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MORPHOLOGICAL AND GENETIC VARIABILITY OF THE POPULATION OF *ANODONTA WOODIANA* (LEA, 1834) OCCURRING IN THE HEATED KONIN LAKES SYSTEM

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ABSTRACT. The bio-morphological (shells, weight) and genetic (izoenzymes) variations were studied in a new mussel species to Poland, *Anodonta woodiana*, which occurs in the heated Konin lakes system. The mussels were collected from three environments - the lenitic lake zones, the water flow-through channel and the lotic areas of a man-made reservoir. Significant bio-morphological differences between the shells of various populations were determined by environmental conditions. The high degree of genetic similarity of the mussels dictated their inclusion within the same species.

Key words: *ANODONTA WOODIANA*, MUSSELS, SHELL MORPHOLOGY, GENETIC SIMILARITY

INTRODUCTION

The Chinese mussel *Anodonta woodiana* (Lea) occurs in the natural habitats of the rivers Amur and Yangtze. It is a new freshwater mollusk in Poland which was recently accidentally introduced (Piechocki and Riedel 1997). The first records of its distribution in the heated Konin lakes system are from 1993 (Protasov et al. 1993). The problem of the taxonomy of the mussel described under the genus *Anodonta* (Zdanowski 1996, Afanasjev et al. 1997) has generated much discussion and remains unsolved. At first, the newly occurring mussels in the heated Licheńskie Lake were identified by J. I. Starobogatov (personal communication) as *Sinanodonta gibba* (Benson). Those inhabiting the warm channels of the cooling water system of the Konin power plants were described as *S. orbicularis* (Zdanowski 1996, Afanasjev et al. 1997). The individuals occurring in the man-made initial cooling reservoir corresponded with regard to both form and size to the typical form of *A. woodiana* studied by Kiss (1995). Finally, the decision was made (Piechocki and Riedel 1997) to classify the mussels from the Konin lakes as the same species of *A. woodiana*.

The mussels from different study sites in the cooling water system of the Konin power plants show significant morphological variations and a great ability to adapt to the increased temperatures of the system (Afanasjev et al. 1997, Soroka 1998, Soroka and Piotrowski 1999, Kraszewski and Zdanowski 2001). They prefer warm, well-oxygenated, lotic habitats with high water turbulence (Afanasjev et al. 2001, Kraszewski and Zdanowski 2001). Genetic studies conducted with izoenzyme electrophoresis and PCR-RFLP of the mitochondrion gene COI (cytochrom C oxidase subunit I) revealed that of all the morphological forms of *Anodonta* belong to one species - *A. woodiana* (Soroka and Skotarczak 1999, Soroka and Stachów 2000).

Thus far, the young populations of *A. woodiana* have had a positive effect on the aquatic ecosystems of the Konin lakes because they accumulate nutrients and heavy metals from the water, thus contributing to the improvement of water purity (Protasov et al. 1993, 1994, 1997, Zdanowski 1996, Sinicyna et al. 1997, Zdanowski et al. 1997, Królak and Zdanowski 2001). Compared to other mollusks, aggregations of this species break down the most organic matter (Protasov et al. 1994, 1997, Sinicyna et al. 1997). When they occur in high densities (up to 50 kg m^{-2}), these mussels, which filter up to 1,600 l per hour, can contribute significantly to the cleanliness of water throughout the entire Konin lakes system (Protasov et al. 1993, Sinicyna et al. 1997). Nutrients are most intensively taken up by young individuals which are experiencing the most intensive growth (Kiss 1995, Afanasjev et al. 2001).

Observation of *A. woodiana* at the first and only Polish sampling station allowed for the study of the effect this species has on freshwater ecosystems. Age variability within a population may indicate that the species is adapting to new environmental conditions (Geller et al. 1994). The *A. woodiana* populations in the Konin lakes system were dominated by three-year-old individuals (43%), while four-year-olds comprised 33% of the total (Soroka 2000, Afanasjev et al. 2001, Kraszewski and Zdanowski 2001).

The aim of the study was to statistically analyze the significant morphological and genetic variations of the mussel *A. woodiana* which inhabits three different environments in the Konin lakes system.

MATERIAL AND METHODS

Samples of *A. woodiana* were obtained in September 1998 from three environments in the Konin heated lakes system which differed in temperature from heating and in water retention. These were Licheńskie Lake, the warmest channel, and the man-made initial cooling reservoir for discharge water from the power plant (Fig. 1).

