.0 mg, Zn – 100.0 mg, J – 0.80 mg, Se – 0.20 mg. Vitamins: vitamin A (E 672) – 13200 IU, vitamin $D_3$ (E671) – 3120 IU, vitamin E – 68.0 mg, vitamin $K_3$ – 4.80 mg, vitamin $B_1$ – 2.2 mg, vitamin $B_2$ – 7.2 mg, vitamin $B_6$ – 5.0 mg, vitamin $B_{12}$ – 44.0 mg, vitamin H (biotin) – 136 mcg, niacin – 44.0, Ca-D-pantothenate – 18.0 mg.

At 12 and 24 wks of age, blood samples were collected from 10 intact cockerels and 10 capons, and the birds were slaughtered (electrical stunning followed by cutting the jugular vein). Blood samples were collected from 10 randomly selected birds from each treatment to determine testosterone levels. Blood was collected into test tubes containing heparin. Freshly collected blood was centrifuged twice (MPW-350R centrifuge, MPW MED INSTRUMENTS; 5 minutes, 10000 rpm), and each time the supernatant was transferred to 1.5 ml Eppendorf Safe-Lock micro test tubes with an Eppendorf automatic electronic pipette. Plasma samples were frozen at -72°C in 1.5 ml Eppendorf Safe-Lock micro test tubes in the Kaltis 390 ultra low temperature laboratory freezer. Testosterone levels were analyzed by radioimmunoassay (RIA) with the use of commercial kits supplied

by DIAsource TESTO – RIA – CT (DIAsource ImmunoAssays S.A., Belgium). Serum Ca and P concentrations, and alkaline phosphatase activity were analyzed using an automatic blood chemistry analyzer and Roche testing kits (COBAS MIRA plus, Roche Diagnostics, Rotkreuz, Switzerland).

Live body weight (BW) was determined before slaughter. Carcasses were chilled for approximately 18 h at a temperature of 4°C, and carcass dissection was performed. Tibiotarsal bones were dissected from individual birds. The length of right bones was measured with a caliper accurate to 1 mm. The structure of bone tissue (BMD – Bone Mineral Density, cortical/compact and cancellous/spongy bone combined) was determined by computed microtomography using the SkyScan 1174 scanner (Bruker – SkyScan, Belgium) and the following software: CT-Analyzer, Nrecon, CT Vox and DataViewer. The data were analyzed using Nrecon 1.6.9.18, CTAnalyser 1.14.4.1+, CTVox 2.1.0 r741 and DataViewer 1.5.1.2 packages. The values of BMD were determined for a Hounsfield units (HU) range of –1000 to 0. The analyzed area was the proximal metaphysis of the tibiotarsal bone.

The statistical analysis involved the determination of arithmetic means ($\bar{x}$) and standard deviations (SD). The data were analyzed by two-way ANOVA (age × sex category; A × B: 2 × 2). The results were processed using Statistica 2010 software.

## Results

At 12 wks of age, Green-legged Partridge cockerels and capons were characterized by similar average body weight of 1194 g and 1206 g, respectively, which increased to 2030.6 g and 2067.6 g at 24 wks of age. Caponization had no significant effect on the body weights of birds, which were influenced only by age ($P \leq 0.01$, Table 2).

At 12 wks of age, tibia weight was similar in cockerels and capons (10.01 g and 9.79 g, respectively), whereas at 24 wks of age, it was higher in cockerels (15.38 g vs. 13.17 g, $P \leq 0.01$, Table 2). Age had a significant effect on tibia weight in both cockerels and capons ($P \leq 0.01$), whereas the effect of castration on this parameter was noted in older birds, at 24 wks of age (age x sex category interaction, $P \leq 0.01$, Table 2).

Tibia length was affected by both the age of birds and castration ($P \leq 0.01$, Table 2). Cockerels had longer tibiotarsal bones than capons, both at 12 and 24 wks of age ($P \leq 0.01$, Table 2).

Table 2
Body weights and selected tibia parameters in 12- and 24-week-old Green-legged Partridge cockerels and capons (mean ± SD)

| Item | Sex category | Age [wks] | | P-value | | |
|---|---|---|---|---|---|---|
| | | 12 | 24 | age | sex category | A · S interaction |
| Body weight [g] | cockerels | 1194.40 | 2030.8 | 0.000 | 0.241 | 0.227 |
| | | ±80.20 | ±107.845 | | | |
| | capons | 1206.00 | 2067.80 | | | |
| | | ±48,44 | ±40.95 | | | |
| Tibia weight [g] | cockerels | 10.01 | *15.38 | 0.000 | 0.005 | 0.023 |
| | | ±0.43 | ±0.76 | | | |
| | capons | 9.79 | 13.17 | | | |
| | | ±0,27 | ±0,48 | | | |
| Tibia length [cm] | cockerels | *12.46 | *14.88 | 0.000 | 0.028 | 0.670 |
| | | ±0.13 | ±0.34 | | | |
| | capons | 11.48 | 13.98 | | | |
| | | ±0.34 | ±0.20 | | | |
| Bone mineral density (BMD) [g $cm^{-3}$] | cockerels | 0.98 | 1.80 | 0.000 | 0.000 | 0.247 |
| | | ±0.09 | ±0.11 | | | |
| | capons | *1.62 | *2.32 | | | |
| | | ±0.15 | ±0.14 | | | |

*– values in columns (cockerels an capons of age group) differ significantly

Caponization had a significant effect on the mineral density of tibiotarsal bones. The tibiotarsal bones of capons had higher BMD values than the bones of cockerels, both at 12 and 24 wks of age ($P < 0.01$, Table 2). BMD values increased with age, from 0.098 g/$cm^2$ and 0.0162 g $cm^{-2}$ in 12-wk-old cockerels and capons, respectively, to 0.180 g $cm^{-2}$ and 0.232 g $cm^{-2}$ in 24-wk-old birds ($P < 0.01$, Table 2).

Age significantly influenced the blood concentrations of Ca and P, and the activity of alkaline phosphatase ($P \leq 0.01$, Table 3). At 12 wks of age, blood Ca concentration reached 13.90 mg $dl^{-1}$ in cockerels and 10.67 mg $dl^{-1}$ in capons ($P < 0.01$), and blood $P$ levels were determined at 6.92 mg $dl^{-1}$ in cockerels and 8.44 mg $dl^{-1}$ in capons ($P < 0.01$). At 24 wks of age, blood Ca concentration was comparable in cockerels and capons (9.38 mg $dl^{-1}$ and 9.00 mg $dl^{-1}$, respectively), and a similar trend was noted in blood P concentration (cockerels – 3.83 mg $dl^{-1}$, capons – 3.85 mg $dl^{-1}$). Caponization increased the blood concentrations of Ca and P, but only at 12 wks of age (age × sex category interaction, $P \leq 0.01$, Table 3). The surgical procedure had no influence on the activity of alkaline phosphatase (Table 3).

Table 3
Effect of caponization on selected blood parameters and testosterone levels in Green-legged Partridge cockerels and capons (mean ± SD)

| Item | Sex category | Age [wks] | | P-value | | |
|---|---|---|---|---|---|---|
| | | 12 | 24 | age | sex category | A · S interaction |
| Ca [mg $dL^{-1}$] | cockerels | *13.91 | 9.38 | 0.000 | 0.021 | 0.027 |
| | | ±0.17 | ±0.18 | | | |
| | capons | 10.67 | 9.00 | | | |
| | | ±0.16 | ±0.17 | | | |
| P [mg $dL^{-1}$] | cockerels | 6.92 | 3.83 | 0.000 | 0.025 | 0.003 |
| | | ±0.062 | ±0.076 | | | |
| | capons | *8.44 | 3.85 | | | |
| | | ±0.086 | ±0.748 | | | |
| Alkaline phosphatase [U $L^{-1}$] | cockerels | 756.3 | 523.2 | 0.000 | 0.321 | 0.247 |
| | | ±0.04 | ±0.06 | | | |
| | capons | 804.3 | 476.8 | | | |
| | | ±0.02 | ±0.01 | | | |
| Testosterone [ng $ml^{-1}$] | cockerels | *0.40 | *1.86 | 0.000 | 0.000 | 0.247 |
| | | ±0.04 | ±0.06 | | | |
| | capons | 0.13 | 0.11 | | | |
| | | ±0.02 | ±0.01 | | | |

*– values in columns (cockerels an capons of age group) differ significantly

The effectiveness of surgical castration was confirmed by an analysis of blood testosterone levels, which increased with age and were significantly ($P < 0.01$) higher in cockerels than in capons (Table 3).

## Discussion

The present study investigated whether surgical castration influences morphometric parameters and the mineral density of tibiotarsal bones in Green-legged Partridge cockerels and capons. Testosterone stimulates the activity of osteoblasts in the bone formation process, as well as the ossification of long bones. Therefore, the absence of sex steroids in cockerels could affect bone metabolism (MAHMUD et al. 2014, MUSZYŃSKI et al. 2017). Our findings indicate that the castration-induced decrease in blood testosterone levels had no effect on the body weights of Green-legged Partridge cockerels and capons, but it significantly affected the analyzed morphometric parameters and mineral density (BMD) of tibiotarsal bones (Table 2).

At 24 weeks of age, the tibiotarsal bones of Green-legged Partridge cockerels were longer and heavier than the bones of capons. In a study of male Taiwan country D × $L_2$ cockerels, CHEN et al. (2006b) noted lower weight of tibiotarsal bones in capons, whereas the length of the analyzed bones was not affected by castration. In an experiment performed on male Single Comb White Leghorns (CHEN et al. 2007), caponization decreased the length of tibiotarsal bones but had no influence on bone weight. The results of our study and the findings of other authors point to different responses of cockerels of various breeds to reduced testosterone levels. It should also be noted that Taiwan country D × $L_2$ cockerels (CHEN et al. 2006b) were caponized at 10 wks of age, Single Comb White Leghorns (CHEN et al. 2007) were caponized at 12 wks of age, and Green-legged Partridge cockerels analyzed in this study were surgically castrated at 8 wks of age.

In the current study, capons were characterized by higher BMD values than cockerels, both at 12 and 24 wks of age. In a study of Polbar chickens caponized at 8 wks of age, no significant differences in the mineral density of tibiotarsal bones were found between intact males and capons (MUSZYŃSKI et al. 2017). It should be stressed, however, that in the cited study, caponization had a beneficial influence on the body weights of birds, which was not observed in the present experiment. The above results suggest that male birds of various breeds may differ in their susceptibility to changes resulting from decreased synthesis of steroid hormones.

CHEN et al. (2006a) demonstrated that caponization increased total blood Ca concentrations. However, in a study by CHEN et al. (2007), caponization had no influence on total blood Ca levels. In the current study, an increase in the blood concentrations of Ca and P was observed in 12-wk-old capons, i.e. 4 wks after castration, whereas 24-wk-old cockerels and capons (16 wks after castration) were characterized by similar blood Ca levels (Table 3). The significant decrease in blood Ca concentrations, noted in capons 4 wks after castration, resulted from declining testosterone levels. The stimulation of osteoclast formation and the release of Ca from bones contributed to the stabilization of blood Ca levels, which were comparable in 24-wk-old cockerels and capons.

LIN AND HSU (2003) analyzed Taiwan country cockerels (TLRL native chicken Taishi No. 13) and found no differences in blood Ca concentrations between 28-wk-old cockerels and capons; capons had only higher blood P levels. According to LIN AND HSU (2003), caponization increases blood ionized Ca concentrations, but not total Ca levels. CHEN et al. (2006a) reported that caponization did not affect plasma alkaline phosphatase levels, which is consistent with our findings.

The results of this study indicate that caponization exerted a significant effect on the analyzed morphometric parameters and mineral density of tibiotarsal bones in Green-legged Partridge cockerels. However, further research involving a higher number of parameters is needed to determine whether the observed changes could pose a risk in capon production.

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# NUTRIENT COMPOSITION OF SOME SELECTED TRADITIONAL FOODS OF IJAW PEOPLE OF BAYELSA STATE

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Key words: traditional foods, proximate composition, mineral contents, antinutrient composition, protein contents.

Abstract

This study evaluated the nutrient composition of traditional dishes of Ijaw people of Bayelsa State, Nigeria. The traditional dishes (Kekefiyai, Kiri-igina, and Opuru-fulou) were prepared using the traditional cooking methods and subsequently analyzed for proximate, antinutrient, and mineral contents, using standard methods. Kekefiyai had the highest carbohydrate content (32.5% ± 0.5) and fibre (31.79% ± 0.18) while Opuru-fulou had the highest protein content (33.69% ± 0.00) and ash (16.02% ± 0.12). Kiri-igina had the highest lipid and moisture content. The saponin, tannin, cyanogenic glycosides, and oxalate content occurred highest in Kiri-igina, while Kekefiyai had the highest alkaloid and phytate content. Opuru-fulou recorded the highest concentration of Mg, P, Mn, Cu, Zn, and Sulphate. Na and Ca occurred highest in Kekefiyai, and K, Fe, and Cl, occurred highest in Kiri-igina. The present study has shown that these selected traditional foods can provide substantial amounts of nutrients adequate to meet the daily requirement of Ijaw people in Bayelsa State.

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# KOMPOZYCJA SKŁADNIKÓW ODŻYWCZYCH W KILKU WYBRANYCH TRADYCYJNYCH POTRAWACH LUDU IJAW ZE STANU BAYELSA

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Słowa kluczowe: tradycyjne potrawy, podstawowy skład, związki mineralne, składniki antyodżywcze, zawartość białka.

Abstrakt

W pracy badano wartość odżywczą tradycyjnych potraw ludu Ijaw ze stanu Bayelsa w Nigerii. Tradycyjne potrawy (kekefiyai, kiri-igina, and opuru-fulou) były przygotowane według oryginalnych receptur i analizowane pod względem podstawowego składu chemicznego, zawartości związków mineralnych i składników antyodżywczych z zastosowaneim standardowych metod oznaczeń. Kekefiyai charakteryzowało się najwyższą zawartością węglowodanów (32.5% ± 0.5) i włókna pokarmowego (31.79% ± 0.18), podczas gdy opuru-fulou cechowało się najwyższą zawartością białka (33.69% ± 0.00) i popiołu (16.02% ± 0.12). Kiri-igina miało najwyższą zawartość lipidów i wilgotność. Saponiny, taniny, glikozydy cyjanogenne i szczawiany występowały w największej koncentracji w kiri-igina, podczas gdy Kekefiyai zawierało najwięcej alkaloidów i związków fitynowych. W przypadku opuru-fulou stwierdzono najwyższą koncentrację Mg, P, Mn, Cu, Zn i siarczanów. Na i Ca występowały w największej ilości w kekefiyai, natomiast K, Fe, i Cl w kiri-igina. W badaniach wykazano, że analizowane wybrane tradycyjne potrawy są w stanie dostarczyć znacznych ilości składników odżywczych odpowiednio do zapotrzebowania w dziennej racji pokarmowej ludu Ijaw ze stanu Bayelsa.

## Introduction

Man has always exhibited a unique survival instinct through his continuous affinity to search and discover foods (MARLOWE 2005). Food has been defined by UWAKWE and AYALOGU (1998) as any edible substance that provides the required nutrients necessary for the proper functioning of the body. It has also been described as any material that gives energy to the body, promotes growth, and repairs worn out cells when ingested, digested and assimilated (OKAKA and OKAKA 2005, OLUSANYA 2008, AMADI et al. 2013). Food, a basic necessity of life (OKAKA and OKAKA 2001) exists in different types but can be firmly rooted on the customs and traditions of the people (AMADI et al.2013). The basic food nutrients, deriva-

ble after the consumption of foods at different concentrations include proteins, carbohydrates, mineral, vitamins, lipids etc (OKAKA and OKAKA 2001). Food is comprised of various chemicals that combine in various properties to provide color, flavor, shape and taste to foods. Previously, the popular conception from some researchers (KYLE and COLE 2001, AMADI et al. 2011) was that the type of food consumed by a group, community, or locality, determines their nutritional status, but nowadays, the existence of quick food makes that idea a very debatable one. However, it is a clear fact especially in Nigeria that the agricultural output of a particular geographical location largely depends on some climatic factors and the vegetative zone, which thus influences the dietary status of the citizenry. Most African foods are peculiar cuisines utilized specifically by a group of people but can also be known with country and continental names like Nigerian foods and African foods (CAYOT 2007). Further, some foods can be attributed to a community, locality or ethnic group. Such foods are referred to as traditional foods. Traditional foods are ancient foods with ancestral heritage rooted on strong foundation of customs, culture and natural environment. They are foods with old historical background continuously evolving to retain the cutlery tradition of the people and to surmount the monotony in the diet (VIJAYALAKSHMI et al. 2005). An obvious advantage of traditional foods is a wide rate of acceptance within the society. This advantageous attribute plays a key role when developing food products and during the establishment of nutritional programs. Further a good knowledge of the nutritive value of traditional foods helps to expand the consumption of these foods. Apart from the primary nutrients derivable from foods, studies have revealed the presence of non-nutritive chemicals with disease preventive and immune boosting properties. These chemicals are referred to as phytochemicals (SOFOWORA 1980, OKAKA and OKAKA 2001). At elevated levels, phytochemicals can act as antinutrients and become harmful to the human body. DURU et al. (2014) have observed that though natural and synthetic foods contain nutrients, the occurrence of phytochemicals is greater in natural foods.

Bayelsa State is a state in Southern Nigeria with a rich cultural heritage and Ijaw as one of the inhabitant ethnic groups. The Bayelsan people have very rich cuisines that include ceremonial dishes, normal day to day delicacies and traditional snacks. Among the numerous rich cuisines of the Bayelsan people, are the "Kekefiyai", "Kiri-igina", and "Opuru-fulou". These delicacies are peculiar to the Ijaw ethnic group, and as well, unify and showcase their great culture. Despite the tremendous potentials of traditional foods to improve both nutrition and food security among the populace, FAO (1995) observed that some traditional foods cause concerns

as a result of microbial contamination and food poisoning through the use of additives and other adulterants.

Bayelsa is a state located in the Niger Delta region of Southern Nigeria between Delta State and Rivers State. Bayelsa was created in 1996 and has Yenagoa as its State capital. The name Bayelsa was coined from a concatenation of the initials of three former comprising local government areas. Bayelsa State is geographically located at latitude $45^{0}45^{l}$ north and longitude $6^{0}05^{l}$ west covering an area of 9415.8 $km^2$ and a population of 1704515, and population density of 158 people/$km^2$ accounting for 1.2% of Nigeria's total population (EDOUMIEKUMO et al.2014). Bayelsa has an estuarine and riverine setting, thus making many of the communities not accessible by road due to large amount of water bodies that surround them. Predominantly, four of the ten languages, Izon, Nembe, Ogbia, and Epie-Atissa are among the languages spoken by the comprising ethnic groups that include Ijaw, Kolokunu, Ekpetiama, Igbiriran, Atissa, and Biseni. Christianity is regarded as the dominant religion, notwithstanding the practice of traditional religion by few. Bayelsa State is one of the largest petroleum producing state in Nigeria because of the massive deposition of crude oil and natural gas in the state. Despite this, the majority of the inhabitants have a poor living status due to an inexistent commerce caused by inadequacy of transportation, education, health, and other infrastructures. Hence, many rural dwellers subsistently and commercially engage in fishing.

Historically, the Niger Delta Ijaw ethnic group is seen as one of the major ethnic group in Nigeria and was given a separate province during the colonial administration. The Ijaw people are considerable regarded as being of antiquity in Niger Delta. They mainly inhabit the local government of Bomadi, Patani, Burutu, Warri South, Warri North, and South-west Warri. Many researchers have postulated that the Ijaws originated from the central Delta but must have migrated towards the west bordered by Delta, and east which is the present day Rivers State.

Therefore, considering the central roles these foods play for the people of Ijaw community in terms of food security, there is need to evaluate the nutritional composition of these three traditional foods. Hence, this study was carried out to evaluate the nutritional properties of Kekefiyai, Kiri-igina, and Opuru-fulou.

## Materials and Methods

### Preparation of Kekefiyai and starch

The ingredients used for the preparation of the Kekefiyai, Kiri-igina, and Opuru-folou were purchased from the local market in Opolo community Yenagoa Local Government Area of Bayelsa State.

The traditional method was used for the preparation of Kekefiyai. It is a pottage made with 500 g of unripe plantain washed, chopped into bits and eventually pounded, and then cooked with 4920 ml of water in a cooking pot for about 50 minutes. As it boils, the following ingredients were added and mixed: 200 g of dried fish, 90 g of sliced onions, 50 g of ground crayfish, 1 g of table salt, 10 g of ground fresh pepper, 100 ml of red palm oil, and a local seasoning called Onga. The mixture was allowed to boil for 10 mins (Figure 1).

The starch was prepared by adding 100 g starch gotten from cassava root to 50 ml water and turned homogenously in a cooking pot. Twenty five milliliters (25 ml) of red palm oil was added before boiling and continuously stirred on a cooking gas until the mixture turned into a paste. This paste is served alongside Kekefiyai for a complete diet.

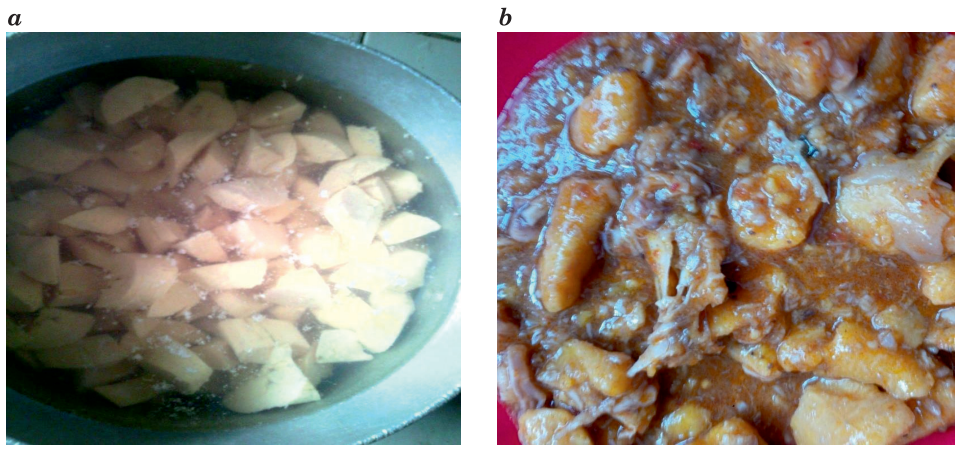


**Fig. 1.** Kekefiyai before (*a*) and after (*b*) preparation

### Preparation of Kiri-igina

This is a special type of soup prepared without cooking on fire. The soup was prepared using the traditional method. One hundred and twenty milliliters (120 ml) of water was boiled in a cooking pot and transferred into a wooden mortar. About 20 g of ground roasted Ogbono (*Irvingia*

*gabonensis* seeds) was added to the contents of the mortar and stirred using a cooking spoon. Two hundred grams (200 g) of smoked fish, 10 g of ground fresh pepper, 90 g of sliced onions, and 1 g of table salt were added successively to the mixture in the mortar and eventually stirred for 3 minutes. The soup is used to serve 'eba' or 'fufu' for a complete dish (Figure 2).

**Fig. 2.** Kiri-igina

## Preparation of opuru-fulou (prawn soup)

This peculiar delicacy is cooked mostly during flood seasons due to the availability of fresh prawns deposited at the water sides after flooding. The soup is normally prepared with sufficient quantities of fresh prawns (Opuru) from where the soup name is gotten. The soup was prepared using the traditional method. The head of one hundred and fifty grams (150 g) of prawn was cut off, washed and blended slightly. This blended prawn head was mixed with 200 ml of water and then filtered using a locally made mesh. The filtrate was transferred into a cooking pot and allowed to boil.

**Fig. 3.** Opuru-fulou before (*a*) and after (*b*) preparation

The remaining part of the prawn was added to the boiling filtrate, and the following ingredients were added: 200 g dried fish, 1 g of table salt, 50 g of ground crayfish, 90 g of sliced onions, and 10 g of ground fresh pepper. The mixture was allowed to boil for 20 minutes after which 120 ml of red palm oil was added and allowed to boil for another 10 minutes. About 150 g of Ogbono (*Irvingia gabonensis* seeds) was added to the mixture and allowed to cook further for 15 minutes, and the soup was ready to be served (Figure 3).

## Preparation of samples for analysis

**Determination of proximate and antinutrient composition.**

The food samples were oven dried individually at $70^0$C for 4 hours. These dried samples were ground into flour using a hand mill machine and then transferred into an airtight container prior to analysis

Proximate analysis of the samples for carbohydrate, crude fat, ash, crude protein, fiber and moisture contents were carried out according to standard methods of AOAC (1990).

The qualitative detection of antinutrients in the samples was carried out following the description of SOFOWORA (1980) and HARBORNE (1973).

Quantitative determination of oxalates, phytates, tannins saponins and cyanogenic glycosides were determined following the method of AOAC (1990).

**Mineral Content Determination.**

Wet digestion of samples (5 ml) using a mixture of concentrated $HNO_3$ and 60% (v/v) $HClO_4$ was carried out according to the method of AOAC (1990) where the organic matter in the sample was digested and afterwards diluted to a final volume of 25 ml with deionized distilled water. The levels of Na, K, Ca, Fe, Mg, P, Mn, Cu, and Zn in the samples were thus evaluated using an atomic absorption spectrophotometer (Buck Scientific model 210 VGP) and flame photometer (Jenway model).

The sulphate contents of the food samples were determined turbidimetrically according to AOAC (1984).

The chloride level was determined titrimetrically using the method of AOAC (1984).

The mineral ratios were determined as described by DAVID (2010). Reference values and ranges used for this study was obtained from DAVID (2010).

## Statistical analysis

All data were subjected to statistical analysis. Values are reported as Mean ± Standard deviation, while one way ANOVA was used for significance testing among the three foods, using Statistical Package for Social Sciences (SPSS) version 20. The results were considered significant at p-values of less than 0.05 ($p < 0.05$).

## Results and Discussion

The carbohydrate content (Table 1) of the cooked sample of the traditional food Kekefiyai, was the highest (32.57%±0.50) followed by Kiri-igina (20.57% ± 0.51) while Opuru-fulou (14.96% ± 0.03) was the lowest. The variations observed in the carbohydrate content were due to the amount and type of different ingredients used in the preparations of the food.

Table 1

Proximate composition of Kekefiyai, Kiri-igina, and Opuru-fulou

| Parametrs | Kekefiyai | Kiri-igina | Opuru-fulou |
|---|---|---|---|
| Carbohydrate [%] | $32.57\pm0.50^a$ | $20.57\pm0.51^b$ | $14.96\pm0.03^c$ |
| Crude protein [%] | $10.94\pm0.20^a$ | $26.88\pm0.36^b$ | $31.69\pm0.00^c$ |
| Lipid [%] | $20.48\pm0.32^a$ | $31.44\pm0.01^b$ | $29.20\pm0.13^b$ |
| Fibre [%] | $29.79\pm0.18^c$ | $4.75\pm0.16^b$ | $6.19\pm0.15^b$ |
| Moisture [%] | $2.82\pm0.98^a$ | $4.60\pm0.46^b$ | $2.60\pm0.11^a$ |
| ASH [%] | $5.40\pm0.16^a$ | $12.02\pm0.17^b$ | $16.02\pm0.12^b$ |

Values are mean ± standard deviation of triplicate determinations. Values in the same row bearing the same superscript letter (*a*, *b*) are not significantly different at 5% level

The reason for the high carbohydrate content of Kekefiyai could be attributed to the basic ingredient starch which is mixed with the dish after preparing the food (shown in Figure 1) and is known to be high in carbohydrate content and provides a good source of energy, while the reason for the equally high carbohydrate content in Kiri-igina could be caused by the ingredient (Ogbono) used in the preparation of the food (Figure 2). The crude protein content of the traditional foods in Table 1, ranged from 10.94% ± 0.20 (Kekefiyai) to 31.69% ± 0.00 (Opuru-fulou). The protein content of Opuro-fulou was significantly higher than those of the other food samples. The high protein content of Kiri-igina could be attributed to the protein content of the ingredients, prawn, smoked fish (Tilapia) ad crayfish (*Cambaridae*

*cambarus*) used in the preparation of the traditional diets. The high level of protein content in Opuru-fulou and Kiri-igina may show that they can be sufficient for the daily requirement for children, adults, as well as individuals suffering from protein deficiency diseases and disorders (ONIGBINDE 2005). Crude lipid content of the food samples was highest in Kiri-igina (31.44% ± 0.01), followed by Opuru-fulou (29.20% ± 0.13) and the lowest in Kekefiyai (20.48% ± 0.32). The fact that prawns, the main ingredient in Opuru-fulou (Figure 3), are particularly rich in protein and oil can be used to explain the high crude lipid content in Opuru-fulou. Since high crude fibre of ingredients reduce digestibility, and can also improve digestive health, fibre also plays a role in the prevention and treatment of disease such as obesity, diabetes, cancer and gastrointestinal disorders. There is also evidence that dietary fibre improves glucose tolerance and is therefore beneficial in treating maturity preset diabetes (OLUSANYA 1991). The moisture contents of the traditional foods assessed showed the highest value for Kiri-igina (4.60% ± 0.46), followed by Kekefiyai (2.82% ± 0.98) and Opuru-fulou (2.60% ± 0.11) as shown in Table 1. However, the moisture content of all the traditional food samples studied were low, compared to that of 'Kantong' (32.47%) as reported by KPIKPI et al. (2009). The relatively low moisture content of these samples, suggests that they may not be liable to bacterial spoilage, during storage. High moisture content of foods has been showed to encourage microbial growth. The ash contents of these evaluated traditional foods, ranged from 5.40% ± 0.16 in Kekefiyai to 16.02%±0.12 in Opuru-fulou. Therefore, Opuru-fulou, has the highest measure of total mineral content, which is a good nutritional attribute. This might be due to the higher proportion of prawn in the diet (Figure 3). The ash content of the traditional food studied were high compared to those of some traditional recipes reported by DAS et al.(2009), and some traditional Kuwati dishes reported by DASHTI et al.(2011). The ash content of all the foods were more than 3.0% and are therefore of more nutritional importance as previous reports (DAS et al.2009) has shown that when leaves are used as food for humans, they should contain an ideal quantity of around 3.0% for ash content.

The saponin content of the cooked samples of Kiri-igina (24.60mg/100 g) and Opuru-fulou (21.86mg/100 g) shown in Table 2 was high, while that of Kekefiyai (15.06mg/100 g) was comparably lower. The decrease in the saponin content of the cooked sample was significant in Kekefiyai. This can be attributed to the effect of an integrated approach that combines a variety of the traditional food processing and preparation practices (HOTZ and GIBSON 2007), such as thermal processing plus household pounding in the case of Kekefiyai (Figure 1). Although without toxicity, saponins are very poorly

absorbed by the body, and so tends to pass through without causing harm (SEZGIN and ARTIK 2010). Alkaloids occurred highest in in Kekefiyai (64.86 ± %0.10) and equal in both Opuru-fulou and Kiri-igina (Table 2). The phytate content in Kekefiyai was found to be (41.25% ± 0.10), while in Kiri-igina and Opuru-fulou, occurred below detection levels (Table 2).

Table 2

Antinutrient [mg/100 g] content of Kekefiyai, Kiri-igina, and Opuru-fulou

| Antinutrients | Keke-fiyai | Kiri-igina | Opuru-fulou |
|---|---|---|---|
| Saponin | $15.06 \pm 0.20^a$ | $26.40 \pm 0.10^b$ | $21.86 \pm 0.10^b$ |
| Alkaloid | $64.86 \pm 0.10^a$ | $16.34 \pm 0.30^b$ | $16.34 \pm 0.20^b$ |
| Phytate | $41.25 \pm 0.10^a$ | ND | ND |
| Tanin | $37.5 \pm 0.10^a$ | $44.03 \pm 0.10^b$ | $31.10 \pm 0.10^c$ |
| Cyanogenic glycosides | $14.67 \pm 0.25^a$ | $22.67 \pm 0.20^b$ | $21.00 \pm 0.20^b$ |
| Oxalate | $82.41 \pm 0.30^b$ | $87.96 \pm 0.20^b$ | $70.40 \pm 0.10^a$ |

Values are means ± standard deviation of triplicate determinations. Values in the same row bearing the same subscript letter (s) (*a*, *b*) are not significantly different at 5% level.

The occurrence of phytate in Kekefiyai was high when compared to some sweet potatoe dishes in Kwara State Nigeria (ABUBAKAR et al.2010) and dishes of *Afzelia africana* processed using different methods (ODENIGBO and OBIZOBA 2010). However, the values are comparably lower than those of some dishes such as potato kuba, lentil soup, and falafel (DASHTI et al. 2011). The high phytate content of Kekefiyai might be attributed to the presence of plantain, one of the basic ingredients used in the preparation of the diet that is known to be very high in its phytate content. PERIAS and GIBSON (2002) reported that soaking cereals in water can result in passive diffusion of water soluble Na, K, or Mg phytate, which can then be removed by decanting the water. There may also be the effect of thermal processing. Phytic acid forms very stable complexes with mineral ions, thereby rendering them unavailable for intestinal uptake because the first step in mineral absorption requires the mineral remains in ionic state (LOPEZ et al. 2002) thus inducing mineral deficiency. The results of the tannin content of the traditional foods in Table 2 showed that Kekefiyai was 37.50mg/100 g, Kiri-igina (44.03mg/100 g) and Opuru-fulou (31.10mg/100g). These values are quite high when compared to those of some sweet potato diets consumed in Kwara State Nigeria that ranged from 0.22mg/100g to 0.86 mg/100g (ABUBAKAR et al.2010). Tannins are reported to have possible anticarcinogenic effect (BUTLER 1989). Also dietary proanthocyanidins (tannins) can contribute to an improved animal health by reducing the

detrimental effects of internal parasites as observed in sheep (NIEZEN et al.1995). The cyanogenic glycosides contents of the cooked samples ranged from 14.67 mg/100 g in Kekefiyai to 22.67 mg/100 g in Kiri-igina which were comparably higher than those of the Beniseed soup cooked for 15, 30, 45, and 60 minutes, that ranged from 0.58 mg/100 g to 0.97 mg/100 g (AGIANG et al. 2010). High dose of HCN pose a serious inhibitory effect on the respiratory cytochrome oxidase activity (ONIGBINDE 2005). The oxalate content in the cooked diets ranged from 70.4 mg/100g in Opuru-fulou and 87.96 mg/100g in Kiri-igina, and these values were higher than the oxalate contents of some sweet potato dishes consumed in Kwara State Nigeria (ABUBAKAR et al. 2010) and Beniseed soup (AGIANG et al. 2010). Oxalate is of concern because high oxalate diets can increase the risk of renal calcium absorption (OSAGIE 1998) with a reported toxic level of soluble oxalate in a range of 2.0–5.0 g.

