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AChE as biomarker of mussels and fish contamination with chemicals in the Gulf of Gdańsk

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1. Introduction

Significant part of the anthropogenic contaminants load reaching the marine environment enters the food chain. Traditionally, in order to monitor the effect of the contaminations on biota, analyses of the contaminants concentration in organisms have been carried out. Recently biomarkers were recognized as useful tool for assessment of the pollution impact on marine organisms. This is due to their sensitivity, low cost and specifity (Huggett et al, 1992; Walker and Livinstone, 1992; WHO, 1993).

For example acetylocholinesterase (AChE) inhibition in bivalvia and fish is used as a biomarker of exposure to neurotoxins (eg.: organophosphorus, and carbamate compounds – present in the environment as pesticides). The role of AChE in cholinergic transmission is to regulate the nervous transmission by reducing the concentration of ACh (acetylocholine) in the junction through AChE-catalysed hydrolisys of ACh. When AChE is inactivated by organophosphorus or carbamate esters the concentration of ACh in the junction remains high in comparison with unaffected organisms (Bocguene et al, 1990; Escartin and Porte, 1997).

2.1. Materials and methods

2.1. Sampling strategy

Organisms from two species of bivalvia (*Macoma balthica*, *Mytilus trossulus*) and flounder *(Platichthys flesus*) were used as biomonitoring organisms of coastal pollution in this study. The distribution of sampling stations where the organisms were collected, in the Gulf of Gdańsk is shown in Fig. 1.

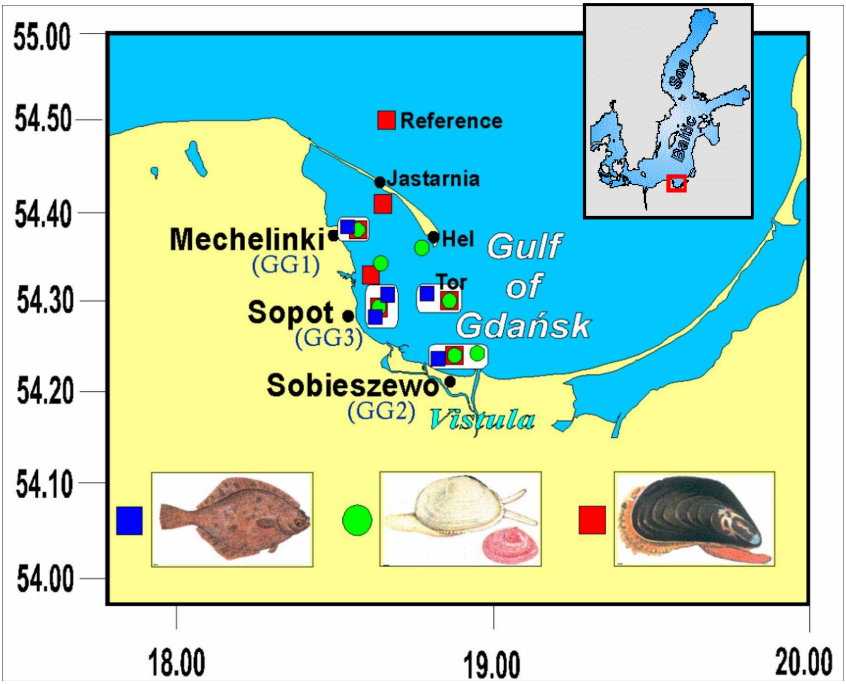

Fig. 1. Distribution of sampling stations in the Gulf of Gdańsk
Rys. 1. Rozmieszczenie stacji pomiarowych w Zatoce Gdańskiej

All sampling grounds are distributed inside the Gulf of Gdańsk, except a reference station – located on the 'open sea' side the Hel Peninnsula. In the Gulf of Gdańsk 4 sampling grounds were selected:

- off Mechelinki – the most polluted site, the sewage outflow from the Gdynia WWTP is located there,
- off Sopot – the recreational and touristic area,
- close to the mouth of the Vistula river – in this site the influence of the Vistula river is at its maximum,
- on the ship route to the ports (TOR).

The organisms were collected in 2001 (March and November), and 2002 (March). The mussels (of uniform shell length: *M. trossulus* 35±5 mm, *M.balthica* >15 mm) were catched using a drag net from the research vessel "Oceania" (IO PAS, Sopot). Catching was towed at 1,5÷2 knots for 15÷20 min so as to minimize stress to catch. The flounder (20 females and 3÷10 males, body length 20÷30 cm) were collected by local fishermans using flounder nets (φ_{mesh} = 60mm).