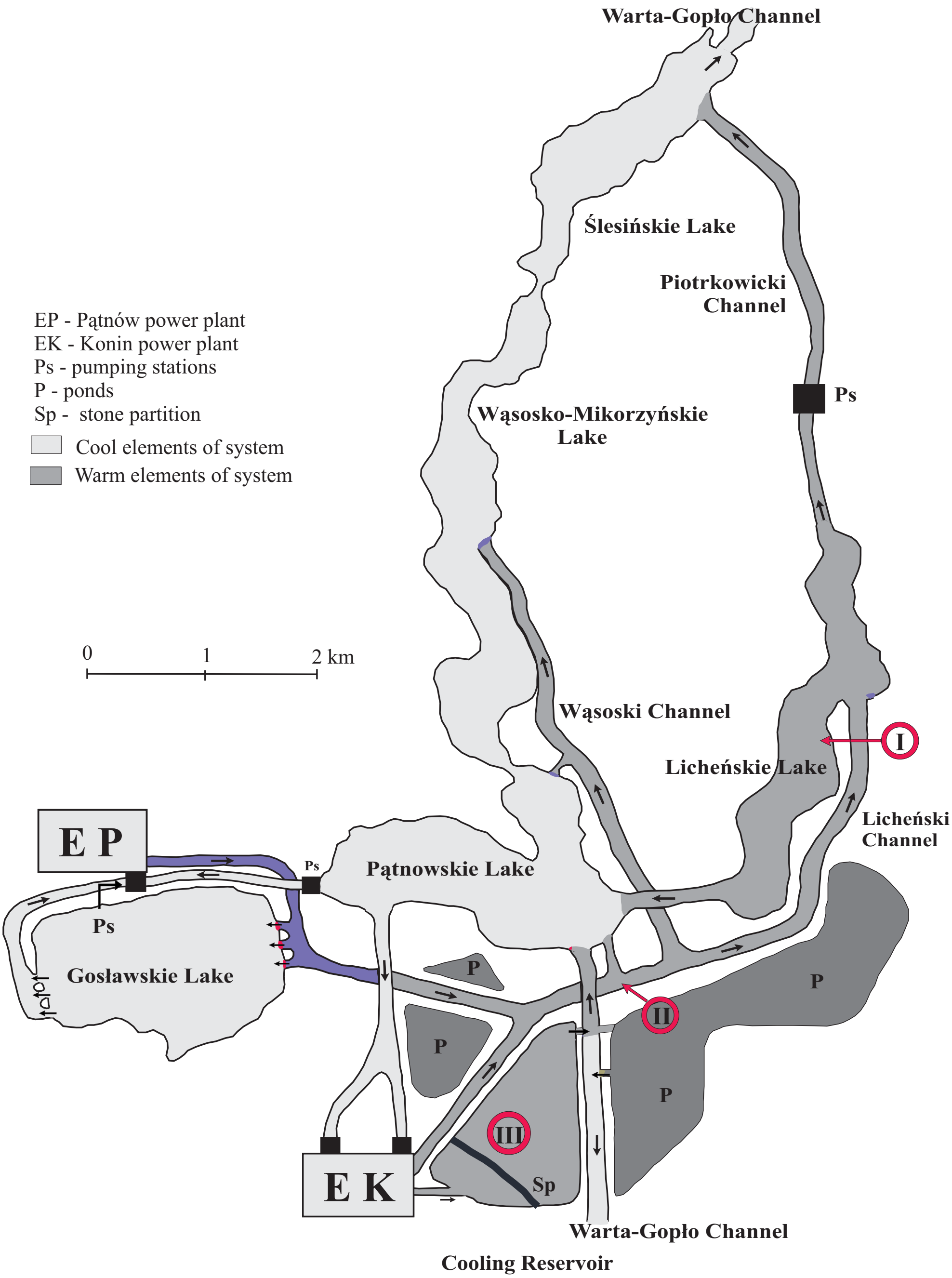

Fig. 1. Konin lakes system. Location of sampling stations.

The average water temperature in the lake during the summer stagnation period was 26°C and in the reservoir it was 32°C. Water exchange in both reservoirs occurred every three days. The mean summer water temperature in the channel was 30°C, and the water flow was 15 $m^3 s^{-1}$ (Zdanowski, unpublished data). The mussels were collected at random: 29 individuals from Licheńskie Lake (Group I); 27 individuals from the warmest channel (Group II); 23 individuals from the initial cooling reservoir (Group III) (Fig. 1).