Table 3

Mineral contents (mg/100g) of Kekefiyai, Kiri-igina", and Opuru-fulou

| Minerals | Keke-fiyai | Kiri-igina | Opuru-fulou |
|---|---|---|---|
| Sodium (Na) | $90.77 \pm 0.10^a$ | $26.21 \pm 0.10^b$ | $22.66 \pm 0.10^b$ |
| Potassium (K) | $68.93 \pm 0.20^b$ | $76.60 \pm 0.30^a$ | $65.10 \pm 0.20^b$ |
| Calcium (Ca) | $261.3 \pm 0.20^a$ | $26.51 \pm 0.25^b$ | $35.90 \pm 0.20^c$ |
| Iron (Fe) | $27.07 \pm 0.05^a$ | $63.63 \pm 0.20^b$ | $41.58 \pm 0.20^c$ |
| Magnesium (Mg) | $44.89 \pm 0.50^a$ | $86.12 \pm 0.20^b$ | $91.59 \pm 0.10^b$ |
| Phosphorus (P) | $49.9 \pm 0.20^a$ | $55.3 \pm 0.10^b$ | $55.3 \pm 0.20^b$ |
| Manganese (Mn) | $0.33 \pm 0.03^a$ | $2.39 \pm 0.12^b$ | $7.74 \pm 0.38^c$ |
| Copper (Cu) | $0.26 \pm 0.03^a$ | $1.34 \pm 0.04^b$ | $3.73 \pm 0.44^c$ |
| Zinc (Zn) | $1.70 \pm 0.21^a$ | $2.44 \pm 0.42^a$ | $5.98 \pm 0.21^b$ |
| Sulfate | $213.28 \pm 0.10^a$ | $333.68 \pm 0.25^b$ | $426.56 \pm 0.20^c$ |
| Chloride | $239.20 \pm 0.20^a$ | $302.11 \pm 0.25^b$ | $216.11 \pm 0.20^a$ |

Values are mean ± standard deviation of triplicate determinations. Values in the same row bearing the same superscript letter(s) (*a*, *b*, *c*) are not significantly different at 5% level

The results for the micronutrient content of the traditional dishes assessed in this study, were presented in Table 3. The sodium content of the traditional foods ranged from 22.66 mg/100g in Opuru-fulou to 90.77mg/100 g in Kekefiyai. The sodium content of the foods in this present study is considered higher than Ikwerre traditional foods evaluated by AMADI et al.(2012). The potassium contents of the diets ranged from 65.10 mg/100 g in Opuru-fulou to 76.60 mg/100 g in Kiri-igina. These values were considered normal compared to those reported in some Came-

roonian household foods (SOP et al. 2008). The potassium levels observed in Opuru-fulou may not be unconnected to the main ingredient (Prawn) – Figure 3. Potassium helps to maintain the osmotic pressure and the acid base balance of the body (OLUSANYA 2008). The calcium content of the traditional foods ranged from 26.50mg/100 g in Opuru-fulou to 261.3 mg/100 g in Kekefiyai. The high level of calcium in Kekefiyai may be as a result of the use of plantain during its preparation. The values were considered moderate when compared to those of some regional recipes reported by DAS et al. (2009) but higher than those for some Cameroonian household foods (Kana Sop et al.2008). The iron contents of the diets were much higher in comparison to those reported by AMADI et al. (2012) for some traditional diets of South Southern Nigeria that ranged from 0.21 mg/100 g to 6.03 mg/100 g. HALLBERG et al. (1979) have reported that 90% of iron taken as food in developing countries is non-haem. Majority of Nigerians consume more of plant foods because of their socio-economic status. LITTER and RIVER (2003) further stated that the absorption state of non--haem iron can be enhanced with the intake of vitamin C rich foods. The magnesium contents of these tradition diets ranged from 44.89 mg/100 g in Kekefiyai to 91.59 mg/100 g in Opuru-fulou. These values are high when compared to some Ikwerre traditional foods that ranged from 38.0 gmg/100 g to 85.04 mg/100 g (AMADi et al. 2012). The phosphorus contents of these evaluated foods ranged from 49.90 mg/100 g in Kekefiyai to 55.30 mg/100 g in Kiri-igina which were found normal when compared to some values of some Ikwerre traditional diets as reported by AMADI et al. (2012). The high level of phosphorus observed in Opuru-fulou may be due to the fact that phosphorus is abundantly found in the main ingredients. In addition, it was observed that Opuru-fulou had the greatest amount of Mn, Cu, and Zn among the three traditional dishes assessed in this study.

Table 4

Mineral element ratio of Kekefiyai, Kiri-igina, and Opuru-fulou

| Ratio | Kekefiyai | Kiri-igina | Opuru-fulou | Reference | Range |
|---|---|---|---|---|---|
| Na/K | 1.32 | 0.34 | 0.35 | 2.4 | 1.4–3.4 |
| Ca/P | 5.32 | 0.48 | 0.64 | 2.6 | 1.8–3.6 |
| Ca/K | 3.79 | 0.35 | 0.55 | 4.2 | 2.2–6.2 |
| Zn/Cu | 6.54 | 1.82 | 1.60 | 8.0 | 4.0–12 |
| Na/Mg | 2.02 | 0.30 | 0.24 | 4.0 | 2.0–6.0 |
| Ca/Mg | 5.82 | 0.31 | 0.39 | 7.0 | 3.0–11.0 |
| Fe/Cu | 104.11 | 47.48 | 11.15 | 0.9 | 0.2–1.6 |

The Na/K ratio in the body is of great concern for prevention of high blood pressure, and Na/K ratio < 1 is recommended (OLAOFE et al. 2009). The Na/K ratios of all the three diets shown in Table 4, were more than 1 hence the food samples may not be considered adequate in reducing the incidence of high blood pressure. If the Ca/P ratio is low (low calcium and high phosphorus intake), a great amount of calcium may be lost in the urine, decreasing the calcium levels in the bone. Food is considered poor if the ratio is less than 0.5 while Ca/P above 2 helps to increase the absorption of calcium in the small intestine (OLAOFE et al. 2009). Only the Ca/P ratio in Kiri-Igina was below 0.5 while in Kekefiyai and Opuru-fulou the Ca/P ratio occurred above 0.5, which suggests that these foods may be considered rich sources of calcium. Also, the result for the mineral element ratio showed that the Fe/Cu ratio of all the three foods evaluated, fell above the standard ranges and values shown in Table 4. This is a worrisome outcome because elevated Fe/Cu ratio can compromise normal cellular activity and lead to mitochondrial damage through increase in free radical production (DAVID 2010).

## Conclusion

These findings show that the traditional diets can provide substantial amounts of nutrients to meet the normal daily dietary requirements of the Bayelsan people.

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# COMPOSITION OF FATTY ACIDS IN DARK FLOUR FROM SPELT AND COMMON WHEAT GRAIN GROWN ORGANICALLY IN POLAND

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Key words: spelt, common wheat, dark flour, organic growing, fatty acids.

Abstract

The aim of the study was to compare the total lipid content, main fatty acids composition and their proportions in dark (high-extracted) flour obtained from the grain of seven spelt wheat (*T. spelta*) cultivars and one (reference) common wheat cultivar (*T. vulgare*), grown organically in Poland during two successive years of cultivation. Husked spelt grain cv. „Ceralio", „Schwabenkorn", „Frankenkorn", „Holstenkorn", „Schwabenspelz", „Ostro" and „Oberkulmer Rotkorn", and common wheat grain cv. „Korweta" were ground in a buhrstone mill on a certified organic farm. The total ash content and the acidity of the produced flour were determined. Total lipid content was evaluated by the Soxhlet method, and fatty acids composition was determined using gas chromatography.

The majority of investigated spelt flours characterized by significantly higher total acidity level than the flour from common wheat cv. „Korweta". In most studied flours, fat acidity exceeded the allowable standard. The average total lipid content of the tested spelt flours, excluding cv. „Ceralio" (from first year of harvest), was significantly higher than in cv. „Korweta" wheat flour. The results of the study indicate that linoleic acid was the dominant fatty acid in all flour types, but significantly lower levels of this acid were determined in spelt flour than in the flour obtained from common wheat cv. „Korweta". All spelt flours were characterized by significantly higher concentration of oleic acid in comparison with cv. „Korweta" wheat flour. The highest total lipid content was reported in cv. „Holstenkorn" spelt flour which, in addition to cv. „Ceralio" flour, was also marked by the most favorable composition of essential PUFAs.

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# SKŁAD KWASÓW TŁUSZCZOWYCH W WYSOKOWYCIĄGOWEJ MĄCE Z ZIARNA ORKISZU I PSZENICY ZWYCZAJNEJ Z PRODUKCJI EKOLOGICZNEJ W POLSCE

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Słowa kluczowe: orkisz, pszenica zwyczajna, wysokowyciągowa mąka, produkcja ekologiczna, kwasy tłuszczowe.

Abstrakt

Celem badań było porównanie zawartości lipidów ogółem oraz składu i proporcji głównych kwasów tłuszczowych w wysokowyciągowej mące uzyskanej z ziarna siedmiu odmian orkiszu (*T. spelta*) oraz z ziarna (wzorca) jednej odmiany pszenicy zwyczajnej (*T. vulgare*), z produkcji ekologicznej w Polsce, z dwóch kolejnych lat uprawy. Odplewione ziarno orkiszu odmian: „Ceralio", „Schwabenkorn", „Frankenkorn", „Holstenkorn", „Schwabenspelz", „Ostro" i „Oberkulmer Rotkorn" oraz ziarno pszenicy zwyczajnej odmiany „Korweta" przemielano w młynie żarnowym zlokalizowanym w atestowanym gospodarstwie ekologicznym. W uzyskanej mące oznaczono zawartość popiołu ogółem i kwasowość. Określono zawartość lipidów ogółem metodą Soxhleta oraz oznaczono skład kwasów tłuszczowych za pomocą chromatografii gazowej.

Większość badanych mąk orkiszowych charakteryzowała się istotnie wyższą ogólną kwasowością niż mąka z ziarna pszenicy zwyczajnej odmiany „Korweta". Kwasowość tłuszczowa dla większości badanych mąk przekraczała dopuszczalny poziom określony w normie. Średnia zawartość lipidów ogółem w badanych mąkach orkiszowych, z wyjątkiem mąki z ziarna orkiszu odmiany „Ceralio" (z pierwszego roku zbiorów), była istotnie wyższa niż w mące z ziarna pszenicy odmiany „Korweta". W analizie składu kwasów tłuszczowych wykazano, że w badanych mąkach dominował kwas linolowy, z tym, że było go istotnie mniej w mąkach orkiszowych niż w mące z ziarna pszenicy odmiany „Korweta". Wszystkie mąki orkiszowe charakteryzowały się istotnie wyższą zawartością kwasu oleinowego w porównaniu z mąką z ziarna pszenicy odmiany „Korweta". Najwyższą zawartością lipidów ogółem charakteryzowała się mąka z ziarna orkiszu odmiany „Holstenkorn", która, obok mąki z ziarna orkiszu odmiany „Ceralio", cechowała się również najkorzystniejszym składem niezbędnych nienasyconych kwasów tłuszczowych (NNKT).

## Introduction

Spelt (*T. spelta*) is one of the oldest wheat species (next to einkorn – *T. monococcum* and emmer – *T. dicoccum*) which has retained the characteristic properties of primitive wheat species and is, therefore, popularly used in organic farming (MAJEWSKA et al. 2007, SUCHOWILSKA et al. 2009, MIKOS and PODOLSKA 2012, BOROS et al. 2015). Most researchers focus

on the advantages and the characteristic attributes of spelt grain, including its high total protein content, wet gluten yield, relatively high micronutrient and macronutrient content, to demonstrate its superior nutritional value to common wheat grain (ABDEL-AAL et al. 1995, GRELA 1996, MOUDRÝ and DVOŘÁČEK 1999, BONAFACCIA et al. 2000, MAJEWSKA et al. 2007, SUCHOWILSKA et al. 2009, ESCARNOT et al. 2010). Many authors have demonstrated that spelt grain and the resulting flour are characterized by a higher total lipid content and slightly different fatty acid composition than common wheat grain and flour (ABDEL-AAL et al. 1995, GRELA 1996, PIERGIOVANNI et al. 1996, RANHOTRA et al.1996, MARCONI et al.1999, RUIBAL-MENDIETA et al. 2004, SUCHOWILSKA et al. 2009, ESCARNOT et al. 2010). RUIBAL-MENDIETA et al. (2004) proposed an analysis of fatty acid composition and proportions as a method of distinguishing spelt flour from common wheat flour and determining any attempts at falsifying the composition of the former.

Spelt flour (light and dark) is successfully used as raw material in the baking industry (BOJŇANSKÁ and FRANČÁKOVÁ 2002, MAJEWSKA et al. 2007, ABDEL-AAL 2008, CACAK-PIETRZAK et al. 2013). Especially dark (high-extracted) common wheat and spelt flour is a source of valuable nutrients that are required by the human body, including carbohydrates, proteins, B group vitamins and mineral compounds (Mg, Fe, Zn, Cu). Dark flour is also a vital source of fiber, including polysaccharides and oligosaccharides, and lignins which are indigestible and thus improve the peristalsis of the large intestine. Those compounds also lower blood cholesterol and glucose levels (ESCARNOT et al. 2010). Dark wheat flour has a greater antioxidant capacity and a lower allergizing potential than white flour (KATINA et al. 2005, MARCINIAK and OBUCHOWSKI 2007).

The lipid content and fatty acid composition of grain is largely determined by cereal species, variety, climate and growing conditions. High temperature and low precipitation lower lipid content and fatty acid saturation (KONOPKA and ROTKIEWICZ 2004). Lipids affect the technological quality and nutritional value of grain and flour (PRABHASANKAR and RAO 1999, RUIBAL-MENDIETA et al. 2002). During dough kneading, the lipid contained in wheat flour binds with proteins and becomes an integral part of the gluten matrix that forms the crumb of bread (PANAZZO et al. 1993). KONOPKA and ROTKIEWICZ (2000) demonstrated that native lipids significantly influence the rheological properties of bread, they affect the starch pasting process, mechanical properties of dough and, ultimately, the quality and shelf-life of bread. PRABHASANKAR and RAO (1999) and PRABHASANKAR et al. (2000) emphasized the role of polar lipids which determine the volume of bread loaf, and of unsaturated fatty acids which improve

gluten quality through the oxidation of –SH groups. The above observations were validated by PANAZZO et al. (1993) and MAGNUS et al. (2000) who suggested that bread loaf volume is determined not only by the content of lipids (free or bound) in flour, but also by lipid type. PRABHASANKAR and RAO (1999, 2001) observed that the lipid content, fatty acid composition and proportions in wheat flour are determined by the grain milling technology (type of mill) and the type of flour.

The most frequently determined fatty acids are linoleic acid (n-6 group), linolenic acid (n-3), arachidonic acid (n-6), eicosapentaenoic acid and docosahexaenoic acid (DHA). Those compounds, abbreviated as EFAs (essential fatty acids), are the precursors of prostaglandins, prostacyclins, thromboxanes and leukotrienes which have hormone-like functions (ČERTÍK et al. 2006). The human body is unable to build those acids because it lacks the enzymes catalyzing the double bond forming reaction in fatty acid chains located further than carbon atom 9. For this reason, EFAs have to be supplied with food (SINGH 2005).

As shown by epidemiological analyses and medical tests carried out worldwide, the consumption of saturated fats increases the risk of cardiovascular disease, while a diet rich in polyunsaturated fatty acids, including linoleic and linolenic acid, lowers the threat of coronary heart disease (CHD). Monounsaturated fatty acids containing oleic acid produce a similar effect by lowering blood glucose and insulin levels, a very important consideration for diabetes patients (ASCHERIO 2002, RYAN et al. 2007). Insufficient consumption of PUFAs (polyunsaturated fatty acids) also maximizes the risk of atopic eczema, circulatory, endocrinological, neurological and immunological disorders. ČERTÍK et al. (2006) and SINGH (2005) claim that n-3 and n-6 acids should be consumed in a balanced proportion. The recommended proportion of omega-6 to omega-3 acids is 5–10:1 (SINGH 2005).

Cereals contain 3.6% d.m. total lipid on average and the predominant fatty acid is linoleic acid, while linolenic acid is found in smaller quantities (ČERTÍK et al. 2006, RYAN et al. 2007). According to SINGH (2005), 10–15% of omega-6 acids are extracted from bound lipids in whole grain kernels. Cereals are the basic food group in an average consumer's diet, and valuable nutrients found in spelt flour, in particular dark flour, can easily supplement any nutritional deficiency.

The objective of the study was to compare the total lipid content, main fatty acids composition and their proportions in dark (high-extracted) flour from the grain of seven spelt wheat (*T. spelta*) cultivars and one (reference) common wheat cultivar (*T. vulgare*), grown organically in Poland (two harvest years). The authors also set out to investigate the nutritional

value of dark spelt flour (from grain milled by traditional way in buhrstone mill) as regards its fatty acids composition.

## Materials and Methods

The experimental material was high-extraction flour obtained from husked winter spelt grain cv. „Ceralio", „Schwabenkorn", „Frankenkorn", „Holstenkorn", „Schwabenspelz", „Ostro" and „Oberkulmer Rotkorn", and common winter wheat grain cv. „Korweta" as the reference. The grain was grown in line with organic farming standards on a certified Polish farm located in the Kujawy-Pomerania Province. The experimental material comprised grain from two successive years of harvest (2007, 2008). After harvest, spelt grain was husked in a Gerbgang (Germany) machine. It was purified, brought to a moisture level of 15% and ground under standard conditions in a buhrstone mill (FP 950, Denmark) in the "Bio" Pasta Factory of the above organic farm.

The produced flour was analyzed to determine its total ash content (*Ziarno zbóż*... PN-ISO 2171: 2010), total acidity (HORUBAŁOWA and HABER 1994), and fat acidity (*Przetwory zbożowe*... PN-ISO 7305: 2001). Fat was extracted by the Soxhlet method (KREŁOWSKA-KUŁAS 1993), and fatty acid composition was determined by gas chromatography according to the method described by ZADERNOWSKI and SOSULSKI (1979). A sample used in the determination of the fatty acid composition (around 10 µg) was placed in a vial, and 2 ml of a methylating mixture (chloroform-methanol-sulfuric acid, 100:100:1 v/v/v) was added. The sample was methylated by heating sealed vials in a drier at a temperature of 70°C for two hours. After methylation, the vials were opened, a small quantity of zinc dust was added (to neutralize sulfuric acid), the solvent was evaporated, and fatty acid methyl esters (FAMEs) were eluted with hexane. The resulting solution was analyzed by gas chromatography (GC) using a J&W Scientific (USA) DB-225 column (20 m × 0.25 mm × 0.15 µm) and helium as carrier gas. The following chromatographic parameters were applied: injection temperature of 250°C, column temperature of 200°C, detector temperature of 300°C. The analysis involved a Carlo Erba (Italy) Fisons 8000 gas chromatograph interfaced with a computer with HP ChemStation software (Palo Alto, USA). Fatty acid composition was determined in three parallel samples.

The obtained results were verified statistically by a one-factorial analysis of variance and Duncan's multiple range test with the use of Statistica 12.0. software. The experimental factor was regarded as significant at $p < 0.05$.

## Results and Discussion

The total ash content of spelt flour from the grain harvested in first year of experiment was within the range of 1.17 to 1.57 % d.m. (for flour made from spelt grain cv. „Ceralio" and „Holstenkorn" respectively), while the total ash content of cv. „Korweta" common wheat flour was 1.03% d.m. (Table 1). As regards second year of grain harvest, the total ash content of spelt flour was determined in the range of 1.06% d.m. (cv. „Schwabenspelz") to 1.21% d.m. (cv. „Holstenkorn"), while the flour from common wheat cv. „Korweta" was characterized by a total ash content of 1.09% d.m. The above results were indicative of dark flour of 1050 and 1400 type (according to the Polish Standard *Przetwory zbożowe*... PN-A-74022: 2003). The year of grain harvest had a profound effect on the reported ash content, in particular of spelt flour.

MARCONI et al. (1999) and BONAFACCIA et al. (2000) (conventional production system) reported higher ash content values for dark spelt flour (1.59–2.10% d.m. and 1.76–1.85% d.m. respectively) than noted in this study. In the work of BOJŇANSKA and FRANČÁKOVÁ (2002) (organic production system), the ash content of dark spelt flour ranged from 1.79 to 2.36% d.m., and it was also affected by spelt grain cultivar, but mostly by the harvest year and growing conditions. It should be noted that the investigated attributes were also largely dependent on the type of the applied mill, grinding parameters, the moisture content and hardness of milled grain.

Table 1

Total ash content and extraction yield of flour obtained from grain of tested wheat cultivars

| Specification | Total ash content [% d.m.] | | Flour extraction yield [%] | |
|---|---|---|---|---|
| Harvest year | I | II | I | II |
| Common wheat | | | | |
| Korweta | $1.03^{a}$ | $1.09^{A}$ | $86.6^{a}$ | $87.6^{A}$ |
| Spelt wheat | | | | |
| Ceralio | $1.17^{b}$ | $1.09^{A}$ | $89.0^{b}$ | $87.6^{A}$ |
| Schwabenkorn | $1.38^{c}$ | $1.08^{A}$ | $92.6^{c}$ | $87.4^{A}$ |
| Frankenkorn | $1.18^{b}$ | $1.07^{A}$ | $89.2^{b}$ | $87.2^{A}$ |
| Holstenkorn | $1.57^{d}$ | $1.21^{B}$ | $95.9^{d}$ | $89.6^{B}$ |
| Schwabenspelz | $1.43^{e}$ | $1.06^{A}$ | $93.5^{e}$ | $87.0^{A}$ |
| Ostro | $1.52^{f}$ | $1.16^{C}$ | $95.0^{f}$ | $88.8^{C}$ |
| Oberkulmer Rotkorn | $1.46^{e}$ | $1.08^{A}$ | $93.9^{e}$ | $87.4^{A}$ |

* Within columns, values followed by the same small and capital superscript letters in a given year do not differ significantly

Wheats are classified into hard and soft grain cultivars. The process of grinding hard wheat is very energy-consuming because the endosperm comprises starch granules that are tightly built into the endosperm matrix. Flour made from soft wheat grain has a tendency to form aggregates, it is more difficult to sieve and, consequently, has a lower extraction rate. The dark flour extraction yield ranged from 86.6 to 87.6% (common wheat) and from 87.2 to 95.9% (spelt wheat) depending on the year of harvest and cultivar (Table 1). It was also positively correlated with total ash content in studied dark flours.

Grain and cereal products have an acidic pH. The acidity of cereals is determined by the level of phosphoric acid, acidic phosphates, organic acids, amino acids and proteins with an acidic pH (KREŁOWSKA-KUŁAS 1993). Acidity increases during long-term storage due to the activity of enzymes that break down organophosphorus compounds, fats and proteins (HORUBAŁA and HABER 1994). The total (potential) acidity of flour from common wheat grain cv. „Korweta" was 3.6°, while it was within the range of 3.7 to 5.4° for spelt flour (first year of harvest) (Table 2). The acidity of spelt flour from grain harvested in second year ranged from 2.7° (cv. „Schwabenspelz") to 4.6° (cv. „Frankenkorn" and „Ostro"), while the total acidity of flour obtained from grain of common wheat cv. „Korweta" reached 2.9°. In most cases, the total acidity of spelt flour was significantly higher than that of cv. „Korweta" wheat flour. The total acidity of all investigated flour types from both harvest years did not exceed the allowable level (not more than 7°) (HORUBAŁA and HABER 1994).

Table 2

Total and fat acidity of high-extraction flour obtained from grain of tested wheat cultivars

| Specification | Total acidity of flour Acidity grades° | | Fat acidity of flour mg KOH/100 g d.m. | |
|---|---|---|---|---|
| Harvest year | I | II | I | II |
| Common wheat | | | | |
| Korweta | $3.6^{a}$ | $2.9^{A}$ | $54.6^{a}$ | $53.3^{A}$ |
| Spelt wheat | | | | |
| Ceralio | $3.7^{a}$ | $3.3^{B}$ | $60.5^{b}$ | $77.1^{B}$ |
| Schwabenkorn | $3.9^{a}$ | $4.0^{C}$ | $51.3^{c}$ | $79.1^{C}$ |
| Frankenkorn | $5.1^{c.d}$ | $4.6^{D}$ | $52.9^{e}$ | $49.6^{D}$ |
| Holstenkorn | $5.0^{d}$ | $4.0^{C}$ | $54.8^{a}$ | $66.2^{E}$ |
| Schwabenspelz | $4.1^{d}$ | $2.7^{E}$ | $49.8^{f}$ | $61.3^{F}$ |
| Ostro | $5.4^{b}$ | $4.6^{D}$ | $60.8^{b}$ | $99.6^{G}$ |
| Oberkulmer Rotkorn | $4.5^{c}$ | $3.8^{F}$ | $66.3^{d}$ | $132.1^{H}$ |

* Within columns, values followed by the same small and capital superscript letters in a given year do not differ significantly

The fat acidity of flour from common wheat grain cv. „Korweta" harvested in first year of experiment reached 54.6 mg KOH/100 g d.m., while the acidity of flour from the investigated spelt cultivars ranged from 49.8 to 66.3 KOH/100 g d.m. (Table 2). The fat acidity of flour from spelt grain harvested in second year of experiment was determined in the range of 49.6 mg KOH/100 g d.m. for cv. „Frankenkorn" to 132.1 mg KO/100 g d.m. for cv. „Oberkulmer Rotkorn". The fat acidity of flour from common wheat grain cv. „Korweta" was 53.3 mg KOH/100 g d.m. When compared with the reference standard indicated in *Przetwory zbożowe*... PN-A-74022: 2003, the obtained results show that the fat acidity of most investigated spelt flours exceeded the allowable level (no more than 50 mg KOH/100 g d.m. for all flour types) to a lesser or greater extent. The above could be attributed to the fact that in comparison with common wheat flour, spelt flour was characterized by a higher ash content and, consequently, a higher content of fat which could have been partially oxidized. Fat acidity is indicative of the quantity of non-esterified fatty acids released by lipase (SZAFRAŃSKA 2007). Shortly after the publication of the above standards, Polish control authorities carried out fat acidity analyses and revealed that the allowable values had been exceeded several-fold even in flour samples collected directly after milling, in particular in flour samples with ash content higher than 0.75% d.m. (SZAFRAŃSKA 2007). The above findings imply a need to revise the guideline values.

The average total lipid content of the studied spelt cultivars ranged from 1.52 to 2.04 % d.m. (harvest I) and from 1.37 to 2.11% d.m. (harvest II), while the lipid content of flour obtained from common wheat grain cv. „Korweta" was estimated at a level of 1.42% d.m. for harvest I samples and 1.54% d.m. for harvest II samples (Table 3). The highest lipid content (in two successive years) was determined for the flour obtained from spelt grain cv. „Holstenkorn", which was also characterized by the highest ash content.

In a study carried out by RUIBAL-MENDIETA et al. (2002) (conventional production system), dark spelt flour was marked by a higher total lipid content (1.80–4.40% d.m.) in comparison with common wheat flour (1.50–2.34% d.m.). According to GRELA (1996) (conventional production system), the flour obtained from spelt grain was also characterized by a higher lipid content than common wheat flour. A higher lipid content in grain of ten spelt cultivars (1.50–2.02% d.m.) in comparison with common wheat grain cv. „Samara" (1.43% d.m.) was also noted by MOUDRÝ and DVOŘÁČEK (1999) (low – input production system). In dark spelt flours studied by MARCONI et al. (1999), total lipid content differed significantly between the tested cultivars, ranging from 3.80 to 4.20% d.m. ČERTÍK et al.

Table 3

Total lipid content and fatty acids composition of high-extraction flour obtained from grain of tested wheat cultivars

| Specification | Total lipid content | | Palmitic acid $C_{16:0}$ | | Stearic acid $C_{18:0}$ | | Oleic acid $C_{18:1}$ | | Linoleic acid $C_{18:2}$ | | Linolenic acid $C_{18:3}$ | | Ratio of oleic to palmitic acid $C_{18:1}/C_{16:0}$ | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | [% d.m.] | | [%] | | [%] | | [%] | | [%] | | [%] | | – | – |
| Harvest year | I | II | I | II | I | II | I | II | I | II | I | II | I | II |
| Common wheat | | | | | | | | | | | | | | |
| Korweta | $1.42^{a}$ | $1.54^{A}$ | $19.9^{a}$ | $16.8^{A.B.D}$ | $0.4^{a.c}$ | $1.1^{A}$ | $14.2^{a}$ | $14.8^{A}$ | $61.9^{a}$ | $61.9^{A}$ | $3.6^{a}$ | $4.6^{A}$ | $0.71^{a}$ | $0.88^{A}$ |
| Spelt wheat | | | | | | | | | | | | | | |
| Ceralio | $1.52^{b}$ | $1.37^{B}$ | $20.8^{b}$ | $17.3^{A}$ | $0.3^{a.b.c}$ | $1.2^{B.C}$ | $17.5^{b}$ | $18.9^{B}$ | $58.9^{b.d}$ | $58.4^{B}$ | $2.5^{b.e}$ | $3.9^{B}$ | $0.84^{b}$ | $1.09^{B}$ |
| Schwabenkorn | $1.79^{c}$ | $1.64^{C}$ | $19.0^{c}$ | $16.7^{A.C}$ | $0.4^{c}$ | $1.3^{B.D}$ | $20.6^{c.d}$ | $21.4^{C}$ | $57.3^{c}$ | $56.4^{C}$ | $2.7^{b.c.e}$ | $3.3^{C}$ | $1.09^{c}$ | $1.28^{C}$ |
| Frankenkorn | $1.71^{e}$ | $1.67^{C}$ | $18.3^{c}$ | $16.5^{C.D}$ | $0.4^{b.c}$ | $1.1^{A}$ | $19.9^{g}$ | $21.1^{C}$ | $59.4^{b.e}$ | $57.1^{D}$ | $2.0^{c.d}$ | $3.4^{D}$ | $1.09^{c}$ | $1.28^{C}$ |
| Holstenkorn | $2.04^{f}$ | $2.11^{D}$ | $17.2^{d}$ | $13.6^{E}$ | $0.4^{a.b.c}$ | $1.1^{A}$ | $20.3^{d.g}$ | $23.1^{D}$ | $59.6^{d.e.f}$ | $57.7^{E}$ | $2.6^{b.d.f}$ | $3.5^{E}$ | $1.18^{d}$ | $1.70^{D}$ |
| Schwabenspelz | $1.84^{d}$ | $1.69^{C}$ | $17.1^{d}$ | $15.6^{F}$ | $0.3^{a.b}$ | $1.0^{A}$ | $20.6^{d.f}$ | $22.1^{E}$ | $57.3^{c}$ | $57.1^{D.F}$ | $3.2^{a.c.f}$ | $3.4^{D}$ | $1.20^{d.f}$ | $1.42^{E}$ |
| Ostro | $1.84^{d}$ | $1.84^{E}$ | $17.3^{d}$ | $16.4^{B.C}$ | $0.3^{b}$ | $1.1^{E}$ | $21.1^{c.e.f}$ | $22.5^{F}$ | $58.3^{b}$ | $55.6^{G}$ | $3.1^{a.b}$ | $3.4^{D.E}$ | $1.22^{f}$ | $1.37^{F}$ |
| Oberkulmer Rotkorn | $1.77^{c}$ | $1.87^{E}$ | $16.8^{d}$ | $16.5^{B.C}$ | $0.4^{c.d}$ | $1.3^{A}$ | $21.2^{e}$ | $22.0^{E}$ | $59.1^{b.f}$ | $56.6^{C}$ | $2.5^{b.d}$ | $3.2^{F}$ | $1.26^{e}$ | $1.33^{G}$ |

* Within columns, values followed by the same small and capital superscript letters in a given year do not differ significantly

(2006) reported a slightly lower lipid content in spelt grain (1.70% d.m.) than in common wheat grain (2.10% d.m.). According to ABDEL-AAL et al. (1995) and MARCONI et al. (1999), spelt grain has a higher lipid content owing to the fact that the germ, the main source of lipids, has a greater share of the grain. A different hypothesis suggests that the lipid content of the aleurone layer of spelt grain is higher than that of common wheat grain (RUIBAL-MENDIETA et al. 2002). It should be noted that lipid content in flour is also determined by the degree of milling and the type of the applied mill, which could explain differences in lipid content values reported by various authors (PRABHASANKAR and RAO 1999, PRABHASANKAR et al. 2000). PRABHASANKAR and RAO (2001) (conventional production system) studied the lipid content of wheat flour processed in four mill types, and reported the highest lipid content in flour ground in a roller mill, followed by a hammer mill, a disc mill and, finally, a buhrstone mill. The above could be due to the fact that lipids bind with other flour components, i.e. starch and proteins, as temperature increases in the milling process. The exact mechanism of this reaction has not yet been fully elucidated.

An analysis of fatty acid composition revealed that linoleic acid was the dominant acid in the studied flours, but it was found in significantly lower quantities in spelt flour than in flour obtained from common wheat grain cv. „Korweta" (Table 3). The linoleic acid content of spelt flour was determined in the range of 57.3 to 59.6% (harvest I) and 56.4 to 58.4% (harvest II), and it reached 61.9% in flour obtained from reference common wheat (both years). A similar trend was noted by RUIBAL-MENDIETA et al. (2004) in an analysis of the fatty acids composition of dark spelt flour and GRELA (1996) who found that the four tested spelt cultivars contained the highest quantities of linoleic acid (54.0–55.7%), followed by oleic acid (20.1–20.8%) and palmitic acid (18.1–18.9%), while the remaining fatty acids were determined in smaller quantities.

The palmitic acid content of the tested spelt cultivars ranged from 16.8 to 20.8% (harvest I), and a significant decrease was reported in spelt grain harvested in the following harvest year, when the palmitic acid content was determined in the range of 13.6 to 17.3%. The palmitic acid content of common wheat flour was 19.9% and 16.8% (I and II harvest respectively). The year of cultivation strongly influenced on the content of this fatty acid, which was unstable within the two years of experiment. In a study carried out by RUIBAL-MENDIETA et al. (2004), the content of palmitic acid in dark flour made from different spelt cultivars ranged from 15.4 to 18.3% (1998 harvest) and from 15.2 to 19.4% (2002 harvest). GRELA (1996) observed that spelt grain contained more palmitic acid (18.1–18.9%) than common wheat grain (16.7%).

An analysis of the oleic acid content of the investigated flour types showed that the flour obtained from common wheat grain cv. „Korweta" contained 14.2% (harvest I) and 14.8% (harvest II) oleic acid, while the oleic acid content of spelt flour was determined in the range of 17.5 – 21.2% (harvest I) and 18.9 – 23.1% (harvest II). All spelt flour types were characterized by significantly higher levels of oleic acid in comparison with the flour obtained from common wheat grain cv. „Korweta" (Table 3, Figure 1 and Figure 2). The above correlation was also noted by GRELA (1996) and RUIBAL-MENDIETA et al. (2004).

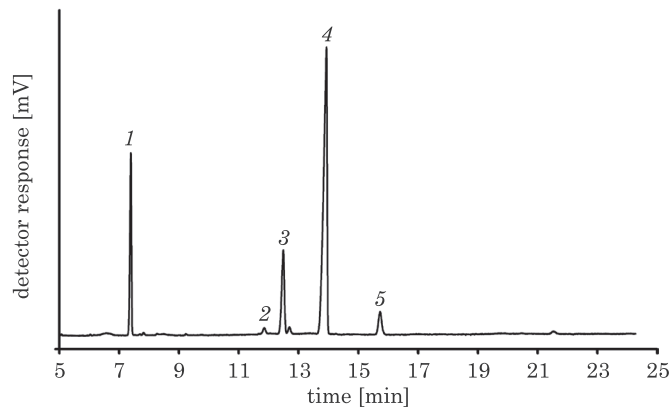


Fig. 1. Fatty acids composition of high-extraction flour obtained from common wheat grain cv. „Korweta" – an example chromatogram (*1* – palmitic acid, *2* – stearic acid, *3* – oleic acid, *4* – linoleic acid, *5* – *α*-linolenic acid)

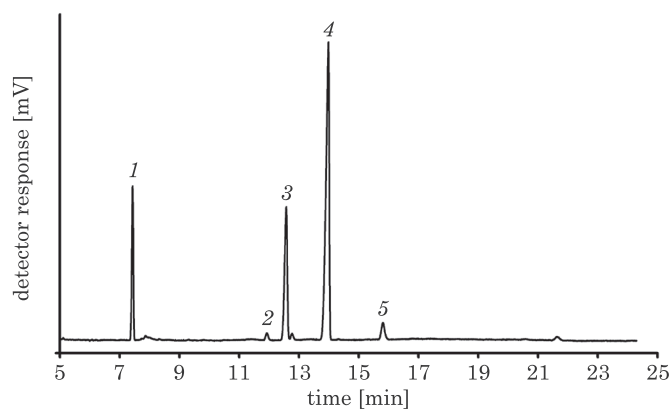


Fig. 2. Fatty acids composition of high-extraction flour obtained from spelt wheat grain cv. „Holstenkorn" – an example chromatogram (*1* – palmitic acid, *2* – stearic acid, *3* – oleic acid, *4* – linoleic acid, *5* – *α*-linolenic acid)

The linolenic acid content of spelt flour from grain harvested in first year of experiment ranged from 2.0 to 3.2%, and of spelt flour from the harvest II – from 3.2 to 3.9%. The flour obtained from common wheat grain cv. „Korweta" contained (in two successive harvest years) 3.6% and 4.6% linolenic acid, respectively. In both years, the linolenic acid content of the reference flour was significantly higher than the values reported in respect of spelt flour. RUIBAL-MENDIETA et al. (2004) also reported lower linolenic acid levels in spelt flour than in common wheat flour. In a study carried out by GRELA (1996), the linolenic acid content of spelt grain was less than half (2.9–3.3%) that reported in common wheat grain (7.1%).

