

## Effect of eutrophication on the distribution and ecophysiology of the mussel *Mytilus trossulus* (Bivalvia) in southern Baltic Sea (the Gulf of Gdańsk)

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### Abstract

The effect of eutrophication on benthos is usually reported as negative. In the case of the Gulf of Gdańsk, eutrophication has increased the availability of food resources to filter feeders such as mussels, affecting their physiology and vertical distribution. Comparative studies of the mussel *Mytilus trossulus* from two depth zones (10 m and 40 m) over a seasonal cycle revealed ecophysiological differences between deep- and shallow-water animals. For the same shell length, the shallow-water mussels were heavier (dry weight =  $0.004L^{2.297}$ ) and showed a higher weight index (dry weight/volume), averaging  $3.8 \pm 1.9 \text{ mg cm}^{-3}$ , than the deep-water mussels (dry weight =  $0.0002L^{1.726}$  and  $2.5 \pm 1.1 \text{ mg cm}^{-3}$ ), primarily because of a nearly twofold greater carbohydrate store. In the shallow zone, females contained more carbohydrates (on average 5.8% dry weight) than males (3.8% dry weight) because females conserve energy for reproduction. Differences in physiologic variables, and subsequently physiologic performance of the mussels, were related to different nutritional conditions in the ambient water. The deep habitat had lower Chl *a*, averaging  $1.1 \text{ mg L}^{-1}$ , total particulate matter (TPM)  $4.3 \text{ mg L}^{-1}$  and particulate organic matter (POM) 1.1% of TPM as compared with the shallow habitat, with average Chl *a*  $2.5 \text{ mg L}^{-1}$ , TPM  $4.9 \text{ mg L}^{-1}$ , and POM 1.5%. The eutrophication of the Gulf of Gdańsk has led to an increase in the food availability in the water column, which allowed efficient colonization of the deep zone by the mussels; however, the dominance of males over females (~3:1) suggests that the food sustains only spawning-related metabolic demands and is not sufficient for energy conservation in this zone. Starch gel electrophoresis of eight enzyme loci showed no statistical differences in the allele and genotype frequencies between the shallow- and deep-water mussels; thus, the differences in ecophysiological traits between depths are due to acclimatization.

Anthropogenic eutrophication has become a global problem, affecting many marine systems (Diaz et al. 2004). The resulting ecologic effects of eutrophication (Cloern 2001), including habitat destruction, shifts in species composition and distribution range, invasion of nonnative species, and changes in food web efficiency, are increasingly apparent in benthic communities in coastal areas and estuaries of a high level of residential and industrial development. The Baltic Sea has been subject to a significant anthropogenic input of nutrients during the last century, and eutrophication now has large-scale implications (Cederwall and Elmgren 1990; Ærtebjerg et al. 2001; HELCOM 2003). Inherent properties of the sea, namely, restricted water exchange with the oceanic system, considerable inflow of freshwater, little depth, and strong halo-thermal water stratification, make the Baltic vulnerable to anthropogenic influences (Wulff et al. 1990), providing a good environment in which to study the consequences of eutrophication at various levels of the ecosystem organization.

Clear-cut alterations, which are convincingly linked to eutrophication, have occurred in the population of the epifaunal

suspension-feeding mussel *Mytilus trossulus*, which, in the Baltic, inhabits predominantly sandy and muddy-sandy bottoms of the littoral zone, showing a patchy or homogenous distribution pattern (Wiktor and Pliński 1992). The mussel plays an important role in the transfer of organic matter from the water column to sediments through filtration and bio-deposition and, thus, contributes in coupling energy fluxes between pelagic and benthic systems (Kautsky and Evans 1987). As a result of approximately doubled primary production (Cederwall and Elmgren 1990), subsequent increase in total particulate matter content (Maksymowska et al. 1997), and a sedimentation rate of up to  $1\text{--}2 \text{ mm yr}^{-1}$  (Witkowski and Pempkowiak 1995) in coastal waters of the southern Baltic over recent decades, the spatial distribution and standing stock of *M. trossulus* have extended toward a deep zone. In the 1960s, the mussel inhabited regions of a maximum depth of 20–25 m and reached highest abundance and biomass at 7–12 m. By the end of the 1980s, the mussel was present in dense beds down to 20 m, making up to 80% of the total macrozoobenthos biomass on the hard substrate, and occurred below 20 m (Osowiecki 2000). The expansion of *M. trossulus* into the deep regions at that time was also reported in other Baltic basins, e.g., in the Baltic archipelago (Jansson and Kautsky 1977). The abundance of the mussels in the Gulf of Gdańsk, southern Baltic, increased substantially in a deep zone of 40–50 m from the 1990s, leading to a currently much more homogenous distribution to the depth of 40 m where the mussel had not been present (Osowiecki 2000). The biomass increased from 13.4 to 326.1 g dry weight  $\text{m}^{-2}$  at 38 m in a central part of the Gulf between 1982 and 1997, possibly mediated by a concurrent reduction

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### Acknowledgments

This work was supported by the University of Gdańsk grant to M.W. (BW-1320-5-0300-3) and the Ministry of Education of Yemen in a form of 3-year scholarship to A.S.B. The authors wish to thank two reviewers for their helpful and valuable comments.

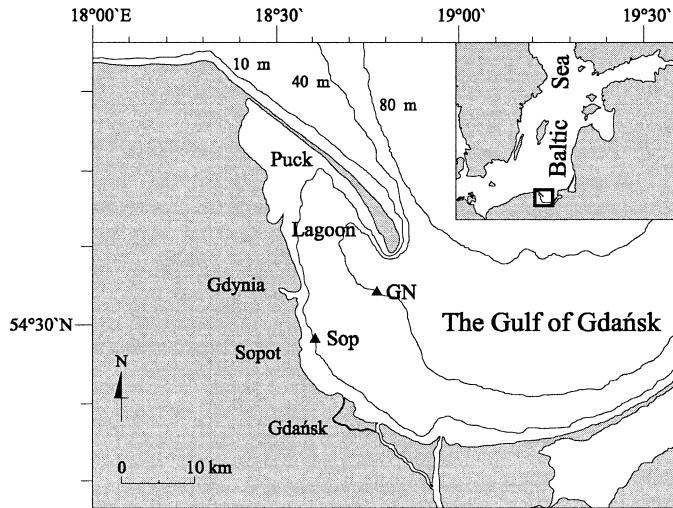


Fig. 1. Location of sampling sites in the Gulf of Gdańsk (southern Baltic Sea).