The mussels were transferred to the laboratory, measured, and subsequently kept in aerated aquariums where they were fed dry Cladocera. A second set of measurements were taken following a half a year of aquarium culture. The shell measurements included the following: length (L) - the maximum anterior-posterior dimension of the shell; height (H) - the maximum dorsal-ventral dimension of the shell; convexity (D) - the maximum lateral dimension of the shell; mollusk weight (W) - the weight of the shell together with the body. The results obtained were used to calculate the coefficients of mollusk height (100 H/L) and convexity (100 D/H) (Piechocki and Dyduch–Falniowska 1993). Variations of shell characteristics in different groups were studied by analyzing the major components (PCA) (Alexandrowicz 1987, Henrion et al. 1987, 1988, Szybiak 1997). Significant differences between the parameters of different mussel groups were analyzed with the t-Student test and analysis of variance - Fisher test (LSD).

Genetic studies were performed with izoenzyme electrophoresis with the use of a starch gel. The variability in seven enzymes was analyzed using three buffer systems (Kośmider 2000). The coefficients of genetic similarity (I_N) and genetic distance (D_N) of the mussels were determined with the Nei method (Nei 1978) and the BIOSYS-1 program (Swofford and Selander 1983).

RESULTS

The highest similarity of the mussels (*A. woodiana*) was noted in their shell convexity (D), while the greatest variations were noted in their wet body weight (W) and shell length (L). The lowest body weight was characteristic for individuals from Group I from Licheńskie Lake, and the heaviest mussels belonged to Group III from the initial cooling reservoir (Fig. 2). The highest values of the statistical coefficients were also found for weight measurements. Comparatively lower values were noted for the other parameters (L, H, D) and the two coefficients (100 H/L, 100 D/H). The degree of body weight variability (V) was more than 27% in all three reservoirs.

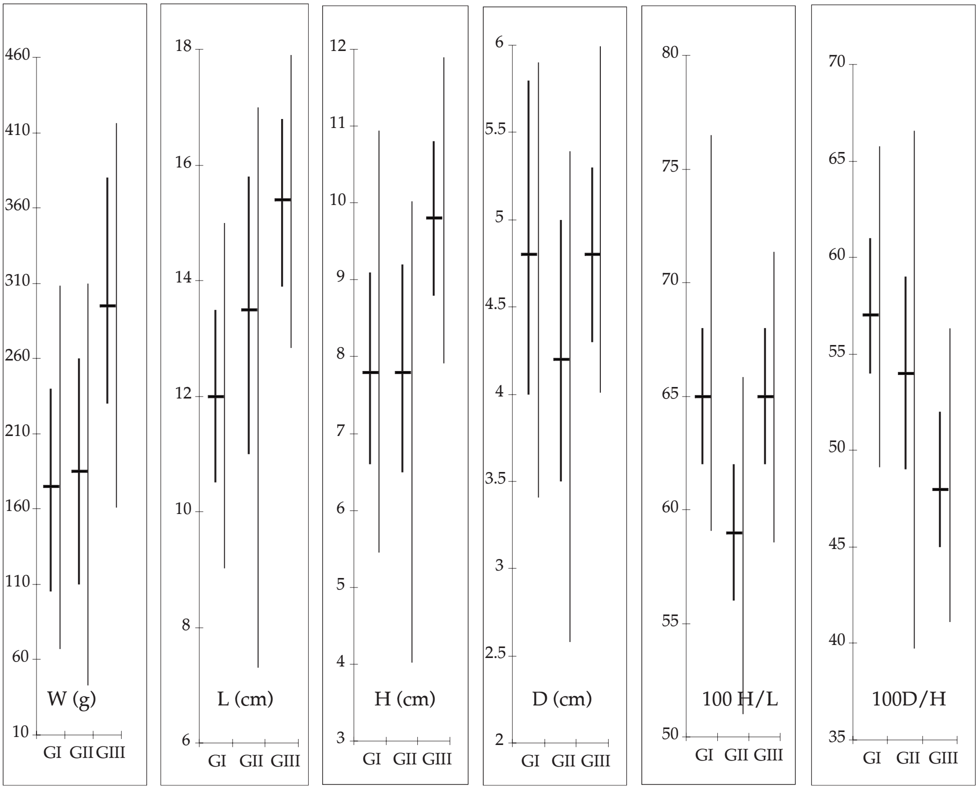

Fig. 2. Diagram of the variations of biometric parameters (W, L, H, D, 100 H/L, 100 D/H) of the mussel *A. woodiana*: from Licheńskie Lake (Group I - GI), from the discharge channel (Group II - GII), from the initial cooling reservoir (Group III - GIII) (range and standard deviation).