Stearic acid was found in the lowest quantities in the tested flours, and the flour obtained from grain harvested in first year of investigation contained nearly three times less stearic acid than the flour from the harvest II. Spelt flours contained from 0.3 to 0.4% stearic acid in the first year, and from 1.0 to 1.3% in the second year. The stearic acid content of common wheat flour reached 0.4% and 1.1%, respectively (harvest I and harvest II). GRELA (1996) also found that from among the five studied fatty acids, stearic acid occurred in lowest quantities in spelt grain, but reported higher stearic acid levels in spelt (1.4–1.6%) than in common wheat (0.8%).

RUIBAL-MENDIETA et al. (2004) observed a certain correlation when studying the composition and proportions of fatty acids in common wheat flour and spelt flour. In these investigations, an analysis of fatty acid proportions revealed an oleate/palmitate ratio of 1.06 and 0.97 in spelt flour from two successive harvest years, while a 0.57 and 0.54 ratio was reported in respect of common wheat flour, respectively.

The oleate/palmitate ratio was greater than 1 in most of the spelt high--extraction flours tested in this study, while it remained below 1 in the dark flour obtained from common wheat grain cv. „Korweta" (Table 3). The importance of these findings should be emphasized as researchers mentioned above pointed to this correlation as a possible method (in addition to more sensitive detection techniques such as PCR – polymerase chain reaction and electrophoresis) of distinguishing spelt flour from common wheat flour.

## Conclusions

The majority of the studied high-extraction spelt flours were characterized by significantly higher acidity (total acidity and fat acidity) and total lipid content than the flour from common wheat grain cv. „Korweta", which could be attributed to a higher ash content of spelt flours. Linoleic acid was the dominant fatty acid in all studied flour types, but its content was signi-

ficantly lower in spelt flours. Stearic acid was found in the lowest quantities, but its presence was not as profoundly affected by the species and cultivar of wheat grain. In the group of tested spelt flours, the highest lipid content was reported in the flour obtained from spelt grain cv. „Holstenkorn" which, next to cv. „Ceralio", showed significantly higher concentrations of essential PUFAs. All investigated spelt flours were characterized by significantly higher oleic acid levels in comparison with flour obtained from common wheat grain. In the majority of studied spelt flours, the oleate/palmitate ratio was above 1, while values lower than 1 were noted for common wheat (reference) flour. The results of this study indicate that the acidity, total lipid content, fatty acid composition and proportions in the investigated flours were significantly affected by wheat species (spelt, common wheat), wheat cultivar and year of harvest, namely climatic and growing conditions. The farming location and the applied production and milling system are also important considerations.

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# EVALUATION OF TECHNOLOGICAL QUALITY IN GRAIN AND FLOUR OF WINTER TRITICALE (*TRITICOSECALE* WITTM.) FROM CONTROLLED CULTIVATION CONDITIONS

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Key words: winter triticale, irrigation, nitrogen fertilisation, flour, dough, bread.

Abstract

The paper presents results of studies from the years 2009–2010, aiming at the determination of quality attributes and technological properties of grain as well as produced bread, depending on sprinkling irrigation and nitrogen fertilisation of winter triticale cv. Gniewko. The first order factor was the water variant (non-irrigated, irrigated by sprinkling), while the second order factor was nitrogen fertilisation (0, 60, 90, 120 kg N $ha^{-1}$). It was found that despite improvement in certain indexes for grain, flour and dough under the influence of sprinkling irrigation and nitrogen fertilisation faults were found in the produced bread, which did not meet requirements for bread for human consumption. A primary cause for poor bread quality was connected with high amylolytic activity of flour.

# OCENA JAKOŚCI TECHNOLOGICZNEJ ZIARNA I MĄKI PSZENŻYTA OZIMEGO (*TRITICOSECALE* WITTM.) Z KONTROLOWANYCH WARUNKÓW UPRAWY

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Słowa kluczowe: pszenżyto ozime, deszczowanie, nawożenie azotem, mąka, ciasto, chleb.

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Abstrakt

W pracy przedstawiono wyniki badań z lat 2009–2010 mające na celu określenie cech jakościowych i technologicznych ziarna oraz uzyskanego pieczywa w zależności od deszczowania i nawożenia azotem pszenżyta ozimego odmiany Gniewko. Czynnikiem pierwszego rzędu był wariant wodny (niedeszczowany, deszczowany), a czynnikiem drugiego rzędu było nawożenie azotem (0, 60, 90, 120 kg N $ha^{-1}$). Stwierdzono, że pomimo poprawy niektórych wskaźników ziarna, mąki i ciasta pod wpływem deszczowania i nawożenia azotem uzyskany chleb był wadliwy i nie spełniał wymogów stawianych pieczywu konsumpcyjnemu. Stwierdzona niska liczba opadania w ziarnie oraz mała zawartość glutenu i niska liczba opadania w mące przyczyniły się do wytworzenia ciasta o słabych cechach farinograficznych, a w dalszej kolejności uzyskania niskiej wydajności i objętości chleba.

## Introduction

Cereals constitute the basic food source worldwide and in terms of energy requirement they cover over 30% requirement of the world human population. The dominant species still include rice, maize and wheat; however, their production is frequently limited by climatic and soil conditions. Poland is a country with a considerable share of light soils, which results from the predominance of soils originating from sand, loamy sand and sandy clays. For this reason an alternative for these soils may be provided by cultivation of cereals in terms of their quality exhibiting comparative traits to wheat, at the same time showing greater adaptability to inferior soil conditions and greater tolerance to stress induced by drought and pathogens (LONBANI and ARZANI, 2011). According to MARCINIAK et al. (2008 a, b), triticale – thanks to its high productivity and stability obtained in recent years – might constitute a regional contribution of Poland to the world food market. In literature many authors indicates the potential use in human nutrition for triticale grain and its composition, resulting in increased interest in this synthetic species among breeders and processing companies (MC GOVERIN et al. 2011, PATTISON and TRETHOWAN 2013). To be used for flour and baking bread triticale grain should be of adequate quality, both in terms of general properties and specific requirements of the milling and baking industries. Many studies (TOHVER et al. 2004, SABOVIC 2014) indicate that it is possible to produce bread of satisfactory quality from triticale flour, but it requires certain modifications of bread production methods. Baking quality of cereals is determined first of all by the amount and composition of gluten reserve proteins (WIESER 2007, MARTINEK et al. 2008). According to VARUGHESE et al. (1996), triticale grain in comparison to wheat grain exhibits lower contents and lesser strength of gluten. For many years breeding work on triticale has aimed at

the generation of new cultivars exhibiting properties comparable to wheat, i.e. good baking value, an increased number of glutelin units (TOHVER et al. 2005). Next to genetic characters, quality attributes are also modified by climatic, soil and cultivation conditions (PATTISON and TRETHOWAN 2013, JANKOWSKI et al. 2014). Among cultivation factors the application rate and date of nitrogen fertilisation have the greatest effect on properties and technological value of grain. Nitrogen is a nutrient having the most marked effect on yield and protein content (DELOGU et al. 1998). However, effects of nitrogen application are very often modified by variable weather conditions (RUITER et al. 1999), particularly availability of water. Many authors indicate that this factor has a stabilising effect on the yield of grain, while it also has an advantageous effect on grain quality (ALBRIZIO et al. 2010).

The aim of this study was to determine the effect of sprinkling irrigation and nitrogen fertilisation on selected quality parameters of grain, flour, dough and bread produced from triticale cv. Gniewko.

## Materials and Methods

The field experiment using winter triticale cv. Gniewko were conducted in the years 2009–2010 at the Department of Agronomy, the Poznań University of Life Sciences on soils at the Department Experimental Teaching Station in Złotniki near Poznań (N: 52°29'0" E: 16°49'53") Poland. Triticale was grown on lessivè soils, formed from light loamy sands, of quality classes IVa and IVb, very good and good rye complex. According to the international WRB classification (2006) these soils are classified as Albic Luvisols, while according to Soil Taxonomy (1999) they are Typic Hapludalfs, while in terms of grain size they are loamy sands underlined by loam. The arable layer has a slightly acid reaction, pH from 5 to 6 (pH measured in 1 M KCl). This soil is abundant in phosphorus (0.78 mg P $kg^{-1}$ soil) and potassium (1.35 mg K $kg^{-1}$ soil), while it is poor in magnesium (0.45 mg Mg $kg^{-1}$ soil). The groundwater table is deposited at a depth from 2.9 to 5.5 m and it is located outside the range of triticale roots, which in years with lower precipitation totals may cause periodical water deficits for crops. The trial was established as a two-factorial experiment in the split–plot design in four replications. The area of plots for crop harvest was 24 $m^2$. Investigated factors included the water variant (non-irrigated and irrigated by sprinkling) and nitrogen fertilisation applied at 0, 60, 120 and 180 kg $ha^{-1}$. Fields were irrigated at a decrease of soil moisture content in the 0-30 cm layer to 70% field capacity at the

period of greatest sensitivity of plants to water deficit. Water application rates in the years of analyses were 120 mm in the first year and 80 mm in the following year (a single dose was 40 mm). Nitrogen fertilisation was applied as ammonium nitrate at 60 kg N $ha^{-1}$ before sowing and in respective treatments at 60 kg N $ha^{-1}$ at the tillering phase (BBCH 21) and 60 kg N $ha^{-1}$ the heading phase (BBCH 51). Phosphorus and potassium fertilisation as 46% superphosphate was applied before sowing at 80 kg $ha^{-1}$ $P_2O_5$ (35 kg P $ha^{-1}$) and as 60% potassium salt at 100 kg $ha^{-1}$ $K_2O$ (83 kg K $ha^{-1}$). Moreover, weed infestation was regulated by autumn spraying with a preparation containing active substances chlorotoluron 80% at 1.0 kg $ha^{-1}$ and fluroxypyr + 2.4-D at 1.2 l $ha^{-1}$. The other cultivation measures were performed in accordance with the recommendations for this species.

Samples of grain produced in a given year were ground in a Quadrumat Senior mill, simultaneously determining flour extraction rates (JAKUBCZYK and HABER, 1983).

Analysis of the basic chemical composition included determinations of contents of dry matter (*Ziarno zbóż…* PN-EN ISO 712: 2012), crude ash (ICC method 104/1), crude fat (ICC-Standard No. 136. 1984), protein according to Kjeldahl (AOAC, 1990) and fibre (VAN SOEST, 1963). Nitrogen-free extractives were denoted as 100% (contents of protein + fat + ash + crude fibre). Results of all assays were converted into dry mass.

Grain quality was evaluated based on determinations of grain moisture content (by the oven dry method), bulk density of grain (PN-ISO 797-2:1998) and grain vitreousness (ICC-Standard No. 129. 1980).

Quality of flour was assessed based on flour colour (PN-A-74029: 1999P), the amount of gluten, sedimentation properties (SDS test) and amylolytic properties (falling number) (PN-ISO 3093: 1996/ AZ1:2000).

Rheological properties of dough were assessed using a Brabender farinograph in accordance with the ICC-Standard No. 115/72. Farinograph analyses determined water absorption of flour (%), dough stability (min.), dough development (min.), dough softening (BU) and Brabender quality number.

Experimental bread was baked using a single phase process in order to determine the yield and bread volume per 100 g bread based on the method developed by KLOCKIEWICZ-KAMIŃSKA and BRZEZIŃSKI (1997).

Recorded results were analyzed statistically using the analysis of variance for orthogonal factorial experiments and the analysis of variance in the split-plot design (ANOVA). The means of treatment were compared by means of Tukey's Multiple Range test and least significant difference (LSD) was declared at $P < 0.01$ and $P < 0.05$.

## Results and Discussion

In the period of analyses mean air temperature ranged from 9.4 to 10.2ºC, at the multiannual mean of 8.8°C, while in the vegetation period for April it was 14.2 and 10.5 ºC, May 15.1 and 12.0ºC, June 16.7 and 19.2ºC, whereas for July it was 21.7 and 23.0ºC, respectively. Precipitation totals in these years ranged from 605.8 mm to 707.3 mm, while for the above mentioned months they amounted to 16.0 and 38.5 mm, 92.3 and 134.6 mm, 129.1 and 26.6 mm, 104.6 and 100.9 mm, respectively (Table 1).

Table 1

Weather conditions at Meteorological Station at Złotniki in 2008–2010

| Months | Years | | | | Average of the multi-year 1951–2006 | |
|---|---|---|---|---|---|---|
| | 2008/2009 | | 2009/2010 | | | |
| | rainfalls [mm] | temperature [°C] | rainfalls [mm] | temperature [°C] | rainfalls [mm] | temperature [°C] |
| IX | 16.8 | 14.4 | 53.9 | 17.0 | 45.8 | 13.8 |
| X | 69.4 | 9.9 | 59.4 | 7.9 | 34.8 | 9.1 |
| XI | 20.5 | 5.4 | 38.2 | 6.6 | 34.7 | 3.7 |
| XII | 25.0 | 1.5 | 31.8 | -0.3 | 39.0 | 0.1 |
| I | 16.3 | -2.4 | 34.4 | -6.5 | 28.9 | -1.4 |
| II | 32.9 | 0.0 | 22.8 | -0.5 | 27.2 | -0.4 |
| III | 56.8 | 4.5 | 33.8 | 4.2 | 30.0 | 3.3 |
| IV | 16.0 | 14.2 | 38.5 | 10.5 | 31.3 | 8.5 |
| V | 92.3 | 15.1 | 134.6 | 12.0 | 48.0 | 14.2 |
| VI | 129.1 | 16.7 | 26.6 | 19.2 | 57.8 | 17.4 |
| VII | 104.6 | 21.7 | 100.9 | 23.0 | 74.5 | 19.1 |
| VIII | 26.1 | 21.4 | 132.4 | 19.6 | 54.2 | 18.4 |
| Total – Average | 605.8 | 10.2 | 707.3 | 9.4 | 506.2 | 8.8 |

Chemical analyses showed that grain of winter triticale cv. Gniewko varied slightly in terms of its composition depending on the experimental factors (Table 2). Sprinkling irrigation caused a reduction of contents of crude protein and ash, as well as an increase in crude fat contents in grain. A decrease in protein contents under the influence of irrigation in wheat was also reported by ERECUL et al. (2012).

Table 2

Chemical composition in grain of winter triticale (in % dry matter)

| Factor | Level | Crude protein | Crude fiber | Crude lipides | N – free extract | Ash |
|---|---|---|---|---|---|---|
| WV | T0 | 11.6 | 4.50 | 1.35 | 72.0 | 1.82 |
| | T1 | 10.9 | 4.56 | 1.54 | 72.4 | 1.68 |
| LSD | | 0,33** | n.s. | 0.15* | n.s. | 0.12* |
| NF [kg N ha$^{-1}$] | 0 | 9.70 | 4.64 | 1.38 | 73.5 | 1.78 |
| | 60 | 9.96 | 4.53 | 1.48 | 73.5 | 1.73 |
| | 120 | 11.7 | 4.49 | 1.44 | 71.7 | 1.72 |
| | 180 | 13.5 | 4.47 | 1.48 | 70.0 | 1.70 |
| LSD | | 0.47** | 0.12* | n.s. | 0.88** | 0.07* |

WV – water variant; T0 – non-irrigation; T1 – sprinkling irrigation; NF – nitrogen fertilization; n.s. – not significant; *$P < 0.05$ and **$P < 0.01$.

Nitrogen fertilisation applied in this experiment significantly modified analysed components. With an increase in nitrogen application rates a significant increase was observed in crude protein contents, but reduced contents of crude fibre, nitrogen-free extractives and ash. KIRCHEV (2014) in the experiments on triticale conducted in southern Bulgaria showed a positive effect of high nitrogen application rates on the content of protein in triticale grain.

Technological quality of triticale grain assessed based on moisture content, grain bulk density, vitreousness and falling number depending on sprinkling irrigation showed not significant effect of this factor (Table 3).

Table 3

Technological quality of winter triticale grain cv. Gniewko depending on sprinkling irrigation and nitrogen fertilization

| Factor | Level | Falling number [s] | Grain vitreousness [%] | Bulk density of grain [kg hl$^{-1}$] | Grain moisture content [%] |
|---|---|---|---|---|---|
| WV | T0 | 62.0 | 1.37 | 64.1 | 11.9 |
| | T1 | 63.1 | 1.62 | 65.1 | 11.7 |
| LSD | | n.s. | n.s. | n.s. | n.s. |
| NF [kg N ha$^{-1}$] | 0 | 62.7 | 1.0 | 63.4 | 11.6 |
| | 60 | 63.0 | 0.0 | 64.8 | 12.1 |
| | 120 | 62.5 | 1.25 | 64.2 | 11.8 |
| | 180 | 62.0 | 3.75 | 65.9 | 11.7 |
| LSD | | n.s. | n.s. | n.s. | n.s. |

WV – water variant; T0 – non-irrigation; T1 – sprinkling irrigation; NF – nitrogen fertilization; n.s. – not significant; *$P < 0.05$ and **P < 0.01.

A trend could also be observed for an increase in values of these parameters in treatments irrigated by sprinkling. In turn, the assessment of the effect of nitrogen fertilisation on these parameters showed a significant effect of nitrogen dose on grain vitreousness. An increase in grain vitreousness was recorded under the influence of increasing nitrogen doses. The greatest value of this parameter was observed following the application of the highest of the applied doses, i.e. 180 kg N $ha^{-1}$. KIRCHEV (2014) showed that increasing of nitrogen doses within the range of 0–180 kg N $ha^{-1}$ contributed to the highest value of this parameter at the highest tested variant.

Analysis of technological quality of flour showed that sprinkling irrigation significantly differentiated only parameters in the sedimentation test, i.e. swelling power and sedimentation of large molecules of gluten proteins and the amount of gluten (Table 4). Irrigation caused a deterioration of flour strength determined based on the sedimentation test and the amount of gluten contained in flour. Presented analyses indicate low contents of gluten proteins, determining dough elasticity, gas retention and as a result of the formation of a spongy crumb structure. A negative effect of irrigation on the value in the sedimentation test and the amount of gluten in wheat was also shown by ERECUL et al. (2012). Those authors recorded the highest values of these parameters in treatments with no sprinkling irrigation applied, while a considerable reduction of their values was observed between water application variants of 0 mm and 40 mm.

Table 4

Technological quality of winter triticale flour cv. Gniewko depending on sprinkling irrigation and nitrogen fertilization

| Factor | Level | Flour yield [%] | Flour colour [% of standard] | Falling number [s] | SDS test [ml] | Amount of gluten in flour (elution manual) [%] | Water absorption of flour [%] |
|---|---|---|---|---|---|---|---|
| WV | T0 | 66.0 | 77.3 | 67.7 | 34.1 | 5.69 | 51.3 |
| | T1 | 66.5 | 77.8 | 76.4 | 28.1 | 2.52 | 51.2 |
| LSD | | n.s. | n.s. | n.s. | 4.05** | 2.99* | n.s. |
| NF [kg N $ha^{-1}$] | 0 | 66.9 | 77.4 | 74.0 | 24.5 | 0.38 | 50.4 |
| | 60 | 66.3 | 78.0 | 74.5 | 27.2 | 0.35 | 50.5 |
| | 120 | 66.3 | 77.6 | 71.7 | 33.0 | 5.04 | 51.3 |
| | 180 | 65.4 | 77.1 | 68.0 | 39.5 | 10.6 | 52.7 |
| LSD | | n.s. | n.s. | n.s. | 5.72** | 4.22** | n.s. |

WV – water variant; T0 – non-irrigation; T1 – sprinkling irrigation; NF – nitrogen fertilization; n.s. – not significant; *$P < 0.05$ and **$P < 0.01$.

In the presented experiment sprinkling irrigation did not significantly differentiate flour yield, flour colour, falling number.

Nitrogen doses significantly modified results of the sedimentation test and wet gluten content. The greatest values of both parameters were recorded for the highest tested application rate of this nutrient, i.e. 180 kg N $ha^{-1}$. ZECEVIC et al. (2010) showed positive effect of increasing nitrogen doses in the cultivation of two winter triticale cultivars, the greatest sedimentation values and washed gluten amounts were recorded at the fertilisation with 120 kg N $ha^{-1}$.

The falling number determined in these tests for flour from triticale cv. Gniewko indicates a high activity of α-amylase, which has a negative effect on the enzyme-protein complex of flour. High amylolytic activity of triticale flour was reported by CEGLIŃSKA et al. (2005).

The yield of flour from triticale grain was 65.4–66.9%, while both sprinkling irrigation and increasing nitrogen doses had no significant effect on this parameter.

Water absorption of flour is an important parameter characterising material for baking of bread and other cereal products. CEGLIŃSKA et al. (2005) reported that an increase in nitrogen doses caused decrease of triticale flour yield.

Table 5

Technology assessment of dough and bread obtained from grain triticale cv. Gniewko depending on sprinkling irrigation and nitrogen fertilisation

| Factor | Level | Farinograph test | | | | Properties of bread | |
|---|---|---|---|---|---|---|---|
| | | dough development [min] | dough stability [min] | dough softening [BU] | Brabender quality [number] | bread yield [%] | bread volume for 100 g of bread [$cm^3$] |
| WV | T0 | 0.99 | 0.94 | 221 | 16.7 | 139 | 442 |
| | T1 | 0.91 | 0.72 | 234 | 13.2 | 137 | 452 |
| LSD | | n.s. | n.s. | n.s. | n.s. | n.s. | n.s. |
| NF [kg N $ha^{-1}$] | 0 | 0.85 | 0.42 | 228 | 10.2 | 135 | 438 |
| | 60 | 0.80 | 0.50 | 214 | 10.0 | 137 | 460 |
| | 120 | 0.97 | 0.77 | 231 | 15.0 | 139 | 450 |
| | 180 | 1.17 | 1.62 | 237 | 24.7 | 140 | 442 |
| LSD | | n.s. | 0.71* | n.s. | 9.95* | 2.93** | 21.4* |

WV – water variant; T0 – non-irrigation; T1 – sprinkling irrigation; NF – nitrogen fertilization;; n.s. – not significant; *$P < 0.05$ and **$P < 0.01$.

In the presented experiment sprinkling irrigation did not significantly differentiate rheological properties of dough (Table 5). Still certain trends may be observed towards a reduction of such parameters as dough development, dough stability and quality number, and an improvement of dough softening. Similar changes in the technological value of dough produced from wheat grain grown using irrigation were shown by SELEIMAN et al. (2011). In turn, nitrogen fertilisation significantly modified such parameters as dough stability and Brabender quality numbers. Increasing nitrogen doses caused a significant increase in values of both parameters. The greatest dough stability and quality number were recorded for the highest analysed nitrogen dose (180 kg $ha^{-1}$), which is also confirmed by an earlier study by CEGLIŃSKA et al. (2005). The recorded values were low for both traits and formed triticale dough produced from grain of cv. Gniewko both after kneading, fermentation and re-kneading was stiff and non-elastic, which shows that it exhibits poor properties and thus limited applicability in baking.

The baking test showed faults of the produced bread, which was of inadequate quality and very limited potential use for human consumption. An excessive amylolytic activity (low falling number) in grain and flour caused obtaining dough with poor farinographic characteristics, as a result of which the produced bread had extent lacked crumb and low yield and bread volume. Sprinkling irrigation did not significantly differentiate yield and volume of bread. Values of both these parameters were determined by nitrogen fertilisation. Bread yield was 135–140% and it increased with an increase in nitrogen application rates, while significant differences were recorded between the treatment with no nitrogen fertilisation and two greatest doses of this nutrient, which was confirmed in a study by BIELSKI et al. (2015). In turn, the greatest bread volume was found from the treatment fertilised with 60 kg N $ha^{-1}$. In the opinion of ZECEVIC et al. (2010), due to the low quality of triticale, a potential improvement in properties of baked bread could be provided by an addition of wheat flour.

## Conclusions

Collected observations showed that flour of winter triticale cv. Gniewko, irrespective of the water and nitrogen fertilisation variants, exhibits low applicability for the baking industry. This results from the fact that despite improvement in certain parameters of grain, flour and dough under the influence of sprinkling irrigation and nitrogen fertilisation, faults were found in the produced bread and it did not meet require-

ments for bread for human consumption. The low falling number in the analyzed grain, low gluten content and falling number in the flour contributed to the formation of dough with poor farinographic characteristics, followed by low yield and bread volume.

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# COLD STORAGE, FREEZING AND LYOPHILISATION AND ITS EFFECT ON TRANSFORMATIONS OF PHENOLIC COMPOUNDS IN LINGONBERRY (*VACCINIUM VITIS-IDAEA* L.)

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Key words: lingonberry, phenolic compounds, phenolic acids, anthocyanidins, low temperature, antioxidative activity.

Abstract

The aim of the study was to determine the extent of changes in the content of bioactive substances in lingonberry fruit following cold storage, freezing and lyophilisation.

In the paper therapeutic properties of lingonberry fruits were presented, and all present in the fruits phenolic compounds were discussed, especially phenolic acids and anthocyanins. The effect of selected technological operations (freezing and lyophilisation)on the content of phenolic compounds in the fruit was described. The antioxidative activity of phenolic compounds present in fruits and juice was also analysed.

The results of the study indicate that lyophilisation had a negative impact on the level of all phenolic compounds in lingonberry fruits. During this operation the total content of phenolic compounds decreased for about 37%.At the same time phenolic acids concentration was decreased by 20%. The greatest change was observed in anthocyanins concentration, in lyophilised fruits anthocyanins concentration decreased by as much for about 97%. Such a significant degradation of polyphenolic compounds caused a substantial (36%) decrease in antioxidant activity.

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# PRZECHOWYWANIE CHŁODNICZE, ZAMRAŻANIE I LIOFILIZACJA ORAZ ICH WPŁYW NA PRZEMIANY ZWIĄZKÓW FENOLOWYCH W BORÓWCE BRUSZNICY (*VACCINIUM VITIS-IDAEA* L.)

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Słowa kluczowe: borówka brusznica, związki fenolowe, kwasy fenolowe, antocyjany, niskie temperatury, aktywność antyoksydacyjna.

Abstrakt

Celem pracy było określenie wielkości zmian w zawartości substancji biologicznie aktywnych w owocach borówki brusznicy poddanych przechowywaniu chłodniczemu, zamrażaniu i liofilizacji.

W pracy przedstawiono właściwości terapeutyczne borówki brusznicy oraz omówiono występujące w niej grupy związków fenolowych, szczególnie kwasy fenolowe i antocyjany. Opisano wpływ wybranych operacji technologicznych (zamrażania i liofilizacji) na zawartość związków fenolowych w owocach. Analizowano również aktywność antyoksydacyjną związków fenolowych obecnych w soku z owoców.

Wyniki badań wskazują, że liofilizacja miała negatywny wpływ na poziom wszystkich związków fenolowych w owocach borówki brusznicy. Podczas tego procesu łączna zawartość związków fenolowych zmniejszyła się o ok. 37%. Jednocześnie zaobserwowano 20% ubytek kwasów fenolowych. Największą zmianę zaobserwowano podczas analizy zmian zawartości antocyjanów, w liofilizowanych owocach zmalała ona aż o ok. 97%. Tak znacząca degradacja związków polifenolowych spowodowała znaczny (ok. 36%) spadek aktywności antyoksydacyjnej.

## Introduction

Lingonberry *(Vaccinium vitis-idaea)* is a shrub which grows naturally in heathers and pine forests in Northern Europe, Asia and North America. Its fruit is picked and processed to obtain products with therapeutic properties. In recent years, the first cultivars have been grown in orchards in The Netherlands which are fit for cultivation as a commodity. Lingonberry fruit are picked in their natural habitat and, despite lively interest in the fruit from the food processing industry, no commodity plantations of the species exist in Poland. Lingonberry provides two kinds of raw materials: leaves and fruit, which are used in herbal medicine, pharmaceutical

and food industries. A number of studies have been carried out on phenolic compounds of lingonberry, especially phenolic acids, anthocyanins and flavonol glycosides. EK et al. (2006), HOKKANEN et al. (2009) found that leaves of lingonberry contain phenolic glycosides: arbutin, methylarbutin, pyranosides, flavonoids, mainly hyperoside), catechin tannins, minerals and organic acids. HOKKANEN et al. (2009) identified 51 different phenolic compounds in lingonberry, including: flavan-3-ols, proanthocyanidins, flavonols and their glycosides and conjugates of various phenolic acids. EK et. at. (2006) identified a total of 28 phenolic compounds, including flavonols, anthocyanidins, catechins and their glycosides, as well as acetylated radicals of caffeic acid and various caffeoyl and ferulic acid conjugates. The authors identified such conjugates as: coumaroyl-hexose-hydroxyphenol, caffeoyl-hexose-hydroxyphenol, coumaroyl-hexose-hydroxyphenol, quercetin-3-O-R arabinofuranoside, kaempferol-pentoside, and kaempferol-deoxyhexoside in the plant, and the flavonolacylglycosides quercetin-3-O-[4″-3-hydroxy-3-methylglutaroyl)]-R-rhamnose and kaempferol-3-O-[4″-3-hydroxy-3-methylglutaroyl)]-R-rhamnose.

IERI et al. (2013) reports that among the identified derivatives of cinnamic acid, chlorogenic acid was identified, which was the main phenolic acid present in fruit, whereas caffeoyl arbutin was the main compound in fruit and leaves of lingonberry. In terms of quantitative composition, hydroxycynnamic acids were the main components and they accounted for 52–84% of the total phenols.

Justification and aim of the study: in recent years, the possibility of using forest fruit in regional food products has been attracting increasing attention. Of the numerous technological options available, those which are the easiest to implement in small processing facilities are selected, such as cold storage, freezing or lyophilisation of fruit. The aim of the study was to determine the extent of changes in the content of bioactive substances in lingonberry fruit following cold storage, freezing and lyophilisation. Changes in the total content of phenolic compounds, anthocyanins and phenolic acids were analysed.

## Material and Methods

Lingonberry fruit picked in forests in the north of Poland (near Łeba) were used as the study material. A five kilos batch of fruit was divided into three parts. One part was stored under refrigerated conditions at 6–8°C, one was frozen and stored at -18°C, and one was lyophilised. All the three fruit batches were vacuum-packed in plastic packaging. Lyophilised fruit

were stored at room temperature away from light. The samples were analysed after six months of storage. In all samples dry matter was measured gravimetrically.

**Dry matter content.** The fruits were determined: dry matter content by weight of *Przetwory owocowe…* PN-A-90 75101 03.

**Total phenolic compounds content.** Total content of phenolic compounds was determined colorimetrically with the use of Folin-Ciocalteu reagent according to the method described by AOAC, (1974) and SHAHIDI AND NACZK (1995). Phenolic compounds were extracted three times with 80% methanol. The collected supernatants were evaporated to dryness. The colour reaction of phenolic compounds was induced by Folin-Ciocalteu reagent solution (Sigma-Aldrich, St. Louis, MO, United States). Absorbance at a wavelength of 765 nm after 60 minutes was measured with the use a UNICAM UV/Vis UV2 spectrophotometer (ATI Unicam, Cambridge, UK). The content of phenolic compounds was expressed as gallic equivalent.

**Anthocyanins content.** Total anthocyanins in samples were analysed according to colorimetric method described by GIUSTI and WROLSTAD (2001), Quantitative analysis was weightd on external calibration curve prepared with the use of cyanidyn-3-glucoside.

**Antioxidant activity.** The DPPH Radical Scavenging Assay (DPPH test) was determined according to MOURE et al. (2001). The DPPH radical scavenging activity was calculated and the antioxidant capacity of samples was expressed as μmol Trolox equivalent per 1 mg of sample.

**Phenolic acids content.** The phenolic acid assay was conducted by the method described by ZADERNOWSKI (1987).The qualitative and quantitative composition of phenolic acids in individual fractions was analysed by high-performance liquid chromatography (HPLC). The compounds under analysis were separated on a Synergi-Fusion column (150 mm x 2 mm; 4 μm) (Phenomenex) with an HPLC 1200 unit manufactured by Agilent Technologies. The chromatogram was developed in a gradient flow of the mobile phase, which consisted of acetonitrile with 0.15% formic acid and water, also acidified with 0.15% formic acid. The mobile phase flow rate was 0.2 $cm^3$ $min^{-1}$. The detection was effected with a photodiode detector (PDA Agilent Technologies) for the wavelength of 260 and 320 nm. Individual compounds were identified by comparing the retention time and an UV-Vis spectrum with the reference standards of the compounds. The quantitative analysis was carried out with the calibration curves for external reference standards of the phenolic acids under analysis.

**Statistical analysis.** The results of all analysis performed in triplicate experiments were statistically analysed using Statistica 12.0 PL software

(StatSoft Inc., Kraków, Poland). In order to indicate the significance of differences between samples, unvaried analysis of variance (ANOVA) with a Duncan test at $p \leq 0.05$ significance level was used.

## Results and Discussion

After the lingonberry fruit was dried at the temperature of 110°C it was shown that water and components of the fruit volatile in steam, i.e. volatile organic acids and flavour compounds accounted for 96% of the fruit weight. Dehydrated components of the pulp, fruit skin and seeds (small pits), defined as dried matter, accounted for the remaining part, i.e. 4%.

Table 1

The dry matter and the extract content in fruit lingonberry

| Fruits | Dry weight [%] | Extract content [%] |
|---|---|---|
| Stored inrefrigeration | 13.96 ± 0.11 | 12.55± 0.13 |
| Frozen | 14.32 ± 0.19 | 13.00± 0.10 |
| Lyophilized | 81.08 ± 1.09 | – |

Lyophilising drying resulted in 5.7-fold concentration of components of the dry matter. Lyophilisation of lingonberry fruit yielded the product containing 81.08% of dry matter (Table 1). ZADERNOWSKI and OSZMIAŃSKI (1994) report that carbohydrates at 5–12% account for the major part of lingonberry fruit dry matter. An extract which determines the percentage of water-soluble components, other than volatile in steam, is another important determinant which characterises the technological value of fruit. The extract value is determined mainly by sugars soluble in cell juice. The extract accounted for 12.55 % of the cold-stored fruit weight, and for 13.00% of the frozen fruit. The slight increase in the percentage of extract in frozen fruit may be caused by inversion of sugars during storage in a freezer.

ZADERNOWSKI and OSZMIAŃSKI (1994), CIOŁKOWSKA-PALUCH (2000), HO et al. (2001), PLISZKA (2003) report that dry matter also contains, apart from carbohydrates, pectins, organic acids (mainly benzoic acid), ascorbic acid, carotenoids and polyphenols: flavans, procyanidines, cinnamic acid, trans-resveratrol and *p*-coumaric acid. Lingonberry fruit contains 11 to 20 mg of vitamin C in 100 g of dry matter. Carotenoids are present mainly as: lutein at 0.36 mg·100 $g^{-1}$ of dry matter, and ß-carotene at 0.2 mg·100 $g^{-1}$ of dry matter. These components are the main source of the therapeutic properties of lingonberry fruit.

There are the two main groups of phenolic compounds: flavonoids (which include anthocyanins) and non-flavonoid compounds, whose main groups include phenolic acids, derivatives of cinnamic and benzoic acids.

The term "total phenolic compounds", which is used in the literature, refers to polyphenolic species isolated from plant material by extraction with methanol or acetone. Most of these compounds react with the Folin--Ciocalteau reagent and they are determined colorimetrically.

The results of the study are given on a dry and fresh weight basis. This makes it easier to interpret the results of this experiment and other results. It was found that the content of phenolic compounds and their antioxidant activity were different and depended on whether the lingonberry fruit was stored under refrigerated conditions, frozen or lyophilised. The total phenolic compounds expressed as gallic acid on a dry basis was 3844.77 mg·100 $g^{-1}$ and 536.73 mg·100 $g^{-1}$ on a fresh weight basis in refrigerated fruit, whereas it was slightly higher in frozen 5283,10 mg·100 $g^{-1}$ on the basis of dry matter weight of frozen fruit and 756.54 mg·100 $g^{-1}$ on fresh matter basis. These findings are consistent with the content of phenolic compounds in fresh fruit, published by KÄHKÖNEN et al. (2001), and SZAJDEK and BOROWSKA (2008). The process of sublimation resulted in a decrease in the total phenolic compounds in lyophilised lingonberry by approx. 37% compared to the cold-stored and 54% to the frozen fruit. The total phenolic compounds content in lyophilised fruit was 2428.81 mg·100 $g^{-1}$ on a dry weight basis (Table 2). This resulted from the fact that lyophilisation degrades phenolic compounds to colourless species, which do not give coloured products in reactions with the Folin-Ciocalteau reagent.

