

Oceanological and Hydrobiological Studies

International Journal of Oceanography and Hydrobiology

Volume 43, Issue 3

ISSN 1730-413X

eISSN 1897-3191

(303–311)

2014



DOI: 10.2478/s13545-014-0145-8
Original research paper

Received:

July 09, 2014

Accepted:

September 05, 2014

Comparative study of ecophysiological and biochemical variation between the Baltic and North Sea populations of the invasive soft shell clam *Mya arenaria* (L. 1758)

Rafał Lasota, Karolina Pierścieniak,
Justyna Miąg, Maciej Wołowicz*

*Department of Marine Ecosystems Functioning
Institute of Oceanography, University of Gdańsk
Al. M. Piłsudskiego 46, 81-378 Gdynia, Poland*

Key words: *Mya arenaria*, acclimatization, adaptation, phenotypic plasticity, ecophysiology, biochemical components, respiration rate

Abstract

Seasonal variations of environmental factors, such as temperature and salinity, require metabolic acclimatization in sedentary benthic fauna distributed over a wide geographical range. The soft-shell clam *Mya arenaria* inhabits the coastal waters of the North Atlantic including North America and Europe. In Europe, *M. arenaria* populations are distributed from Iceland to the Mediterranean Sea, including the North Sea, the Baltic Sea and the Black Sea. Seasonal changes in physiological parameters (gonad index, condition index, biochemical composition and respiration rate) of *M. arenaria* from the Baltic Sea (the Gulf of Gdańsk, Poland), and the North Sea (Versee Meer, the Netherlands) were studied. The sex ratio of both populations did not differ from 1:1 and the seasonal gonad index was higher in the Baltic population. The average condition index changed seasonally at both studied sites, and was also higher in the Baltic population (except the autumn) compared to the North Sea. In

both studied populations, the content of proteins, lipids and carbohydrates in the soft tissue followed the seasonal variations, and it was higher in the Baltic population. The respiration rate was lower in the Baltic population, and seasonal changes in the respiration rate seem to be correlated with changes in the water temperature. Based on the results obtained in the present study, we suggest that *Mya arenaria* is characterized by a large phenotypic plasticity and differences in the observed physiological traits are due to acclimatization to ambient environmental conditions.

INTRODUCTION

The soft-shell clam *Mya arenaria* (L. 1758) is believed to be the first marine non-native/invasive species introduced into European waters by human (probably by the Vikings) from the Atlantic coasts of the North America in the 13th century (Petersen et al. 1992, Beets et al. 2003, Behrends et al. 2005). Its present wide distribution range in European boreal waters reflects the repeated human-mediated introductions and natural colonization, and extends from the White Sea, through the British Isles, the North Sea to the Bay of Biscay; the soft-shell clam is present also in the Black Sea and was even found in two Mediterranean lagoons (Stora et al. 1995, Strasser 1999, Wołowicz – own observations). *Mya arenaria* is an eurytopic species which is able to withstand a wide range of environmental conditions. It can live in the salinity between 4-35 PSU and easily survives a short term salinity fluctuation of 15 PSU. The northern limit of the distribution is determined by the temperature of 12-15°C required for the larval development, whereas the southern distribution limit is probably determined by the temperature of 28°C (Strasser 1999 and references therein).

Adaptation to different environmental conditions can occur either through phenotypic plasticity (acclimatization) or local genetic adaptation (changes in allele frequencies). Genetic studies on the soft-shell clam *Mya arenaria* based on protein-coding

* Corresponding author: ocemw@ug.edu.pl

markers, i.e. allozymes and mtDNA, showed a relatively low level of genetic polymorphism and a weak genetic structure on a large geographical scale as compared to other marine eurytopic invertebrates (Lasota et al. 2004, Strasser & Barber 2009). Thus, adaptation to environmental heterogeneity in the soft-shell clam may occur through physiological acclimatization. The aim of the present study was to determine differences in the ecophysiological traits and biochemical composition between two populations of *Mya arenaria* living in the Baltic and North Sea, and to identify links between those differences and local environmental conditions with special emphasis on salinity and temperature. The populations analyzed in the present study were previously subject to genetic analysis, which showed no significant geographical genetic differentiation of isoenzymes and COI mtDNA gene sequences (Lasota et al. 2004; Lasota et al. – in preparation).