In the laboratory notes were taken on flounder condition, length, sex, weight (whole and empty fish, liver, gonads and spleen) and degree of parasitisms.

Different tissues sectioned from the 5 pooled organisms were used as source of samples: gills from *M. trossulus*, foot from *M.balthica* (Mora et al, 1998; Mora et al, 1999), and muscle tissues from flounder (Kirby et al, 2000; Schneider et al, 2000). The sectioned organs were immediately transferred to liquid nitrogen and then kept in deep freezer -80°C.

2.2. Extraction – fraction S9

100÷200 mg wet weight aliquots of the desired tissue samples were homogenized in 0,02M phosphate buffer (pH = 7,0; 0,1% Triton X-100) in ratio 1/4 (weight/volume) using an electric homogenizer (homogenization was performed twice, each time for 20 sec, in glass vessels kept in ice). Then the homogenate was centrifuged at 10000 g in the temperature of 4°C for 20 min. The supernatant (fraction S9) was stored at -80°C before the biochemical measurements. The procedure is described in detail by Bocquene and Galgani (1998).

2.3. Protein determination

The method described by Bradford (1976) was used for quantitative determination using BSA (bovine serum albumin) as the protein standard, after having been adapted to be used with a microplate reader "Genios" (TECAN). For each microplate well the following solutions were added: 10 µl of diluted S9 extract (dilution factor with destilated water applied to the samples was – for fish: 1/50; – for mussels: 1/10), 90 µl destilated water and 280 µl Bradford reagent (diluted 1/5). Absorbance was read at λ = 595 nm and the protein concentration were calculated from the standard curve.

2.4. Measurement of AChE

The method for measurements of AChE activity in the microplate reader, adopted from Bocquene and Galgani (1998), was used. The following proportions of solutions were applied.

Solutions – (all were adapted to room temperature)	Blank	Mussel sample	Fish sample
	5 replicates	3 replic.	3 replic.
0,02M Phosphat buffer + 0,1% Triton X-100	350 µl	330 µl	340 µl
Sample – fraction S9	–	20 µl	10 µl
0,01M DTNB (in 0,1M Tris/HCL, pH = 8.0)	20 µl	20 µl	20 µl
Incubation	5 min		
0,1M ACTC (in Aqua dest)	10 µl	10 µl	10 µl

This was followed by absorption measurement at λ=405nm in 4 kinetic intervals (0, 1, 2, 3 min)

3. Results and discussion

All pooled samples of mussels and individuals fish had AChE activity [nmol/min · mg protein] in the following ranges: 29÷83 (in *M.balthica*), 12÷38 (in *M.trossulus*) and 94÷185 (in flounder). The obvious, and already noticed species dependence of the levels, Bocquene and Galgani (1998) can be noticed.

AChE activities in *M. balthica* were the lowest in sites close to the Hel Peninsula in all sampling periods (Fig. 2).

In this area the highest concentrations of pollution in sediment and water had been measured (Sapota, 2000). The highest mean values of AChE were found in whole tissue of *M. balthica* from the Tor station (80÷83 nmol/min · mg protein).

The lowest mean value of AChE activity in *M. trossulus* were observed near the Mechelinki and Jastarnia stations (15÷20 nmol/min · mg protein) – Fig. 3.

This area is regarded as a very polluted one (Sapota, 2000, Potrykus et al., 2003) – due to sewage dicharges. In the sampling site near Sopot (GG3) the higher values of AChE activity in *M. trossulus* (ca 38 nmol/min · mg protein) were observed.

In female flounder mean values of AChE activity higher, than in males, were found (Fig. 4).