The content of anthocyanins in cold-stored fruit was 489.61 mg·100 $g^{-1}$ on a dry weight basis and it was slightly higher in frozen fruit – 549.37 mg·100 $g^{-1}$ on a dry weight basis. Converted to the fresh weight basis, the content of anthocyanins was: 68.35 mg·100 $g^{-1}$ in cold-stored fruit and 78.67 mg·100 $g^{-1}$. (Tab. 2.). LEE and FINN (2012), VOLLMANNOVA et al. (2009), KÄHKÖNEN et al. (2001) report that the content of anthocyanins in lingonberry fruit ranges from 17 to 50 mg·100 $g^{-1}$ on afresh weight basis. The slightly higher content of phenolic compounds and anthocyanins in the frozen fruit was caused by a higher content of dry matter. Anthocyanins accounted 12,73% of the total phenolic compounds in the cold-stored fruit, for 10.40% in the frozen fruit and for only 0.62% in the lyophilised fruit.

The process of sublimation was found to have a destructive effect on the total phenolic compounds, especially on anthocyanins. The anthocyanin contents in lyophilised fruit was 10.37 mg·100 $g^{-1}$ on a dry weight basis (Table 2). Compared to the anthocyanin content in the cold-stored

fruit, the process of sublimation reduced the anthocyanin content in the lyophilised fruit by up to 97%. A visual observation showed that the red colour of the lyophilised lingonberry fruit was much brighter than cold-stored and frozen fruit.

Table 2

The total amount of phenolic compounds, anthocyanins and phenolic acids in fruit lingonberry stored inrefrigeration, frozen and lyophilized

| Fruits | Total phenolic compounds | | Anthocyanins | | Phenolic acids | |
|---|---|---|---|---|---|---|
| | mg·100 g $^{-1}$ fresh weight | mg·100 g$^{-1}$ dry weight | mg·100 g $^{-1}$ fresh weight | mg·100 g$^{-1}$ dry weight | mg·100 g$^{-1}$ fresh weight | mg·100 g $^{-1}$ dry weight |
| Stored inrefrigerator | 536.73 ±32.20 | 3844.77$^{a}$ ±32.13 | 68.35 ±8.35 | 489.61$^{a}$ ±28.12 | 108.35 ±8.12 | 756.68$^{a}$ ±20.00 |
| Frozen | 756.54 ±52.60 | 5283.10$^{b}$ ±88.00 | 78.67 ±9.60 | 549.37$^{a}$ ±37.02 | 109.22 ±6.19 | 760.00$^{a}$ ±18.45 |
| Lyophilized | 1969.33 ±121.43 | 2428.81$^{c}$ ±149.76 | 10.37 ±1.94 | 15.12$^{b}$ ±2.15 | 492.62 ±10.13 | 607.54$^{b}$ ±25.00 |

*a*, *b*, *c* – mean values indicated by the same letter do not differ significantly ($p \leq 0.05$)

The destructive effect of lyophilisation on phenolic compounds, especially on anthocyanins, was confirmed by MAZUR and BOROWSKA (2007), who examined the effect of sublimation drying on transformations of phenolic compounds in cranberry.

The lyophilisation process resulted in a 50% reduction in the polyphenol content and a 7-fold reduction in the anthocyanins content compared to fresh cranberry fruit. MAZUR and BOROWSKA (2007) claim that adverse transformations of anthocyanins during the process of berry fruit processing are affected by oxidation and formation of brown-red polymerised compounds.

Phenolic acids and their derivatives were another group of phenolic compounds in lingonberry fruit. Free phenolic acids are present in small amounts in plants and their presence usually results from the fact that they have not been transformed into bound forms during physiological processes which take place during fruit ripening. Phenolic acids were usually found to occur mainly in a bound form as components of low- or high--molecular weight polyphenolic compounds. For example, free phenolic acids and various forms of depsides are low-molecular polyphenols. High--molecular polyphenols include lignins, procyanidins, hydrolysing tannins, with which phenolic acids form conjugates bound by ester or glycosidic bonds (SHAHIDI and NACZK 1995).

GAWLIK-DZIKI (2004) reports that some derivatives of cinnamic acid are commonly present as free forms or as depsides, and they also form esters with carboxylic acids or glucose, while derivatives of benzoic acid usually occur as glycosides. Most depsides occur in bound forms, for example, they are parts of hydrolysing tannins and they form complexes with proteins and polysaccharides. Apart from the structures described above, phenolic acids bind with lipids, sterols, polysaccharides, peptides.

The total content of phenolic acids in cold-stored and frozen lingonberry fruit was, respectively: 756.68 ± 20 mg·100 $g^{-1}$ on the d.m. (108.35 ± 8.12 mg·100 $g^{-1}$ on the fresh weight) and 760.00 ± 18.45 mg·100 $g^{-1}$ on the d.m.(109.22 ± 6.19 mg·100 $g^{-1}$ on the fresh weight) and the mean content was 758.34mg·100 $g^{-1}$ on the dry weight (108.35mg·100 $g^{-1}$ on the fresh weight (Table 2). This comprised free phenolic acids (15%) and phenolic acids bound by glycosidic bonds with other components (67%) and phenolic acids bound by ester bonds (18%). Total phenolic acids accounted for about 20% of the total phenolic compounds (Figure 1). The process of sublimation reduced the content of phenolic acids in the lyophilised fruit to 607.54 ± 25 mg·100 $g^{-1}$ on the dry weight or 492.62 ± 10.13 mg·100 $g^{-1}$ on the fresh weight. The total phenolic acids accounted for approx. 25% of total phenolic compounds present in lyophilised fruit (Table 2, Figure 1.). Lyophilisation caused destruction of ester and glycosidic bonds and, as a result, the percentage of free phenolic acids increased to 34% while the content of phenolic acids bound by ester bonds decreased to 52% and those bound by glycosidic bonds – to 14% (Figure 1).

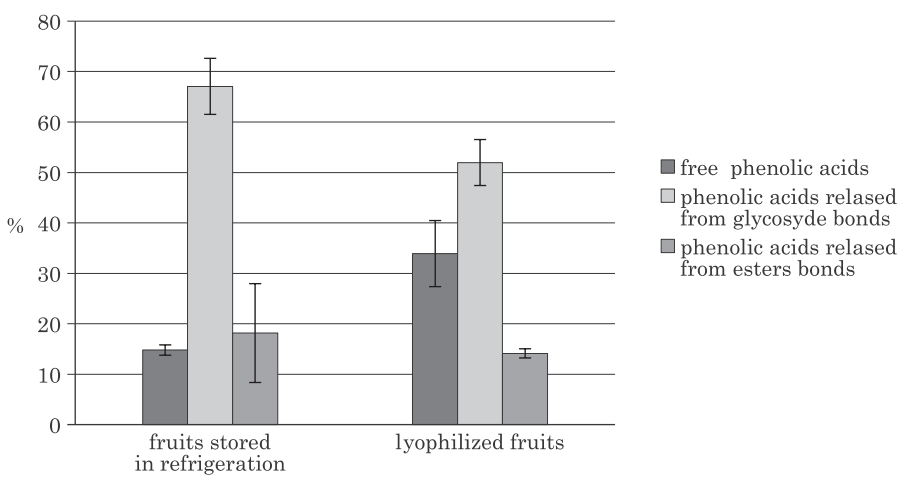


Fig. 1 The percentage of free phenolic acids and released from the ester and glycosidec bonds

Table 3

The amount of free phenolic acids and released from the ester and glycosidic bounds, founded in fruits

| Phenolic acids | Free phenolic acids | | Phenolic acids released from the bounds | | | | Total phenolic acids | |
|---|---|---|---|---|---|---|---|---|
| | | | esters | | glicosides | | | |
| | mg·100 g $^{-1}$ fresh weight | mg·100 g$^{-1}$ dry weight | mg·100 g $^{-1}$ fresh weight | mg·100 g$^{-1}$ dry weight | mg·100 g$^{-1}$ fresh weight | mg·100 g $^{-1}$ dry weight | mg·100 g$^{-1}$ fresh weight | mg·100 g$^{-1}$ dry weight |
| Protocatechuic | 1.08 ± 0.23 | 7.57 ± 1.59 | 30.75 ± 5.12 | 214.71 ± 35.75 | 12.01 ± 4.84 | 83.85 ± 33.82 | 43.84 | 306.13 |
| *p*-OH-Benzoic | 1.69 ± 0.01 | 11.79 ± 0.10 | 3.89 ± 0.07 | 27.18 ± 0.48 | 3.69 ± 0.19 | 25.80 ± 1.31 | 9.27 | 64.77 |
| Vanillic | 3.33 ± 0.12 | 23.23 ± 0.83 | 1.08 ± 0.00 | 7.52 ± 0.01 | 2.54 ± 0.09 | 17.73 ± 0.65 | 6.95 | 48.48 |
| Caffeic | 0.00 ± 0.00 | 0.00 ± 0.00 | 1.47 ± 0.52 | 10.25 ± 4.61 | 0.54 ± 0.27 | 3.80 ± 1.38 | 2.01 | 14.05 |
| *p*-Coumaric | 4.56 ± 0.08 | 31.86 ± 0.58 | 19.98 ± 0.91 | 139.50 ± 6.37 | 0.57 ± 0.09 | 4.01 ± 0.66 | 25.11 | 175.37 |
| Ferulic | 5.35 ± 0.54 | 37.39 ± 3.74 | 15.51 ± 0.13 | 108.34 ± 0.89 | 0.31 ± 0.05 | 2.15 ± 0.37 | 21.17 | 147.88 |
| Total | 16.01 | 111.84 | 72.68 | 507.50 | 19.66 | 137.34 | 108.35 ± 8.12 | 756.68 ± 20.00 |

Table 4

The amount of free phenolic acids and released from the ester and glycosidic bounds, contained in the lyophilized fruits

| Phenolic acids | Free phenolic acids | | Phenolic acids released from the bounds | | | | Total phenolic acids | |
|---|---|---|---|---|---|---|---|---|
| | | | esters | | glicosides | | | |
| | mg·100 g $^{-1}$ fresh weight | mg·100 g$^{-1}$ dry weight | mg·100 g $^{-1}$ fresh weight | mg·100 g$^{-1}$ dry weight | mg·100 g$^{-1}$ fresh weight | mg·100 g $^{-1}$ dry weight | mg·100 g$^{-1}$ fresh weight | mg·100 g$^{-1}$ dry weight |
| Protocatechuic | 29.63 ± 4.36 | 36.54 ± 5.38 | 97.21 ± 30.48 | 119.89 ± 37.60 | 34.22 ± 1.48 | 42.20 ± 1.82 | 161.06 | 198.64 |
| *p*-OH-Benzoic | 18.41 ± 0.60 | 22.71 ± 0.74 | 14.01 ± 2.42 | 17.28 ± 2.99 | 13.34 ± 0.92 | 16.45 ± 1.13 | 45.76 | 56.44 |
| Vanillic | 15.88 ± 0.42 | 19.59 ± 0.52 | 4.53 ± 0.60 | 5.59 ± 0.74 | 17.95 ± 0.56 | 22.14 ± 0.70 | 38.36 | 47.28 |
| Caffeic | 0.00 ± 0.00 | 0.00 ± 0.00 | 5.74 ± 1.25 | 7.08 ± 1.54 | 0.00 ± 0.00 | 0.00 ± 0.00 | 5.74 | 7.08 |
| *p*-Coumaric | 63.63 ± 2.99 | 78.47 ± 3.69 | 81.00 ± 17.71 | 99.90 ± 21.84 | 2.29 ± 0.20 | 2.83 ± 0.24 | 146.92 | 181.2 |
| Ferulic | 39.51 ± 6.08 | 48.72 ± 7.50 | 53.50 ± 17.57 | 65.98 ± 21.67 | 1.76 ± 0.64 | 2.17 ± 0.79 | 94.77 | 116.87 |
| Total | 167.06 | 206.03 | 255.99 | 315.72 | 69.56 | 85.79 | 492.61 ± 10.13 | 607.54 ± 25.00 |

Protocatechuic acid is the dominant phenolic acid in lingonberry fruit with the content of 306.13 mg·100 $g^{-1}$ d.m. (40.5% of all the acids). *p*-Coumaric acid was present in the amount of 175.37 mg·100 $g^{-1}$ d.m. (23.2%), and ferulic acid – 147.88 mg·100 $g^{-1}$ d.m. (19.5%) – Table 3. The ratio of these acids changed after lyophilisation: the content of protocatechuic acid decreased to 198.64 mg·100 $g^{-1}$ d.m. (32.7%) and that of ferulic acid to 116.87 mg·100 $g^{-1}$ d.m. (Table 3, Table 4, Figure 2).

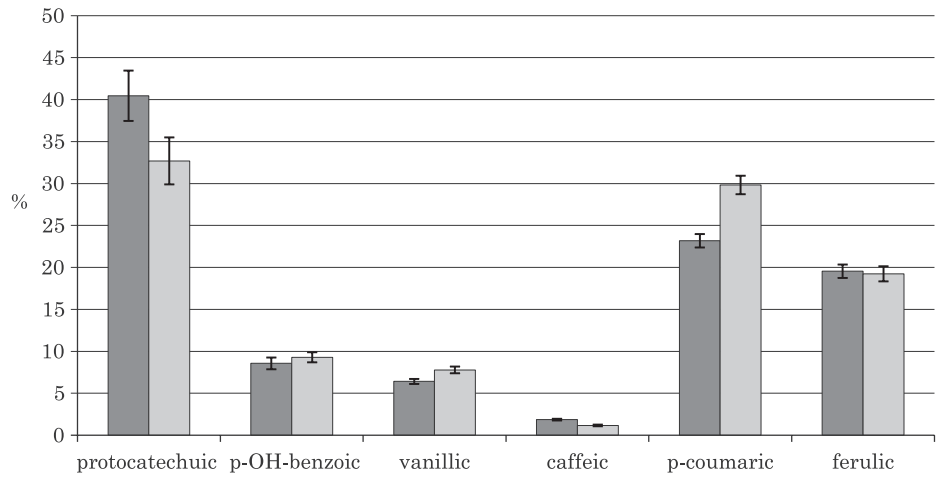


Fig. 2. The percentage of phenolic acids in fruit lingonberry cold storage and freeze-dried

The content of *p*-coumaric acid increased to 181.2 mg·100 $g^{-1}$ d.m. (29.8%). The content of caffeic acid was the lowest: 14.05 mg·100 $g^{-1}$ d.m. (1.9%) in cold-stored fruit and 7.08 mg·100 $g^{-1}$ d.m. in lyophilised fruit (1.2%) (Table 3, Table 4, Figure 2).

The anti-oxidative activity of phenolic compounds in cold-stored and frozen lingonberry fruit was similar: 5.36 µM $DPPH^{\cdot}$ scavenged by 1 mg of phenolic compounds for cold-stored fruit and 5.31 µM $DPPH^{\cdot}$ scavenged by 1 mg of phenolic compounds in frozen fruit. The figure was lower by approx. 36% for the lyophilised fruit: 3.43 µM $DPPH^{\cdot}$ scavenged by 1 mg of phenolic compounds (Table 5).

Table 5

The antioxidant activity of phenolic compounds present in the studied fruits lingonberry

| Specification | Fruits stored in refrigerator | Frozen fruits | Lyophilized fruits |
|---|---|---|---|
| µM $DPPH^{\cdot}$ / 1 mg phenolic | 5.31±0,11 | 5.38±0,06 | 3.43±0,20 |

## Conclusions

An analysis of the findings showed that lingonberry fruit, like other berry fruit, is a rich source of phenolic compounds. Anthocyanins were found to account for approx. 21% and phenolic acids for 20% of the phenolic compounds in fresh lingonberry fruit. The greatest portion of phenolic acids present in fresh lingonberry fruit was bound by ester bonds and the smallest portion occurred as free phenolic acids (15%). Protocatechuic acid was the dominant phenolic acid (40.5% of all the acids) present in lingonberry fruit. Lyophilisation reduced the content of all the phenolic compounds under analysis in lingonberry fruit and especially reduced the content of anthocyanins. The content of phenolic compounds was found to decrease by 37%, those of phenolic acids – by 20% and anthocyanins – by 97%. Lyophilisation cleft ester and glycosidic bonds in lingonberry, thereby increasing the content of free phenolic acids. Moreover, the study found that lyophilisation decreased the antioxidative activity of the phenolic compounds present in lingonberry fruit by 36%.

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# THE FIRST DATA ON THE GENETIC DIVERSITY OF RIVER LAMPREY *LAMPETRA FLUVIATILIS* (LINNAEUS, 1758) FROM THE VISTULA RIVER AND VISTULA LAGOON IN POLAND*

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Key words: conservation, environment, genetic diversity, microsatellite DNA, population.

Abstract

The genetic characteristic of seriously threatened river lamprey *Lampetra fluviatilis* L. from Vistula river and Vistula lagoon (Poland) was described in the present paper. The study, based on the nine microsatellite markers, was conducted to determine the genetic diversity and population structure of river lamprey in Vistula river and Vistula lagoon in Poland.

Observed heterozygosity ranged from 0.158 to 0.974 in lamprey from Vistula river, and 0.040 to 0.990 in lamprey from Vistula lagoon. The expected heterozygosity in river lamprey ranged 0.177–0.673 in specimens and 0.213–0.670 in population from Vistula lagoon. Eight loci appear to be diagnostic, due to occurrence of private alleles, for distinguishing the Vistula river and Vistula lagoon populations. The estimated effective population size (*Ne*) for the studied populations of river lamprey form Vistula river and Vistula lagoon equalled 72.4 (95% CI = 14.9–∞) and 25.8 (95% CI = 8.7–151.1), respectively. Constructed individual's tree based on DAS genetic distances and the Principal Coordinates Analysis (PCoA) exhibited three main genetic groupings within studied fish group.

The presented genetic characteristic of studied lamprey populations is important and necessary to develop and implement conservation actions of river lamprey in Poland.

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# ZMIENNOŚĆ GENETYCZNA MINOGA RZECZNEGO Z RZEKI WISŁY I ZALEWU WIŚLANEGO – BADANIA WSTĘPNE

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Słowa kluczowe: minóg rzeczny, Wisła, Zalew Wiślany, zmienność genetyczna.

Abstrakt

Prezentowane badania dotyczą analizy genetycznej minoga rzecznego *(Lampetra fluviatilis)* z dwóch stanowisk w zlewni Wisły (z Wisły – okolice Czatkowy, woj. pomorskie oraz Zalewu Wiślanego). Analizę genetyczną prowadzono z zastosowaniem dziewięciu par starterów mikrosatelitarnego DNA.

Obserwowana heterozygotyczność (*Ho*) w badanych loci u minoga rzecznego z Wisły i Zalewu Wiślanego przyjmowała odpowiednio wartości 0,158–0,974 i 0,040–0,900. Oczekiwana heterozygotyczność (*He*) u minoga rzecznego z Wisły i Zalewu Wiślanego wynosiła odpowiednio 0,177–0,673 oraz 0,213–0,670. Osiem loci mikrosatelitarnego DNA określono jako diagnostyczne z uwagi na fakt występowania w nich alleli prywatnych. Efektywna liczebność populacji (*Ne*) minoga rzecznego z Wisły i Zalewu Wiślanego przyjmowała wartości odpowiednio 72,4 (95% CI = 14,9–∞) oraz 25,8 (95% CI = 8,7–151,1). Drzewo pokrewieństw filogenetycznych, skonstruowane na podstawie genetycznego dystansu DAS oraz analizy PCoA, wykazywało trzy podgrupy.

Prezentowane wyniki badań stanowią wstępne informacje na temat zmienności genetycznej minoga rzecznego zasiedlającego Wisłę i Zalew Wiślany, które mogą być wykorzystane w ochronie tego gatunku. Uzyskane wyniki zostaną wykorzystane w trakcie opracowywania metod przechowywania i kriokonserwacji nasienia minoga rzecznego w celu utworzenia banku nasienia.

## Introduction

The European river lamprey *Lampetra fluviatilis* L. is a threatened species of anadromous ancient jawless vertebrates (agnathans), formerly widespread throughout western Europe (MAITLAND 1980, HARDISTY 1986). In Poland, the European river lampreys spend the adult phase of their life cycle in Baltic sea, where they feed parasitically on a wide variety of bony fish. Adults return to rivers for reproduction, where they become sexually mature, spawn and die. During last few decades this species have undergone a significant decline throughout Europe because of rivers and estuaries pollution, overexploitation, loss of spawning and larval habitat and by physical barriers to migration (LELEK 1987, OJUTKANGAS et al. 1995, NUNN et al. 2008, MAITLAND 2003). The drastic decline of the most river

lamprey populations initiated the development of conservation programs in Europe through the Bern Convention and the European Habitats Directive 92/43/EEC, indicating the river lamprey as a species requiring the designation of Special Areas of Conservation (SACs) (COUNCIL OF THE EUROPEAN COMMUNITIES 1992, MATEUS et al. 2012). Additionally, this species was listed in the IUCN Red List of Threatened Species 2013 (FREYHOF 2013). In Poland river lamprey was included in the Polish Red Data Book of Animals as an endangered species (WIESER 1992) and the populations of this species are very rare. In Poland, the river lamprey inhabit only a few rivers, for example the lower part of the Vistula river, and are under strict protection The adults of river lamprey were identified in ten Polish rivers (Wda, Drwęca, Wierzyca, Pasłęka, Łupawa, Radew, Wiepsza, Parsęta, lower parts of Oder and Vistula), Vistula and Szczecinski Lagoons (WITKOWSKI 2010). Accordingly, the obtaining of research samples is very difficult and requires many permits both national and local.

During the river lamprey conservation the year-round protection of this species, including a fishing ban, was enacted in Poland to counteract the steady decline of Polish river lamprey populations. This species is seriously threatened and effective protection together with supportive reproduction programs, should be designed and implemented in the most natural river lamprey populations. The existence of endangered populations is often dependent on supportive breeding (with use the innovative biotechnologies in aquaculture condition), rearing of juveniles under controlled conditions and releasing them into the natural environment. Furthermore, during the implementation of restoration plan of seriously endangered species/population the genetic screening, based on molecular analyses should be included as the key point in successful realization of conservation activity (FOPP-BAYAT 2008, FOPP-BAYAT and CIERESZKO 2012). Moreover the conservation of endangered river lamprey populations should involves the species or hybrid identification together with monitoring of important population genetic indices (e.g. gene diversity indicators, phylogeny, inbreeding coefficient, bottleneck effect). To date there is no information about genetic data of river lamprey from Vistula river estuarium in Poland. Therefore the main objective of the present study was the application the molecular analysis, based on microsatellite DNA markers, in highly endangered river lamprey from Vistula river and Vistula lagoon in Poland.

## Material and Methods

This study was carried out in strict accordance with the recommendations in the Polish ACT of 21 January 2005 of Animal Experiments, Dz.U. z 2005 r. nr 33, poz. 289. The protocol was approved by the Local Ethical Committee for the Experiments on Animals in of the University of Warmia and Mazury in Olsztyn, Poland (Permit Number: 78/2012). The river lamprey were captured in fall (October) and spring (April) during spawning migration in the Vistula river near Czatkowa, Poland: N 54°8'4.18"; E 18°49'43.67" (49 specimens), and Vistula Lagoon, Poland: N 54°25'12.67"; E 19°44'8.99" (38 specimens) with the use of tunnel nets.

The fin clips were sampled and stored in 96% ethanol. Then the genomic DNA was extracted during the Chelex 100 procedure (WALSH et al. 1991). The nine microsatellite loci (*Lri-1*, *Lri-2*, *Lri-3*, *Lri-4*, *Lri-5*, *Lri-7*, *Lri-8*, *Lri-9* and *Lri-10*) were amplified during Polymerase Chain Reaction PCR (LUZIER et al. 2010). The PCR amplification was performed in 25-μL reaction volumes with approximately 40 ng genomic DNA, 1x PCR reaction Buffer (50 mM KCL, pH 8.5; Triton X-100), 3.3 mM $MgCl_2$, 0.25 mM of each deoxynucleotide triphosphate (dNTP), 0.4 μM of each primer, and 0.6 U Go *Taq* Flexi Polymerase (Promega, Madison, WI, USA). Re-distilled water was used to bring the reaction mixture to the desired final volume. PCR conditions were as follows: an initial denaturation at 94°C for 3 min; 35 cycles of denaturation at 94°C for 30 s, primer annealing at 50–62°C for 30 s, extension at 72°C for 30 s, and a final extension at 72°C for 5 min. Amplification was conducted with a Mastercycler gradient thermocycler (Eppendorf, Germany). In order to enable genotyping of PCR products with an Applied Biosystem 3130 Genetic Analyser, forward primers were 5'–labeled with different fluorescent reporter dyes (Lri-1-VIC, Lri-2-FAM, Lri-3-NED, Lri-4-PET, Lri-5-FAM, Lri-7-VIC, Lri-8-NED, Lri-9-FAM and Lri-10-VIC). The lengths of the amplified DNA fragments were determined using the Applied Biosystems 3130 Genetic Analyser sequencer with application the GeneScan 600 [LIZ] size standard. Individual microsatellite fragments, amplified using primers with different attached fluorescent dyes, were arranged into sets and analyzed in multiplex mode. Fragment size and allele determination was performed using GeneMapper 4.0 genetic analyser software (Applied Biosystems), following the manufacturer's recommendations.

## Data analysis

Before microsatellite DNA analysis, the received genotype raw data was checked for microsatellite null alleles, inconsistent values, scoring errors and large drop-out in samples using Micro-Checker software (version 2.2.3) (VAN OOSTERHOUT et al. 2004). For genetic characteristics of studied populations of river lamprey and genetic cluster recognition, a set of data analysis approaches were utilized. At the beginning, microsatellite allele frequencies, number of observed allele per locus ($A_o$), allelic range, number of effective alleles ($A_e$), allelic richness ($A_r$), number of private alleles ($A_p$), Shannon's index (*I*), the polymorphism information content (*PIC* value) and inbreeding coefficient (*Fis*) for each loci within tested population of river lamprey were calculated with PowerMarker (version 3.25), Fstat (version 2.9.3.2) and GenAlEx (version 6.5) (GOUDET 2002, LIU and MUSE 2005, PEAKALL and SMOUSE 2012). Additionally, the observed and (*Ho*) and expected heterozygosity (*He*) as well as Hardy-Weinberg (H-W) equilibrium for each locus were computed using Arlequin software (version 3.5) (EXCOFFIER and LISCHER 2010). The effective population size (*Ne*) was also assessed for examined fish by NeEstimator computer program (version 2.01) (DO et al. 2013). The linkage disequilibrium method was used for computing *Ne*, where the lowest allele frequency was 0.02. Calculated *Ne* values were subsequently corrected for underestimation from sampling errors with jackknifing 95% parametric confidence intervals (CIs). To delineate historical demography of the studied populations the test for the bottleneck assessment was conducted using the Bottleneck software (version 1.9), which tests for departure from mutation drift equilibrium based on heterozygosity excess or deficiency (Piry et al. 1999). For this purpose, an Infinite Allele Model (IAM), Stepwise Mutation Model (SMM) and two-phase model of mutation (TPM) were tested for sampled fish group. This method is based on the assumption that in non-bottlenecked broodstock (close to mutation drift equilibrium) the value of expected heterozygosity (*He*) is equal to Heq (heterozygosity expected in a mutation-drift equilibrium). The excess of *He* over Heq is the evidence of severe reduction in broodstock effective size that may occur because of a bottleneck event. Statistical tests were performed using the one-tailed Wilcoxon signed rank test. Additionally, the allele frequency distribution analysis was performed. In order to test genetic divergence between studied populations of river lamprey, the analysis of molecular variance (AMOVA), assessment of the allele sharing distances (*DAS*), Nei's standard genetic distance (*Ds*) (NEI 1972), genetic differentiation index (*Fst*) index, overall number of migrants as well as Bayesian clustering analysis

were utilized. The Arlequin software was used to perform the AMOVA test and to compute the overall genetic differentiation index (*Fst*) within tested populations. Computed individual pair-wise the allele sharing distances matrix was used to construct Neighbour-Joining tree of individuals with an application of Populations computer software (version 1.2.32) (LANGELLA 2002). The Principal Coordinates Analysis (PCoA) was performed on the basis of individual pair-wise matrices of Nei's standard genetic distance (*Ds*) using GenAlEx software (version 6.5). Overall number of migrants between studied populations of river lamprey was estimated by the Private allele method of SLATKIN (1985) and corrected for size using GenePop computer software (version 4.2.1) (ROUSSET 2008). Moreover, Bayesian clustering analysis implemented in computer software Structure (version 2.3.4) (PRITCHARD et al. 2000) was performed to estimate the most likely number of genetic clusters (*K*) in the studied populations. *K* was tested from one to 10 with 10 iterations. The admixture model was used with 30,000 burn-in periods and 1,000,000 Markov chain Monte Carlo (MCMC) replicates in each run. The most probable number of genetic clusters for analyzed microsatellite data set was estimated on the basis of obtained ln Pr(X | K) values and EVANNO at al. (2005) method ($\Delta K$). For this purpose, the Structure Harvester online software (version 0.6.94) was used (EARL and VON HOLDT 2011). For accommodate the obtained genotypic data to the requirements of used computer software, every tetrasomic locus was examined as two disomic loci and as result the mean frequency was considered for estimation of genetic variation.

## Results

Among the nine microsatellite DNA fragments applied in the present study, eight (*Lri-1*, *Lri-2*, *Lri-3*, *Lri-5*, *Lri-7*, *Lri-8*, *Lri-9*, *Lri-10*) were polymorphic, whereas one of them (*Lri-4*) was monomorphic. Moreover, all examined microsatellite loci were considered as disomic with the exception of *Lri-1*, which was considered as tetrasomic. Overall, 39 different alleles were found in the tested populations of river lamprey. A total length of identified alleles in the studied loci varied between 95 and 360 base pairs (bp).

Table 1 and Table 2 show all of evaluated genetic diversity parameters (*Ho*, *He*, $A_r$, $A_o$, $A_e$, *I* and PIC) for the studied populations of river lamprey from Poland. The average number of alleles per locus ranged from 3.8 (Vistula) to 3.4 (Vistula lagoon). The mean values of polymorphic information content (PIC) and the rate of Shannon's index (*I*) in examined populations of

river lamprey from Vistula and Vistula lagoon were 0.390, 0.796 and 0.366, 0.741, respectively (Table 1). Private alleles were identified in both analyzed populations, where Vistula population was characterized by the highest number of the private alleles (8) within the studied microsatellite loci (Table 1).

Table 1

Genetic diversity parameters of two investigated populations of river lamprey from Poland

| Population | Locus | $A_r$ | $A_o$ | $A_e$ | $I$ | PIC | $A_p$ |
|---|---|---|---|---|---|---|---|
| Vistula | *Lri-1* | 4.000 | 5 | 3.044 | 1.189 | 0.612 | – |
| | *Lri-2* | 3.000 | 3 | 1.546 | 0.654 | 0.316 | 1 |
| | *Lri-3* | 6.000 | 6 | 2.034 | 1.016 | 0.470 | 1 |
| | *Lri-4* | 1.000 | 1 | MONO | MONO | MONO | MONO |
| | *Lri-5* | 5.000 | 5 | 1.512 | 0.698 | 0.316 | 2 |
| | *Lri-7* | 3.000 | 3 | 1.416 | 0.550 | 0.268 | 1 |
| | *Lri-8* | 4.000 | 4 | 1.206 | 0.381 | 0.163 | 2 |
| | *Lri-9* | 3.000 | 3 | 1.989 | 0.768 | 0.397 | – |
| | *Lri-10* | 4.000 | 4 | 2.826 | 1.111 | 0.577 | 1 |
| | **Mean** | **3.667** | **3.8** | **1.946** | **0.796** | **0.390** | – |
| Vistula lagoon | *Lri-1* | 4.380 | 6 | 3.032 | 1.225 | 0.612 | 1 |
| | *Lri-2* | 1.944 | 2 | 1.041 | 0.098 | 0.038 | - |
| | *Lri-3* | 4.944 | 5 | 1.981 | 1.011 | 0.4673 | |
| | *Lri-4* | 1.000 | 1 | MONO | MONO | MONO | MONO |
| | *Lri-5* | 3.759 | 4 | 1.571 | 0.683 | 0.329 | – |
| | *Lri-7* | 2.000 | 2 | 1.523 | 0.527 | 0.284 | – |
| | *Lri-8* | 2.000 | 2 | 1.268 | 0.367 | 0.189 | – |
| | *Lri-9* | 3.975 | 4 | 2.218 | 0.912 | 0.453 | 1 |
| | *Lri-10* | 4.520 | 5 | 2.709 | 1.108 | 0.559 | 2 |
| | **Mean** | **3.169** | **3.4** | **1.918** | **0.741** | **0.366** | – |

Explanations: $A_r$ – allelic richness, $A_o$ – observed alleles, $A_e$ – expected alleles, $I$ – Shannon's index, PIC – polymorphism information content, $A_p$ – number of private alleles, MONO – monomorphic locus

The mean values of observed heterozygosity ranged from 0.441 (Vistula) to 0.436 (Vistula lagoon) and were close to the mean values expected under H-W equilibrium (*He*) – Table 2. Chi-Square test for Hardy-Weinberg Equilibrium (H-WE) showed that three (Vistula river) and four (Vistula lagoon) microsatellite loci deviated significantly from Hardy-Weinberg equilibrium (H-WE). In turn, the average values of *Fis* were negative in studied populations, ranging between -0.074 (Vistula) and -0.122 (Vistula lagoon). Moreover, the performed bottleneck test did not reveal any statistically significant *He* > Heq differences under all mutation models used in both of studied river lamprey populations (Table 2). The analysis of allele

frequency distribution revealed an L-shaped distribution. The estimated effective population size (*Ne*) for the studied populations of river lamprey form Vistula river and Vistula lagoon equalled 72.4 (95% CI = 14.9–∞) and 25.8 (95% CI = 8.7–151.1), respectively.

Table 2

Comparison of observed (*Ho*) and expected (*He*) heterozygosity, expected heterozygosity (Heq) in a Infinite Allele Model (*IAM*), Stepwise Mutation Model (*SMM*) and two-phase model of mutation (*TPM*) as well as fixation index (*Fis*) in examined populations of river lamprey

| Population/ Locus | *Ho* | *He* | *P* | IAM | | SMM | | TPM | | *Fis* |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | Heq | *P* | Heq | *P* | Heq | *P* | |
| Vistula | | | | | | | | | | |
| *Lri-1* | 0.974 | 0.673 | 0.000 | 0.436 | 0.050 | 0.571 | 0.217 | 0.502 | 0.098 | -0.463* |
| *Lri-2* | 0.158 | 0.358 | 0.000 | 0.341 | 0.486 | 0.467 | 0.202 | 0.400 | 0.378 | 0.562***** |
| *Lri-3* | 0.553 | 0.515 | 0.423 | 0.597 | 0.238 | 0.734 | 0.012 | 0.668 | 0.076 | -0.074 |
| *Lri-4* | MONO | – | – | – | – | – | – | – | – | – |
| *Lri-5* | 0.342 | 0.343 | 0.421 | 0.534 | 0.140 | 0.672 | 0.003 | 0.607 | 0.042 | 0.003 |
| *Lri-7* | 0.289 | 0.298 | 0.065 | 0.342 | 0.425 | 0.466 | 0.144 | 0.397 | 0.289 | 0.029 |
| *Lri-8* | 0.158 | 0.173 | 0.029 | 0.434 | 0.075 | 0.595 | 0.003 | 0.524 | 0.022 | 0.090 |
| *Lri-9* | 0.368 | 0.504 | 0.099 | 0.337 | 0.244 | 0.465 | 0.494 | 0.395 | 0.327 | 0.271 |
| *Lri-10* | 0.684 | 0.655 | 0.902 | 0.445 | 0.103 | 0.597 | 0.339 | 0.517 | 0.154 | -0.046 |
| **Mean** | **0.441** | **0.440** | **–** | **0.433** | **–** | **0.571** | **–** | **0.501** | **–** | **-0.074** |
| Vistula lagoon | | | | | | | | | | |
| *Lri-1* | 0.990 | 0.670 | 0.000 | 0.453 | 0.094 | 0.596 | 0.234 | 0.516 | 0.182 | -0.493* |
| *Lri-2* | 0.040 | 0.040 | 1.000 | 0.194 | 0.299 | 0.225 | 0.196 | 0.218 | 0.239 | -0.010 |
| *Lri-3* | 0.380 | 0.500 | 0.004 | 0.517 | 0.389 | 0.669 | 0.046 | 0.588 | 0.218 | 0.242* |
| *Lri-4* | MONO | – | – | – | – | – | – | – | – | – |
| *Lri-5* | 0.360 | 0.367 | 0.145 | 0.417 | 0.391 | 0.580 | 0.054 | 0.507 | 0.185 | 0.019 |
| *Lri-7* | 0.320 | 0.347 | 0.680 | 0.183 | 0.225 | 0.231 | 0.337 | 0.212 | 0.284 | 0.078 |
| *Lri-8* | 0.200 | 0.213 | 0.528 | 0.184 | 0.356 | 0.224 | 0.480 | 0.201 | 0.412 | 0.063 |
| *Lri-9* | 0.560 | 0.555 | 0.234 | 0.434 | 0.287 | 0.586 | 0.311 | 0.505 | 0.452 | -0.010 |
| *Lri-10* | 0.640 | 0.637 | 0.803 | 0.511 | 0.254 | 0.666 | 0.288 | 0.595 | 0.460 | -0.004 |
| **Mean** | **0.436** | **0.416** | **–** | **0.362** | **–** | **0.472** | **–** | **0.418** | **–** | **-0.122** |

Explanations: *P* – level of significance; all statistically significant ($P < 0.05$) deviations Ho ≠ He and *He* > Heq were underlined, MONO – monomorphic locus

The estimated overall gene flow (*Nm*) between the populations was at the level 5.09 individuals per generation. Analysis of the genetic structure with AMOVA method showed that only 0.60% of the genetic variation was distributed among studied populations. Moreover, assessed genetic

differentiation between examined populations of river lamprey was at the level *Fst* = 0.006, being statistically insignificant ($P < 0.01$). Received results on individual multilocus genotype, based on structure analyses, did not find any signs of the further genetic clustering within the studied fish group (GIGHER et al. 2013, SCHEDINA et al. 2014, BRACKEN et al. 2015). Utilized method developed by EVANNO et al. (2005) for computation of $\Delta K$ did not reveal any specific value. The maximum value of ln Pr(X | K) was observed for K = 1, where the clear plateau of L'(K) values was present after K = 1. Contrastingly, constructed individual's tree based on DAS genetic distances and the Principal Coordinates Analysis (PCoA) exhibited three main genetic groupings within studied fish group (Figure 1 and Figure 2).