MATERIALS AND METHODS

Sampling

Samples were collected from two locations (Fig. 1): sampling in the Gulf of Gdańsk (GG), the Baltic Sea, Poland ($54^{\circ}37'43,91''\text{N}$, $18^{\circ}31'36,54''\text{E}$), was performed by dredging at a depth of 5 m, and sampling in the Veerse Meer (VM), the North Sea, the Netherlands ($51^{\circ}34'57''\text{N}$, $3^{\circ}37'41''\text{E}$), was conducted manually from a depth of about 1 m. Sampling was performed seasonally. Dates of

sampling in the Gulf of Gdańsk were as follows: 16.07.2005, 04.10.2005, 09.02.2006, 22.05.2006; whereas in Veerse Meer: 02.08.2006, 13.11.2006, 04.02.2007, 28.05.2007, 07.08.2007 (the last sampling was done only for respiration rate measurements). About 50 specimens were collected at each sampling site in each season and immediately transported alive to a laboratory for further analysis. During each sampling event, temperature and salinity of the overlying bottom water were measured.

The Gulf of Gdańsk is a brackish water body with the average annual salinity around 6–7 PSU (Cyberska 1990). It is characterized by high levels of eutrophication and suspended matter in water, soft sediments and the dominant position of filter-feeders, deposit-feeders, and detritivorous species in the benthic community (Wiktor 1985, Kruk-Dowigiallo and Dubrawski 1998). The samples of *Mya arenaria* were collected in the inner part of the Gulf (Puck Bay) where the benthic community consists mainly of mollusks (*Macoma balthica*, *Mytilus trossulus*, *Mya arenaria* and *Hydrobia* spp.), polychaetes (*Harmothoe sarsi*, *Hediste diversicolor*) and crustaceans (*Pontoporeia femorata*, *Corophium volutator*, *Saduria entomon*) (Szaniawska et al. 1999).

The Veerse Meer is a man-made brackish water lake in the south-west Netherlands. It is a former branch of the Eastern Scheldt closed off by the construction of two dams in the 1960s, and turned into a stagnant brackish lake facing a continuous degradation process (Raat 2003, Wijnhoven et al. 2009). Certain connection with the Eastern Scheldt



Fig. 1. Location of the sampling sites

still exists through locks. The area of this water body is 20 km² and the salinity after completing and closing the dams varied from 29 PSU in summer to 20 PSU in winter. These values are rather stable because of the inward percolation of salt through the dykes. The Veerse Meer is an eutrophic reservoir with the green algae (*Ulua* sp.) blooms observed from May to October. Macrozoobenthos is dominated by *Mytilus edulis* and *Molgula manhattensis* (Tunicates) (Kamermans et al. 2002).

Histological examination and gonad index

The sex and the development of gonads were determined by observations of the gonadal tissue under a microscope (400× magnification) and classified according to a 5-grade scale; 1-initiation of gametogenesis (immature), 2-development, 3-ripe gonad, 4-spawning, and 5-post-spawning (resting), adapted after Chipperfield (1953) and Sunila (1981). The gonad index (GI) was defined as:

$$GI = \frac{\sum \frac{\text{the number of specimens of each sexual stage} \times \text{the numerical value of a stage}}{\text{the total number of specimens in a sample}}}{\text{the total number of specimens in a sample}}$$

The numerical values of the maturity stages were as follows: 1 – immature; 2 – developing; 3 – ripe; 4 – spawning; 0 – resting (Chipperfield 1953). The sex ratio in the populations was estimated based only on samples collected in spring and summer, when gonads were well developed, thus the determination of gender was possible for all specimens. In autumn and winter, proper identification was not possible for many specimens due to the lack of oocytes and spermatocytes. The problem with sex determination in the soft-shell clam after the breeding season had been already showed in the study of Miac et al. (1997).