in the biomass of the Baltic clam *Macoma balthica* from 245.1 to 160.6 g dry weight  $m^{-2}$  at this depth, due to the shift of the clam to deeper regions (HELCOM 2003). Divergent hydrologic regime and ecologic conditions in the shallow and deep habitats that result from the seasonal water stratification (halocline, thermocline) occurring at a depth of 20 to 50 m may be expected to affect the ecophysiological performance of the mussel through acclimation to specific environmental situations. Such a depth-related physiologic difference has been documented for *M. balthica*, whose deep-water subpopulation in the Gulf of Gdańsk demonstrated considerably different ecophysiological features (e.g., condition index, biochemical composition, gonadal development cycle) than the shallow-water subpopulation (Bonsdorff and Wenne 1989; Hummel et al. 2000).

The present study assesses the effect of eutrophication on the ecophysiology of the mussel *M. trossulus* from two depth zones in the seriously eutrophicated Gulf of Gdańsk (Łysiak-Patuszak et al. 2004), using ecophysiological variables and biochemical components. Simultaneous records of hydrologic parameters of the overlying bottom water during the seasonal cycle have identified the principal environmental factors that account for the observed differences. The genetic variability of the mussels was also measured, using isoenzyme electrophoresis, to determine whether the observed ecophysiological differences are based on ecophysiological acclimatization or due to local genetic adaptation.

## Materials and methods

**Mussels *M. trossulus***—The mussels were dredged monthly from February 1997 to February 1998 at two sublittoral sites situated at a distance of 10.9 km from each other: a shallow site “Sop” and a deep site “GN” to depths of 10 and 40 m, respectively (Fig. 1). Only individuals of a restricted size range (20.1–30.0 mm) were taken. In the laboratory, mussels were depurated in aquaria with glass-fiber filtered (GF/C; 1.2  $\mu m$ ) seawater at a temperature and salinity corresponding to the actual environmental situation for

24 h. Eighty additional individuals of the same size range were collected at the two sites for genetic constitution analyses in December 2003.

**Morphometry, dry weight, and sex**—Individual shell length (along the longest anterior–posterior axis) of 15 to 21 specimens from each site on each sampling occasion was measured to the nearest 0.01 mm using a digital caliper. The mussels were then deshelled and sexed by the appearance of the gonad and reproductive cells under the microscope to determine sex ratio. After lyophilization (for 48 h at a temperature of  $-40^{\circ}C$  and pressure of  $2 \times 10^{-2}$  bar), the soft tissue was weighed to record individual dry weight, homogenized in a commercial mortar, and pooled in triplicate with respect to sex. Pools of five to seven individuals were kept frozen in polyethylene vials at  $-20^{\circ}C$  until the time of analysis.

Relationship between shell length ( $L$ ) and soft tissue dry weight of *M. trossulus* was described using exponential function according to the formula  $dry\ weight = aL^b$ , where  $a$  denotes dry body weight of an organism of unit length and  $b$  is the allometric exponent. The graphical representation of this function produces a curve for which the goodness of fit was checked according to Sokal and Rohlf (1995).

**Weight index (CI) and biochemical measurements**—The weight index was calculated for females and males separately as the weight after lyophilizing per volume (volume calculated from  $length^3$ ) (Beukema and de Bruin 1977). The protein content was determined according to the method of Lowry et al. (1951). Lipids were extracted as described by Bligh and Dyer (1959) and measured following the method of Marsch and Weinstein (1966). The contents of total carbohydrates and glycogen were determined according to the method of Dubois et al. (1956).

**Genetic constitution**—Mussels collected for genetic constitution were dissected, and a small part of the mantle, homogenized with 0.2 ml of gel buffer, was used for starch gel electrophoresis of seven enzyme systems: Glucosephosphate isomerase (*Gpi* E.C 5.3.1.9), Isocitrate dehydrogenase (*Idh* E.C 1.1.1.42), Leucine aminopeptidase (*Lap* E.C 3.4.1.1.1), Octopine dehydrogenase (*Odh* E.C 1.5.1.11), Phosphoglucuronate dehydrogenase (*Pgd* E.C 1.1.1.44), Phosphoglucumutase (*Pgm* E.C 5.4.22), and Malic enzyme (*Me* E.C 1.1.1.40). Electrophoresis and enzyme visualization was performed according to methods described in Hummel et al. (2001). Allele frequencies, observed heterozygosity ( $H_o$ ), and expected heterozygosity ( $H_e$ ; the unbiased estimate of Nei 1978) were calculated using the BIOSYS program (Swofford and Selander 1981). Allelic and genotypic differentiation was tested with a Fisher exact test (Raymond and Rousset 1995a) and a log-likelihood ( $G$ ) based exact test (Goudet et al. 1996), using the GENEPOP 3.1d program (Raymond and Rousset 1995b). An unbiased estimate of  $p$  values was performed using a Markov chain method (standard errors associated with the  $p$  values were kept  $<0.01$ ). Differences in mean observed and expected heterozygosity were tested with Wilcoxon’s signed-ranks test.

Table 1. Significance of the effect of month and site on environmental parameters, and month, site, and sex on tissue dry weight, weight index (CI), and biochemical components in the mussel *M. trossulus* from the Gulf of Gdańsk, southern Baltic Sea (two- and three-way ANOVA).

	Month	Site	Sex
(a) Environmental parameters			
Temperature			
Salinity		**	
O <sub>2</sub>			
Chl <i>a</i>		*	
TPM		*	
POM		*	
(b) Ecophysiological features of <i>M. trossulus</i>			
Dry weight		***	
CI	*	***	
Protein	**	*	
Lipid	***		
Carbohydrates	***	***	
Glycogen	***	***	*

Empty cell, no effect; \*  $p < 0.05$ ; \*\*  $p < 0.01$ ; \*\*\*  $p < 0.001$ .

**Hydrologic parameters**—At each site, basic hydrologic parameters of overlying bottom water were recorded over the same period. Overlying bottom water was collected with a GoFlow universal water sampler (Niskin type, 5-liter capacity) at a constant distance of 30 cm over the sea bottom to avoid sediment resuspension due to the sampler action. Temperature, salinity, and dissolved oxygen concentration (O<sub>2</sub>) were measured immediately after sampling with a WTW Multiline P4 analyzer. Chlorophyll *a* concentration (Chl *a*), total particulate matter (TPM), and particulate organic matter (POM) were determined according to standard methods (Kramer et al. 1994).