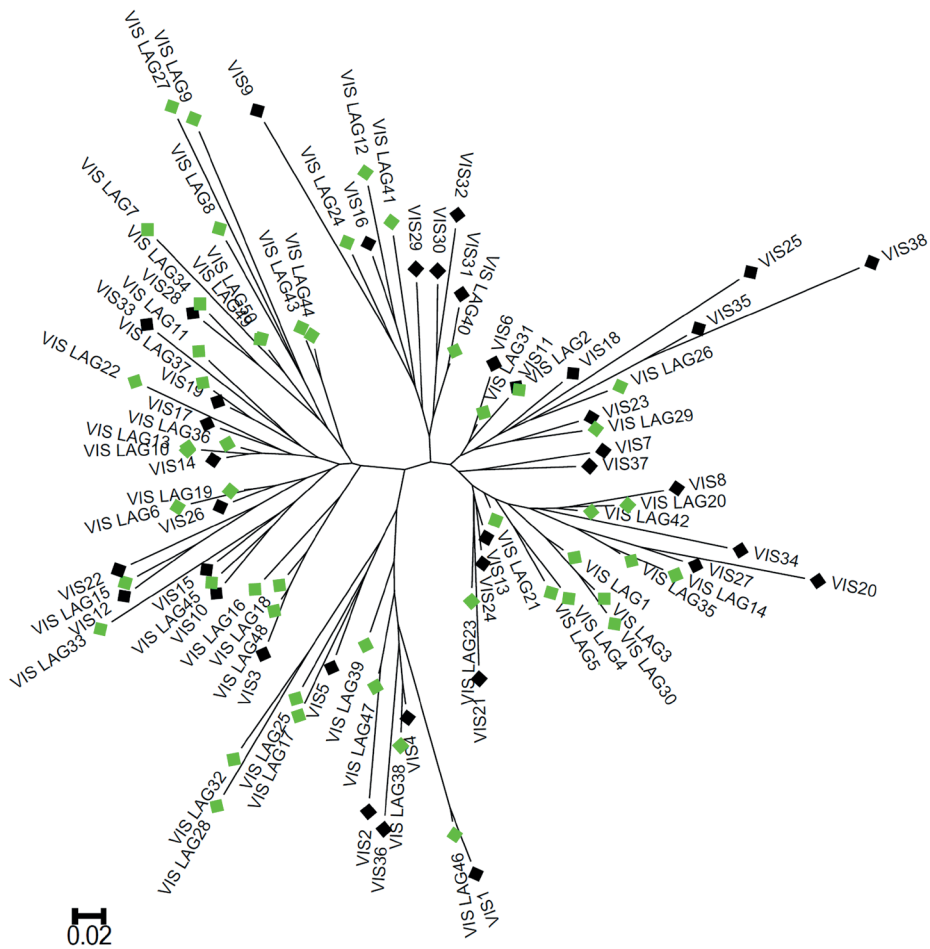


Fig. 1. The unrooted Neighbor-Joining tree of examined river lamprey (*Lampetra fluviatilis*) individuals based on allele sharing distances (*DAS*)

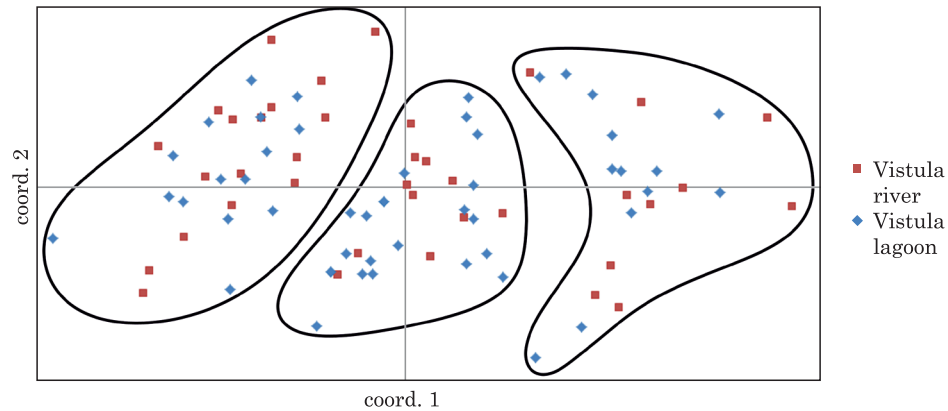


Fig. 2. Scatter plot of the Principal Coordinates Analysis (PCoA) based on an individual pair-wise Nei standard (*Ds*) genetic distance for examined specimens of river lamprey (*Lampetra fluviatilis*)

## Discussion

The river lamprey populations in Poland inhabit an environment greatly affected by negative human impact. These pressures may have a strong, adverse impact on population viability need to be carefully assessed as well as managed at scales that are consistent with the population structure of river lamprey. However the decline of river lamprey is still significant in Vistula river and Vistula lagoon in Poland, but the established conservation plan (river lamprey is under partial protection: fishing of this species is forbidden) is realized without any information about genetic structure of this species. Indeed, scientific assessment of population structure, and implementation of conservation and management measures should be conducted based on genetic studies of conserved species/population.

In the present study the microsatellite DNA analysis was applied for identification of genetic diversity of two river lamprey populations in Poland. Such markers were sufficient and useful tools for delineating population structure of studied river lamprey populations. Application of microsatellite markers was helpful and allowed obtaining valuable data that will provide the starting point for the restoration programe of river lamprey in Vistula river estuarium.

To date, the applied genetic analyzes allowed the identification and characterization of the significant diversity of lampreys in the Iberian Peninsula area, a refugium region during the Pleistocene glaciations (Mateus 2013). Moreover, this study enabled understanding the historical

processes of glacial colonization together with an assessment of the contemporary gene flow between species *Lampetra* sp. in Europe (MATEUS 2013). The application of sequencing analysis of entire genome has significantly contributed to the better understanding of the lampreys taxonomy and enabled the identification of differences between the common species of lampreys (MATEUS 2013). This is very important that the genetic analysis conducted on lamprey enabled to broaden the knowledge about their evolution providing the model to study the processes of speciation (GIGHER et al. 2013).

Population genetic studies of lampreys were frequently conducted on: i) comparison of distinct populations of one species, ii) migration analysis of lampreys, and iii) the estimation of relationship between separate taxa. Currently, the lampreys have become a model organisms in the studies of evolutionary biology associated with an anadromous and resident life styles (BRACKEN et al. 2015). The sea lamprey was the most frequently studied species because of its interesting evolutionary history and invasive character in Great Lakes. Based on genetic analysis, researchers tried to resolve the problems related with the species migration rate, dispersion abilities and its invasive impact on water ecosystems in order to derive genetically (DEROSIER et al. 2007, SPICE et al. 2012, TAYLOR et al. 2012). In turn, LUZIER et al. (2010) developed the microsatellite markers for brook lamprey (*Lampetra planeri*), and then applied them in study of the genetic population structure of other lamprey species. SPICE at al. (2011) conducted genetic studies on the Pacific lamprey (*Entosphenus tridentatus*), using 12 polymorphic microsatellite DNA that enabled indication the diagnostic loci distinguishing *Entosphenus* and *Lampetra* (SPICE et al. 2011). These markers were also used for the population analysis and making decisions in conservation of *Entosphenus tridentatus* (SPICE et al. 2011). Genetic research based on mitochondrial DNA fragment (*ATPase* subunit 6 and 8 and a part of *cytochrome b*) was carried out to identify the river lamprey and brook lamprey in Portugal (MATEUS et al. 2011). This analysis enabled the characteristics of haplotypes and phylogenetic analysis of studied lamprey species together with verification of its species (MATEUS 2011).

The above examples of analyzes, conducted in different species of lampreys, clearly indicate the importance of application of molecular markers in study of endangered organisms that are protected. It should be emphasized that in Poland, the genetic research on lampreys have not been conducted so far. Considering the lack of information about the genetic structure and biodiversity of lampreys in Poland, proposed in this paper, genetic research contribute to increased based information on the Polish populations of the river lamprey.

In the present study, examined fish group was characterized by similar levels of the genetic diversity compared to the lamprey populations across Europe and North America (McFarlane and Docker 2009, Gaigher et al. 2013, Schedina et al. 2014). However, observed genetic variability in this species seems to be explicitly lower than commonly described in teleost fish (Fopp-Bayat and Woznicki 2006, Fopp-Bayat et al. 2015, Kucinski et al. 2015). It is believed that hagfish and lampreys are an ancient group of vertebrates, being characterized by a ancient structure of their genomes. Recent evolutionary studies informed that a genome duplication event took place after the divergence between Cyclostomata (lampreys and hagfish) and gnathostomates (Holland et al. 1994, Thornton 2001). Therefore, it may explain lower variability of microsatellite DNA loci in lampreys with comparison to teleost fish species.

The applied, in the present research, sensitive genetic population tests showed a lack of significant genetic structure between the two examined populations of river lamprey. This result is consistent with described studies on anadromous lampreys from Japan, North America and Europe, where very little or no genetic structure were found (Goodman et al. 2008, Yamazaki et al. 2011, Bracken et al. 2015). The absence of population genetic structure seems to be a general rule in anadromous lamprey species, which is associated with the lack of natal homing (Waldman et al. 2008, Spice et al. 2012). Instead, it was proven that lampreys use pheromones released by stream inhabiting larvae to localize suitable spawning grounds (Fine et al. 2004). Both of the studied populations of river lamprey (Vistula river and Vistula lagoon populations) migrate annually upstream Vistula river for spawning sites. Despite the lack of clear signs for genetic structure within the studied populations, some evidences for asymmetric gene flow and limited panmixia were also found. Higher number of private alleles in population from Vistula river might suggest that the gene flow occurs unidirectional mainly from Vistula lagoon to Vistula river population. Additionally, the presence of private alleles in fish from both sites (Vistula river and Vistula lagoon) might be the evidence for the presence of few cryptic populations. Similarly, the obtained results on individual DAS genetic distances and the Principal Coordinates Analysis (PCoA) might suggest the existence of three mixed populations of river lamprey within studied region. Currently, this phenomenon can be connected to migratory behavior of lampreys. After the larval stage, lampreys metamorphose to young adults and then to sea phase. The sea migration of river lampreys is connected to host-fish on which lamprey parasitize. During this time, different populations of lampreys may mix because they can parasitize on one stock/population of sea fish (for example on cod).

After the sea phase, mature lampreys start spawning migration to rivers and during this time they could also mixed. Our results may indicate a situation where three populations of lampreys were mixed. Probably all three studied populations of river lamprey migrated to spawning sites, and some individuals did not complete their spawning migration.

In conclusion, the described data are very important in interpreting the phylogeographic, genetic and population context and they are the key point during development of management strategies and protection of this lamprey species in Poland. The primary genetic data will be valuable also in biodiversity monitoroing or supplementation plan of river lamprey during protection of endangered populations.

Tranlaslated by DOROTA FOPP-BAYAT

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# CLIMATE-RELATED VARIABILITY AND STOCK – RECRUITMENT RELATIONSHIP OF THE NORTH PACIFIC ALBACORE TUNA*

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Key words: recruitment, spawning stock biomass, sea surface temperature, Pacific decadal oscillation, El Niño southern oscillation.

Abstract

The stock-recruitment relationship is biological in nature and it is evident that one affects the other. However, fish life cycle, growth, abundance and distribution is dependent on a complex relation with various biotic and abiotic variables inclusive of environmental factors. The stock-recruitment relationship and environmental influences was investigated for the North Pacific albacore tuna (*Thunnus alalunga*). Statistical investigation revealed the presence of different density-dependent effects in the relationships of recruits per spawning biomass (RPS) and recruitment (R) against the female spawning stock biomass (SSB). Significant relationship of R, RPS and SSB were determined with the sea surface temperature (SST), Pacific Decadal Oscillation (PDO) and multivariate El Niño Southern Oscillation (ENSO), with SST being the principal variable. This makes the stock recruitment behavior multidimensional. The results suggest significant influence of the environmental conditions on the stock recruitment relationship of the North Pacific albacore tuna including a possible regime shift.

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# WPŁYW KLIMATU A ZALEŻNOŚĆ STADO – UZUPEŁNIENIE NA PRZYKŁADZIE TUŃCZYKA PÓŁNOCNOPACYFICZNEGO *THUNNUS ALALUNGA*

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Słowa kluczowe: uzupełnienie stada, biomasa stada tarłowego, temperatura powierzchni morza, pacyficzna cyrkulacja powietrza, El Niño – oscylacja południowa.

Abstrakt

Modele stado – uzupełnienie wyjaśniają jedynie pewną część obserwowanej wariancji rekrutacji. Cykl życiowy, tempo wzrostu, liczebność i rozmieszczenie ryb wiążą się z różnymi zmiennymi biotycznymi i abiotycznymi, w tym z czynnikami klimatycznymi. W pracy zbadano proces naturalnej rekrutacji w zależności od zmieniających się parametrów środowiska. Wyniki wskazują na istotny wpływ warunków klimatycznych na zależność stado – uzupełnienie.

## Introduction

The North Pacific albacore is a temperate tuna species distributed in the North Pacific which is of significant economic importance to the Pacific Island Nations, territories as well as various fishery industries that obtain license to harvest albacore stock from these waters (SUND et al. 1981, MIYAKE et al. 2004, GILLETT 2009, CHILDERS et al. 2011, SPC 2012, BELL et al. 2013). Albacore stock in the Northern Pacific has been shown to be as discrete and reproductively isolated (UEYANAGI 1969, SUZUKI et al. 1977, CHOW and USHIAMA 1995, RAMON and BAILEY 1996, TAKAGI et al. 2001, ICHINOKAWA et al. 2008). Tuna fisheries provides employment to over 13,000 Pacificic islanders and represents a significant proportion of governmental revenue (more than 40% in some cases) from tuna licensing fees alone (GILLETT 2009). In the Western and Central Pacific Ocean (WCPO), longline landings of albacore represents approximately 1/3$^{rd}$ of annual tuna catch. The North Pacific albacore accounts for ~50% of total global albacore harvests (LAURS and POWERS 2010, WILLIAMS and TERAWASI

2013). The ecological properties and behavior of albacore is not well understood despite having a long fishery history in the Pacific.

Oceanic regime shifts are long lasting and sudden large scale spatial changes in ecological conditions often characterized by changes in abundance and spatial variability of various aquatic species (MÖLLMAN et al. 2014). A regime shift in a commercial fish species stock abundance and/or distribution characteristics across a time series can be in response to high levels of anthropogenic activities such as fishing pressure or environmental and climatic regime shifts (LEHODEY et al. 2003, CAHUIN et al. 2009, LITZOW et al. 2016). LEHODEY et al. (2015) showed that the stock abundance of the South Pacific albacore tuna is significantly affected by both the applied levels of fishing pressure and alteration in environmental and climatic conditions. The spawning behavior of albacore was shown to be related to the spatial variability of the optimal ambient spawning sea surface temperature (SST). LEHODEY et al. (2003) compared the annual recruitment patterns over decadal scales in the Western and Central Pacific Ocean (WCPO) for skipjack (*Katsuwonus pelamis*), yellowfin (*Thunnus albacares*) and the South Pacific albacore tuna and detected long-term sustained trend change in the recruitment time series for all three species. The changes or regimes were shown to have responded to regime shifts in the inter-decadal climatic variability of the El Niño Southern Oscillation (ENSO).The spatial distribution and abundance of the South Pacific albacore tuna has been shown to be related to the environmental and climatic variables of the SST, Multivariate El Niño Southern Oscillation Index (ENSO) and the Pacific Decadal Oscillation (PDO) (LEHODEY et al. 2003, ZAINUDDIN et al. 2004, GANACHAUD et al. 2011, LE BORGNE et al. 2011, LEHODEY et al. 2011, SPC 2012, BELL et al. 2013, GANACHAUD et al. 2013, PHILLIPS et al. 2014, ZHANG et al. 2014, LEHODEY et al. 2015, SINGH et al. 2015, SINGH et al. 2017). Multivariate El Niño Southern Oscillation Index (ENSO) is a sub-decadal time scale and important climatic variability with effects spanning over most parts of the tropics and sub-tropics. Multiple variables of surface wind indexes, atmospheric conditions, sea-level pressure, SST and surface air temperature are used for the calculation of ENSO (WOLTER and TIMLIN 1998).

PHILLIPS et al. (2014) determined an abrupt change (regime shift) in the spatial distribution of the juvenile North Pacific albacore in the waters of the US west coast in response to alteration in the condition of the SST and PDO. SINGH et al. (2017) determined significant influence of the SST, PDO and ENSO variability on the recruitment and spawning biomass of the North Pacific albacore from 1970 to 2012. In the North Pacific Ocean there is a major dominant mode of climate index called the Pacific decadal

oscillation (PDO) which occurs over decadal time scales and affects oceanic temperatures both in the Northern and the Southern Pacific (MANTUA et al. 1997, ZHANG et al. 1997, DESER et al. 2004, CHHAK et al. 2009, LINSLEY et al. 2015).

The objectives of this study were 1) to elucidate the relationship between the recruitment (R), female spawning stock biomass (SSB) and the reproductive success (RPS) of the North-Pacific stock of the albacore tuna, 2) to determine if the albacore R, SSB and RPS time series experienced a regime shift due to a regime shift in environmental conditions, 3) to determine if inter-decadal environmental and climatic variation of the sea surface temperature (SST), El Niño Southern Oscillation (ENSO), and the Pacific decadal Oscillation (PDO) have significant impact on the stock-recruitment relationship of the North Pacific albacore tuna.

## Materials and Methods

### Data sources

Stock assessment of commercial North Pacific albacore tuna harvests are compiled and reported by the Albacore Working Group of the International Scientific Committee for Tuna and Tuna-like Species (ISC) (ISC 2014). Data is gathered from the ISC member countries of Japan, USA, Korea, Chinese Taipei and Canada. Also included is information from Inter-American Tropical Tuna Commission (IATTC) and China as well as some countries under the Western and Central Pacific Fisheries Commission (WCPFC). Fishing gears and methods used include gillnet, pole and line, purse seine, troll, longline including harpoons, handlines and recreational gear. From 1970 to 2012, the annual recruitment (R) and female spawning stock biomass (SSB) data was estimated by the ISC using an age-structure, length-structure and sex-structured Stock Synthesis assessment model fitted to time series of catch and size structure data (ISC 2014). R and SSB data from the base case assessment model wereextracted from the ISC report and the annual recruits per spawning biomass was calculated from this. Further details on the techniques and methods for the collection and calculation of the annual R and SSB data are described in ISC (2014). The R and SSB estimates by the ISC are assumed to be reliable with the deviations noted in ISC (2014). The geographical range for the albacore stock distribution was restricted to the North Pacific region defined within the coordinates of 50°N – 120°E, 10°N – 120°E, 50°N

– 120ºW, 10ºN – 120ºW presented in Figure 1. Previous studies have identified the North Pacific albacore stock as discrete and reproductively isolated from other albacore stocks (UEYANAGI 1969, SUZUKI et al. 1977, CHOW and USHIAMA 1995, RAMON and BAILEY 1996, TAKAGI et al. 2001, ICHINOKAWA et al. 2008).

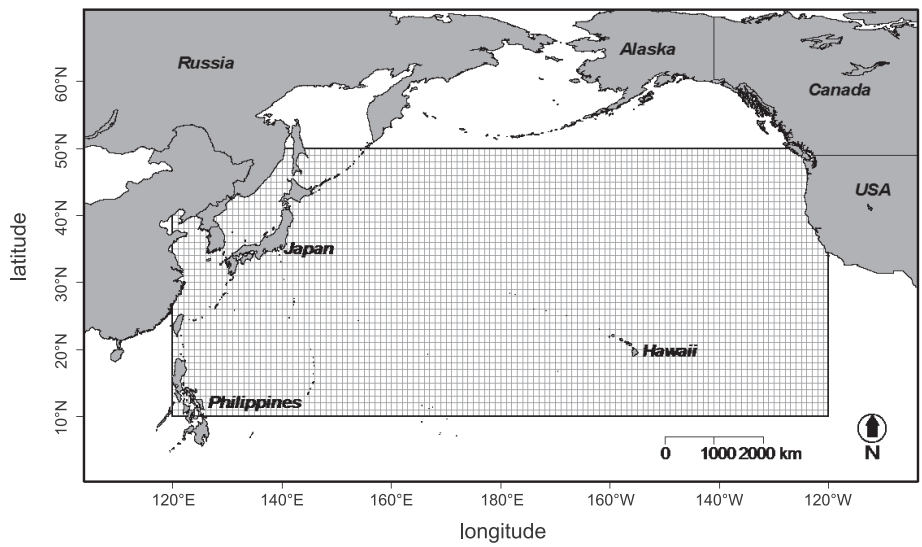


Fig. 1. Area of the study of North Pacific albacore tuna recruitment and spawning stock

For each year the recruitment is estimated as the number fish at age-0 and the SSB represents the total weight of reproductively mature female specimens at the initiation of the spawning season. Pacific albacore tuna recruitment and spawning period typically occurs only once per year as determined by CHEN et al. (2010). UEYANAGI (1957) determined that 50% of the North Pacific albacore reaches maturity at age-5 and 100% by age-6 and this maturity ogive was used for the calculation of the SSB as reported in ISC (2014). Recent work of FARLEY et al. (2014) and assessments by ISC (2011) also support these estimations. The spawning zone for albacore occurs in the North Pacific between Taiwan, Hawaii and Philippine waters within the latitudes of 10ºN and 25ºN and longitudes of 155ºW and 120ºE (UEYANAGI 1957, OTSU and UCHIDA 1959, YOSHIDA 1968, CHEN et al. 2010) – Figure 1. Like most fishery populations, the stock-recruitment pattern of the North Pacific albacore displays a scattered distribution (Figure 2).

For comparing the North Pacific albacore tuna stock variability with environmental variables, the SST and other temperature related climatic anomalies data set were obtained. SST monthly data on a 1º by 1º resolution for the years 1970 to 2012 was extracted for the study area (Figure 1)

from Hadley Centre Sea Ice and Sea Surface Temperature data set (HadISST) with further description and compilation details available from RAYNER et al. (2003). The NOAA/ESRL Physical Sciences Division

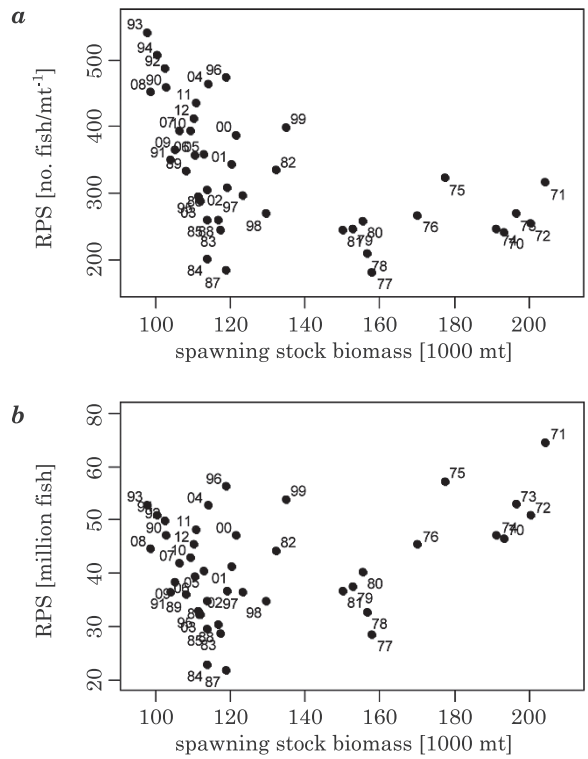


Fig. 2. Pattern of the recruits per spawning biomass (RPS) against the spawning stock biomass (SSB) (*a*) and the stock-recruitment distribution (*b*) for the albacore tuna (*T. alalunga*) in the North Pacific Ocean for the years ranging from 1970–2012

(Boulder, Colorado, USA), website (http://www.esrl.noaa.gov/psd last accessed 02 March 2016) was accessed to obtain monthly grouped time series data on the climatic indexes of the Pacific decadal oscillation (PDO) and the Multivariate El Niño Southern Oscillation Index (ENSO).

## Regimes and density-dependent effects

SAKURAMOTO (2012), SINGH et al. (2014) and WADA and JACOBSON (1998) outlined the method for identifying different regimes in the fish stock parameters and determining the presence of density dependent effects in time series data. North Pacific albacore R, SSB and RPS time series were explored for observable and sustained differences in trend over

the years from 1970 to 2012. Existence of any such trends would indicate a possible "regime shift". An observable regime shift in a fish stock usually results in response to changes in environmental conditions and as such a regime shift should also be present in environmental conditions which have relationship with the North Pacific albacore stock time series.

We carried out statistical analysis to investigate the stock recruitment relationship of albacore tuna for the presence of different density-dependent effects. Simple least-squares regression was applied for log(RPS) against log(SSB). This technique has limitations as the presence of possible observational errors in R and SSB can cause overestimation and underestimation of the RPS (SAKURAMOTO and SUZUKI 2012, STÖCKL et al. 1998). For validation of the results we applied regression of log(R) against log(SSB) as this method is more robust and less sensitive to observational errors. Results indicating slopes which are significantly different from unity at $p < 0.05$ were considered positive for the presence of different density dependent effects and a regime shift in the albacore tuna stock-recruitment relationship.

Simple least-squares regression has some drawbacks as it does not assume the presence of observation errors in the independent variable and without observation error the parameter estimates derived from least squares regression will have high tendency to exhibit bias according to STÖCKL et al. (1998). The Deming regression method (MARTIN 2000) assumes the presence of errors in both the independent and the dependent variables. The Deming regression algorithm developed by AOKI (2012) was applied to log(RPS) against log(SSB) and log(R) against log(SSB), with the assumption that the variance ratio in R and SSB is equal to 1. Variance ratios of 1, 2, 3, …, 5 were also used for sensitivity tests. The parameters of Deming regression were compared with the least-squares regression.

The Ricker model (RICKER 1954) shown below (Equation 1) was used to further investigate the stock recruitment relationship. An error term was included as an assumption for the presence of observational and/or process errors. A multiplicative error term was added to the model instead of an additive one as the variability was expected to be different in different areas of data.

$$R = a \cdot SSB exp^{-bSSB} \cdot exp^{\epsilon} \tag{1}$$

Where $a$ and $b$ represent the density independent and density dependent parameter respectively. The term  is the assumption of variability around the model. To fit the model using the R statistical software the linearized form of Ricker model (Equation 2) was used (QUINN and DERISO 1999):

$$\log(R) = \log(a \cdot SSB exp^{-bSSB}) + \epsilon \tag{2}$$

The R and SSB data was divided into different year ranges between 1970 to 2012 to determine if density dependent patterns exist in the stock recruitment relationship. Different stock-recruitment models using environmental variables were also investigated and discussed. All statistical analysis was performed using the language "R" software version 3.4.0 (R CORE TEAM 2017).

## Results

### Regimes and density-dependent effects

The stock trajectory of the albacore tuna RPS in Figure 3 shows a sustained change in the time series trend which occurs between 1987 to 1990 with a lower RPS average from 1970 to 1988 and a higher average from 1989 to 2012 which coincides with the trend for R where a difference in trend can also be between 1970 to 1988 and 1989 to 2012. For SSB there is a trend difference between the years 1970 to 1982 and 1983 to 2012.

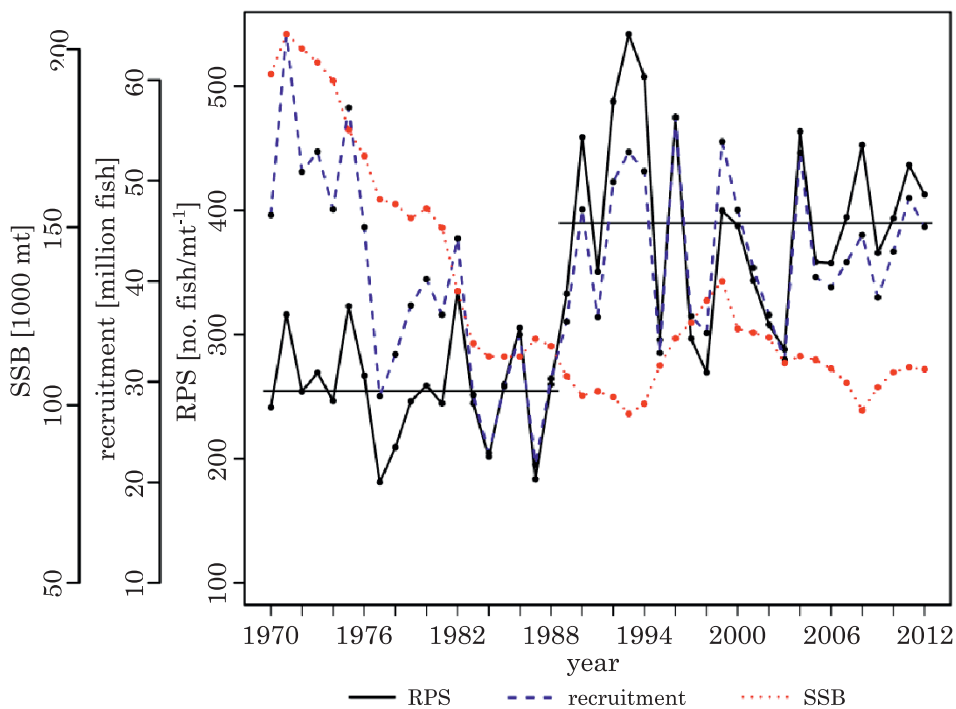


Fig. 3. The recruitment (R), spawning stock biomass (SSB) and recruits per spawning biomass (RPS) time series trajectory of the albacore tuna (*T. alalunga*) in the North Pacific Ocean for the years ranging from 1970–2012

Such changes in the stock trend indicate the possibility of different density-dependent effects and a possible regime shift. The changes or regimes of the stock parameters is possibly resulting from changes in environmental conditions and should coincide with environmental conditions exhibiting relationship with the albacore stock (SAKURAMOTO 2012).

From Figure 3, it can be seen that for some years, when the SSB is low, R is high and so is RPS. This may be a result of the smaller number of larvae which experience an abundance of food and significantly reduced competition and cannibalism, which would lead to a larger survival rate of the larvae from the initial high natural mortality. Figure 4 shows

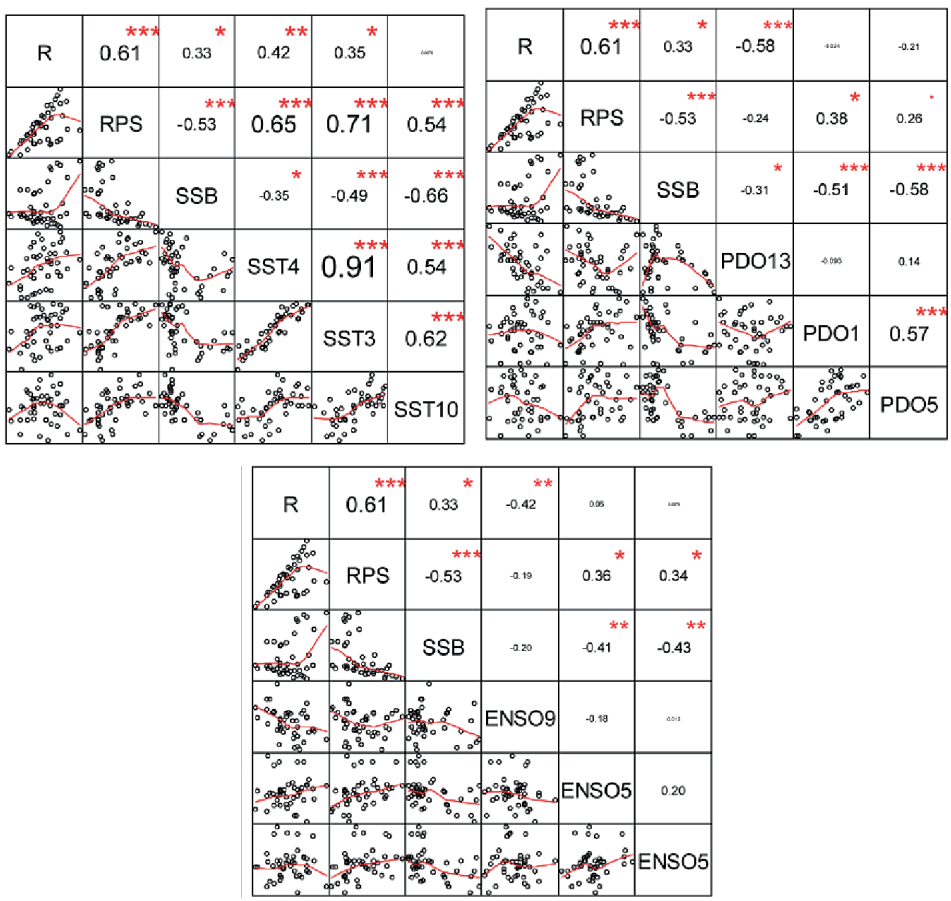


Fig. 4. Scatterplot matrix showing absolute correlations, kernel density overlays with significance asterisks ($p < 0.05$*, $p < 0.01$**, $p < 0.001$***) of the relation between the recruitment (R), recruits per spawning biomass (RPS) and spawning stock biomass (SSB) of the North Pacific albacore tuna (*T. alalunga*) with the environmental variables of SST, PDO and ENSO for the years ranging from 1970–2012. The numbers refer to the months (1, 2, …, 12) and the number 13 is the annual average

the correlation between the R, RPS and SSB of the North Pacific albacore tuna with the environmental factors of the SST, PDO and ENSO. SST of the study area (Figure 1) was identified as the principle environmental factors correlating with the North Pacific albacore tuna stock. Figure 5

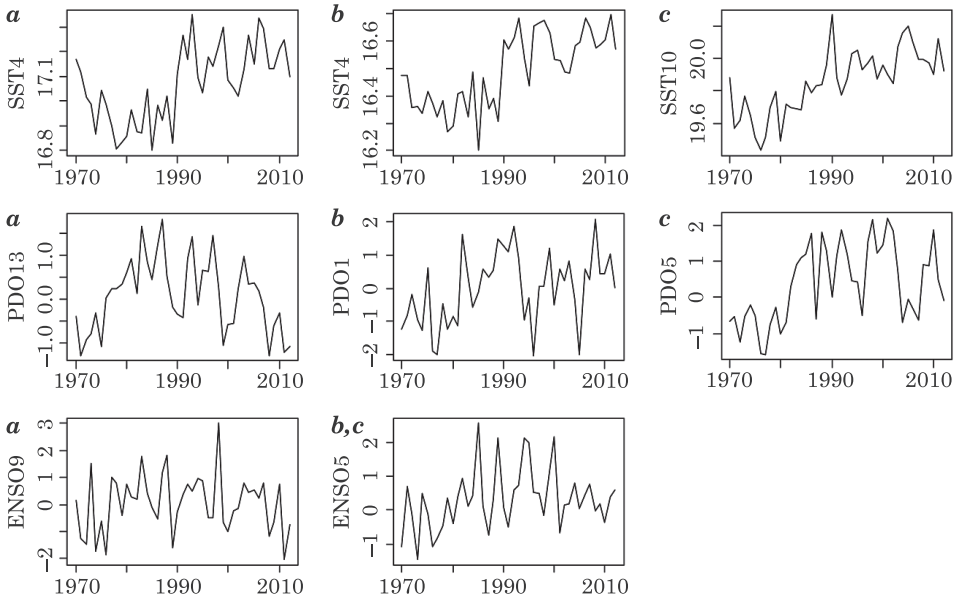


Fig. 5. Environmental conditions time series trend for SST, PDO and ENSO from 1965 to 2012 which exhibited significant correlation with R, SSB, and RPS as shown in Figure 4. The letters refer to the stock parameter for which the particular environmental condition shows the highest correlation (*a* – R, *b* – RPS, *c* – SSB).The numbers refer to the months (1, 2, … 12) and the number 13 is the annual average.

shows the time series trend of the environmental variables from Figure 4, where it can be seen that there is a visible change in the trend of the SST time series around the years 1987–1989 which corresponds to the change in trend for the R and RPS.R and RPS trajectories plots with their respective correlated SSTs are shown in Figure 6. The changes in trajectories coincide quite well indicating that significant proportion of changes in stock behavior of the North Pacific albacore tuna are in response to SST time series dynamics including the observed regime shift.