Condition index and biochemical composition

The shell length was measured with slide callipers to the nearest 0.01 mm. To determine the dry weight of specimens, soft tissues were frozen at –80°C and freeze-dried for 72 hours. The shells were dried at 55°C for 48 hours. The morphometric condition index (CI) was calculated as follows: soft tissue dry weight (mg) per shell volume calculated from (length)³ (cm) (Beukema & De Bruin 1977, Wenne & Styczyńska-Jurewicz 1985). Dry soft tissues were homogenized with a planetary micro mill (Fritsch Pulverisette 7). The content of protein (Lowry et al. 1951), lipid (Bligh & Dyer 1959, Marsh & Weinstein

1966), carbohydrate and glycogen (Dubois et al. 1956) was determined for 30 specimens per season per site (3×10 pooled individuals).

Respiration rate

The respiration rate was measured seasonally. Within 24 h after sampling, soft-shell clams were gently removed from aquaria where they were purged overnight in filtered water from the sampling site at a temperature corresponding to the ambient temperature at a sampling site. The purpose of this procedure was to prevent any disturbance in the oxygen consumption due to the possible presence of micro-flora and fauna, and pseudo feces. The specimens were transferred to the temperature-controlled respiration chambers of 0.3 dm³ volume. The chambers were filled with filtered habitat water, previously aerated to 100% of oxygen saturation and were kept in a dark room to avoid light stress. The measurements continued until the oxygen tension in the chambers had decreased by 20–30%, i.e. from 50 to 120 minutes. Respiration rates were measured at water temperatures of 4, 10, 16 and 22°C in the case of samples from the Gulf of Gdańsk, and 4, 10, 17 and 24°C in the case of the Veerse Meer population, in a separate, temperature-controlled respiration chambers. The decrease in oxygen tension was measured with YSI 5331 oxygen probes (Clark-type polarographic electrodes). The measurements were performed in three replicates (3 respiration chambers with several clams of the same length classes in each chamber); control measurements were carried out using the same experimental setup without animals. After each measurement, animals were frozen in –80°C and freeze-dried for 72 hours to a stable weight. The soft tissue dry weight (in mg) was determined, and the mass-specific respiration rates were calculated for each experimental temperature.

Statistical analyses

The sex ratio of the clams was compared to the 1:1 ratio using the χ^2 test. Statistical differences in seasonal and geographical variability of the condition index, biochemical composition and the respiration rate were tested by Kruskal-Wallis non-parametric ANOVA. Analyses were performed using the STATISTICA® software (version 10, StatSoft, Inc.) and Microsoft® Office Excel software.

RESULTS

Environmental parameters

The measured value of seasonal temperature and salinity at sampling sites represents the local conditions during the experiments. The water in the Gulf of Gdańsk is much more brackish. The average temperature was also lower in the Gulf of Gdańsk, located further to the north, except the autumn season when the recorded temperature was a little higher than in the Veerse Meer. Despite minimal differences in the depth, both sites can be regarded as shallow, and temperature changes at both sites seemed to follow local meteorological trends with a temperature decrease in cold seasons and an increase in warm seasons (Table 1).

Sex ratio

The males to females' sex ratio at both sampling sites did not differ significantly from 1:1 ($p < 0.05$, χ^2 -test).

Gonad index (GI)

In the Gulf of Gdańsk, after fast winter gonad development, the majority of specimens (70%) were mature in May (Fig. 2). At the beginning of September, all specimens were already after

Table 1

Temperature (°C) and salinity (PSU) measured at each sampling site in each season

	Sampling point		VM		GG	
	Parameters		T	S	T	S
Season	Summer		21	29.0	19	7.2
	Autumn		10	28.5	12	6.4
	Winter		3	22.5	0.4	7.3
	Spring		15	27.3	11	6.2

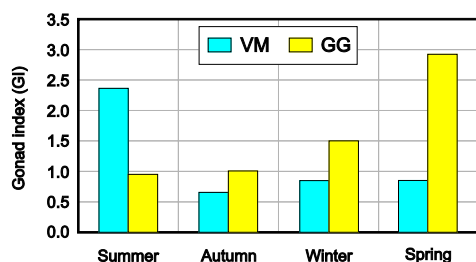


Fig. 2. Seasonal changes in the gonad index (GI) at sampling sites VM and GG

spawning, at the first stage of gonad development, which suggests the reproduction in late spring/early summer. In the Veerse Meer, the development of gonads was slower, but the reproduction occurred also in summer as in the case of the GG population.