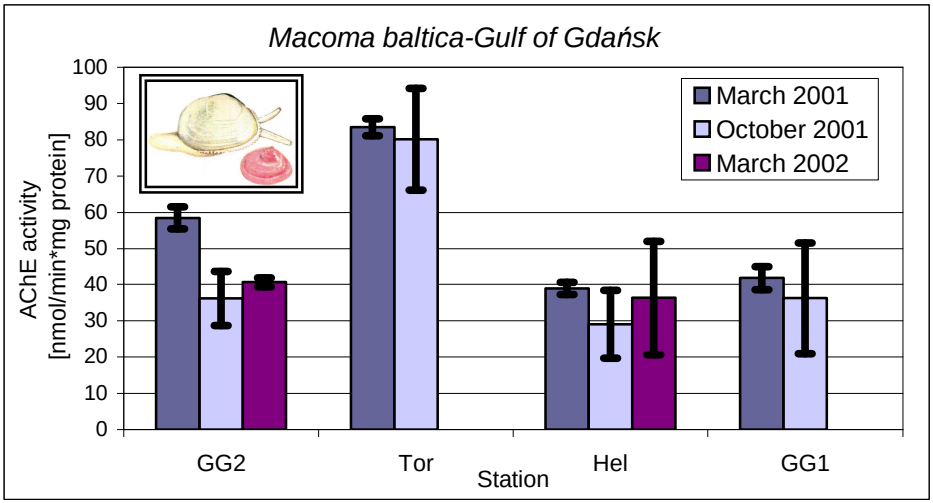

Fig. 2. Levels of AChE activity in *Macoma balthica* from the Gulf of Gdańsk
Fig. 2. Aktywność AChE w małżu *Macoma balthica* z Zatoki Gdańskiej

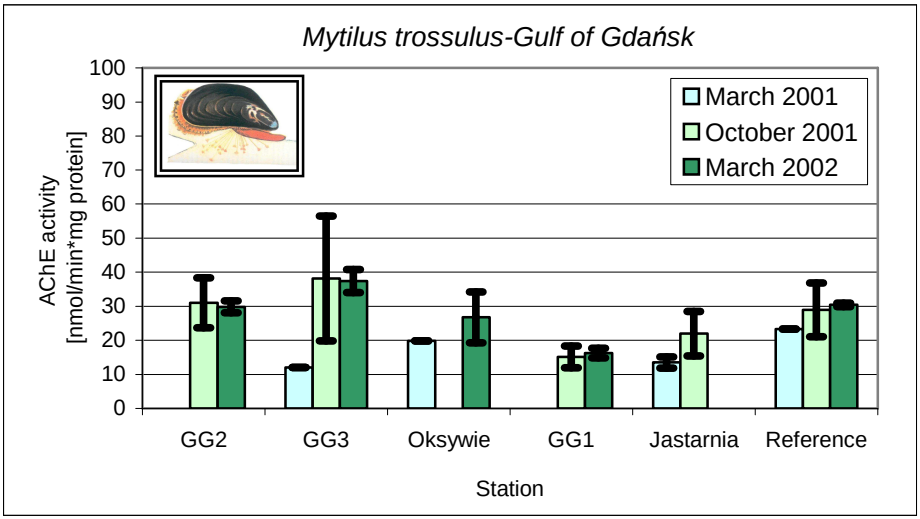

Fig. 3. Levels of AChE activity in *Mytilus trossulus* from the Gulf of Gdańsk
Rys. 3. Aktywność AChE w małżu *Mytilus trossulus* z Zatoki Gdańskiej

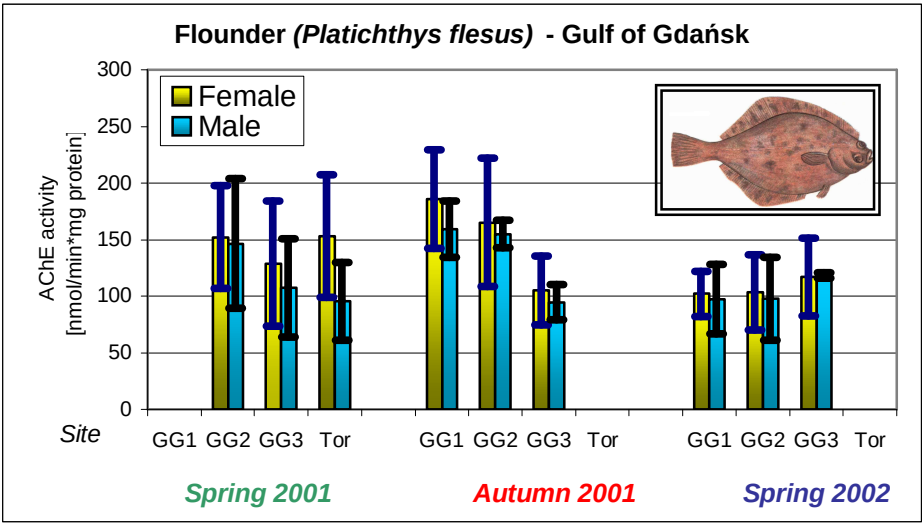

Fig. 4. Level of AChE activity in flounder from the Gulf of Gdańsk
Rys. 4. Aktywność AChE w storni z Zatoki Gdańskiej

The lowest mean values in flounder were found in spring 2002 in all sampling sites. At that time very low water temperature was recorded. It could have influenced level of AChE activity (Bocquene and Galgani, 1998). AChE activities in individual flounders were different in all sites and periods (this explains high standard deviations). The phenomenon can be also attributed to the age, sex, and environmental factors.