The longest, average shell length (L) was in Group III; the variation of this parameter was moderate (9%). The shortest shell lengths were observed for individuals from Groups I and II at rather high parameter variabilities of 12 and 18%, respectively. The average shell height (H) was the greatest for Group III; it was equally low for Groups I and II. The highest variation (V) of this parameter was noted for Group II, while it was moderate for Group III. The average shell convexity (D) was similar in all the groups. This was in spite of considerable variation (11-18%) of the parameter among the three groups.

The highest shell convexity was observed in individuals from Group III occurring in the initial cooling reservoir (Fig. 2). The highest average values of the height coefficient (100 H/L) were found for mussels from Groups I and III. The variation (V) of this coefficient was low in all the groups and did not exceed 5%. The highest average

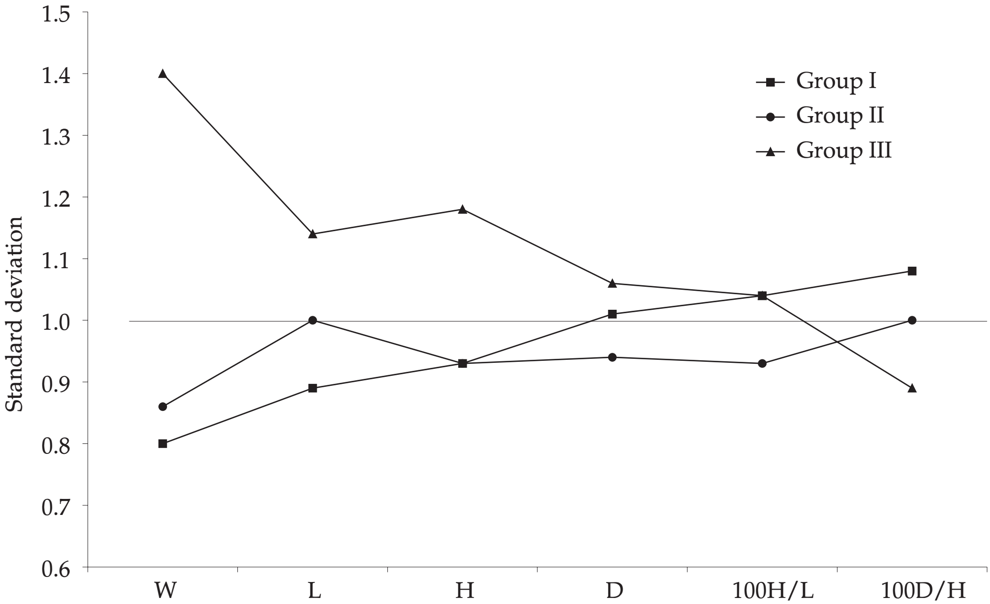

Fig. 3. Standard deviation of the biometric characteristics (W, L, H, D, 100 H/L, 100 D/H) of *A. woodiana* from: Licheńskie Lake (Group I), the discharge channel (Group II), the initial cooling reservoir (Group III).

value of the convexity coefficient (100 D/H) was noted for Group I mussels. The coefficient variation in this group was low and did not exceed 6%, while for Groups II and III it was moderate, but not higher than 10%.

The highest standard deviation among the three groups was found to be for mussel weight (W) (Fig. 3). Animals from Groups I and II were similar with respect to both body weight and shell weight, as well as shell height (H). All the groups had similar shell convexities (D) and the two indicators, 100 D/H and 100 H/L, were also similar.