Figure 7 shows the results of regression analysis applied to log(RPS) against log(SSB) and log(R) against log(SBB). The slope of the regression line adopted for log(RPS) against log(SSB) was -0.714 ($p = 1.12 \cdot 10^{-4}$) and 95% confidence interval of (-1.052, -0.377). The negative slope was statistically significant and positive for the presence of different density dependent effects and indicated a regime shift. According to the simulations by

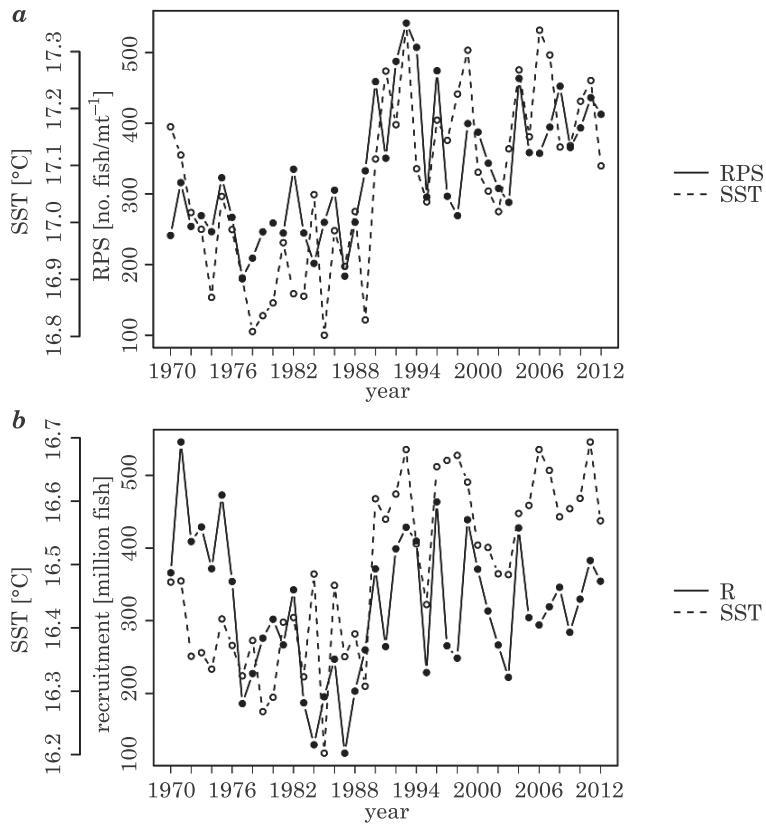


Fig. 6. The recruitment (R) and recruits per spawning biomass (RPS) time series plots of with the respective correlated SSTs from Figure 4 and Figure 5. The similarities in the trend and coinciding shift in trend pattern can be seen indicating significant influence of the SST on albacore tuna (*T. alalunga*) R and RPS trajectory

(SAKURAMOTO 2012) and as shown by STÖCKL et al.(1998), using RPS for regression analysis can lead to potentially flawed results in cases where observation errors are present. To validate the results, we applied regression analysis to log(R) against log(SSB) which is a more reliable method. The regression line slope was 0.110 ($p = 3.01\times10^{-2}$) with 95% confidence interval of (0.010, 0.192) which was significantly less than unity.

When Deming regression was applied to log(RPS) against log(SSB) the slope was significantly different from unity with a slope of -1.560 at 95%confidence interval of (-2.618, -1.094). For log(R) against log(SSB) the slope was 1.475 and not significantly differ from 1.0 as the 95% confidence interval was -12.160, 7.127). The ratio of variance for Deming regression of log(R) against log(SSB) was assumed to be 1.0 and when the variance ratio was increased to 2.0 (Figure 8) the results did not significantly differ

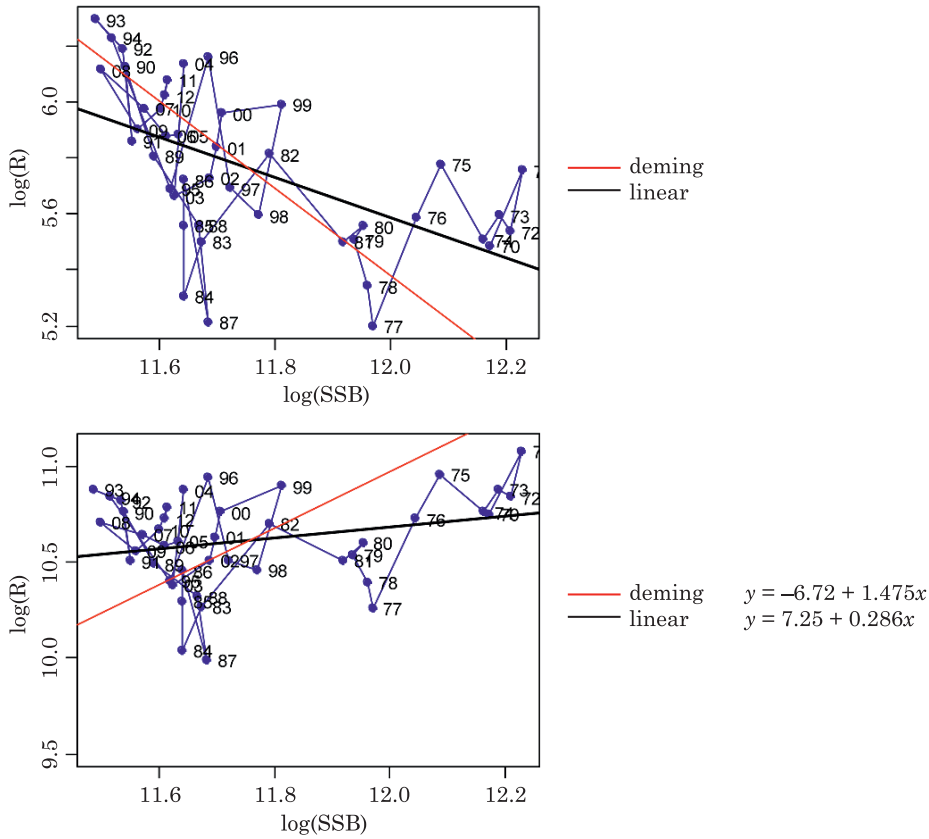


Fig. 7. The relationship between log(RPS) against log(SSB) and log(R) against log(SSB) using Deming regression and simple linear regression. For log(RPS) against log(R) the correlation coefficient was (cc = -0.555), for log(R) against log(SSB) (cc = 0.258)

indicating the absence of different density dependent effects and a proportional stock-recruitment relationship shown in Equation 3 and Equation 4

$$\log(R) = \log(l) + \log(SSB) + \varepsilon \tag{3}$$

$$R = l \cdot SSB + \varepsilon \tag{4}$$

where $l$ is a parameter estimate and $\varepsilon$ is an unexplained variable. However, when the ratio of variance was augmented to ≥ 3.0, the slope was significantly different from 1.0 (Figure 8). For the variance ratio of 3.0 the slope was 0.451 with 95% confidence interval of (-0.292, 0.842). By raising the variance ratio to ≥ 3.0 the slope was significantly different from unity indicating that a proportional model such as Equation 3 and Equation 4 cannot be used to describe the stock-recruitment relationship of the North

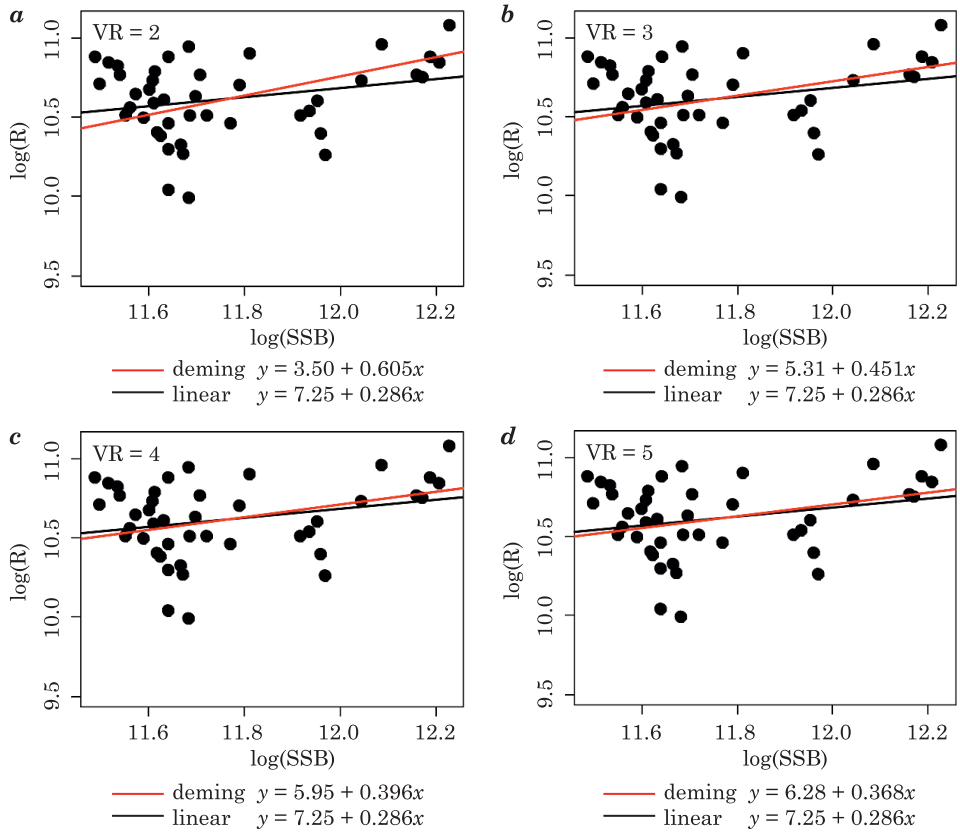


Fig. 8. For Deming regression of log(R) against log(SSB) with the slopes for different variance ratios (VR = 2, 3, …, 5) are shown. Correlation coefficient is (cc = 0.258)

Pacific albacore tuna. The estimated SSB and R by the ISC had significant annual standard deviations (SD) (ISC 2014). For example, the SD for SSB was 30,203 mt for 1993 and 73,551 mt for 1971. The SD for R was 7.4 million fish for 1987 and 18.8 million fish 1971. This would mean that the stock-recruitment variance would be expected to be high.

When stock recruitment relationship was fit using the Ricker model (Equation 2), a density dependent pattern was shown with $p < 0.001$ (Figure 9*a*). When Ricker model was applied to the different regimes, regime 1 (1970–1988)had a large $p$-value ($p = 0.450$) suggesting the density independent model as a better fit (Figure 9*b*) whereas regime 2 (1989–2012) showed density dependent pattern ($p < 0.001$) (Figure 9*b*).

The inability of the proportional models from Equation 3 and Equation 4 to describe the stock-recruitment relationship of the North Pacific albacore tuna and the presence of different density dependent effects indi-

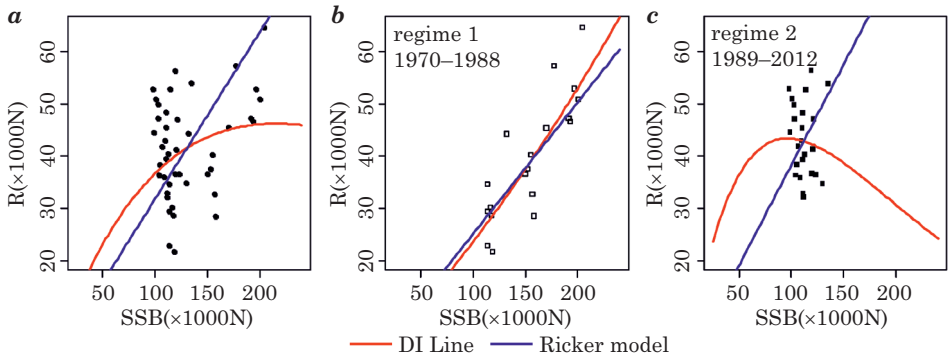


Fig. 9. The Ricker model fit to the stock recruitment relationship of the North Pacific albacore tuna. DI is the density independent line; (*a*) Data from 1970 to 2012 show density dependent relationship with $p < 0.001$; (*b*) regime 1 data from 1970 to 1988 had a large *p*-value ($p = 0.450$) suggesting the density independent model as a better fit; (*c*) regime 2 data from 1989–2012 had a small *p*-value ($p = 0.001$) suggesting the density dependent model as a better fit

cates strong influences of environmental condition on its stock characteristics. Equation 3 and Equation 4 can be modified to incorporate the environmental factors resulting in Equation 5 and Equation 6

$$\log(R_t) = \log(l) + \log(SSB) + f(y_{i,t-n}) + \varepsilon \tag{5}$$

$$R_t = l \cdot SSB_t, e^f \cdot f(y^{i,t-n}) + \varepsilon \tag{6}$$

where $f(.)$ denotes a function that takes into account the influence of the environmental conditions in the year $t$-n. $y_i$ denotes the environmental factors as well as the ecological factors with $y_i=[y_1,y_2,\ldots,y_k]$. $k$ is the number of environmental and ecological factors exhibiting relationship to the albacore tuna stock parameters. This case is apparent from the work of SINGH et al. (2017) where the environmental factors of SST and PDO were incorporated into the generalized additive models to explain the R trajectory of the North Pacific albacore tuna as shown in Equation 7

$$\log(R_t) = 1{,}284 \cdot \log(SSB_t) + 0.450 \cdot SST4_{t-2} - 11.423 \cdot PDO13_t + \\ + 0.661 \cdot (SST4_{t-2} \cdot \mathrm{PDO}_{u,t}) + \varepsilon \tag{7}$$

where $R$ represents the albacore recruitment for year $t$, $SST$ and PDO are the independent variables of the sea surface temperature and the Pacific decadal oscillation and ε is a normally distributed unsolved random variable. In SINGH et al. (2017), the individual and combined effects of the environmental conditions and SSB were tested using the equation 5 type. The combined effect of environmental conditions and SSB resulted in the most significant model through AIC and *p*-value comparison resulting in

the selection of Equation 5, $R^2 = 0.606$ ($p = 1.41 \cdot 10^{-10}$). PHILLIPS et al. (2014) also came up with a threshold generalized additive mixed model (tGAMM) for the North Pacific albacore tuna from 1961 to 2008 in the US West coast which incorporated the environmental variables of SST and PDO with $R^2 = 0.290$ ($p \leq 1.00 \cdot 10^{-4}$). This provides further evidence that environmental factors of SST and PDO have significant influence on the stock-recruitment relationship of the North Pacific albacore tuna.

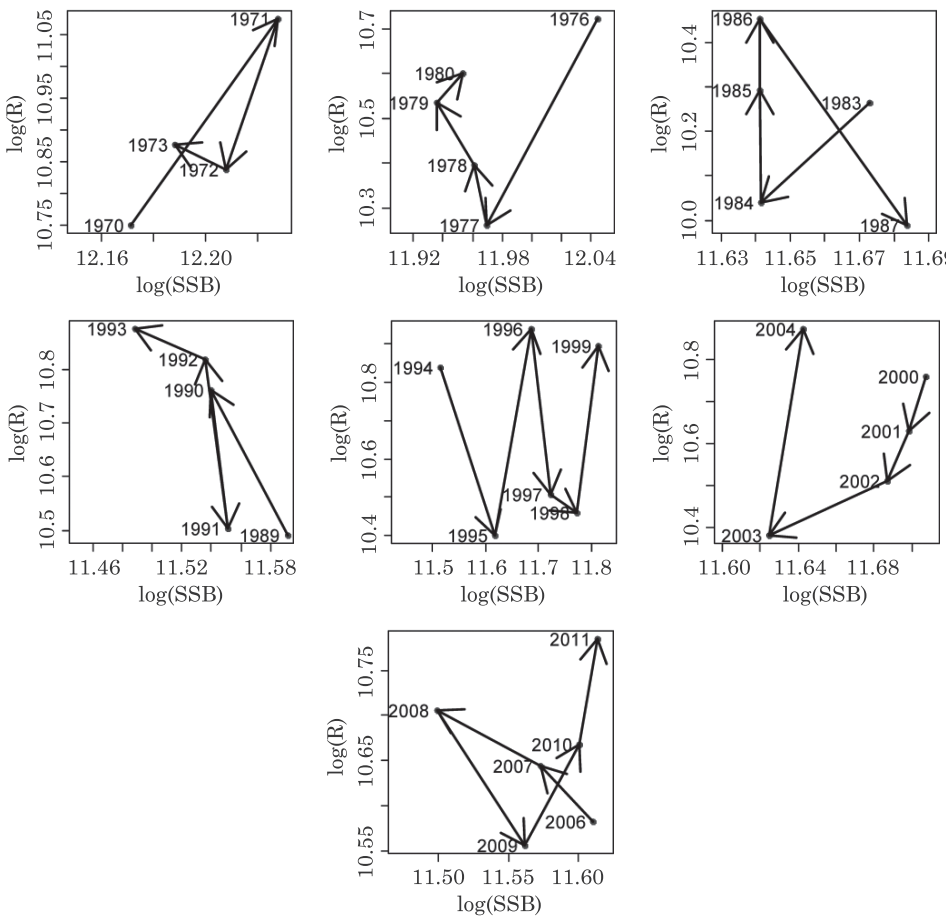


Fig. 10. Stock-recruitment relationship of the North Pacific albacore tuna. Log(R) and Log(SSB) denote the logarithm for the recruitment and female spawning stock biomass in year *t*. The clockwise and anticlockwise loops can be seen. (i) 1970–1973; (ii) 1976–1980; (iii) 1983–1987; (iv) 1989–1993; (v) 1994–1999; (vi) 2000–2005 (vii) 2006–2011

SAKURAMOTO (2015) studied the stock-recruitment relationship of the Pacific stock of the bluefin tuna (*Thunnus thynnus*) and Japanese sardine (*Sardinops melanostictus*). The study showed that the long-termcyclic

fluctuations in environmental conditions result in clockwise and anticlockwise loops in the stock recruitment relationship. The characteristics of such relationships are dependent upon the cycle of the environmental conditions and the reproductive cycle of the fish species. Indeed, the presence of clockwise and anti-clockwise loops can be seen for the stock recruitment relationship of the albacore tuna (Figure 10).

If we take Equation 5 as our main model then the stock recruitment relationship of albacore tuna will be more than 2-dimensional. Figure 11*a* shows the 3-dimensional plot when log(R) is plotted with log(SSB) and SST in April from Equation 5. Due to the large number of data points, a clear relationship cannot be observed. Figure 11*b* shows that increase in SSB and SST causes R to increase. As it can be seen, the complete relationship is better represented by the 3-dimensional construct compared to the

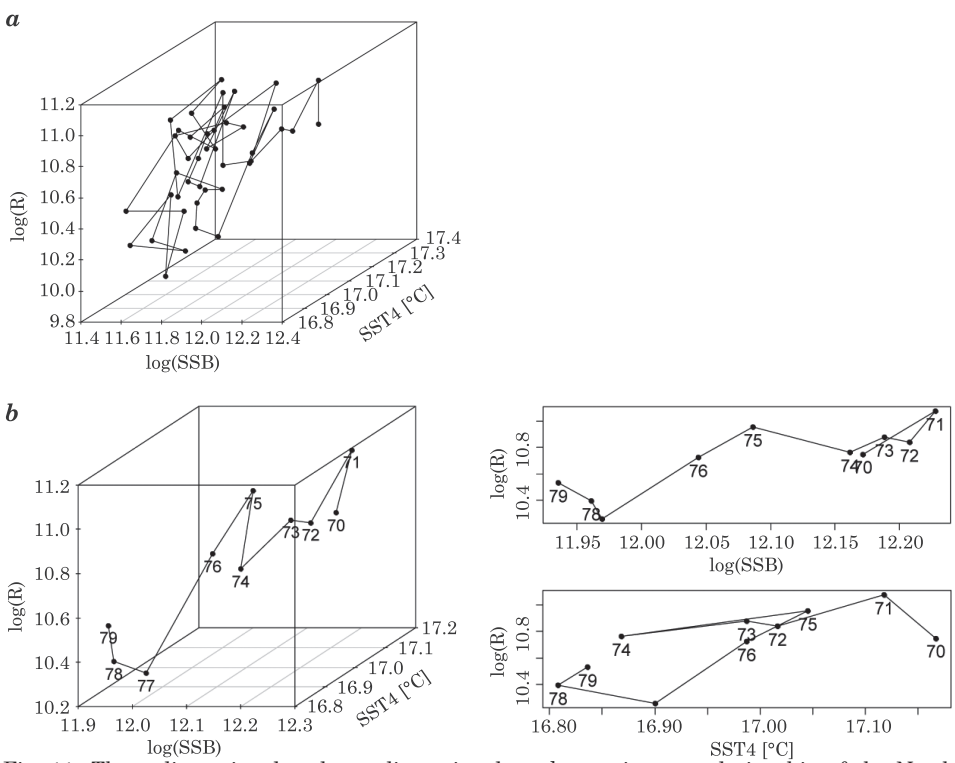


Fig. 11. Three dimensional and two dimensional stock-recruitment relationship of the North Pacific albacore tuna; (*a*) R, SSB and SST in April from 1970–2012; (*b*) 1970–1979

2-dimensional construct. Figure 12*a* shows the 3-dimensional construct of log(R) against log(SSB) and annual average PDO. In Figure 12*b* the looping characteristics of the stock-recruitment relationship in relation to

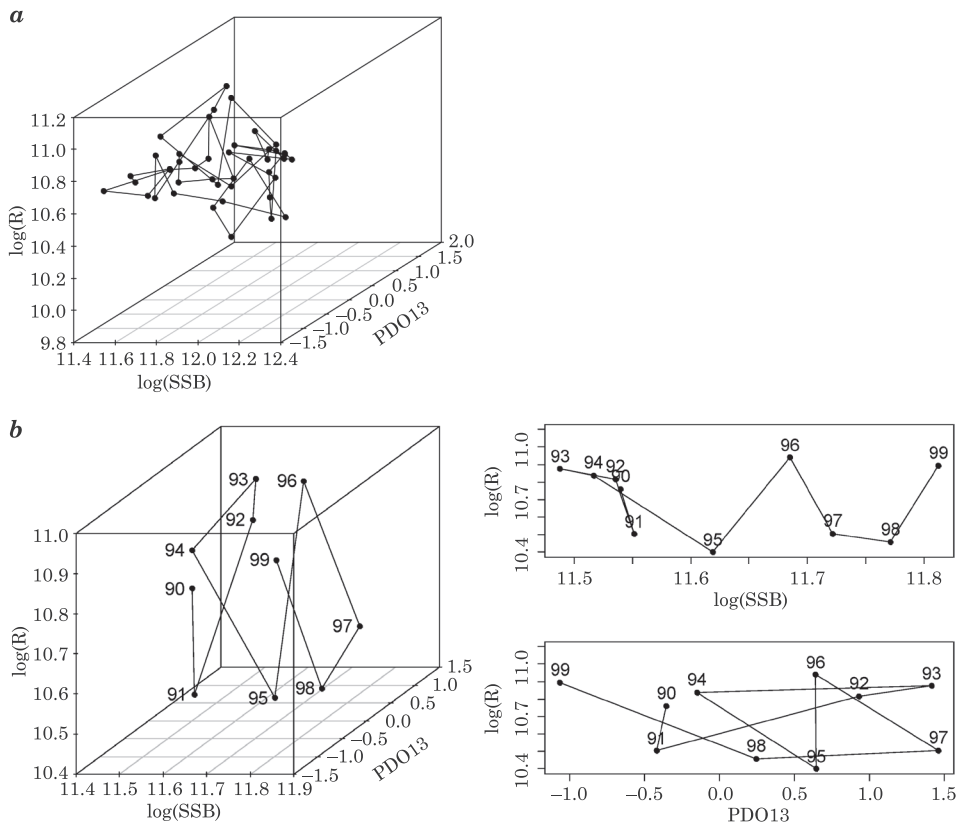


Fig. 12. Three dimensional and two dimensional stock-recruitment relationships of the North Pacific albacore tuna; (*a*) R, SSB and annual average PDO from 1970–2012; (*b*) 1990–1999

the PDO can be seen. This cannot be seen in the 2-dimensional models. Figure 11 and Figure 12 make it evident that the recruitment dynamics of the North Pacific albacore tuna cannot be determined by the SSB alone but together with the environmental conditions as shown by Equation 7.

## Discussion

This work was undertaken to identify if the stock-recruitment relationship of albacore tuna exhibit density-dependent effects and indicate a possible regime shift in the environmental conditions. Significant density-dependent effects of the SSB were detected on the North Pacific albacore tuna R and RPS. The changes in R and RPS pattern coincide with the annual trajectory of the SST (Figure 5, Figure 6) where a shift in the trajectory pattern was observed for the same period as that for the R and RPS

(Figure 3). This indicates that a possible regime shift in the North Pacific SST for the geographical coordinates of 50ºN – 120ºE, 10ºN – 120ºE, 50ºN – 120ºW, 10ºN – 120ºW (Figure 1) has occurred and has had significant impact on the stock-recruitment relationship of albacore tuna. Indeed, highly significant relationship between R, RPS, SSB and SST can be seen in Figure 4. PHILLIPS et al. (2014) investigated the correlation between the spatial distribution of the North Pacific albacore tuna and the local environmental variability of the SST in the US West Coast for a period of 48 years from 1961 to 2008. SST had significant and spatially variable correlation with albacore tuna CPUE and a significant geographical shift in the relationship was identified before and after the threshold year of 1986. Most significant tGAMM model was formed using SST and PDO as the independent variables. SST was the principal component that exhibited a regime shift which was mostly responsible for the shift in the spatial distribution of the albacore tuna juvenile CPUE before and after the threshold year.

With reference to Figure 3 and Figure 6, SST trend and R, RPS trends show changes in the same period from 1987 to 1989. Since the albacore R is estimated as age-0 fish, it makes sense that albacore R as well as RPS level will be affected in same period of the SST regime-shift. For the SSB, a change in the trend can be seen before and after 1982 for SSB which is a lag of 5–7 years when comparing with trend change of the R and RPS. This lag period coincides with the maturity ogive for North Pacific albacore tuna which is between 5–6 years (UEYANAGI 1957, ISC 2011, FARLEY et al. 2014). LEHODEY et al. (2003) showed shifts in trajectory patterns and the presence of regime shift indicators in the recruitment time series of skipjack (*Katsuwonus pelamis*), yellowfin (*Thunnus albacares*) in the Western and Central Pacific Ocean (WCPO) and the South Pacific albacore tuna from the early 1960s to the late 1990s. These patterns were shown to correlate to the positive and negative PDO phases and the ENSO time series pattern. The relationship of the climatic variables of PDO and ENSO with South Pacific albacore tuna has also been shown by SINGH et al. (2015). Indeed, significant correlations were detected between the albacore tuna R, RPS and SSB and the climatic variability of PDO and ENSO (Figure 5).

When simple regression analysis was applied, density-dependent effect was detected for log(RPS) versus log(SSB) as well as for log(R) versus log(SSB). When Deming regression was applied assuming the presence of observation errors, density-dependent effect was detected for log(RPS) versus log(SSB) but not for log(R) versus log(SSB). If we assume a variance ratio of < 3.0 in R and SSB then the findings of simple linear regression

and Deming regression do not synchronize. However, if we assume a variance ratio of $\geq 3.0$ then the results for simple linear regression and Deming regression coincide well with each other and both indicate the presence of different density-dependent effects in the stock-relationship. The Ricker model revealed different stock recruitment relations for the two albacore stock regimes. This further supports the occurrence of different density dependents effects in the stock recruitment relationship of the North Pacific albacore tuna stock indicating the likely influences of extrinsic factors on the trajectory of the albacore tuna.

The relationship between R and SSB is biological in nature and there is no doubt that one affects the other. Environmental factors influence the stock characteristics of albacore tuna between the successive generations of R and SSB. It is evident that using environmental factors leads to the improvement of the stock-recruitment relationship (Equation 7) for the North Pacific albacore tuna. The stock-recruitment relationship can be represented by Equation 5. That is,

$$\log(R_t) = \log(l) + \log(SSB) + f(y_{i,t-n}) + \varepsilon \tag{5}$$

The stock recruitment relationship in this case is multidimensional, incorporating the influences of various biotic and abiotic variables.

The results presented here still remain semi-conclusive and form the platform for further studies. The models presented are based on the best estimates of variables that are available and come with their own sources of possible errors. The stock-recruitment variability and uncertainties are expected as the estimates of R and SSB are based on fishing activity data from different countries with fishing methods differing between as well as within countries. The fishing season, period and area also differ among different data sources used for the estimates (ISC 2014). The actual stock--recruitment relationship can be masked by observation and/or process errors (SAKURAMOTO and SUZUKI 2012) which likely exist in the data used in this study and further work is needed to elucidate this. Different fishing regimes will have variable influence on albacore population structure inclusive of the spawning stock structure, spawning success and various other variables under the stock recruitment variability. The key uncertainties noted in the data used in this study are insufficient sex-specific size data and the unavailability of updated parameters of maturity and natural mortality (ISC 2014). The time series data ranges over a period of more than four decades. Over this period there has been changes in fishing technology and data recording system have also developed. The efficiency of one unit of effort would be expected to improve with improvement of technology. For example, in the 1970s the efficiency of a fishing hook

would be expected to have been lower compared to a fishing hook in 2010. As such, a unit of effort in 1970 may not be the same as a unit of effort in 2010. Data recording and storage systems have also seen improvement. One would expect higher reliability (lower stock-recruitment variability) of recent data in comparison to earlier recorded data.

Estimates derived from the stock-recruitment relationships are fundamental for fisheries stock management. Quantities derived from the relationship are used to set fishing quota limits and overexploitation reference points. Stock-recruitment parameter estimates are significantly influenced by measurement errors in recruitment and spawning biomass including time series bias. Such uncertainties make the determination of stock-recruitment relationships is a challenging task for fisheries stock assessors (HILBORN and WALTERS 1992, SAKURAMOTO and SUZUKI 2012).

The SST data for the study area was based on the HadISST as described in RAYNER et al. (2003). HadISST was based on a combination of SST data from a diversity of methods. The necessary bias adjustments were made to the data. For the SST up to 1981 the reduced space optimal interpolation (RSOI) method was applied (KAPLAN et al. 1997). For SST from 1982 to 2012, RSOI was applied to the combination of in situ and satellite SST. Further bias adjustments were made to the data following the method of JONES et al. (2001) to homogenize the SST grid-scale variance. PDO index is estimated using the SST in the Pacific north of 20ºN (MANTUA et al. 1997). ENSO calculation is determined using the variables of wind indexes, sea-level pressure, SST, atmospheric conditions and surface air temperature (WOLTER and TIMLIN 1998) with each variable having its own possible sources of bias such as that mentioned in ZHANG et al. (2013).

WANG and LIU (2005) tested the reliability of AIC in selecting the model fit for various stock-recruitment relationships. The results ascertained the validity of AIC in selecting the most suitable relationship. SINGH et al. (2017) used the AIC for model selection, resulting the model represented by Equation 7. This gives validity to the stock-recruitment relationship presented in this work represented by Equation 5. Log transformation of the relationship represented by Equation 5 stabilizes the residual variance of the dependent and independent variables and reduces the effects of possible observation and process errors present in the variables (HILBORN and WALTERS 1992).

The stock–recruitment models presented here may are based on dependent and independent variables. As outlined previously, each variable comes with its own bias. The estimated variables are adjusted for bias as efficiently as presently possible. Based on this and the modeling techniques used, the models can be expected to have sufficient reliability,

however bias is still expected to be present in the stock-recruitment relationship. Detailed studies of the North Pacific albacore tuna age-class and its relation to the SST are needed to conclusively determine the stock-recruitment relationship of the North Pacific albacore tuna and the environmental regime shift. With the results presented here, albacore tuna fishery managers need to be wary of the influences following an environmental regime shift especially for the SST where albacore R and RPS trajectory pattern closely follow the SST behavior. Such events can influence stock abundance both with time and distribution and managers need to adjust their harvesting plans accordingly.

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# CHANGES IN LAND USE IN THE DIRECT CATCHMENT OF LAKE GĄSAWSKIE IN THE PERIOD OF 1945–2011 IN VIEW OF ITS ECOLOGICAL STATUS*

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Key words: ecological status, changes in lake area, land use, macrophytes.

Abstract

The aim of this study was to evaluate land use in the sub-catchment (i.e. direct catchment) of Lake Gąsawskie in the period of 1945–2011, focusing on its effect on the current ecological status of the lake.

The rate of lake overgrowing and changes in its area were assessed over the period of 66 years. The extent of sewerage cover in the Gąsawa commune located in the direct catchment of the lake was taken into consideration. The lake catchment is characterised by the predominance of arable land. Analysis of cartographic materials from 1945, 1991 and 2011 showed an increase in building development in the catchment of the lake at the expense of the percentage of arable land. At the same time the area of this lake was found to decrease from 105.85 ha to 94.98 ha, i.e. by 10.3%. The rate of its depletion is 0.15 ha annually.

Studies of the ecological status conducted using macrophytes index ESMI and physico-chemical parameters of water showed a poor condition of the lake. This is also evidenced by a limited number of plant communities, a slight share of immersed macrophytes, particularly hornwort (*Ceratophyllum demersum*). High concentrations of nutrients promote an intensive development of phytoplankton, as indicated by low water transparency.

Results of this study showed that undertaking effective actions in the lake catchment is necessary to limit further degradation of Lake Gąsawskie waters.

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# ZMIANY SPOSOBU UŻYTKOWANIA GRUNTÓW W ZLEWNI BEZPOŚREDNIEJ JEZIORA GĄSAWSKIEGO W LATACH 1945–2011 W KONTEKŚCIE JEGO STANU EKOLOGICZNEGO

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Słowa kluczowe: stan ekologiczny, zmiana powierzchni jeziora, użytkowanie terenu, makrofity.

Abstrakt

Celem badań była ocena sposobu użytkowania gruntów w zlewni bezpośredniej Jeziora Gąsawskiego w latach 1945–2011 ze szczególnym uwzględnieniem jego wpływu na aktualny stan ekologiczny jeziora.

Oceniono tempo zarastania jeziora oraz zmiany jego powierzchni w ciągu 66 lat. Zlewnia jeziora charakteryzuje się dominacją gruntów ornych. W analizie materiałów kartograficznych z lat 1945, 1991 i 2011 wykazano wzrost zabudowy w zlewni jeziora kosztem udziału gruntów ornych. Jednocześnie stwierdzono zmniejszenie się powierzchni jeziora z 105,85 ha do 94,98 ha, czyli o 10,3%. Tempo jego zaniku wynosi 0,15 ha rocznie.