Condition index (CI)

Values of the condition index differ significantly between the regions (Kruskal-Wallis ANOVA, $p < 0.05$) and during the year at each sampling site (Kruskal-Wallis ANOVA, $p < 0.0001$ in both regions). The condition index of the soft-shell clams from GG gradually increased from summer (after spawning) through autumn and winter and reached a higher value in spring (Fig. 3). Seasonal changes in the VM population CI was different as compared to GG, the highest CI value was observed in autumn (Fig. 3).

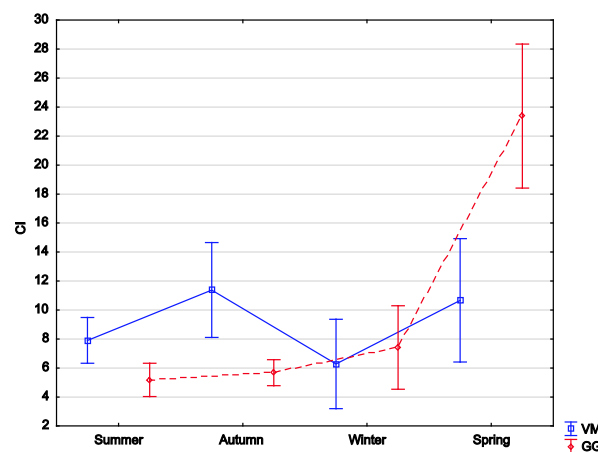


Fig. 3. Seasonal changes in the condition index (CI) at sampling sites VM and GG (average $\pm$ standard deviation)

Biochemical composition of soft tissues

The average annual values for all the components were higher in the population from the Baltic Sea (Fig. 4), however, statistically significant differences between sites were found only for the content of proteins and lipids (Kruskal-Wallis ANOVA, $p < 0.001$).

The mean seasonal content of proteins varied between 47.3% and 59.3% at GG, and between 22.8% and 34.1% at VM. The content of lipids was between 4.2% and 6.3% at the GG site, and from 1.4% to 2.4% at the VM site. Seasonal differences in