Following a half a year of aquaculture, an increase in the measured parameters of *A. woodiana* was observed at 0.1–0.5 cm for L, H, D, and 15-23 g for W. The recorded variation of the biometric parameters of the mussels belonging to the three groups was still similar with regard to shell convexity, and it was most variable with respect to individual weight. All the basic parameters showed higher values in comparison with the initial measurements, and lower indicator values (100 H/L, 100 D/H). The same degree of variability for the parameters was observed, i.e. the highest was for weight and the lowest for the 100 H/L coefficient. The values that were the most similar to the average results obtained for all the mussels were noted for Group II specimens from the discharge channel. The highest standard deviation was found for wet weight (W), with the extreme values lower than 0.17 or higher than 0.41. Groups I and II were similar with regard to shell height (H). All three groups were the most similar with respect to the height coefficient (100 H/L), and they were the least similar with regard to both shell convexity (D) and the shell convexity coefficient (100 D/H).

The results of the t-Student test revealed that differences in the initial measurements of the mollusks from Licheńskie Lake (Group I) and the discharge channel (Group II) were significant only for length (L) and both coefficients (100 H/L and 100 D/H). In the second measurement, these differences were also detected for shell convexity (D) and the two coefficients. In both measurements, significant differences between the mussels from Licheńskie Lake (Group I) and the initial cooling reservoir (Group III) were found for weight (W), length (L), height (H) and the convexity coefficient (100 D/H). For mussels from Groups II and III, significant differences were found in all the parameter values obtained during both measurement periods.

From the analysis of the major components (PCA) used for all the parameters, three significantly different subgroups were distinguished (Fig. 4). Subgroup 1 was represented by the youngest individuals aged 2+ and 3+ and contained Group I mussels (59%) from Licheńskie Lake and Group II mussels (38%) from the discharge channel. Subgroup 2 was comprised of older mussels (aged 3+, 4+, 5+) from Group II (59%) and Group I (31%). Subgroup 3 included the oldest mussels (aged 4+, 5+) mainly from Group III (86%) from the initial cooling reservoir for discharge water. The three subgroups differed significantly with regard to shell length and height.

Subgroup 1 from Licheńskie Lake differed significantly from the other groups in its lower shell convexity, while subgroup 3 differed in its higher height coefficients (100 H/L) and a lower shell convexity coefficient (100 D/H). The relationships of the bio-metric characteristics which were observed following a half a year of aquaculture under laboratory conditions were very similar. Subgroup 1 was comprised of the same individuals, while subgroup 2 was supplemented by individuals which had previously been in subgroup 3, but which now exhibited different height and convexity coefficients.

The variability of the morphological characteristics of the mussel *A. woodiana* indicates the considerable flexibility of this species and its ability to adapt to environmental conditions. This was not confirmed by genetic studies of the izoenzymes . The genetic analysis of seven enzymes revealed the presence of 12 loci containing from