**Statistical analysis**—The amplitude of the seasonal variation was described as the “seasonal factor” and calculated as the ratio of the highest value of a respective parameter to the lowest value (Bordin et al. 1992). Untransformed data were included into all statistical models followed by analysis of normality (the Kolmogorov–Smirnov test for goodness of fit) and homogeneity of variances as prerequisites to the parametric approach. The functional relation between pairs of variables was assessed by correlation analysis; the significance of individual differences in length and soft tissue dry weight of the mussels between sites was estimated with discriminant function analysis and post hoc classification probability. Mean data were compared with respect to month, site, and sex by analysis of variance (ANOVA). Multiple regression analysis was used on combined data from the two sites to allow for effects of environmental parameters on ecophysiological features. The level of significance for all tests was set as  $p < 0.05$ . Statistical analyses were carried using Statistica (Sokal and Rohlf 1995).

## Results

**Hydrologic parameters**—Salinity, Chl *a*, TPM, and POM in the overlying bottom water were significantly different

Table 2. Amplitude of seasonal variation (highest value/lowest value) for (a) environmental parameters and (b) ecophysiological features of *M. trossulus* at two sites in the Gulf of Gdańsk (southern Baltic Sea).

	Sop	GN
(a) Environmental parameters		
Temperature	8.80	11.20
Salinity	1.10	1.05
O <sub>2</sub>	1.82	1.56
Chl <i>a</i>	11.90	9.70
TPM	2.50	2.10
POM	3.30	3.10
(b) Ecophysiological features of <i>M. trossulus</i>		
Dry weight	6.56	4.84
CI	3.19	3.15
Protein	1.38	1.32
Lipid	3.34	2.90
Carbohydrates	7.16	5.05
Glycogen	29.45	19.77

between sites (ANOVA  $p < 0.05$ ; Table 1). All environmental parameters, except salinity, tended to vary with season. Hydrologic conditions in the water over the sea bottom at the site GN, which is located in a central part of the Gulf of Gdańsk and away from direct terrestrial inputs, were generally less variable than at Sop, which is located in a shallow coastal zone and subject to a lower seasonal factor (Table 2). At the shallow site, water temperature ranged from 2.2 to 19.4°C, averaging 8.3°C, and generally followed local meteorologic condition trends. At the deep site, temperature was lower, averaging 5.4°C (range 1.2–13.4°C) (Fig. 2). Dissolved oxygen concentration ranged between 6.2 and 11.3 mg L<sup>-1</sup> at Sop and between 7.1 and 11.1 mg L<sup>-1</sup> at GN, i.e., within normoxic values, and showed a similar seasonal pattern at the two sites (correlation analysis,  $p < 0.05$ ). Salinity varied slightly throughout the year and was lower at Sop (average 7.3) than at GN (7.6). Chlorophyll *a*, TPM, and POM differed between sites, averaging 2.5 and 1.1 mg Chl *a* L<sup>-1</sup>, 4.9 and 4.3 mg TPM L<sup>-1</sup>, 1.5% and 1.1% of TPM at Sop and GN, respectively. The total particulate matter exhibited two peaks at Sop, namely, in early summer (May through July) and autumn (November), when the maximum of 8.47 mg L<sup>-1</sup> was recorded. One major peak in late summer/autumn (September through November), with the maximum of 5.80 mg L<sup>-1</sup> in September, was observed at GN. At the two sites, variations in TPM in the overlying bottom water reflected the presence of organic particles (Fig. 2), which accounted for 16.4% to 54.4% of TPM. A seasonal change, similar to the TPM pattern, was also observed for Chl *a* and POM, with elevated values in May and November declining in December. A significant correlation was found between TPM and POM at GN ( $p < 0.05$ ) and between Chl *a* and POM at Sop ( $p < 0.05$ ), suggesting that phytoplanktonic cells are the principal source of organic matter in the water column, particularly at the shallow site. During intense biological production, the contribution of Chl *a* (phytoplankton) to TPM reached 98.8% (at Sop in October) and 43.7%