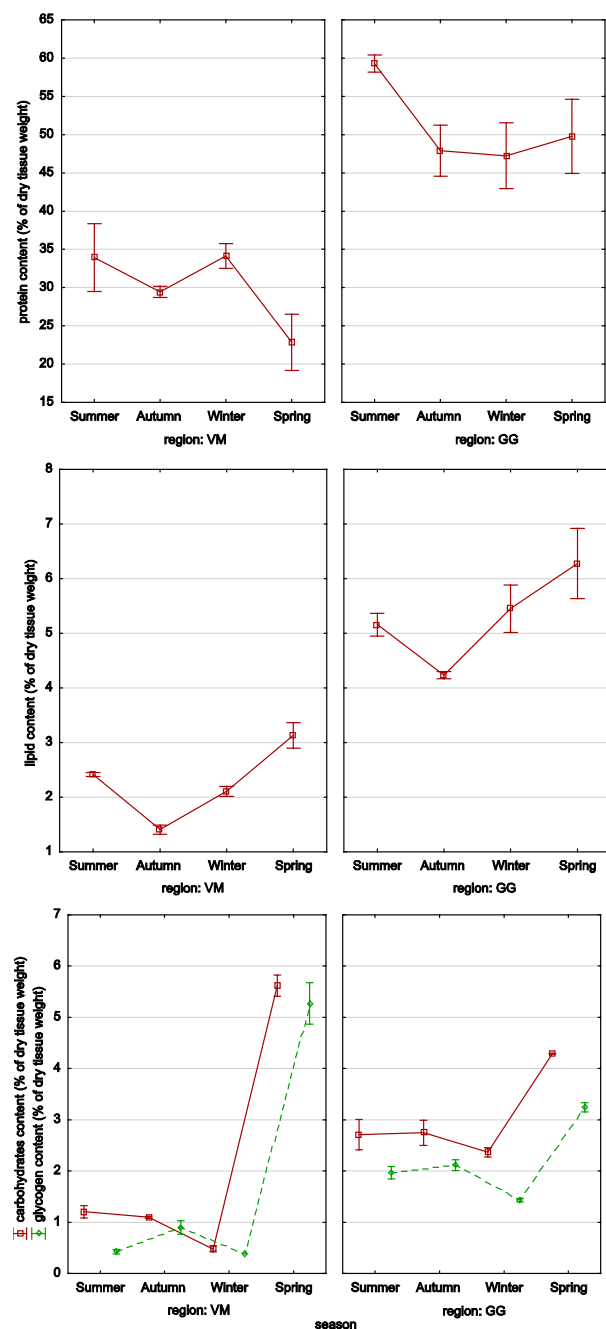


Fig. 4. Seasonal changes in the content of biochemical components at sampling sites VM and GG (average $\pm$ standard deviation)

the content of proteins and lipids in each of the studied regions were statistically non-significant (Kruskal-Wallis ANOVA, $p > 0.05$). The content of carbohydrates was between 2.4% and 4.3% at GG, and between 0.5% and 5.6% at VM. In both cases, 70% to almost 100% of carbohydrates were

represented by glycogen (Fig. 4). Seasonal differences in the content of carbohydrates and glycogen were statistically significant in both regions at the level of 0.05, mainly due to a major increase in spring (Fig. 4).

Respiration rate

In general, the respiration rate of the soft-shell clam from VM was higher than that from GG (Fig. 5) (not statistically tested since measurements were performed in slightly different temperatures). Seasonal differences in the respiration rate were statistically significant in the population from the Veerse Meer (Kruskal-Wallis ANOVA, $p < 0.01$). The respiration rate in both populations increased with temperature (GG $p < 0.0001$, VM $p < 0.01$). The highest respiration rate was recorded in spring and autumn, at the most elevated temperature, reaching

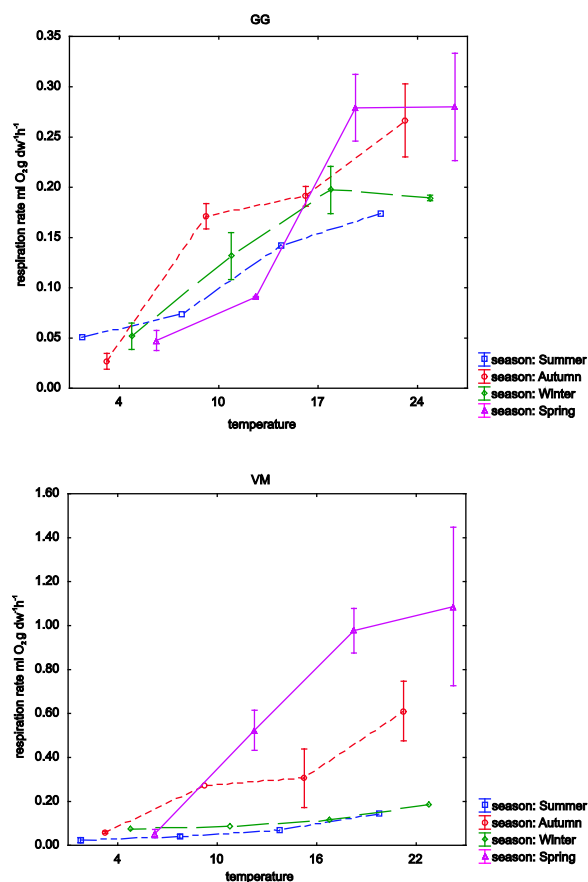


Fig. 5. The respiration rate at different water temperatures in each season (the values are average of two or three replicates of measurements, $\pm$ standard deviation, except summer in GG and winter in VM, where there were no replicates)

the value of 0.28 ml O₂ g dw⁻¹ h⁻¹ in GG in spring, and 1.1 ml O₂ g dw⁻¹ h⁻¹ in VV, respectively (Fig. 5).