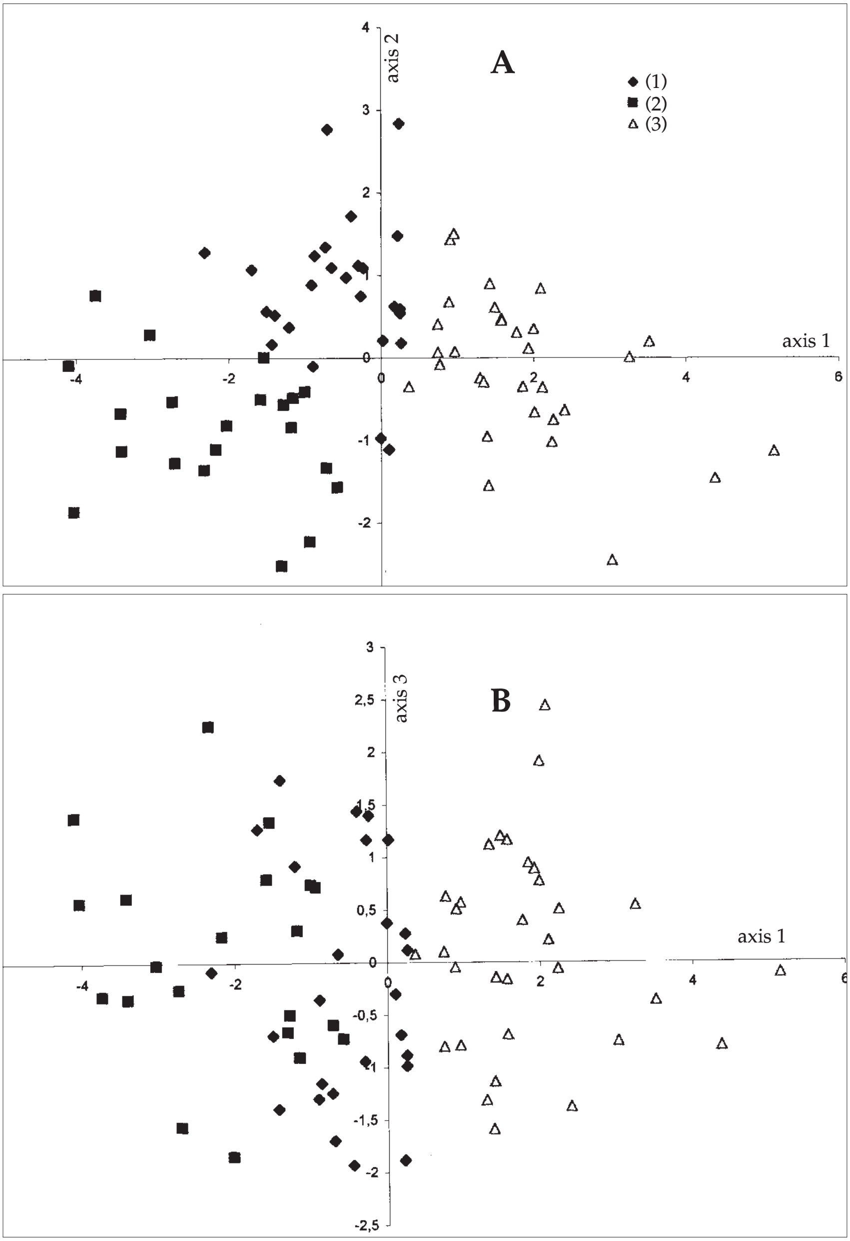

Fig. 4. Diagram of the *A. woodiana* collection arrangement into three significantly different subgroups that were determined by major component analysis (PCA) of the biometric characteristics; (A) according to axis 1 and axis 2, (B) according to axis 1 and axis 3. Mussel domination from the Konin lakes: 1 - Licheńskie Lake, 2- the discharge channel, 3 - the initial cooling reservoir.

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                         Similarity

.90        .92        .93        .95        .97        .98       1.00
+----+----+----+----+----+----+----+----+----+----+----+----+

                                                              ***** Group I
                                                              *
                                                              *   * Group II
                                                              *****
                                                                  * Group III

+----+----+----+----+----+----+----+----+----+----+----+----+
.90        .92        .93        .95        .97        .98       1.00
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Fig. 5. Diagram of the genetic similarity (I_N), (Nei 1978) between the *A. woodiana* mussel collections from Licheńskie Lake (Group I), the discharge channel (Group II), the initial cooling reservoir (Group III).

1 to 4 alleles. The values of the genetic similarity coefficient I_N between the *A. woodiana* groups were found to be within the range of 0.99–1.00, and thus were very high (Fig. 5). Conversely, the values of the genetic distance coefficient (D_N) were low (0.000-0.007) for the mussel groups.

DISCUSSION

The mussels *A. woodiana* are characterized by large shells and considerable individual weight. The biomass of the population of this species in the Thames River has been recorded to be 3,000 kg ha^{-1} (Kiss and Pekli 1988). In Hungary, this species is the largest of the native mussels, and its biomass in the Koros River contributed 75% to the total mussel biomass, i.e. 20-25 thousand kg ha^{-1} (Kiss 1992). Considering that the species invaded Hungary in 1962 (Kiss 1995), its high rate of growth is unusual. However, the growth of these mussels is not linear; during hot summer seasons it may even double in comparison with colder summers (Kiss 1995).

Considering the increased water temperatures in the Konin lakes, it could be expected that between the Polish species *A. woodiana* and populations of the same species in Hungary there are comparable growth rates and percentage contributions to the ecosystem. Such profound changes in the bottom community composition may have significant effects on the ecological equilibrium of the natural environment. The destabilization of trophic relations caused by the invasion of foreign species has been previously observed not only in mussels, but also in other invertebrates and vertebrates (Hebert et al. 1989, Borcherding 1991, Strayer 1991, Spidle et al. 1994, Zdanowski 1994, Marsden et al. 1996, Goldschmidt 1999, Lee 1999).