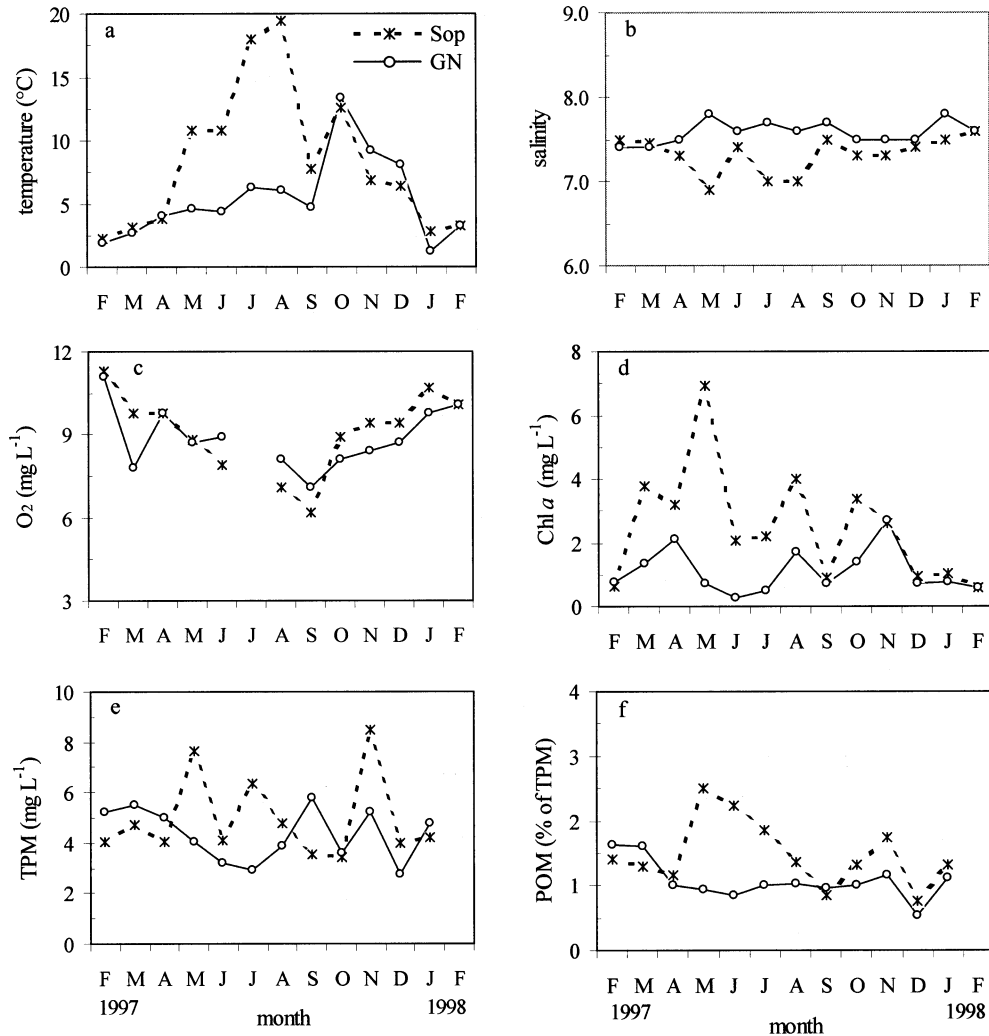


Fig. 2. Seasonal variations in (a) temperature, (b) salinity, (c) concentrations of dissolved oxygen (O<sub>2</sub>), (d) chlorophyll *a* (Chl *a*), (e) total particulate matter (TPM), and (f) particulate organic matter (POM) in overlying bottom water at two sites (Sop and GN) in the Gulf of Gdańsk (southern Baltic Sea).

(at GN in August), in the cold months it diminished to 15.9% and 14.3% (in February) at Sop and GN, respectively.

*M. trossulus*—Morphometry, dry weight, and sex ratio: Soft tissue dry weight varied significantly between sites (AN-OVA  $p < 0.001$ ), but no difference was apparent for month or sex (Table 1). The pattern of seasonal changes was similar at the two sites for the combined data (males and females pooled), and when males and females were considered as separate groups within a single site (correlation analysis  $p < 0.05$ , Fig. 3). The mussel tissue dry weight increased gradually in spring/early summer (from March to June) followed by a sharp decrease in July, indicating the end of gamete release at that time and the beginning of a new gametogenetic cycle to extend over autumn and winter. The pattern was more pronounced at Sop, as indicated by the seasonal factor (6.56 and 4.84 at Sop and GN, respectively) (Table 2). Spatial differences in tissue weight were clearest during the spring,

when average dry weight (for both sexes) nearly doubled between February and April at Sop, but increased only by a factor of 1.6 at GN (Fig. 3). The length and soft tissue dry weight showed a significant difference between sites (discriminant analysis  $p < 0.001$ ). For equal shell length, mussels from Sop were heavier (two-sample  $t$ -test,  $p < 0.005$ ; individual dry weight averaged 0.067 g) than those from GN (0.045 g). On the basis of the classification coefficients of both groups, 65.7% of the mussels could be (post hoc) correctly assigned to one of the sites. The regression equations estimating the relationship between shell length ( $L$ ) and tissue dry weight of *M. trossulus* were dry weight =  $0.004L^{2.297}$  ( $r = 0.730$ ,  $p < 0.001$ ,  $n = 426$ ) and dry weight =  $0.0002L^{1.726}$  ( $r = 0.662$ ,  $p < 0.001$ ,  $n = 428$ ) at Sop and GN, respectively. The remarkable dominance of males over females was observed at GN. The ratio of males to females averaged 2.9 (range 0.7–8.5) as compared with Sop where the sex ratio averaged 1.4 (range 0.8–3.0).

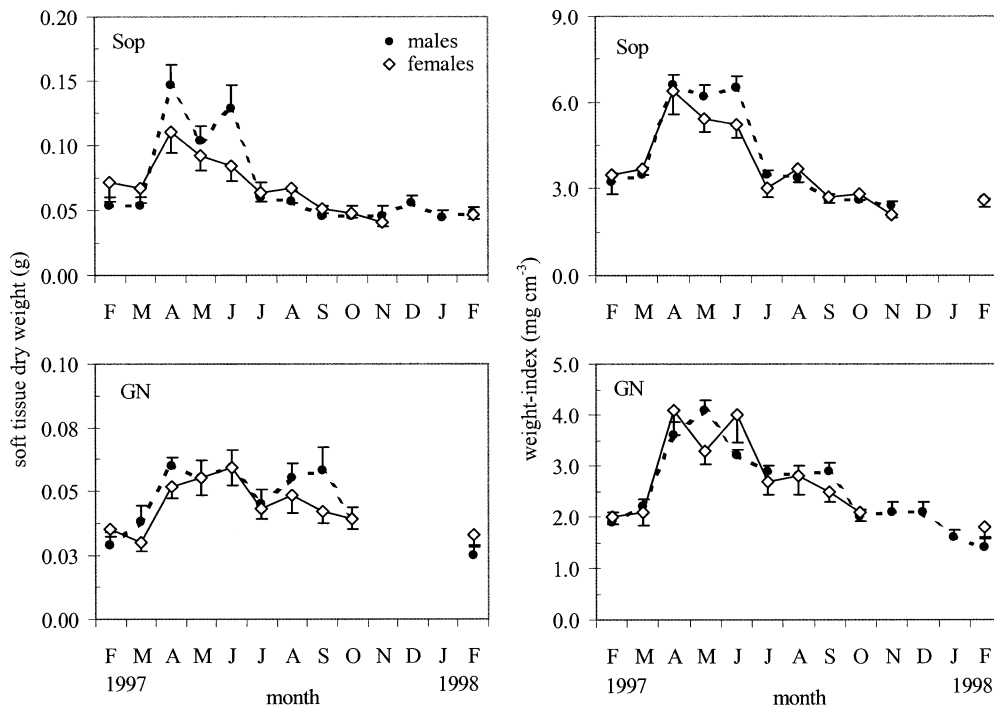


Fig. 3. Seasonal variations in soft tissue dry weight and weight index in two sex groups of the mussel *M. trossulus* at two sites, Sop and GN, in the Gulf of Gdańsk (southern Baltic Sea). Data are presented as mean  $\pm$  standard error ( $n_{\text{pool}} = 3$ , five to seven individuals in each pool).