DISCUSSION

Seasonal variations in seawater temperature require metabolic acclimatization of organisms inhabiting the coastal, shallow waters of north Europe. In the case of the two studied areas, also the difference in the water salinity affects the physiology of benthic organisms. It is because of higher energy demands for the regulation of active intracellular ion transport and particularly osmoregulation. Organisms under osmotic stress have to expend additional energy to maintain the haemolymph osmolality above that of the environment (Schmidt-Nielsen 1997). Environmental temperature and salinity influence important physiological processes like activity, including the filtration rate and (depending on the feeding rate and food absorption), respiration, excretion, reproduction etc. Environmental conditions play an important role in the energy budget of ectotherms, but also in the food availability and finally in the growth rate of organisms.

The bivalves from the northern European populations have a monocyclic reproductive cycle with the initialization of gametogenesis at the end of autumn and the beginning of winter (September/October), the development of gonads and the maturation of oocytes and spermatozoa in spring, and the spawning period at the turn of spring and summer. The spawning in GG and VM populations occurs in summer, while in VM it appears to be a little later. A similar observation was made on *Cerastoderma glaucum* populations from the same sites (Tarnowska et al. 2009) and on *Macoma balthica* and *M. trossulus* from the Gulf of Gdańsk (Pierścieniak et al. 2010). The gonad index (GI) followed a similar pattern for both populations, with higher values in the reproduction period (spring-summer; in the case of GG, the summer sample was collected already after breeding, thus the value of the gonad index was low) and lower values during the resting time (autumn and winter). As evidenced by Newell et al. 1982, and Navarro et al. 1989, the reproductive cycle is stimulated also by food availability. In the case of boreal bivalve species, the water temperature in spring correlates with the phytoplankton development and is a crucial environmental factor initiating the reproduction. The temperature and food availability is critical for spawning time, which was also proven for many

bivalve species from the boreal zone, including *Macoma balthica*, *Mytilus edulis*, *Cardium edule* (Honkoop & Van der Meer 1998) and *Cerastoderma glaucum* (Kingston 1974).

The condition index depends mainly on the growth, the metabolism and the gonad development stage of bivalves (Lucas & Beninger 1985, Hummel et al. 2000). In the northern populations of bivalves with a monocyclic reproduction pattern, the highest values of the condition index were reported in spring, before spawning. In the Gulf of Gdańsk, such a pattern is followed by the populations of *Cerastoderma glaucum* (Tarnowska et al. 2009), *Macoma balthica* (Hummel et al. 2000) and *Mytilus trossulus* (Wołowicz et al. 2006). The CI of GG population reached the highest value in spring, whereas in VM – in autumn, which reflects the favorable trophic conditions resulting from spring and autumn phytoplankton blooms (Pliński 1995).

Similarly to other invertebrates, carbohydrates and/or lipids are the main sources of energy reserves in marine bivalves. Lipids are the main energy reserves in the northern populations, whereas carbohydrates, i.e. glycogen – in the southern ones. The trophic conditions depend on the seasonal food availability, and in northern boreal zone they determine seasonal changes in the main biochemical components. Variation in biochemical constituents are also affected by reproductive cycles. In the VM and GG populations, the pattern of biochemical components (proteins, lipids and carbohydrates) shows similar seasonal changes, but their content (except carbohydrates in spring) was about two times higher in GG. This may be due to the fact that the population of VM could be exposed to poorer trophic conditions than the population of GG. This can be confirmed by larger changes in protein levels of the population from the North Sea compared to the population from the Baltic Sea. In unfavorable trophic conditions, the energy reserves accumulated in carbohydrates are used first, then reserves in lipids, and finally in structural protein (Beninger & Lucas 1984).

The highest lipid content in the soft tissue of *M. arenaria* in both populations studied was recorded just before spawning, as lipids are the major component of oocytes (Holland 1978, Gabbott 1983, Miąg 1997). After spawning, the lipid content in both populations decreased, followed by a slight increase in winter, which reflects higher food availability during the autumn phytoplankton bloom and development of oocytes (Houlihan 1991).