The Group III mussels *A. woodiana* from the initial cooling reservoir were comparable with regard to both form and size to the mussels described by Kiss (1992, 1995). They were morphologically separate from other populations occurring in different environments of the Konin lakes. They showed the highest average values of all the bio-parameters analyzed and were characterized by the least convex shape and the lowest convexity coefficients (100 D/H). This is due to the great shell height (H) in this group, and a shell convexity of about 4.5 cm, which was similar to that of other groups. The mussels of Groups I and III had round shells and exhibited the highest height coefficient values (100 H/L). The most elongated shells were observed in mussels from Group II which inhabited the sandy and muddy bottom of the warm, flow-through channels.

The shell parameters of individual mussel groups correlated with the thermal conditions of the environments. The largest sizes were reached by Group III individuals from the initial cooling reservoir, while the lowest were noted for Group I from Licheńskie Lake. A similar correlation was observed for *Dreissena polymorpha* (Pall.) In more intensely warmed environments, the body weight increase of this mussel in the Konin lakes was higher than shell growth (Stańczykowska 1976). Probably for the same reason, the *A. woodiana* mussels from Licheńskie Lake (Group I) were lighter and had the lowest height and the shortest length.

The variability of the biometric features of the shells did not show a distinct separation between the three mussel groups. More than 93% of the individuals of each group were contained within the significant range of standard deviation. The ranges of variability of particular characteristics would coincide with one another. Similarly high biometric variation of shells within one species has been noted in other mollusks, e.g. the snail *Macrogastra plicatula* or *Vestia elata* (Szybiak 1997, Abraszewska-Kowalczyk and Sulikowska 1998). Entirely atypical populations of *M. plicatula* from distant geographical regions have been observed (Szybiak 1997).

The variation of morphological features such as length, height, and shell convexity depend on many factors, i.e. the age of individuals, environment and access to food (Kraszewski and Zdanowski 2001). The analysis of a mussel population inhabiting a warm channel (Afanasjev et al. 2001) showed an allometric increase of young individuals and isometric growth of individuals more than five years old. The highest changeability in the three mussel groups was observed for wet weight. This is shown by very high values of the variation coefficient of 27 to 40% within groups and 43% for the entire collection. Such values are typical for an asymmetric distribution (Szybiak 1997). Variation of this extent may result from the extension of the shell and body weight observed with the mussels' age (Soroka 2000). The decrease of calcium carbonate in shells with age is also likely to be responsible for this phenomenon.

A. woodiana from the Konin lakes were characterized by a higher average shell length (L) than populations of this species from Hungary (Kośmider 2000). As reported by Kiss (1995), Hungarian three-year-old individuals were from 70 to 95 mm long, four-year-olds were 85-100 mm, five-year-olds 95-115 mm, and six-year-olds 110-120 mm long. The Polish mussels measured as follows: three-year-olds - 101-162 mm; four-year-olds - 128-176 mm; five-year-olds - 145-168 mm; six-year-olds - longer than 162 mm (Kośmider 2000). Young individuals up to 55 mm long inhabited the shallower parts of the channels, while older ones measuring 120-170 mm in length occurred in deeper areas (Protasov et al. 1997). The largest individuals of *A. woodiana* were observed in France and they were 250-270 mm long. In Hungary, they were 180-210 mm and in Hong-Kong 163 mm (Kiss and Pekli 1988, Kiss 1995).

Although the existence of mussels in extreme environmental conditions regarding salinity, temperature and water eutrophication may significantly affect their morphological characteristics, as was shown for *D. polymorpha* (Stańczykowska 1976). These conditions do not necessarily change the genetic structure of a population (Scott and Koehn 1990, Wenne 1993, Kilgour et al. 1994, Soroka 1996). The morphological variations of the shells of the three mussel groups studied were not corroborated by the genetic results. The morphological variation of the shells of this species may only have an environmental basis. Isoenzyme electrophoresis revealed the high genetic similarity of the three mollusk groups, which is characteristic for populations of the same species (Ayala 1982). The highest genetic similarity was shown between individuals from Groups II and III, both of which occurred in similar thermal conditions, i.e. in water with a temperature 4-6°C higher than that in Licheńskie Lake.