Weight index and biochemical composition: The weight index (CI) of *M. trossulus* was related to month and site (ANOVA  $p < 0.05$ ; Table 1). The pattern of seasonal changes was similar at the both sites (correlation analysis  $p < 0.05$ ) and closely followed that of dry weight (correlation analysis  $p < 0.05$ ), with elevated values in late spring/early summer, i.e., just before spawning when gonads are mature, and lower values in July through January after the main spawning period (Fig. 3). At Sop, additional peak of the weight index occurred in October/November (up to  $2.78 \text{ mg cm}^{-3}$ ) as a result of increased food resources from the autumnal phytoplankton bloom, when Chl *a* reached  $3.36 \text{ mg L}^{-1}$  and POM  $1.73\% \text{ TPM}$  (Fig. 2). Mussels were generally in a better physiologic state at Sop (CI averaged  $3.8 \text{ mg cm}^{-3}$ ; two-sample *t*-test,  $p < 0.005$ ) as compared with GN ( $2.7 \text{ mg cm}^{-3}$ ).

Proteins varied over time and between sites (ANOVA  $p < 0.05$ ; Table 1). Although a similar pattern of seasonal changes was observed for the two sites (correlation analysis,  $p < 0.005$ ), with elevated values during autumn and winter and a decline in spring (Fig. 4), mussels from the deep zone contained more proteins over the growing period (February through July) as compared with the mussels from the shallow zone (two-sample *t*-test  $p < 0.05$ ). The lipid content varied with time (ANOVA  $p < 0.001$ ) but not between sites and sex groups (Table 1). For the two sites, peak values were noted in late spring and summer and low values during winter, when high-energy lipid reserves were stored for gamete development. However, in the deep zone a rise in lipids was smaller (approximately 1.6-fold) and protracted over a full summer (from April to August) followed by a gradual de-

cline in September–October. In the shallow zone, lipids increased more rapidly (approximately twofold) between March and May and remained high until June, then decrease steeply afterward (Fig. 4). The pattern of seasonal changes at Sop displayed a close mirror image to the temporal variations of proteins (correlation analysis,  $p < 0.001$ ). The content of total carbohydrates in the mussels varied significantly with time and between sites (ANOVA  $p < 0.001$ ; Table 1). There was a prominent seasonal cycle, similar at the two sites (correlation analysis,  $p < 0.05$ ), with maximum values in midsummer (June–August) and minimum values during winter (January–March; Fig. 5), temporal variation being more pronounced at Sop (Table 2). The winter decrease in the carbohydrate reserves explained the loss in body weight during the cold months (Fig. 3). Total carbohydrate content in the shallow-water mussels was more than twice as great as carbohydrate content in the deep-water mussels (two-sample *t*-test,  $p < 0.001$ ; carbohydrates averaged  $14.7\% \text{ dry weight}$  and  $6.5\% \text{ dry weight}$  at Sop and GN, respectively). At Sop, females recovered carbohydrate reserves faster after winter and stored more carbohydrates than males (two-sample *t*-test,  $p < 0.05$ ; on average  $16.0\% \text{ dry weight}$  and  $13.2\% \text{ dry weight}$  for females and males, respectively). At GN females tended to have a lower carbohydrate content (the annual value averaged  $6.9\% \text{ dry weight}$ ) than males ( $7.3\% \text{ dry weight}$ ). The glycogen content in *M. trossulus* was strongly affected by month, site, and sex (ANOVA  $p < 0.05$ ; Table 1). The seasonal changes followed the patterns described for the total carbohydrates at a respective site (correlation analysis  $p < 0.05$ ) and were more distinct at Sop (Table 2). Elevated values occurred in sum-

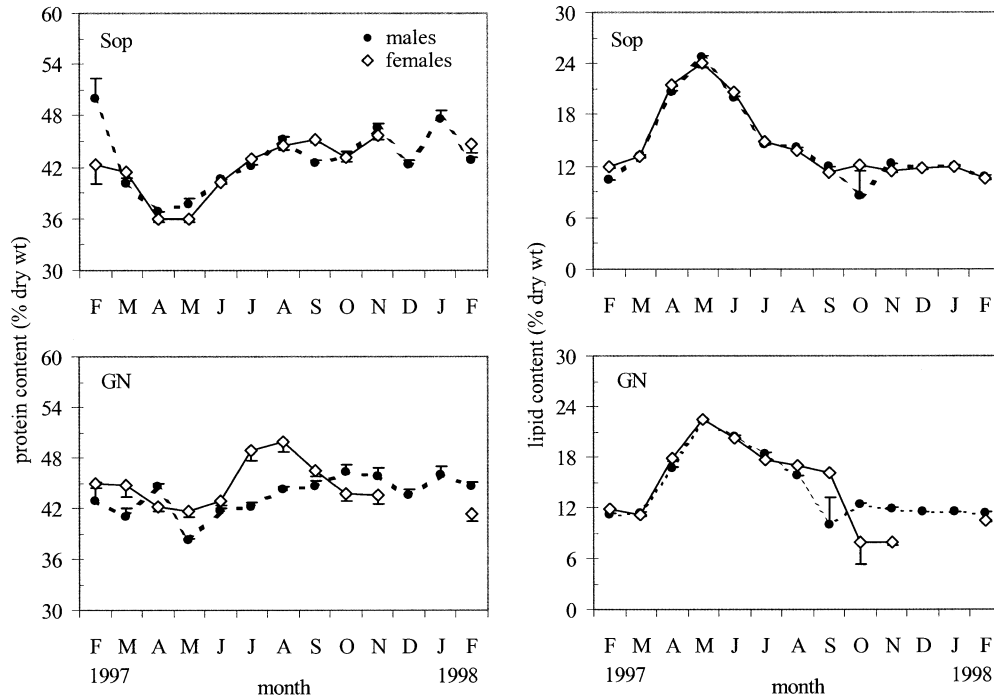


Fig. 4. Seasonal variations in the contents of protein and lipids in two sexes of the mussel *M. trossulus* at two sites, Sop and GN, in the Gulf of Gdańsk (southern Baltic Sea). Data are presented as mean  $\pm$  standard error ( $n_{\text{pool}} = 3$ , five to seven individuals in each pool).