W badaniach stanu ekologicznego wykonanych metodą ESMI i parametrów fizyczno-chemicznych wody wykazano słaby stan jeziora. Świadczy o tym również niewielka liczba zbiorowisk roślinnych oraz nieznaczny udział makrofitów zanurzonych, w szczególności rogatka sztywnego (*Ceratophyllum demersum*). Wysokie stężenie biogenów sprzyja intensywnemu rozwojowi fitoplanktonu, o czym świadczy niska przezroczystość wody.

Wykazano, że podjęcie skutecznych działań w zlewni jeziora jest niezbędne, aby ograniczyć dalszą degradację wód Jeziora Gąsawskiego.

## Introduction

Lakes are a significant component of landscape, as well as an important element of the ecosystem, on which their existence is dependent. Water bodies are responsible for the modification of microclimate, hydrogeological conditions, while also being a reservoir of water for all living organisms. In relation with the increasing demand for water and climate change, the accelerating incidence of droughts and torrential rains, water resources are becoming increasingly depleted. The present-day society faces a very difficult challenge connected with the extension of water discharge from the catchment and ensuring its best possible quality.

In order to maintain good water quality it is necessary to undertake effective actions aiming at the limitation of its further degradation and finally improvement of its condition. This was the objective for the Water Framework Directive (European Commission Directive 2000), on the

power of which the approach to evaluation of water and its resources was changed, treating water as the environment for living organisms and not as it was the case previously – in view of its economic use. Biological evaluation of the ecological status of waters was introduced, comprising the quantitative and qualitative status of phytoplankton, macrophytes, phytobentos, bentic macroinvertebrates and ichthyofauna. Hydromorphology and physico-chemical parameters of waters supplement analyses of the biological condition of waters.

Major source of water pollution are area sources (GARDNER et al. 2002, JASIEWICZ and BARAN 2006, DURAND et al. 2011, PYTKA et al. 2013), primarily of agricultural origin. They result e.g. from the application of excessive doses and inappropriate dates of mineral and artificial fertilisation, crop monoculture and failure to apply crop rotation (GROCHOWSKA et al. 2014, LAWNICZAK et al. 2016). Agricultural land use has also influenced water retention in soil, caused decreased soil infiltration capacity and faster water drainage from the catchment (KĘDZIORA 2007).

Land use in the catchment is crucial for the maintenance its good quality. Frequently the share of point pollution is considerable in water pollution. Nevertheless, in recent years we have been observing an increased effect of area pollutants in water pollution, which is connected with improved sewage disposal in catchments and elimination of point pollution sources (BOROMISZA 2013).

Point sources connected with agricultural land use include illegal dumps, inappropriate disposal of animal waste, artificial fertilizers or pesticides. Although the development of sewerage systems has been intensive in recent years, it is necessary to ensure adequate sewage treatment and maintain adequate leak tightness of sewerage systems. All these pollutants may penetrate to surface waters through underground flow, subsurface flow and surface runoff.

A significant threat may also result from the recreation management of land directly adjacent to the water body. Recreation infrastructure facilities often have no regulated water supply and sewage disposal systems, or municipal waste disposal, which leads to problems with an increased nutrients inflow (ŁAWNICZAK et al. 2015).

Inflowing pollutants accelerate the natural, slow eutrophication process. These changes lead to disappearance of lakes, which is manifested in two simultaneous processes. One of them is connected with the decreasing lake depth due to the deposition of plant residue and animal waste on the bottom. The other process is related with overgrowing, which as a result of the decreasing depth of the water body increases the potential area for colonization by aquatic vegetation. Thus it is absolutely crucial to ensure

proper catchment management so as to minimize the risk of deterioration of the condition of its waters.

The aim of this study was: 1) to evaluate land use in the direct catchment of Lake Gąsawskie in the period of 1945–2011, focusing on the effect on the current ecological status of the lake; and 2) to assess the rate of overgrowing and disappearance of the lake during the analyzed period.

## Methods

Catchment use was evaluated using the vector Georeference Database of Topographic Objects (GBDOT 2011). That map presents the status of 2011 at a 1:10000 scale. This study takes into consideration all elements from the class of land cover objects and the class of rushes and marshes. For the purpose of this study these data were divided into the following categories: built-up areas, meadows and pastures, forests, wetlands, arable lands, surface waters, the littoral zone within the analyzed lake including emergent vegetation and vegetation with floating leaves. Cartographic analyses were conducted in the ArcGIS environment. The lake catchment was based on the Map of Hydrographic Division of Poland at a 1:10000 scale (MPHP 2010). The littoral zone of the lakes was analyzed using additionally an orthophotomap with a 0.25 m resolution (ISOK 2010).

The historical cartographic material used in this study comprises topographic maps from the 1990's in the Polish Coordinate System of 1965, as well as German topographic maps at a 1:25000 scale (Mestichiblatte 2nd edition) of 1945. The rate of the water body overgrowing was also assessed using black-and-white aerial photographs taken in 1966. Preparation of archival materials consisted in the provision of georeferences in their original system of geographic coordinates, followed by their transformation to the current national geodetic coordinate system (PUWG 1992) system. The next process comprised digitalization and classification of land use in accordance with the adopted categories.

The rate of lake overgrowing was assessed based on maps referring the area of the pelagic zone free from emergent vegetation in the analyzed years to the shoreline of the lake in the 1940's.

The degree of sewerage system cover in the direct catchment of the lake was evaluated based on data from the Local Data Bank (GUS 2016) in relation to the Gąsawa commune.

The ecological status of Lake Gąsawskie was evaluated based on the Ecological Status Macrophyte Index (ESMI) (Ciecierska 2008) as well as physico-chemical parameters of water.

In-situ studies comprised analyses of the lake vegetation condition (CIECIERSKA 2008), consisting in:

– determination of transects of 30 m in width and length determined by the depth of plants, located perpendicular to the shoreline;

– identification of cover for plant communities found in a given transect;

– estimation of total bottom cover with vegetation in a given profile;

– estimation of cover for individual communities in the Braun-Blanquet scale;

– determination of the depth range of macrophytes (for each community).

Analyses were conducted from a dinghy and from the lake shore. An anchor was used to identify submerged vegetation.

Based on the collected data the ESMI values were calculated:

$$\mathrm{ESMI} = 1 \quad \exp\left(\frac{H}{H_{\max}} \cdot Z \cdot \exp\left(\frac{N}{P}\right)\right)$$

where:
ESMI – Ecological Status Macrophyte Index
H – phytocenotic diversity index
$H_{\max}$ – maximum phytocenotic diversity
$Z$ – colonization index
$N$ – total littoral area (100%)
$P$ – lake area [km$^2$].

Percentage of plant cover was evaluated base on Braun-Blanquet scale (Table 1) (WIKUM and SHANHOLTZER 1978)as required by United States government regulatory agencies, vegetation studies are conducted using a variety of methods. Density measurement (stem counts. A total of 20 transects, uniformly distributed in the water body, were prepared. Analyses were conducted in July 2014.

Table 1

Braun-Blanquet cover-abundance scale (WIKUM and SHANHOLTZER 1978)

| Braun-Braunquet scale | Range of cover [%] |
|---|---|
| 5 | > 75 |
| 4 | 50–75 |
| 3 | 25–50 |
| 2 | 5–25 |
| 1 | < 5; few individuals |
| + | < 10, few individuals |
| r | < 5, few individuals |

Water samples for quality testing were collected in deepest part of the lake from the subsurface layer of the lake and comprised the determination of the following indexes:

– total phosphorus – by mineralization using persulfate in an acid environment (HACH DR/2800);

– ammonia nitrogen – by colorimetry using the salicylate method (HACH DR/2800);

– nitrite nitrogen – by spectrophotometry using the diazotization method (HACH DR/2800);

– nitrate nitrogen – by spectrophotometry using the cadmium reduction method (HACH DR/2800) (HACH 1992).

The ecological status of the lake was evaluated in relation to the Regulation of 21 July 2016 (Rozporządzenie Rady Ministrów z 21 lipca 2016… Dz.U. 2016 poz. 1187).

## Study area

Lake Gąsawskie is located in the Kujawsko-Pomorskie province, the Żnin county, the Gąsawa commune (Figure 1). Its direct catchment occupies the area of 1148.78 ha. The central part of the catchment is covered by legal protection as the Protected Landscape Area of Żnińskie Lakes, which aims at the protection of the landscape of the lacustrine-riverine channel as well as the cultural and historical value of the area. The lake is elongated in shape from the south-east to north-west with an enlarged part in its southern part. The shoreline is poorly developed ($k$ = 1.51) with a length of 5.3 km. Lake Gąsawskie constitutes a Uniform Surface Water Body, code PLLW 10455. Its abiotic type is 3a, which means that it is a lake with a high calcium content, with a considerable effect of the catchment, stratified. Lake area is 99 ha, while its maximum depth is 10.5 m and mean depth is 5.8 m (*Bathymetric map*… 1958). The Gąsawka River, a left tributary of the Noteć, flows through the lake. This watercourse flows through several lakes, of which Lake Gąsawskie is the second in the river course, fed with waters of the Gąsawka from Lake Oświęcickie. The Gąsawka next flows through Lakes Godawskie, Biskupińskie, Skrzynka, Weneckie, Skarbińskie, Żnińskie: Małe and Duże, Dobrylewskie and Sobiejuskie, to flow into the Noteć near Rynarzewo. Boulder clay predominates in the lake catchment (Kozłowska and Kozłowski 1992).

In the assessment of lake susceptibility degradation according to the Lake Quality Assessment System (Kudelska et al. 1994) Lake Gąsawskie was classified to category II with the total score of 2.43, i.e. a lake

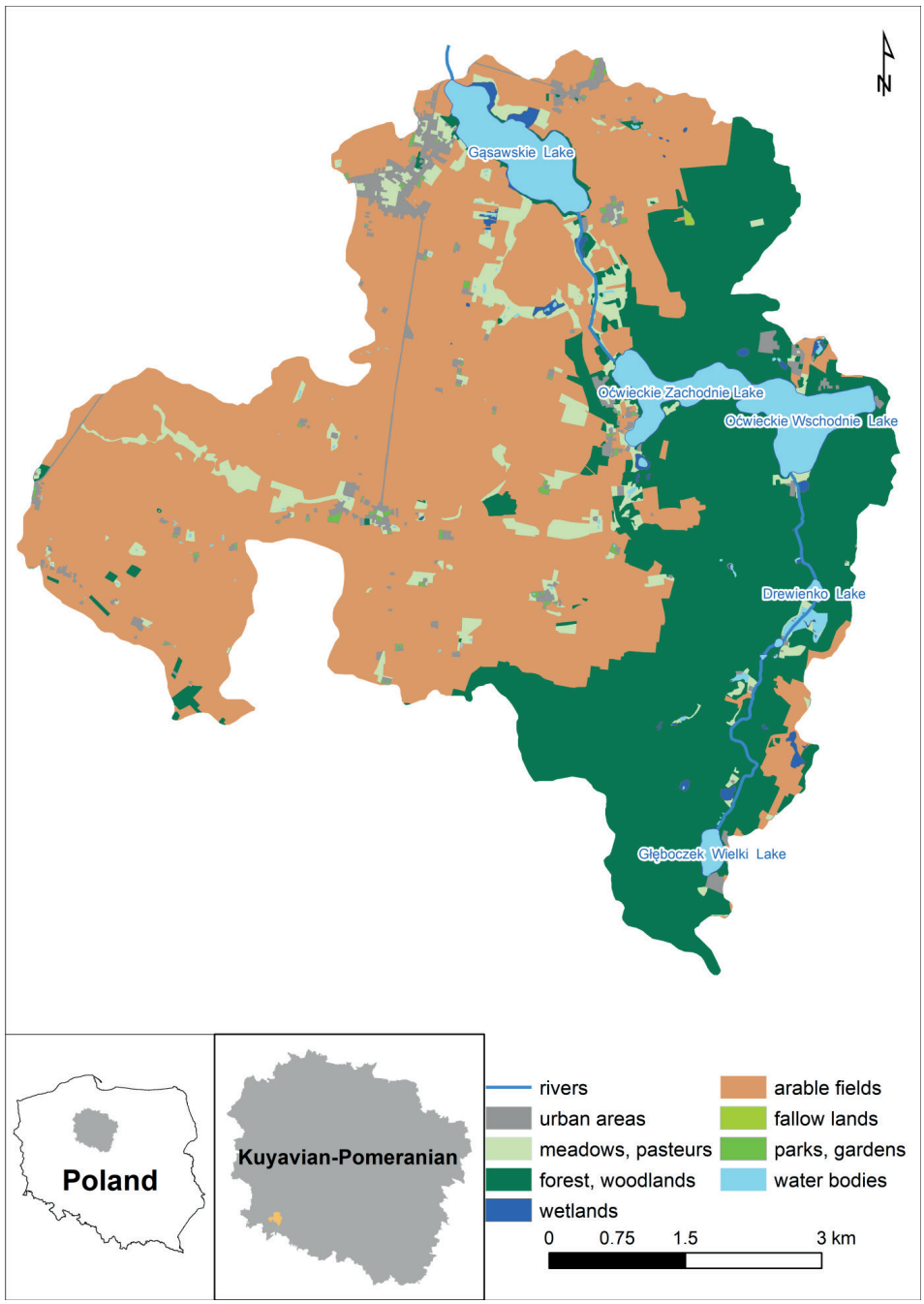


Fig. 1. Location and land use in total catchment of the Gąsawskie Lake

moderately resistant to degradation. A low ratio of lake volume to the length of its shoreline (1.07 thousand $m^3\ m^{-1}$) and the agricultural character of the catchment to the greatest extent determine the reduced lake resistance to degradation. The most advantageous parameter is the ratio of active bottom area and the volume of the epilimnion (of 0.10 $m^2\ m^{-3}$), which shows internal enrichment of the lake with nutrients. The Schindler index, i.e. the ratio of total catchment area with the area of the lake to lake volume, indicates the considerable role of the catchment in the modification of quality parameters in waters of Lake Gąsawskie ($H = 8.3$) (MARKIEWICZ 2005).

## Results and Discussion

### Land use structure in the catchment

In the catchment of Lake Gąsawskie arable land predominates, covering the area of 780.2 ha, i.e. 67.9% (Table 2, Figure 2). In comparison to the 1940's the area of arable land decreased slightly by 2.6% (i.e. by 21 ha).

Table 2
Area of analysed land use forms in the direct catchment of Lake Gąsawskie in 1945, 1990 and 2011 years

| Land use forms | Area [ha] | | |
|---|---|---|---|
| | 1945 | 1990 | 2011 |
| Urban areas | 40.0 | 62.3 | 67.4 |
| Meadows, pasteurs | 84.4 | 77.8 | 73.7 |
| Forest, woodlands | 110.6 | 112.7 | 112.5 |
| Wetlands | 4.1 | 16.2 | 11.6 |
| Arable fields | 801.1 | 776.8 | 780.2 |
| Fallow lands | 0.0 | 0.2 | 0.2 |
| Parks, gardens | 0.6 | 5.7 | 5.7 |
| Water bodies | 2.1 | 2.8 | 2.6 |

It is a similar result to the changes taking place in the Wielkopolska region (2.0%) within the last 100 years, where a relatively limited area of utilized agricultural area is replaced by forests and anthropogenic areas (MATYKA 2012). In terms of land use area forests rank second in the lake catchment (112.54 ha, i.e. 9.8%; Table 2), located mainly at the eastern boundary of the catchment and overgrowing a narrow belt along the southern lake shore (Figure 2). Their area in the analyzed period did not change markedly, as

it decreased by as little as 1.9 ha. Such a low share of tree stands in the lake catchment is not an advantageous phenomenon, particularly as forested areas exhibit positive properties in the pollutant filtration and purification, thus being highly desirable elements in catchments (HEFTING et al. 2005, RANALLI and MACALADY 2010). Along the 2/3 length of the shoreline a narrow belt of trees is found, with a mean width of 25 m. As it was shown by studies of HEFTING et al. (2005) and AGUIAR et al. (2015), in order to ensure high effectiveness in pollution reduction its width should be min. 60 m, particularly since the large share of farmland is connected with an inflow of pollutants with surface runoff, subsurface flow and underground runoff (CARPENTER 1998, LAWNICZAK et al. 2016). This pertains particularly to fields cropped to maize (LAWNICZAK et al. 2016), found in the direct vicinity of the lake.

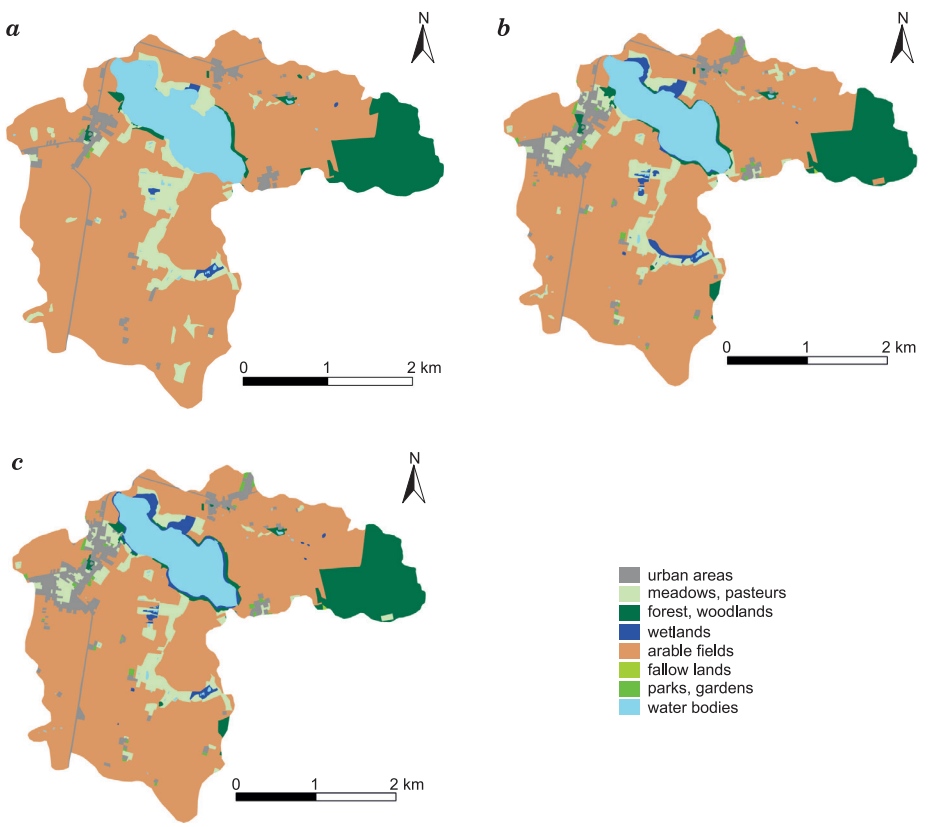


Fig. 2. Land use in the direct catchment of Lake Gąsawskie in: a) 1940 b) 1990 c) 2011 years

Building development is concentrated mainly in the northern part of the catchment and it comprises first of all the villages of Gąsawa and Łysin, which joint area is 67.4 ha, i.e. 5.9% of the catchment (Table 2, Figure 2). Within the 66 years their area increased by 27.4 ha, i.e. by 2.4% area of the entire catchment. The increase in built-up areas leads to a faster runoff of surface waters to lakes (ŁAWNICZAK et al. 2015). Rainwater from built-u areas is drained to surface waters, causing their contamination with municipal pollution, heavy metals, sparsely soluble motor oils (SŁOWIK et al. 2008, PIEKUTIN 2016). The tendency towards an increase in urbanized areas may be observed worldwide (PAUL and MEYER 2001), which has an adverse effect on water quality and a reduction of natural and seminatural areas.

In the catchment of Lake Gąsawskiego surface waters occupy approx. 7.81% (i.e. 97.6 ha) and they are located mainly in its central part (Figure 2). The greatest complex of small water bodies is found surrounded by meadows, which take an area of 2.6 ha. In the course of 66 years their area decreased by as little as 0.5 ha. Meadows and pastures decreased in area by 10.7 ha and currently they cover 73.3 ha, which largest share is found in the southern part of the catchment.

## Water supply and sewage disposal

In the Gąsawa commune the water supply and sewage disposal system is poorly developed. The degree of sewerage system cover in the last decade increased by as little as 13%. Moreover, only 43% population use the sewerage system, at an almost complete cover of the commune area by the water supply system (Table 3). Most farms use drainless tanks with an unknown degree of leak tightness.

Table 3

The percentage of population using the water supply and sewerage system in the Gąsawa commune in the years 2003–2014 (GUS 2016)

| System | 2003 | 2004 | 2005 | 2006 | 2007 | 2008 | 2009 | 2010 | 2011 | 2012 | 2013 | 2014 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Water supply [%] | 92.6 | 92.1 | 92.3 | 92.1 | 91.9 | 92.6 | 93.0 | 93.6 | 93.7 | 93.4 | 93.5 | 99.9 |
| Sewerage [%] | 29.9 | 30.1 | 33.7 | 33.9 | 34.4 | 34.7 | 35.2 | 35.5 | 36.7 | 38.5 | 40.1 | 43.0 |
| Ratio sewerage/water supply | 0.32 | 0.33 | 0.37 | 0.37 | 0.37 | 0.37 | 0.38 | 0.38 | 0.39 | 0.41 | 0.43 | 0.43 |

**Overgrowing rate and changes in lake area.** The analysis of changes in lake area in the period of 1945–2011 showed a decrease in its area by 10.3% (i.e. 10.9 ha) in relation to the reference year of 1945 (Table 4), which is equivalent to the decrease rate of 0.15 ha annually. Slight fluctu-

ations of 0.5 ha between 1990 and 2011 may result from changes in water levels, at which analyses were conducted, or from the difference in the scale of examined maps. The results in relation to studies by other authors are very similar. For the Wielkopolska region the process of lake disappearance is 15.21% from 1920 to 1975 (CHOIŃSKI and PTAK 2008), which is one of the most significant in Poland. For example, for the Pomerania Lake District this rate is 12% in the class of water body size of 50–100 ha (PTAK 2013).

Table 4

Changes in area of Lake Gąsawskie in the period of 1945–2011

| Year | Area [ha] | Changes in area of lake [%] in comparison to the year 1940 |
|---|---|---|
| 1945 | 105.85 | – |
| 1966 | 97.58 | 7.8 |
| 1990 | 94.47 | 10.8 |
| 2011 | 94.98 | 10.3 |

The overgrowing rate for Lake Gąsawskie in the 1940's was 14%. In 1990 the area covered by emergent macrophytes decreased to 12.03%. At present the share of the emergent littoral in the lake in comparison to the 1940's increased by 18.7%. The rate of lake overgrowing in the period of 1940–1990 was 0.04 ha/year in the period of 1940–2011 was 0.05 ha/year.

**Evaluation of the ecological status of the lake.** Phytosociological studies carried out in 2014 showed in Lake Gąsawskie the existence of only five well-developed macrophyte communities. Vegetation did not cover only a short section of the shoreline, on which a bathing beach was located. The greatest percentage in the littoral zone of the lake was recorded for the community with common reed (*Phragmites australis*), occupying 88.3% (i.e. 5.21 ha) of the littoral, characterised by greatest frequency, since it was the only one found in all examined transects. The reed rush was found over the entire length of the shoreline except for the short section of the beach. The other taxa accounted for a much lesser percentage in the lake. Macrophytes with floating leaves showed lesser frequency. They were recorded in 5 transects (i.e. 25% analysed transects), but only in one they were abundant. They covered an area of 0.56 ha, i.e. 9.48% littoral zone (Figure 2). Submerged plants were represented by only one taxon – hornwort (*Ceratophyllum demersum*), which covered an area of 0.12 ha (i.e. 2% total littoral and they were found along the rush belt.

Macrophytes were found to a mean depth of 0.9 m. The most distant locality was colonized by nymphoid-water forms and it was found at a depth of 1.6 m. Only in three analysed transects the depth reached by vegetation exceeded 1 m. The Shannon diversity index, reflecting the actual taxonomic composition, was low amounting to 0.48, similarly as the maximum phytocenotic diversity at 1.61. The colonization index, which describes the ratio of the actual littoral area to the potential area which may be colonised by plants, i.e. a depth of 2.5 m, was found to be 0.36 m. These results indicate adverse conditions determining the development of litoral in Lake Gąsawskie.

Ecological groups of plants and their quantitative ratios are presented in Figure 3. A vast majority of littoral vegetation (89% littoral) and a very small number of elodeid-form species (2%) is characteristic to lakes of poor ecological status (CIECIERSKA et al. 2013, PEŁECHATY and PRONIN 2015). This is particularly evident, since this group was represented only by hornwort, which is a species characteristic to waters with high trophic levels (MURPHY 2002).

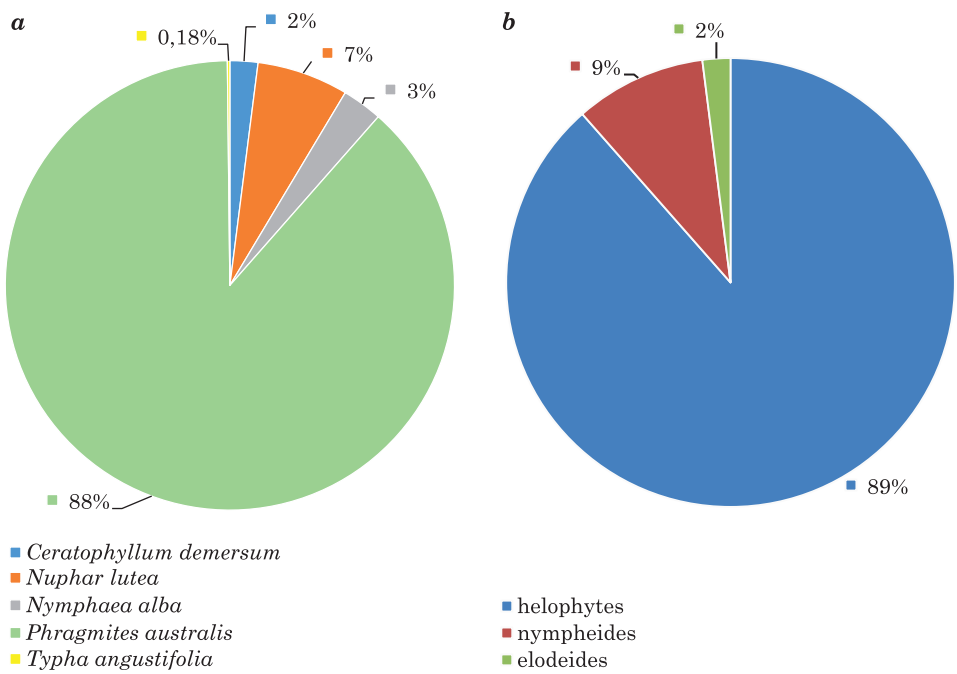


Fig. 3. Percentage of *a*) dominant macrophytes *b*) plant growth forms in the littoral zone of Lake Gąsawskie

The Ecological Status Macrophyte Index was 0.107, which indicates a poor ecological status of this lake. Results of water quality analyses showed high concentrations of nitrogen and total phosphorus as well as poor water transparency indicated by low visibility in the Secchci disc test (Table 5). In view of biological and physico-chemical parameters the condition of the lake was evaluated to be poor.

Table 5

Evaluation of ecological status of Lake Gąsawskie in 2014 according Polish Regulation (Rozporządzenie Rady Ministrów z 21 lipca 2016… Dz.U. 2016 poz.1187)

| Indicator | Unit | Value | CWQ* |
|---|---|---|---|
| Biological elements | | | |
| ESMI | – | 0.107 | IV |
| Physico-chemical parameters | | | |
| Transparency of water – Secchi depth | m | 0.45 | <II |
| Total nitrogen | mg N $dm^{-3}$ | 7.078 | <II |
| Conductivity | μS $cm^{-1}$ | 647 | I/II |
| Total phosphorus | mg P $dm^{-3}$ | 0.075 | <II |
| The ecological status based on biological parameters | | poor | |
| The ecological status | | poor | |

*CWQ – class water quality; I/II – very good and good, < II – ecological status under good

In comparison to the 1980's the condition of the lake deteriorated (MAKAREWICZ 2005). In 1987 it was classified to water quality class III, i.e. such that may be used as a source of water supply for industrial plants except for those requiring classes I and II, as well as for watering agricultural and horticultural areas (Rozporządzenie Rady Ministrów z 9 czerwca 1970… DZ.U. 1970 no. 17 poz.144).

A bad lake condition was also found in Lake Oświęcickie, located above Lake Gąsawskie (MAKAREWICZ 2005). Despite the considerable share of forests in the direct catchment of the lake the quality of its water is not satisfactory. As it was indicated by (MAKAREWICZ 2005, STACHNOWICZ and NAGENGAST 2010), the primary cause is the inappropriate sewage disposal in the catchment of this lake.

## Conclusions

The analyses of the condition of Lake Gąsawskie showed an unsatisfactory water quality and poor ecological status based on the macrophyte index and physico-chemical parameters of water. In the course of 66 years

the area of this lake decreased from 105.85 ha to 94.98 ha, i.e. by 10.87 ha. The rate of its disappearance is high, but comparable to other lakes located in agricultural areas of the Wielkopolska region. The most significant changes which have taken place in the land use structure in the catchment of Lake Gąsawskie in the years 1945–2011 include an increase in anthropogenic areas, particularly building development, at the expense of arable land. This is particularly important, since the sewerage system cover in the Gąsawa commune, where Lake Gąsawskie is located, is only 43%. The effect of agricultural land use in the catchment and the lack of regulated sewage disposal in the catchment is manifested in the poor condition of the lake. This is evidenced by the very low number of plant communities, the slight share of submerged macrophytes, including the species characteristic of fertile habitats, i.e. hornwort. High nutrient concentrations promote intensive development of phytoplankton resulting in limited water transparency, effectively limiting development of submerged vegetation. At the same time we may observe an intensive development of emergent vegetation, influencing the gradual overgrowing of the lake. Results of this study showed that undertaking effective actions in the lake catchment is crucial for any improvement of water quality in Lake Gąsawskie.

Translated by Anna Binczarowska

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# ANALYSIS OF TOURIST ATTRACTIVENESS OF COMMUNES SITUATED ON THE KRZNA RIVER

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Key words: tourist attractiveness, the Krzna river, statistical and econometric methods.

Abstract

The paper presents an analysis of conditions affecting the tourist attractiveness of communes situated along the banks of the Krzna river. The aim of the study was to present the characteristics of tourist traffic recorded in the area of the communes through which the Krzna River flows. The objective of the work was to carry out multi-trait comparisons of communes located on the Krzna river in terms of tourist attractiveness, to determine factors which are the major source of differences between the communities analysed, and to group the communes with similar tourist attractions. The evaluation of commune tourist attractiveness in terms of tourist traffic intensity was based on basic indicators of tourism function (Baretje-Defert index, Schneider index and other) as well as selected variables describing the natural environment status of communes (forests and woodlands, area covered by water). The analysed data is for the year 2015 and comes from the Central Statistical Office of Poland *Local Data Bank*. Principal component analysis (PCA) was used in order to determine multidimensional associations between the characteristics studied. The communes examined differed the most in terms of the characteristics which were the most strongly correlated with the first principal component (PC1). The communes were assigned to 3 groups with different tourist attractiveness properties based on cluster analysis. PCA made it possible to distinguish factors which were the strongest determinants of tourism conditions in the study area. Cluster analysis used to group communes is a useful tool to evaluate the potential of tourism development in communes.

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# ANALIZA ATRAKCYJNOŚCI TURYSTYCZNEJ GMIN POŁOŻONYCH NAD RZEKĄ KRZNĄ

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Słowa kluczowe: atrakcyjność turystyczna, rzeka Krzna, metody statystyczne i ekonometryczne.

Abstrakt

W artykule przeanalizowano uwarunkowania atrakcyjności turystycznej obszaru gmin, przez które płynie rzeka Krzna. Celem pracy było wielocechowe porównanie gmin leżących nad rzeką Krzną pod względem atrakcyjności turystycznej oraz wskazanie czynników, które w największym stopniu różnicują analizowane gminy, a także pogrupowanie gmin o podobnych walorach turystycznych. Do oceny atrakcyjności turystycznej gmin ze względu na intensywność ruchu turystycznego wykorzystano podstawowe wskaźniki funkcji turystycznej (wskaźnik Baretje'a-Deferta, Schneidera i in.) oraz wybrane zmienne opisujące stan środowiska przyrodniczego gmin (tereny leśne i zalesione, tereny pokryte wodami). Dane do analizy zaczerpnięto z *Banku Danych Lokalnych* GUS za 2015 rok. W celu ustalenia wielowymiarowych zależności między badanymi cechami zastosowano analizę składowych głównych (PCA).

Badane gminy w największym stopniu różnicowane były przez cechy najsilniej skorelowane z pierwszą składową główną (PC1). Na podstawie analizy skupień pod względem atrakcyjności turystycznej gminy można podzielić na trzy grupy. Zastosowana metoda składowych głównych pozwoliła na wskazanie czynników, które w największym stopniu różnicują warunki turystyczne na badanym obszarze.

Analiza skupień, jako metoda klasyfikacji gmin, jest użytecznym narzędziem służącym do oceny potencjału rozwoju turystyki.

## Introduction

Various definitions of tourist attractiveness are found in the literature of the subject. According to KUREK (2007), it may be universal when the area is attractive to the general public or relative when referring to certain forms of tourism (water tourism, cycling, etc.). ROGALEWSKI (1974) mentions tourist attractions – nature and culture and tourist development. There are different methods of assessing the attractiveness of a given area, depending on the outcome of the different classifications, categorization (e.g. class 0 monument, five-star hotel, etc.); as a result of the application of a specific research technique (e.g. attractiveness of the area determined by point boning method). The assessment of attractiveness in this case is called valorisation (POTOCKA 2009). The standardized assessment of tourist

attractiveness is difficult because apart from the objectively existing conditions of the natural, cultural and social environment, psychological factors play an important role in the perception and valuation of the object (WARSZYŃSKA 1970, WARSZYŃSKA and JACKOWSKI 1978)

Factors which determine the tourist attractiveness of a given area are dependent on various determinants, eg: natural environment effectiveness, leisure, cultural and tourist facilities visitors are interested in, the size and diversity of accommodation and gastronomic facilities and theirs prices, public transport service availability, entertainment, cultural and sports events. Attractiveness of the natural environment, including its quality, beauty of the surrounding landscape, access to a river or lake, are perceived as main assets of tourist facilities in rural areas (CHMIEL et al. 2012).

The source of the Krzna river, the largest left-hand tributary of the Bug river, is in the areas of mid-forest marshes located in the Jata Sanctuary, in the north-west of Łuków (51°58′56″N 22°45′59″E). The Krzna flows into the Bug in the proximity of the village Neple (52°07′56″N 23°31′19″E), in the enchanting sanctuary called Szwajcaria Podlaska located 7 km off Terespol. The Krzna river is 119.9 km long. Initially, there are two streams: the Northern Krzna and the Southern Krzna whose parallel riverbeds are about 1 km apart. The streams join after the distance of around 55 km before they reach Międzyrzec Podlaski. The Southern Krzna is thought to be the main branch as it is several kilometres longer (*Studium uwarunkowań...* 2010). Based on the regional division of Poland (KONDRACKI 2002), the Krzna flows along the boundaries of two macroregions: Southern Podlasie Lowland and Western Polesie. The Krzna is a typical lowland river with a slope of 0.35‰ (*Studium uwarunkowań...* 2010) whose flow has been regulated along most of its course. It is the final element of the Wieprz-Krzna Canal which is the longest water canal in Poland built in mid-20$^{th}$ century. Only the final part of the river has many meanders which make it very picturesque. In the valley of the Krzna river there can be found various nature conservation forms (sanctuaries, Natura 2000 areas) as well as localities associated with cultural heritage of the area (eg: Łuków, Międzyrzec Podlaski, Biała Podlaska, Neple).

The aim of the study was to present the characteristics of tourist traffic recorded in the area of the communes through which the Krzna River flows. The objective of the work was to carry out multi-trait comparisons of communes located on the Krzna river in terms of tourist attractiveness, to determine factors which are the major source of differences between the communities analysed, and to group the communes with similar tourist attractions.