The great morphological variability of *D. polymorpha* shells has not been yet confirmed by genetic studies (Hebert et al. 1989, Marsden et al. 1996, Protasov and Sinicyna 2000). In spite of the considerable variation of the shape and size of the shell, as well as its color pattern, the single basic indicator of the *D. polymorpha* albino morphological type appears to be the lack of a black zigzag over the white background of the shell (Świerczyński 1994). Genetic studies using izoenzymatic electrophoresis of both the typical and albino morphological types of *D. polymorpha* revealed a high genetic similarity of both forms (I_N = 0.974) and the presence of the same alleles of similar frequencies of occurrence (Soroka 1996). Zebra mussels discovered in the Great Lakes region of the USA by May and Marsden (1992), named quagga, differed essentially from *D. polymorpha* in shell shape and the occurrence and frequency of alleles. Due to the low degree of the genetic similarity coefficient (I_N = 0.30), it was classified as the separate species *D. bugensis* (Spidle et al. 1994). The genetic analysis of a zebra mussel from the deeper parts of Erie Lake (the Great Lakes), tentatively called profunda, showed a distinctly white coloring of the shell; it occurred to be a phenotype of *D. bugensis* (Spidle et al. 1994).

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STRESZCZENIE

ZRÓŻNICOWANIE MORFOLOGICZNO-GENETYCZNE POPULACJI *ANODONTA WOODIANA* (LEA, 1834) W SYSTEMIE PODGRZANYCH JEZIOR KONIŃSKICH

Zbadano różnice biomorfologiczne i genetyczne (izoenzymy) nowego dla Polski gatunku małża *A. woodiana*, występującego w kompleksie podgrzanych jezior konińskich. Materiał do badań zebrano z trzech środowisk różniących się stopniem podgrzania i retencją wody (rys. 1): lenitycznych stref podgrzanego Jeziora Licheńskiego (26°C, grupa I), przepływowego kanału zrzutowego wód podgrzanych (30°C, grupa II) i lotycznych stref zbiornika wstępnego schładzania wód zrzutowych z elektrowni (32°C w okresie letnim, grupa III). Pomiary biometryczne małży powtórzono po półrocznej hodowli w warunkach laboratoryjnych.

Analiza statystyczna danych wykazała, że znaczna zmienność analizowanych parametrów biometrycznych małży trzech zbadanych zbiorów była podobna (rys. 2 i 3). Poszczególne grupy w najmniejszym stopniu różniły się między sobą pod względem wypukłości muszli (D), a w największym pod względem masy ciała i muszli (W) oraz długości muszli (L). Cechami biometrycznymi najbardziej zbliżonymi do średnich wyróżniały się podobne do siebie małże grupy I i II, pochodzące z Jeziora Licheńskiego i kanału zrzutowego, a wyraźnie różniącymi się - małże grupy III, pochodzące ze zbiornika wstępnego schładzania.

Uporządkowanie cech biometrycznych całego zbioru małży, dokonane metodą analizy głównych składowych (PCA), pozwoliło wydzielić trzy istotnie różniące się podzbiory (rys. 4). Do podzbioru 1 należały małże grupy I (59%) z Jeziora Licheńskiego i grupy II (38%) z kanału zrzutowego, do podzbioru 2 - małże grupy II (59%) z kanału zrzutowego i grupy I (31%) z Jeziora Licheńskiego, a do podzbioru 3 - głównie małże grupy III (86%) ze zbiornika wstępnego schładzania wód zrzutowych. Parametrem istotnie różnicującym wydzielone podzbiory była waga małży oraz długość i wysokość muszli. Podzbiór 1 z Jeziora Licheńskiego od pozostałych różnił się istotnie niższą wypukłością muszli, a podzbiór 3 - wyższymi wskaźnikami wysokości (100H/L) i niższym wskaźnikiem wypukłości muszli (100D/H).

Zmienność cech morfologicznych małża *A. woodiana* ma podłoże środowiskowe i wskazuje na znaczną plastyczność tego gatunku do warunków środowiska. Nie została ona potwierdzona badaniami genetycznymi izoenzymów. Analiza genetyczna 7 enzymów ujawniła obecność 12 loci posiadających od 1 do 4 alleli. Wartości współczynnika podobieństwa genetycznego I_N, według Nei'a (1978), pomiędzy analizowanymi grupami *A. woodiana* były bardzo wysokie i mieściły się w zakresie od 0,993 do 1,00 (rys. 5). Wartości współczynnika odległości genetycznej (D_N) dla analizowanych grup małży były bardzo niewielkie i przyjmowały wartości od 0,000 do 0,007.

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