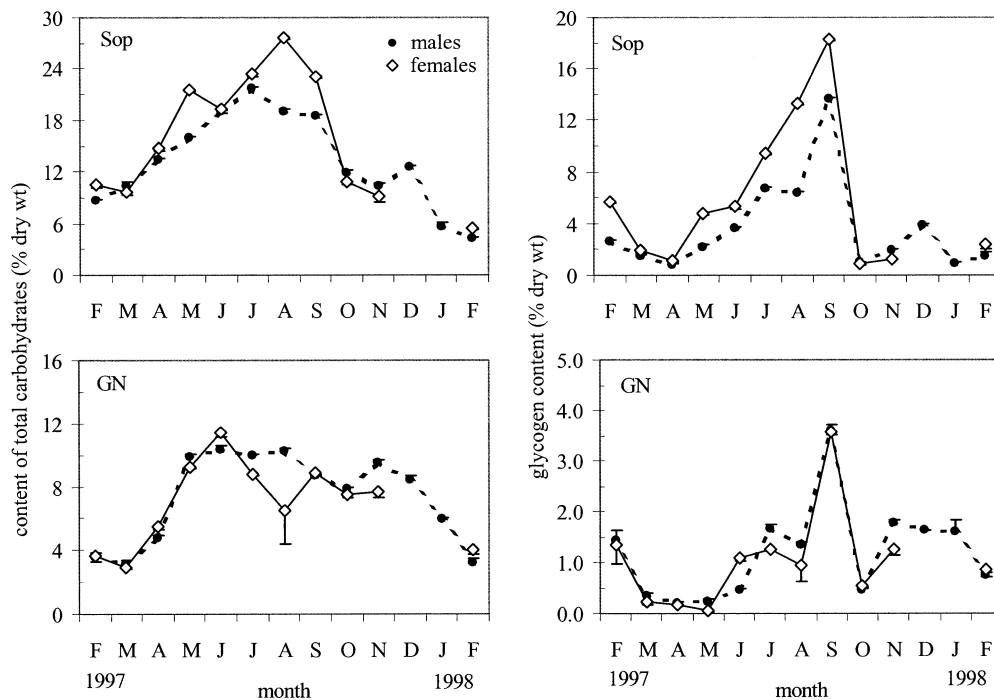


Fig. 5. Seasonal variations in the contents of total carbohydrates and glycogen in two sexes of the mussel *M. trossulus* at two sites, Sop and GN, in the Gulf of Gdańsk (southern Baltic Sea). Data are presented as mean  $\pm$  standard error ( $n_{\text{pool}} = 3$ , five to seven individuals in each pool).

Table 3. Results of multiple regression analysis of the environmental data (only factors of significant relationships were included); T, temperature; Chl *a*, concentration of chlorophyll *a* and dry weight, weight index (CI), and biochemical components of the mussel *M. trossulus* from the Gulf of Gdańsk, southern Baltic Sea with respect combined data for the two sites (\*  $p < 0.05$ ; \*\*  $p < 0.01$ ; – negative relation; + positive relation; open fields are nonsignificant relations).

	Dry wt	CI	Lipid	Protein	Carbo- hydrates	Glyco- gen
Temperature					**/+	*/+
Chl <i>a</i>	*/+	*/+	*/+	*/-		

mer (July–September) and in midwinter (February), and lower values occurred in spring and autumn (Fig. 5). This overall pattern was also valid for the contribution of glycogen to the total carbohydrates that ranged from 5.8% to 79.1% in the shallow zone and from 0.5% to 41.6% in the deeper zone. The shallow-water mussels contained fourfold as much glycogen (4.8% dry weight) as compared with the deep-water mussels (1.2% dry weight). The glycogen content was sex dependent at Sop, females contained more glycogen (on average 5.9% dry weight) than males (3.6% dry weight) (two-sample *t*-test  $p < 0.05$ ), while the reverse tended to be true at GN (1.0% dry weight and 1.2% for females and males, respectively).

Multiple regression analysis of all data (combined results for the two sites) tested the effects of environmental parameters on the ecophysiological features measured. The main factors were temperature, with a positive effect on the total carbohydrates and glycogen, and Chl *a*, which was positively related to dry weight, CI, and lipid but negatively to protein (Table 3).

**Genetic constitution:** The allele frequencies, observed heterozygosity, and expected heterozygosity per locus are shown in Table 4. All eight detected loci were polymorphic according to the 0.99 criterion. Different frequencies of the alleles 1 and 2 at the locus *Odh* occurred between the two sites, although no statistically significant allelic and genotypic differentiation at any locus was found ( $p > 0.1$ ). Average observed and expected heterozygosity across all loci (Sop, Ho  $0.207 \pm 0.060$ , He  $0.292 \pm 0.072$ ; GN, Ho  $0.221 \pm 0.058$ , He  $0.279 \pm 0.063$ ) did not differ between the shallow- and deep-water mussels ( $p > 0.1$ ).

## Discussion

*Ecophysiological strategy of M. trossulus in eutrophicated waters*—Ecophysiological differentiation of the mussel *M. trossulus* from the two depth zones in the southern Baltic Sea was noted. This indicates biochemical acclimatization to the specific environment at each site (Hochachka and Somero 1984). The observed differences included weight, weight index, and carbohydrate (glycogen) reserves, and their temporal variations provided insight into the physiologic strategy of the mussels in eutrophicated waters.

A relevant parameter for comparison of spatial growth and

Table 4. Allele frequencies in the mussel *M. trossulus* from two depth zones (Sop and GN), the Gulf of Gdańsk (southern Baltic Sea). (*n*, number of individuals; He, expected heterozygosity; Ho, observed heterozygosity).