4. Conclusions

Levels of AChE activity are thought to be a useful indicator of biological responses in organisms (mussels and fish) to pollution.

Inhibition of AChE activity in tissue has been proposed as a useful biomarker of an effective exposure to organophosphates and carbamates (Bocquenė and Galgani, 1998; Schneider et al, 2000). Since the sources of contaminants are rather localised (mouth of the Vistula due to runoff, ports, sewage discharges) well-developed gradients of organic pollutants in organisms have been recorded. This is well documented and confirmed in this, biomarker oriented, study.

5. Acknowledgement

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AChE jako biomarker skażenia chemikaliami małży i ryb z Zatoki Gdańskiej

Streszczenie

W ciągu ostatnich kilkunastu lat udokumentowano w środowisku Morza Bałtyckiego spadek stężenia WWA, PCB, dioksyn i metali ciężkich. Jednak ze względu na wysoką toksyczność w odniesieniu do organizmów oraz ze względu na trwałość w środowisku morskim i akumulację w łańcuchu troficznym, należą one do najczęściej badanych zanieczyszczeń w programie środowiskowym. Jako metodę oceny stanu środowiska tradycyjnie stosuje się pomiar stężenia zanieczyszczeń w tkance miękkiej organizmów morskich. Jednak pomiar ten nie wiąże się bezpośrednio z oceną skutków ich akumulacji w organizmach żywych

W ciągu ostatnich 15÷20 lat jako metodę toksykologii środowiskowej służącą do bardziej kompleksowej oceny stanu środowiska, wprowadzono pomiar biologicznych skutków zanieczyszczenia biocenozy – tzw. biomarkerów. Jako wskaźniki stosuje się tu zmiany w funkcjonowaniu organizmów na poziomie biochemicznym, fizjologicznym i histologicznym. Pozwalają one bezpośrednio określić wpływ zanieczyszczeń na organizmy żyjące w środowisku wodnym i mogą być one stosowane do pomiarów reakcji organizmów na chemiczne czynniki stresujące. Najwcześniej skutki biologiczne ujawniają się na poziomie komórkowym. Na poziomie organów i organizmów indywidualnych można zauważyć zmiany chorobowe, zaburzenia odporności i fizjologii. Śmierć organizmów, a tym samym spadek populacji, mniejsza bioróżnorodność, i w efekcie – degradacja środowiska przyrodniczego spowodowana jest długoterminowym efektem ekspozycji organizmów na zanieczyszczenia.

Celem tej pracy było określenie poziomu Acetylocholinoesteracy (AChE) – enzymu współodpowiedzialnego za przekazywanie impulsów w systemie nerwowym w małżach i rybach z Zatoki Gdańskiej. Aktywność AChE ulega obniżeniu w organizmach eksponowanych na związki (np. pestycydy) fosforoorganiczne, i karbaminianowe.

Organizmy pobierano z rejonów przybrzeżnych Zatoki Gdańskiej: Mechelinki, Sopot, ujście Wisły, z centralnej części Zatoki oraz punktu referencyjnego położonego na zewnątrz Półwyspu Helskiego. Badania wskaźników substancji szkodliwych objęły organizmy z dwóch gatunków małży (*Macoma balthica* i *Mytilus trossulus*) i jednego gatunku ryb – storni (*Platichthys flesus*). Analizie poddano wybrane tkanki (skrzela w *M. trossulus*, noga w *M. balthica* oraz mięśnie w storni) zostały zmierzone aktywności AChE (acetylocholinoesterazy).

Analiza zbioru wyników aktywności (AChE) wykazała zmienność gatunkową stężenia AChE, różnice w AChE w organizmach męskich i żeńskich, i znaczną zmienność indywidualną. Nie stwierdzono statystycznie istotnych zmian w zmienności geograficznej i sezonowej.

Natomiast w aktywności AChE w małżach nie obserwuje się istotnych różnic.