Carbohydrates were characterized by the highest rate of changes over the study period. It is assumed that carbohydrates are the primary source of energy for bivalves, especially during the non-productive time (Gabbott & Bayne 1976). Organisms that live in the environment characterized by the high abundance of nutrients, first store carbohydrates, which are converted into lipids when the water temperature decreases (Giese 1966). Thus, an increase in the content of lipids was observed after the autumn phytoplankton bloom. The lowest content of carbohydrates and glycogen was observed in GG and VM from summer to winter. A large increase in carbohydrates and glycogen was noted at both sites in spring, which can be associated with the phytoplankton bloom (Pliński 1995, Wasmund et al. 1998). It means that good trophic conditions delivered enough carbohydrates as a source of immediate energy for basic physiological processes, but also for the development of gonads in females, as they are converted into lipids in the oocytes. The lipids are better reserves of energy because their energy value is two times higher (37.8 kJ g^{-1}) compared to carbohydrates (16.8 kJ g^{-1}).

The respiration rate reflects the actual metabolic activity of an organism connected with various physiological processes (e.g. activity, osmoregulation, gametogenesis, growth). The respiration rate depends on ambient temperature (Clarke 1991), and could be reduced as an effect of exposure to stress, for example limited food availability (Oeschger 1990). The respiration rate in the studied populations increased with temperature. The respiration (at 22°C) of GG soft-shell clams reached the highest rates in spring and autumn and exceeded $0.24 \text{ ml O}_2 \text{ g dw}^{-1} \text{ h}^{-1}$. In VM, the highest respiration rates were noted also in spring and autumn (at 24°C) but reached much higher values (maximally $1.1 \text{ ml O}_2 \text{ g dw}^{-1} \text{ h}^{-1}$). These values are much lower compared to those observed in cockle *Cerastoderma glaucum* from the same sites, with the highest values in spring for the GG population – $3.2 \text{ mg O}_2 \text{ g dw}^{-1} \text{ h}^{-1}$, and $1.4 \text{ mg O}_2 \text{ g dw}^{-1} \text{ h}^{-1}$ in summer for cockles from VM, at the experimental temperature of 24°C ($1 \text{ ml O}_2 = 0.7 \text{ mg O}_2$) (Tarnowska et al. 2009). The higher respiration rate in cockles from the Gulf of Gdańsk is in contrast to the present study and may suggest that *Mya arenaria* is very well adapted to low salinity, which does not cause elevated energetic costs of osmoregulation and reduced energy expenditures for other metabolic processes (Newell 1969). The results obtained for ten populations of *Mytilus* spp., and six

populations of *M. balthica* from the European waters showed that the respiration rates in experimental temperature of 24°C have never exceed $2.5 \text{ mg O}_2 \text{ g dw}^{-1} \text{ h}^{-1}$, with the exception of *M. balthica* from the Westerschelde estuary, NL (Jansen et al. 2007). Although the respiration rate in *M. arenaria* is in the range reported for other marine bivalves, it can be regarded as relatively slow. High respiration rates at a considerably elevated temperature, observed in spring and autumn in both populations, could be explained by low energy required for enzyme catalyzed reactions in these seasons (Hochachka & Somero 2002), or by additional energy required for gonad development. The lowest respiration rates observed in both populations in summer could be explained as metabolic down-regulation of energy saving in marine cold-blooded invertebrates (Jansen et al. 2007). This saving energy mechanism was observed at high water temperatures in southern populations during summer (Wilson & Elkaim 1991).

A key issue to consider is whether the potential differences in the physiological traits of specimens from different populations reflect a local genetic adaptation, resulting from allele frequency changes by natural selection, or they reflect acclimatization related to adaptive changes in intrasystemic processes. Based on the physiological data obtained in the present study and taking into account low genetic differentiation between the populations studied (Lasota et al. 2004; Lasota et al. – in preparation), we can suggest that differences in the observed physiological traits are rather due to acclimatization to ambient environmental conditions. The soft-shell clam *Mya arenaria* may be characterized by large phenotypic plasticity. The term phenotypic plasticity refers to the ability of an organism to acclimatize to varying conditions through changes in morphology, behavior and physiological condition within one genotype (Bayne 2004).

ACKNOWLEDGMENTS

We would like to thank H. Hummel and J. Jansen from The Netherlands Institute of Ecology (NIOO-KNAW) and A. Sokolowski from the Institute of Oceanography UG for their help in sampling and respiration experiments.

The research was founded by the Polish State Committee for Scientific Research (KBN) under grant N N304 033 31/1167.

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