Locus/ alleles	Sop	GN	Locus/ alleles	Sop	GN
<i>Pgd</i>			<i>Lap</i>		
<i>n</i>	75	83	<i>n</i>	82	78
1	0.014	0.000	1	0.585	0.583
2	0.033	0.024	2	0.006	0.006
3	0.947	0.964	3	0.268	0.314
4	0.007	0.012	4	0.140	0.096
He	0.103	0.071	He	0.569	0.555
Ho	0.080	0.072	Ho	0.439	0.397
<i>Gpi</i>			<i>Me-1</i>		
<i>n</i>	85	83	<i>n</i>	82	84
1	0.024	0.018	1	0.043	0.048
2	0.000	0.012	2	0.927	0.917
3	0.941	0.934	3	0.030	0.036
4	0.018	0.030	He	0.139	0.157
5	0.018	0.006	Ho	0.061	0.095
He	0.114	0.127			
Ho	0.118	0.108			
<i>Pgm</i>			<i>Me-2</i>		
<i>n</i>	83	84	<i>n</i>	80	81
1	0.129	0.162	1	0.625	0.698
2	0.841	0.794	2	0.044	0.019
3	0.029	0.044	3	0.331	0.284
He	0.276	0.344	He	0.501	0.435
Ho	0.106	0.138	Ho	0.412	0.481
<i>Idh</i>			<i>Odh</i>		
<i>n</i>	61	78	<i>n</i>	77	75
1	0.934	0.929	1	0.058	0.027
2	0.049	0.064	2	0.643	0.740
3	0.016	0.006	3	0.273	0.207
He	0.125	0.133	4	0.026	0.013
Ho	0.066	0.115	5	0.000	0.013
			He	0.512	0.411
			Ho	0.377	0.360

production is the relationship between length and dry weight that also can be employed to assess environmental conditions. Morphometric and weight measures of *M. trossulus* revealed nonisometric growth and evident differences between the two depth zones. The higher allometric exponent *b* in the shallow-water mussels than in the deep-water mussels suggests a smaller increase in tissue of the latter, also indicated by differences in dry weight (Fig. 3). This can be generally explained by lower food resources at GN as evidenced by Chl *a* content.

The physiologic condition of the mussels over an annual time scale can be described by changes in tissue dry weight or using weight indices, the measures being often closely interrelated. According to Smaal and Stralen (1990), a weight index (CI) can be interpreted as an index of growth; thus, variations in soft tissue dry weight of *M. trossulus* reflected the overall condition of an organism. The pattern of seasonal changes of CI (and also dry weight) was similar at Sop and GN, with elevated values in late spring and early

summer (April–May) when mussels were maintaining mature gametes. At the shallow site the spring increase in body weight and subsequent improvement of a physiologic state was directly enhanced by assimilation of food, which was abundant due to spring phytoplankton bloom. At the two sites, a decline in body weight and the weight index of *M. trossulus* in summer coincided with spawning that in the Gulf of Gdańsk usually begins in May/June when water temperature rises (Jurga and Wołowicz 1994). During a winter period of starvation and gametogenic quiescence the weight index remained low at the two sites, reflecting the inevitable seasonal decrease in metabolic rate in adaptive response to low temperature and food shortage (Honkoop and Beukema 1997). Similar spatiotemporal changes of CI were also shown for the Baltic clam *M. balthica*, another predominant bivalve species in the Baltic Sea (Bonsdorff and Wenne 1989).

A more dynamic picture of physiologic fitness of the mussel populations to the ambient environment is given by seasonal variations of the main biochemical components. The depletion of protein reserves in *M. trossulus* in winter (Fig. 4) was inevitably due to their catabolism to meet metabolic demands of starvation (Bayne and Newell 1983), while in spring protein served as an important energy source for oocyte lipid formation (Bayne et al. 1982; Beninger and Lucas 1984). This was clearly visible in the shallow zone, where a seasonal cycle of protein opposed the lipid cycle. The protein breakdown was of lesser importance in the deep zone where protein levels during spawning remained relatively high and stable, indicating a more “careful” energetic strategy of the mussels that are facing reduced food availability. Lipids constituted the principal energy storage reserves. The seasonal variation in lipids, with elevated values during gametogenesis in spring (May) and a decline to a minimum in summer/autumn following spawning, was typical for the genus *Mytilidae*. Boreal zone mussels accumulate lipids mainly in gonads (oocytes) to form the main component of reproductive material (Zandee et al. 1980) as energy reserves for the larvae during the first life stages (Pieters et al. 1980). A spring lipid build up was greater (nearly twofold) and earlier (in March) at Sop as compared with GN (by a factor of 1.6 in April), which likely resulted from the earlier increase of the food availability in the shallow zone, corresponding to temperature-stimulated spring diatom bloom as described by Chl *a* (Table 3; Fig. 2). The increased lipids in this period might have also resulted from conversion of carbohydrates, specifically glycogen, to lipids, which are essential for oocyte maturity (Zandee et al. 1980). In the deep zone a spawning-induced decline in the lipids extended over a full summer (until August), reflecting the prolonged and protracted gamete release. The physiologic strategy of *M. trossulus* in the deep zone may be that the mussels time their reproductive cycle according to the ambient environmental situation and use lipid reserves primarily to serve the needs of maintenance metabolism under limited food conditions. According to Seed and Suchanek (1992), spawning in *Mytilus* spp. can be initiated only when energy reserves are enough to cover the basic metabolism and gamete production requirements. In addition, relatively low temperature of the overlying bottom water at GN between May and July might

have not been sufficient to stimulate gonads to complete spawning over a short time. Carbohydrates, and particularly glycogen, are an energy source with relatively fast metabolic turnover and can be used to meet enhanced energy demands, e.g., during poor nutritional conditions, therefore reflecting the actual physiologic state of an organism (Beukema and de Bruin 1977; Beninger and Lucas 1984). The low carbohydrates and glycogen contents at GN compared with shallow Sop indicate inevitably a worse performance of the mussels in the deep zone. The increase in food abundance sufficient for carbohydrate storage was delayed until May in the deep region (compared with March in the shallow region) when phytoplankton production started and POM from the water column sedimented to the bottom (Figs. 2, 5). This effect was not apparent for glycogen, the content of which remained low until June (i.e., prior to spawning and during spawning) possibly due to conversion of muscular glycogen to lipids and thus energy allocation in the gonads and to the catabolism of glycogen to provide energy for gamete maturation (Thompson 1984). Since glycogen is a minor component of gametes in *Mytilidae* (ca. 2.5–3.2% dry weight of oocytes; Pieters et al. 1980; Bayne et al. 1982), carbohydrate formation in the mussels during maturation comprised primarily nonglycogen compounds. During spawning, the increased metabolic costs are covered mainly from the glycogen reserve breakdown. However, in *M. trossulus*, spawning was not followed by a drop in carbohydrates and glycogen that can be explained by an overlap of spawning and a peak of feeding activity. In the postspawning period, the metabolic rate declined but, since food was still available, the animals continued to gather reserves and the maximum levels of carbohydrates and glycogen occurred in mid-summer. Additional support for the concept of food-induced differences in physiologic performance between shallow- and deep-water mussels is shown by the lower glycogen content in females at GN, while at Sop the reverse was true (Table 1; Fig. 5). The cost of reproduction in females is a reduction in the energy reserves available for maintenance, in an extreme case this leads to death of the organism when energy demands are in excess of energy acquisition from food (Bayne and Newell 1983). In the deep zone, the sex ratio was clearly in favor of males (~3:1), indicating a higher mortality in females likely due to energy allocation to reproduction under reduced food availability. Ansell (1982) interprets similar behavior in *Polinices* as an opportunistic adaptation maximizing reproduction when food resources diminish.