## Materials and Methods

Data for the year 2015 was used to compare the tourist attractiveness of communes located along the banks of the Krzna river. The data was obtained from the online *Local Data Bank* for the following communes: Łuków, the town of Łuków, Trzebieszów, Kąkolewnica, Międzyrzec Podlaski, the town of Międzyrzec Podlaski, Drelów, Biała Podlaska, the town of Biała Podlaska, Zalesie, Terespol, the town of Terespol (Figure 1).


Fig. 1. Location of analyzed communes upon the Krzna river

The analysis was based on the following variables: $X_1$ – Baretje-Defert index, which indicates the number of beds available for tourists per 100 inhabitants of a tourist-oriented locality, in literature also called the index of tourist function of a locality (CHUDY-HYSKI 2006), $X_2$ – Schneider index expressing the number of tourists using accommodation facilities per 100 permanent inhabitants of the area, $X_3$ – Charvart index which is the number of overnight stays per 100 inhabitants of the area, $X_4$ – index of accommodation capacity utilisation which is the number of overnight stays per one bed, $X_5$ – index of the development of accommodation facilities which is the number of tourists per one bed in the area, $X_6$ – index of tourist traffic density which denotes the number of tourists per 1 km$^2$, $X_7$ – index of accommodation density which denotes the number of beds available for tourists per 1 km$^2$ of the area (BĄK and WAWRZYNIAK 2008, 2009), $X_8$ – forests and woodlands in hectares, $X_9$ – area covered by water in hectares. Due to the fact that the variables describing natural conditions were expressed in different units, they were standardised according to the following formula (PANEK and ZWIERZCHOWSKI 2013):

$$z_{ij} = \frac{x_{ij} - \bar{x}_i}{s_i}$$

where:
$z_{ij}$ – value of standardised variable,
$x_{ij}$ – value of $i$-th variable and $j$-th commune,
$\bar{x}_i$ – arithmetic mean of $i$-th characteristic,
$s_i$ – standard deviation of $i$-th characteristic.

In order to determine multidimensional relationships between the characteristics studied, principal component analysis (PCA) was used to reduce the number of diagnostic variables to obtain a limited number of formal variables called principal components. The number was chosen based on the Kaiser criterion according to which only the components whose eigenvalues are greater than one are subjected to analysis. The principal objective of cluster analysis is to establish groups of objects which are similar in terms of many characteristics (variables). The analysis was based on the Euclidean distance, which is a measure of the distance between objects, and Ward's method as an agglomeration method. The stopping rule applied was Mojena's rule (STANISZ 2009). Calculations were performed using Statistica 12.0 software.

## Results and Discussion

The possibilities of tourism development in a given area are determined by a number of factors which differ in character. Of these, tourist resources and assets in the study area are of primary importance (MEYER 2010). Tourist attractiveness of a given area is to a great extent dependent on the quality of natural environment, recreational interests of visitors as well as the size and diversity of accommodation (CHMIEL et al. 2012). Principal component analysis demonstrated that the tourist attractiveness of communes located on the Krzna river was affected by characteristics associated with the first two principal components: PC1 and PC2 which accounted for over 77% of the total variance being the overall multidimensional variation of characteristics (Table 1).

The first principal component, accounting for 47.16% of the total variation, correlated the most strongly with the index of accommodation facilities (-0.970), Charvat index (-0.963), Schneider index (-0.948) and index of accommodation capacity utilisation (-0.947). In terms of these indicators, the communes analysed differed the most as far as tourism function was concerned. The use of these indicators makes possible to compare the

use of accommodation facilities in the communes of the region and applies to tourists in the strict sense of the term, excluding hikers who visit the area and whose number is not included in the resulting value (SZROMEK 2012).

Table 1
Eigenvalues, percent of variance and cumulative percent of variance of the components obtained

| Principal components | Eigenvalues | Part of the multi-trait variation being explained | Cumulative part of the multi-trait variation |
|---|---|---|---|
| PC1 | 4.244 | 47.160 | 47.16 |
| PC2 | 2.695 | 29.947 | 77.11 |
| PC3 | 0.952 | 10.581 | 87.69 |
| PC4 | 0.703 | 7.813 | 95.50 |
| PC5 | 0.355 | 3.946 | 99.45 |
| PC6 | 0.037 | 0.413 | 99.86 |
| PC7 | 0.011 | 0.127 | 99.99 |
| PC8 | 0.001 | 0.012 | 100.00 |
| PC9 | 0.000 | 0.002 | 100.00 |

Source: authors' calculations based on data from the *Local Data Bank* (2015)

Communes which made the most use of their accommodation facilities had the highest Schneider index and Charvat index. These relationships agree with findings reported by SZROMEK (2012) who has demonstrated an existence of associations between Baretje-Defert index, Schneider index, Charvat index and accommodation density.

The second principal component was strongly associated with the index of accommodation density (-0.893), forested area and woodlands (0.839), index of tourist traffic density (-0.701) and area covered by water (0.611); it accounted for almost 30% of the total variation. This component is related both to natural values and to tourist development, which is the basic element determining the attractiveness of tourism (ROGALEWSKI 1974). In communes with larger forested and woodland areas as well as areas covered by water, the index of accommodation density was lower (Table 1 and Table 2, Figure 2). A similar correlation of forested and woodland area with the first principal component (0.931) was reported by RYMUZA et al. (2015). All the communes with a high Chavart index also had the highest index of accommodation capacity utilisation as well as index of accommodation development. Moreover, it is evident from Figure 2 that communes with high values of Baretje-Defert index, tourist traffic density and accommodation density had the smallest forested and woodland areas as well as areas covered by water.

Table 2

Factor load values of the first three principal components as well as diagnostic characteristics, eigenvalues and cumulative eigenvalues of components

| Characteristics | PC1 | PC2 |
|---|---|---|
| Baretje-Defert index | -0.589 | -0.348 |
| Schneider index | -0.948 | 0.293 |
| Charvat index | -0.963 | 0.253 |
| Index of accommodation capacity utilisation | -0.947 | 0.147 |
| Index of accommodation development | -0.970 | 0.190 |
| Index of tourist traffic density | -0.370 | -0.701 |
| Index of accommodation density | -0.164 | -0.893 |
| Forests and woodlands [ha] | 0.099 | 0.839 |
| Areas covered by water [ha] | 0.247 | 0.611 |

Source: authors' calculations based on data from the *Local Data Bank* (2015)

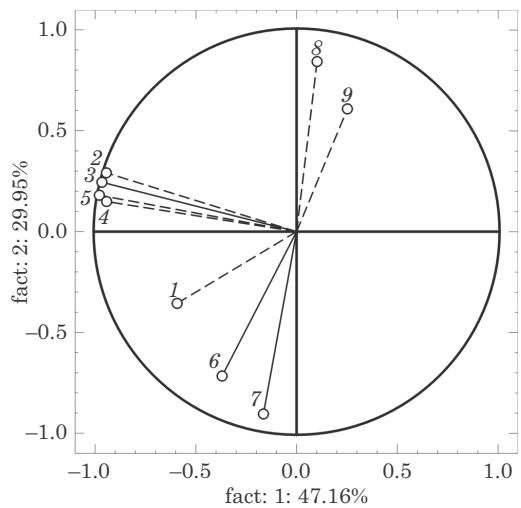


Fig. 2. Location of diagnostic characteristics in the system of the first two principal components: *1* – Baretje-Defert index; *2* – Schneider index; *3* – Charvat index; *4* – index of accommodation capacity utilisation; *5* – index of accommodation facility development; *6* – index of tourist traffic density; *7* – index of accommodation density; *8* – forests and woodlands [ha]; *9* – areas covered by water [ha]

Source: authors' calculations based on data from the *Local Data Bank* (2015)

Cluster analysis performed based on principal components yielded 3 groups of communes with different tourist attractiveness (Figure 3). The first group was composed of the following rural communes: Łuków, Międzyrzec Podlaski, Terespol, Biała Podlaska, Drelów, Kąkolewnica and Trzebieszów. The first group of communes had the lowest values of Baretje-

-Defert index, Schneider index, Charvat index, index of accommodation capacity utilisation, index of tourist traffic density and index of accommodation density. Also, the communes had the largest forested areas and areas covered by water (Table 3).

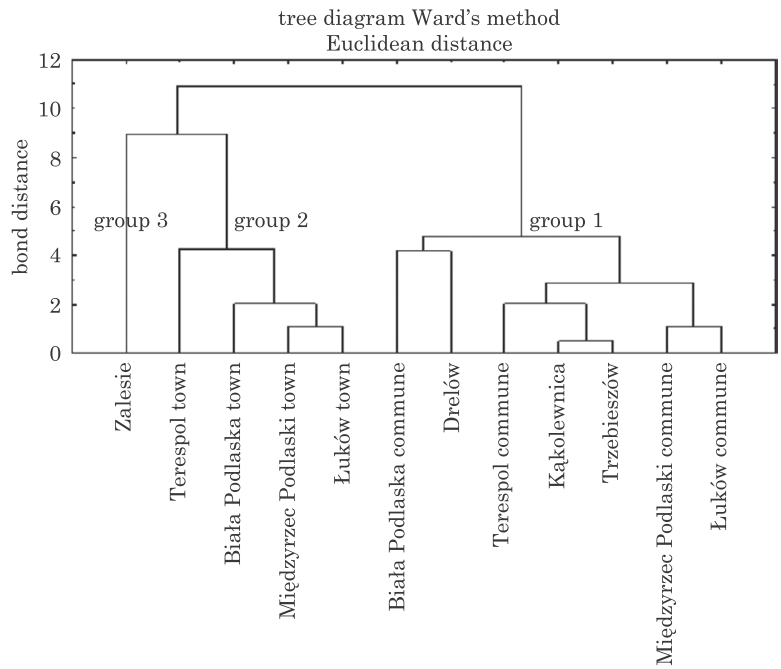


Fig. 3. Dendrogram demonstrating groups of communes with similar natural conditions
Source: authors' calculations based on data from the *Local Data Bank* (2015)

Table 3

Mean values of diagnostic variables in individual groups

| Variable | Group 1 | Group 2 | Group 3 |
|---|---|---|---|
| Baretje-Defert index | 0.59 | 1.21 | 1.63 |
| Schneider index | 19.93 | 38.85 | 395.26 |
| Charvat index | 23.31 | 53.86 | 396.70 |
| Index of accommodation capacity utilisation | 25.68 | 63.16 | 243.92 |
| Index of accommodation development | 16.29 | 46.54 | 243.03 |
| Index of tourist traffic density | 8.50 | 347.59 | 119.03 |
| Index of accommodation density | 0.28 | 8.98 | 0.49 |
| Forests and woodlands (ha) | 6401.57 | 382.75 | 5609.00 |
| Areas covered by water (ha) | 126.57 | 23.00 | 79.00 |

Source: authors' calculations based on data from the *Local Data Bank* (2015)

The course of agglomeration (clustering) indicates that, in this group, Trzebieszów and Kąkolewnica, which formed a cluster at the first step, were the most similar in terms of tourist conditions (Table 4). Group 2 included the following urban communes: Łuków, Międzyrzec Podlaski, Biała Podlaska and Terespol. Within this group, Łuków and Międzyrzec Podlaski were the most similar communes as they were the first ones to form a cluster (step 2). As shown in Table 3, the communes had the highest mean index of tourist traffic density, the highest index of accommodation density but the lowest forested area and area covered by water. A separate group was formed by Zalesie which had the highest values of Baretje-Defert index, Schneider index, Charvat index, index of accommodation capacity utilisation and index of accommodation development. These values indicate that this commune is well developed in terms of tourism (SZROMEK 2012). A similar division of the communes into groups was obtained by means of principal components analysis (PCA). Figure 4 shows spatial variation of the communes in the system of the first two principal components which accounted for over 77% of the total variation. Distribution of communes in the system of the first two principal components indicates that Kąkolewnica and Trzebieszów had the highest mean values of variables associated with the first principal component, that is index of accommodation density, forested and woodland areas as well as area covered by water.

Table 4

Course of agglomeration of communes into clusters

| Step | Communes | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | T | K | – | – | – | – | – | – | – | – | – |
| 2 | ŁGW | MPGW | – | – | – | – | – | – | – | – | – |
| 3 | ŁGM | MPGM | – | – | – | – | – | – | – | – | – |
| 4 | ŁGM | MPGM | BPGW | – | – | – | – | – | – | – | – |
| 5 | T | K | TGW | – | – | – | – | – | – | – | – |
| 6 | ŁGW | MPGW | T | K | TGW | – | – | – | – | – | – |
| 7 | D | BPGW | – | – | – | – | – | – | – | – | – |
| 8 | ŁGM | MPGM | BPGM | TGM | – | – | – | – | – | – | – |
| 9 | ŁGW | MPGW | T | K | TGW | D | BPGW | – | – | – | – |
| 10 | ŁGM | MPGW | BPGM | TGM | Z | – | – | – | – | – | – |
| 11 | ŁGW | MPGW | T | K | TGW | BPGM | ŁGM | MPGM | BPGM | TGM | Z |

T – Trzebieszów, ŁGW – Łuków rural commune, ŁGM – Łuków urban commune, D – Drelów, K – Kąkolewnica, MPGW– Międzyrzec rural commune, MPGM – Międzyrzec urban commune, BPGW – Biała Podlaska rural commune,TGW – Terespol rural commune, TGM– Terespol urban commune, Z – Zalesie

Source: authors' calculations based on data from the *Local Data Bank* (2015)

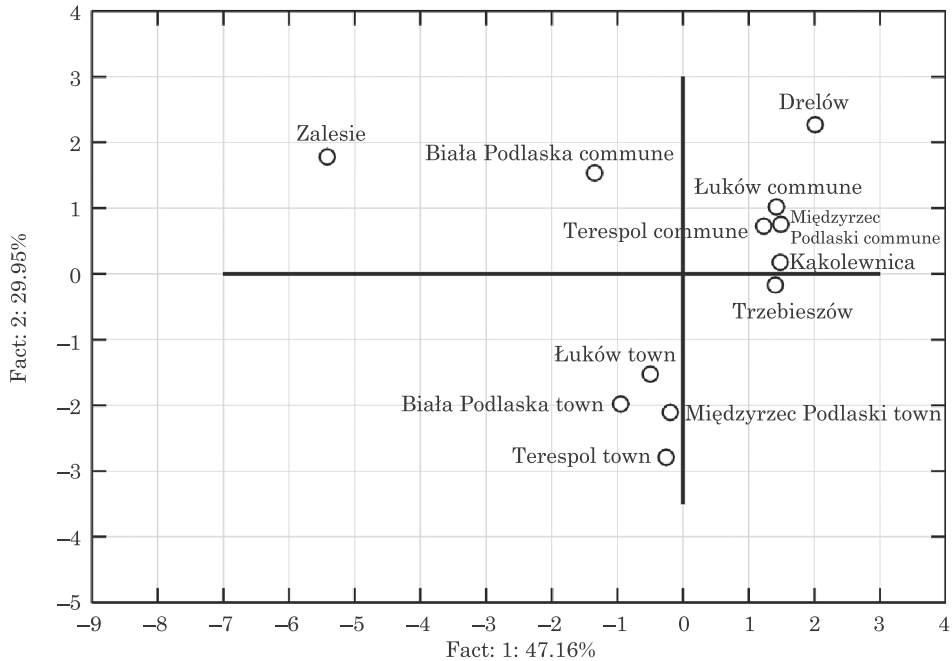


Fig. 4. Distribution of communes in the system of the first two principal components
Source: authors' calculations based on data from the *Local Data Bank* (2015)

It can be inferred from the location of Drelów in the system of principal components that the commune had a low index of accommodation density, index of accommodation capacity utilisation, Schneider index and Charvat index but large forested and woodland areas and an area covered by waters. The following communes: Terespol, Międzyrzec Podlaski, Biała Podlaska and Łuków had average values of the index of accommodation capacity, Schneider index as well as Charvat index. In these communes, the forested area and area covered by water were below the average level. It is worth stressing that the commune Zalesie had high values of variables associated with both the first and second component.

The present study revealed that the tourist attractiveness of areas located along the banks of the Krzna river depends on many factors, the main ones being the development and accessibility of tourist facilities as well as attractiveness of the environment. Similar findings were obtained based on research into the tourism competitiveness and attractiveness of communes located on the Liwiec river (STARCZEWSKI et al. 2014).

## Conclusions

1. In terms of tourist attractiveness, communes located on the Krzna river were the most affected by the characteristics which were the most strongly correlated with the first principal component PC1 (that is the index of accommodation development, index of accommodation capacity utilisation, Schneider index and Charvat index).

2. Based on cluster analysis, the communes were divided into 3 groups in terms of tourist attractiveness. Urban and rural communes formed two separate clusters which mainly differed as to the values of their indicators, forested areas and areas covered by water. Moreover, a separate cluster was formed by Zalesie, a commune which had the highest values of Baretje-Defert index, Schneider index, Charvat index, index of accommodation capacity utilisation and index of accommodation development.

3. Principal component analysis made it possible to indicate the factors which had the greatest effect on the tourism conditions of communes located on the Krzna river. Cluster analysis, a method of grouping communes based on variables describing tourism conditions, is a useful tool to evaluate the potential of tourism development in communes. The methods may be used in the process of management and undertaking necessary steps while creating and carrying out the strategy of commune development.

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# EFFECTIVENESS OF CONTROL MEASURES TAKEN BY THE VETERINARY INSPECTION IN POLAND

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Key words: Veterinary Inspection, control, supervised entities, inconsistencies.

Abstract

The Veterinary Inspection (VI) has been, by virtue of law, equipped with a number of controlling competencies. This evaluation involved the operational activities of 50 district veterinary inspectorates in 2008–2012 in the following provinces: Warmińsko-Mazurskie, Śląskie, Wielkopolskie, and Lubelskie. The effectiveness of administrative decisions on the discovered cases of undesired events in the controlled entities was performed. The results are representative for the whole country. The analysis of the data indicates a need for an increased number of controls and for improving controls and for improving their quality. Continuous monitoring of supervised entities and analysis of the data provide a basis to reduce undesired events.

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# OCENA SKUTECZNOŚCI DZIAŁAŃ KONTROLNYCH INSPEKCJI WETERYNARYJNEJ W POLSCE

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Słowa kluczowe: inspekcja weterynaryjna, kontrola, podmioty nadzorowane, niezgodności.

Abstrakt

Zgodnie z przepisami Inspekcję Weterynaryjną wyposażono w liczne kompetencje kontrolne. Badaniami objęto 50 powiatowych inspektoratów weterynarii działających w latach 2008–2012 w województwach warmińsko-mazurskim, śląskim, wielkopolskim i lubelskim. Oceniono skuteczność decyzji administracyjnych dotyczących ujawnionych zdarzeń niepożądanych w kontrolowanych podmiotach. Wyniki badań są reprezentatywne dla całego kraju.

Analiza uzyskanych danych wskazuje na potrzebę zwiększenia liczby kontroli i ich jakości. Dzięki ciągłemu monitorowaniu podległych jednostek oraz analizie otrzymywanych danych zdecydowanie można ograniczyć rozwój zdarzeń niepożądanych.

## Introduction

The strategic aim of the operational activities of the Veterinary Inspection (VI) involves the protection of public health encompassed in the overall supervision of the safety of animal-origin food (BABIŃSKA et al. 2008a,b 2017, D.U.2015.1482, KWIATEK 2007, KWIATEK and KOWALCZYK 2010, LISIOWSKA 2010, LISIOWSKA et al. 2011, PŁÓKARZ and RUDY 2015, SZAREK et al. 2008). To this end, the VI bodies have been equipped with a number of controlling competencies (BIAŁEK et al. 2012, LISIOWSKA 2013, MALINOWSKA 1996, 2003, POPŁAWSKI et al. 2011, RUDY 2010). The notion *control* is commonly associated with a set of measures implemented by legally authorized inspectors to determine whether there are any inconsistencies between the actual state and the state required by law and the potential causes for such inconsistencies (BABIŃSKA et al. 2015, BOĆ 2004).

The set of VI activities includes comprehensive controls and ad hoc controls; the former translate into periodical controls according to the control schedules. The plans and schedules of such controls are designed by

the Province Veterinary Officer (PVO), who is guided by the District Veterinary Officer (DVO), based on the risk analyses for the previous years (KWIATEK 2007, KWIATEK and KOWALCZYK 2010, TRUSZCZYŃSKI and PEJSAK 2011). An ad hoc control means a controlling procedure over a supervised entity that is introduced beyond the control schedule and in situations with real or potential hazards (DOBKOWSKI 2007). The risk analysis should include clear, interactive and documented exchange of information between the risk assessing party and the risk managing party as well as common communication with all interested parties, covering all process aspects (KWIATEK and KOWALCZYK 2010).

The objective of the paper is to analyse the effectiveness of control measures taken by the VI in four provinces in Poland. The analysis included the administrative decisions issued in 2008 –2012, imposing the following: to eliminate infringements, to suspend operations until any infringements are eliminated, to limit the scope of a specific business operation and to ban business activities in the entities supervised by the VI.

## Material and Methods

The evaluation involved 50 district veterinary inspectorates in four provinces: Warmińsko-Mazurskie, Śląskie, Wielkopolskie, and Lubelskie. The Silesian and Wielkopolska provinces represent economically well-developed regions, and Warmińsko-Mazurskie and Lubelskie are poorer in this regard. Moreover their location and the history of these regions gives a good guarantee of Poland's character. These administrative regions are representative of the whole country. It was deliberately decided not to cover all entities supervised by the VI and limit the scope to selected production animal species: cattle, pigs, and poultry. The sample was selected to make it representative for the desired objective of the study. The evaluation covered cases of inconsistencies diagnosed in production plants supervised by the VI in 2008–2012, including cases of illegal slaughtering. The VI-supervised entities were subject to both comprehensive and ad hoc controls.

The analysis covered the following: identification of the supervised entities, the number of controls, the number of slaughtered animals, the number of illegally slaughtered animals and the number of decisions on meat trade. Importantly, the evaluation also included cases of suspected illegal activities that were reported to the VI and liable to prosecution.

## Results

In the Lubelskie province, all districts (15) were included in the study. In total, 3,193 controls were performed, with the most (506) in the Lubelski district and the least (8) in the Opolski district. The control procedures uncovered 1,177 inconsistencies in this province, of which the most (238) were detected in Puławski district and the least (4) in the Opolski district. The inconsistencies recorded during the controls resulted in issuing decisions requiring the entities to eliminate the infringements and to suspend operations until the infringements were eliminated. The Lubelski district had the highest number of decisions requiring the elimination of infringements; in total, seven decisions suspending operations until the infringements were eliminated were issued, with the most (3) in the Hrubieszowski district (Table 1).

Table 1

A numerical compilation of the results of the control procedures carried out in 2008–2012 in the entities supervised by the Veterinary Inspection in the Lubelskie province

| Lubelskie province – district | Number of | | | |
|---|---|---|---|---|
| | controls | detected inconsistencies | decisions | |
| | | | imposing the elimination of infringements | suspending the operation until the infringements are eliminated |
| Hrubieszowski | 111 | 49 | 4 | 3 |
| Janowski | 182 | 67 | 0 | 0 |
| Lubelski | 506 | 81 | 72 | 0 |
| Łęczyński | 17 | 16 | 0 | 0 |
| Tomaszowski | 257 | 76 | 7 | 0 |
| Włodawski | 471 | 226 | 22 | 0 |
| Zamojski | 451 | 30 | 5 | 1 |
| Bialski | 423 | 48 | 7 | 0 |
| Kraśnicki | 204 | 48 | 5 | 0 |
| Krasnostawski | 55 | 74 | 4 | 0 |
| Lubartowski | 180 | 47 | 15 | 0 |
| Łukowski | 155 | 110 | 22 | 0 |
| Opolski | 8 | 4 | 5 | 2 |
| Puławski | 139 | 238 | 19 | 0 |
| Rycki | 34 | 63 | 3 | 1 |
| **Total** | **3193** | **1177** | **190** | **7** |
| **Percentage** | – | **100** | **16** | **1** |
| **Total percentage of the number of effectuated controls** | – | – | – | **0** |

In the Warmińsko-Mazurskie province, 17 districts were included in the evaluation. In total, 2,501 controls were carried out, with the most (648) in the Olsztyński district and the least (10) in Lidzbarski district. The control procedures uncovered 1,108 inconsistencies in this province, with the most (247) detected in Iławski district and the least (7) in the Lidzbarski district. The controls resulted in issuing 251 decisions requiring the entities to eliminate the infringements and 21 decisions suspending operations until the infringements were eliminated. Decisions imposing the elimination of infringements were most numerous in the Olsztyński district (47) although the Ostródzki district had the most decisions (7) suspending operations until the elimination of infringements (Table 2).

Table 2

A numerical compilation of the results of the control procedures carried out in 2008–2012 in the entities supervised by the Veterinary Inspection in the Warmińsko-Mazurskie province

| Warmińsko--Mazurskie province – district | Number of | | | |
|---|---|---|---|---|
| | controls | detected inconsistencies | decisions | |
| | | | imposing the elimination of infringements | suspending the operation until the infringements are eliminated |
| Bartoszycki | 96 | 102 | 20 | 3 |
| Działdowski | 58 | 71 | 9 | 5 |
| Elbląski | 332 | 107 | 9 | 0 |
| Ełcki | 184 | 29 | 13 | 3 |
| Giżycki | 59 | 37 | 22 | 0 |
| Gołdapski | 68 | 25 | 4 | 0 |
| Iławski | 318 | 249 | 16 | 0 |
| Kętrzyński | 49 | 35 | 1 | 2 |
| Lidzbarski | 10 | 7 | 0 | 0 |
| Mrągowski | 44 | 23 | 9 | 1 |
| Nidzicki | 27 | 14 | 4 | 0 |
| Nowomiejski | 121 | 42 | 23 | 0 |
| Olecki | 137 | 21 | 14 | 0 |
| Olsztyński | 648 | 97 | 48 | 0 |
| Ostródzki | 269 | 170 | 45 | 7 |
| Szczycieński | 64 | 33 | 13 | 0 |
| Węgorzewski | 17 | 46 | 1 | 0 |
| **Total** | **2501** | **1108** | **251** | **21** |
| **Percentage** | – | **100** | **23** | **2** |
| **Total percentage of the number of effectuated controls** | – | – | – | **1** |

In the Śląskie province, the evaluation included 19 districts and 2,731 controls, with the most carried out in the Tyski district (476) and the least (26) in the Bielski district. The control procedures uncovered 2,146 inconsistencies; with the highest number (572) reported in the Gliwicki district and the lowest number in the Bielski district. The number of decisions requiring the elimination of infringements (320) was highest in the Śląskie province and involved three districts (Będzin, Dąbrowa, Sosnowiec). The lowest number of such decisions was issued in the Bielski district (9). In total, there were 29 suspending decisions, with the most (6) issued in the Gliwicki district (Table 3).

Table 3

A numerical compilation of the results of the control procedures carried out in 2008–2012 in the entities supervised by the Veterinary Inspection in the Śląskie province

| Śląskie province – district | Number of | | | |
|---|---|---|---|---|
| | controls | detected inconsistencies | decisions | |
| | | | imposing the elimination of infringements | suspending the operation until the infringements are eliminated |
| Będziński, Dąbrowski, Sosnowiecki | 163 | 396 | 320 | 5 |
| Bielski | 26 | 9 | 9 | 0 |
| Cieszyński | 146 | 22 | 22 | 0 |
| Częstochowski | 86 | 18 | 15 | 0 |
| Gliwicki | 237 | 572 | 76 | 6 |
| Katowicki | 154 | 97 | 74 | 2 |
| Kłobucki | 63 | 276 | 252 | 0 |
| Lubliniecki | 53 | 45 | 27 | 4 |
| Myszkowski | 139 | 31 | 16 | 0 |
| Pszczyński | 51 | 13 | 10 | 0 |
| Raciborski | 82 | 63 | 24 | 1 |
| Rybnicki | 261 | 48 | 43 | 0 |
| Tarnogórski | 222 | 81 | 2 | 2 |
| Tyski | 476 | 99 | 44 | 1 |
| Wodzisławski | 298 | 85 | 56 | 3 |
| Zawierciański | 99 | 197 | 50 | 5 |
| Żywiecki | 175 | 94 | 58 | 0 |
| **Total** | **2731** | **2146** | **1098** | **29** |
| **Percentage** | – | **100** | **51** | **1** |
| **Total percentage of the number of effectuated controls** | – | – | – | **1** |

In the Wielkopolskie province, the evaluation included 31 districts. In total, 7,203 controls were executed, of which the Ostrzeszowski district had the most (1,694) while the Turecki district had the least (11). The control procedures uncovered 2,994 inconsistencies in the province. The highest number of such inconsistencies (614) was recorded in the Słupecki district, whereas none were reported in the Turecki district. The controls in the Wielkopolskie province resulted in issuing 1,173 decisions requiring the entities to eliminate the infringements, with the most (128) in the Ostrzeszowski district, while none were issued in the Turecki district. In total, there were 41 decisions suspending operations until the infringements were eliminated and the Kaliski district had the most (13) – Table 4.

Table 4

A numerical compilation of the results of the control procedures carried out in 2008–2012 in the entities supervised by the Veterinary Inspection in the Wielkopolskie province

| Wielkopolskie province – district | Number of | | | |
|---|---|---|---|---|
| | controls | detected inconsistencies | decisions | |
| | | | imposing the elimination of infringements | suspending the operation until the infringements are eliminated |
| Grodziski | 147 | 100 | 61 | 1 |
| Rawicki | 262 | 204 | 39 | 4 |
| Turecki | 11 | 0 | 0 | 0 |
| Średzki | 30 | 15 | 5 | 1 |
| Wolsztyński | 668 | 143 | 71 | 1 |
| Gnieźnieński | 100 | 19 | 15 | 0 |
| Koniński | 24 | 15 | 0 | 0 |
| Jarociński | 167 | 25 | 19 | 0 |
| Ostrowski | 31 | 36 | 7 | 0 |
| Nowotomyski | 97 | 62 | 11 | 2 |
| Złotowski | 239 | 67 | 13 | 0 |
| Szamotulski | 236 | 111 | 42 | 0 |
| Kaliski | 206 | 98 | 116 | 13 |
| Poznański | 85 | 74 | 47 | 0 |
| Słupecki | 206 | 614 | 69 | 1 |
| Pleszewski | 65 | 121 | 24 | 1 |
| Krotoszyński | 174 | 35 | 31 | 3 |
| Wągrowiecki | 195 | 104 | 10 | 1 |

| | | | | |
|---|---|---|---|---|
| Kolski | 73 | 44 | 104 | 0 |
| Pilski | 142 | 30 | 2 | 0 |
| Kościański | 180 | 138 | 71 | 1 |
| Leszczyński | 198 | 81 | 12 | 0 |
| Międzychodzki | 79 | 24 | 17 | 0 |
| Śremski | 199 | 247 | 18 | 1 |
| Kępiński | 215 | 253 | 33 | 0 |
| Gostyński | 1 198 | 110 | 95 | 8 |
| Wrzesiński | 50 | 68 | 67 | 2 |
| Chodzieski | 25 | 10 | 2 | 0 |
| Ostrzeszowski | 1 694 | 98 | 128 | 1 |
| Obornicki | 152 | 18 | 37 | 0 |
| Czarnkowsko-Trzcianecki | 55 | 30 | 7 | 0 |
| **Total** | **7203** | **2994** | **1173** | **41** |
| **Percentage** | – | **100** | **39** | **1** |
| **Total percentage of the number of effectuated controls** | – | – | – | **1** |

In 2008–2012, the Veterinary Inspection carried out 18,821 controls in total in these four provinces: 3,193 in the Lubelskie province, 2,501 in the Warmińsko-Mazurskie province, 2,731 in the Śląskie province, and 7,203 in the Wielkopolskie province. The number of detected inconsistencies amounted to 7,425 in total, of which 1,177 were in the Lubelskie province, 1,108 were in the Warmińsko-Mazurskie province, 2,731 were in the Śląskie province and 7,203 were in the Wielkopolskie province. In the discussed period of time, the VI issued a total of 2,712 decisions requiring the elimination of infringements: 190 in the Lubelskie province, 251 in the Warmińsko-Mazurskie province, 1,098 in the Śląskie province and 1,173 in the Wielkopolskie province. The number of suspending decisions was 98, of which seven were issued in the Lubelskie province, 21 in the Warmińsko-Mazurskie province, 29 in the Śląskie province and 49 in the Wielkopolskie province (Table 5, Table 6).

Table 5

A numerical compilation of the results of the control procedures carried out in 2008–2012 in the entities supervised by the Veterinary Inspection in four selected provinces

| Province | Number of | | | |
|---|---|---|---|---|
| | controls | detected inconsistencies | decisions | |
| | | | imposing the elimination of infringements | suspending the operation until the infringements are eliminated |
| Lubelskie | 3193 | 1177 | 190 | 1 |
| Warmińsko-Mazurskie | 2501 | 1108 | 251 | 4 |
| Śląskie | 2731 | 2146 | 1098 | 0 |
| Wielkopolskie | 7203 | 2994 | 1173 | 1 |

Table 6

Results of the control procedures carried out in 2008–2012 in the entities supervised by the Veterinary Inspection in four selected provinces, including the percentage of the decisions requiring the elimination of infringements, decisions suspending the operation until the infringements are eliminated and decisions suspending operations until the infringements are eliminated in relation to all control procedures

| Province | Number of | | Percentage of decision | | |
|---|---|---|---|---|---|
| | controls | detected inconsistencies | decisions requiring the elimination of the infringements in relation to all decisions | suspending operations until the infringements are eliminated in relation to all decisions | suspending operations until the infringements are eliminated in relation to all controls |
| Lubelskie | 3193 | 1177 | 16 | 1 | 0 |
| Warmińsko-Mazurskie | 2501 | 1108 | 23 | 2 | 1 |
| Śląskie | 2731 | 2146 | 51 | 1 | 1 |
| Wielkopolskie | 7203 | 2994 | 39 | 1 | 1 |

## Discussion

To accommodate the analysis, it was assumed that the notion of control means "comparing the actual state with the required state and investigating potential inconsistencies as well as checking whether something complies with the current regulations" (JAGIELSKI 2007). Therefore, it is necessary to consider whether the undertaken control measures are consistent with methodology (Instrukcja GLW nr GIWhig-500-4/08 z 1 kwietnia

2008 r.) and the frequency (Instrukcja Głównego Lekarza Weterynarii nr GIWbż-500-2/2011 z 1 września 2011 r.) assumed by the VI. Furthermore, it is emphasized that the notion *control* is commonly associated with a set of measures implemented by legally authorized inspectors and aimed at determining whether there are no inconsistencies between the actual state and the state required by law and the potential causes for such inconsistencies (GIEDROJĆ-BRZANA 2015, MALINOWSKA 2007, RUDY 2000).

Our investigations have shown that the more controls are performed, the more unfavourable decisions are made. This fact indicates the need to increase the number of VI employees and to increase the control of the VI of the veterinary administration in relation to the duties of business entities. It has also been shown that VI controls should be carried out in various forms, such as surveillance, monitoring, verification, audits, sampling and testing.

The control measures of the VI were subject to an investigation carried out by the auditors of the Supreme Audit Office (nr 46/2016/P/15/050/KRR) who, based on an analysis of the documents dated from January 1, 2013, to June 30, 2015, and earlier, arrived at a number of post-auditing conclusions. It was required to secure a continuous updating of the registers and supervised entities, to execute the proper procedure for recording the documents that support the qualifications of inspectors and to introduce controls at the frequency specified in the instruction issued by the Chief Veterinary Inspector. Eliminating such inconsistencies may significantly improve the effectiveness of the control measures of the VI (GIEDROJĆ-BRZANA 2015, LISIOWSKA et al. 2012, ZAKRZEWSKI et al. 2013).

Effective operational activities require highly qualified staff in the VI who are authorized to conduct audits and are involved in a uniform program of continuous education. The controlling bodies that employ personnel dedicated to official controls are required to provide professional development of employees and systematically update knowledge (MALINOWSKA 2007). The scope of training sessions for the employees involved in official controls, such as in the VI, is detailed in the Annex to the Regulation No. 882/2004/WE (Dz.U. UE L 191 z 30.4.2004).

## Conclusions

Based on the analysis of the results, it is concluded that the number of the decisions, suspending the operation of supervised entities until the infringements are eliminated, is in direct proportion to the number of controls carried out the VI.

Undesired events may be reduced by intensifying the control measures imposed by the VI on the supervised entities. Continuous and uniform education of inspectors is critical to improving the effectiveness of such control procedures.

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