*Physiologic acclimatization and/or local genetic adaptation?*—Genetic studies of marine invertebrates provided evidence that a number of enzymes are under strong selection pressure reflecting environmental heterogeneity (e.g., Johannesson et al. 1995). Different environmental conditions and/or low migration rates may promote local adaptation, which will be evident as differences between populations in the allele and genotype frequencies. In addition, positive correlation between multilocus heterozygosity and fitness parameters was found in several bivalve species, suggesting selective advantage of more heterozygous individuals (e.g., Myrand et al. 2002). Highly heterozygous individuals were

found to have lower maintenance metabolism resulting from a lower protein turnover, so that they have more energy for growth, reproduction, and survival (Hawkins and Bayne 1992), i.e., show better fitness to adverse conditions. The allozyme survey on the shallow- and deep-water mussels provided information on whether genetic differentiation could be an additional explanation of the observed ecophysiological traits. Since no allelic and genotypic differences in the studied loci were observed between the shallow- and deep-water mussels, environmental conditions in the two depth zones did not cause a sufficient selection pressure to create local adaptations in *M. trossulus*. This, together with a high gene flow due to large dispersal capacity of the larvae (Lutz and Kennish 1992), is reflected in the genetic homogeneity. The short time period of the observed shift of the mussels to deep waters also should be taken into account. Ecophysiological differences between the mussels studied are due to acclimatization to the ambient environment. The lack of differences in the mean heterozygosity did not provide evidence for better performance of heterozygotes and/or different selection pressure at the two sites. As in the present study, differences in ecophysiology resulting from acclimatization and not genetic adaptation were found between the shallow- and deep-water populations of *M. balthica* from the Gulf of Gdańsk (Hummel et al. 2000). However, in our study the allele frequencies at the locus *Odh* showed a similar pattern to the results of Hummel et al. (2001), who demonstrated significant differences in the allele frequencies at this locus (chi-square test performed by us using data of Hummel et al. 2001  $p < 0.001$ ) between *M. trossulus* from a depth of 10 and 40 m in the Gulf of Gdańsk. It is possible that *Odh* may play a role in adaptation to the deep-water habitats.

*The influence of environmental parameters*—The relationship between the ecophysiological variables of dry weight, weight index, protein, carbohydrate/glycogen, temperature, and Chl *a* in the ambient water was shown (Table 3), indicating that the thermal and food conditions have most profound influences on the animal performance. This relation is well known in physiologic energetics and has been widely described in many bivalve species from temperate regions (Beukema and de Bruin 1977; Bayne and Newell 1983). In our study, however, no apparent differences in temperature between the shallow and deep zone were noticed (Table 1); thus, performance of the mussels is primarily related to the food availability and the length of a food season. Anthropogenic eutrophication, which led to elevated TPM content (Maksymowska et al. 1997) and a sedimentation rate (Witkowski and Pempkowiak 1995) in the southern Baltic Sea over last decades, caused an increase in the food availability across a range of depths, creating favorable food conditions for suspension feeders in deep regions. Accordingly, the vertical distribution of *M. trossulus* extended into the deep zone and the biomass of the mussels can currently reach up to 500 g dry weight  $m^{-2}$  between 20 and 54 m, often exceeding the respective biomass of the mussels in the shallow zone (Osowiecki 2000). However, the metabolic costs of sustaining adverse environmental conditions in the deep habitats are so high that relatively little energy can be allocated into

gametogenesis and spawning. It is therefore hypothesized that in the southern Baltic the deep-water mussels live at the limit of their physiologic capability, and the enhanced energetic expenditure during spawning may cause increased mortality. Further diversification of ecophysiological performance of the deep-water mussels can induce physiologic isolation (e.g., through timing of spawning), highlighting the effect of eutrophication at an ecophysiological level. Similar diversification of ecophysiological variables was found in the sub-Arctic populations of *Mytilus edulis*, which are facing harsh Arctic conditions, i.e., low temperature and food shortage (Hummel et al. 2001).

In the disturbed ecosystem of the Gulf of Gdańsk, the changes in the distribution range and diversification of the bivalve population features itself may be considered a natural feedback response of the system to eutrophication. The increased abundance of suspension feeders in deep waters results in enhanced filtration and removal of suspended particles from the water column and, thus, an improvement of water quality. Ecologic consequences include a change in species composition of benthic macrofauna and presumably subsequent shift in the diet of bottom-feeding organisms (Cloern 2001), affecting the structure and functioning of the benthic–pelagic food loop and, further, a stock of living resources. However, ecophysiological studies showed that in deep waters mussels live at their limit of physiologic capability and are sensitive to any alterations in the environment (e.g., changes in food supply, toxic contaminants, diseases). Currently observed population and ecophysiological phenomena caused by eutrophication are therefore not sustainable and can be reversed with a change in environmental conditions (nutrients and primary production). Although significant trends of decreasing nutrient concentrations (specifically phosphorus) have been reported solely in several areas of the Baltic over the last years (Ærtebjerg et al. 2001), management actions taken in Poland to curtail anthropogenic nutrient loading to the Gulf of Gdańsk, in response to legislative mandates, HELCOM recommendations, and EU Water Framework Directive implementation, provide an optimistic prognosis. For example, clear reductions in diffuse nutrient losses from agricultural areas due to changes in the numbers of livestock units and a reduction of the use of manure and mineral fertilizers already have been noted in many Polish regions. However, effects of these nutrient-reduction actions on benthic communities in the Baltic Sea are estimated to be delayed on the order of 5 to 10 yr (Nausch et al. 1999).

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*Received: 15 March 2004*

*Accepted: 18 November 2004*

*Amended: 20 January 2005*