AChE levels in mussels and fish collected off Lithuania and Poland (southern Baltic)*

OCEANOLOGIA, 46 (3), 2004.
pp. 405–418.

KEYWORDS
Blue mussels (*Mytilus trossulus*)
Flounder (*Platichthys flesus*)
Acetylcholinesterase (AChE)
Gulf of Gdańsk
Klajpėda

Justyna Kopecka[1]
Aleksandras Rybakowas[2]
Janina Baršienė[2]
Janusz Pempkowiak[1]

[1] Institute of Oceanology,
Polish Academy of Sciences,
Powstańców Warszawy 55, PL–81–712 Sopot, Poland;

e-mail: kopecka@iopan.gda.pl

[2] Institute of Ecology,
Vilnius University,
Akademijos 2, LT–08412 Vilnius, Lithuania

Manuscript received 6 May 2004, reviewed 2 August 2004, accepted 9 August 2004.

Abstract

AChE activities were measured in blue mussels gills and flounder muscles samples collected off Poland – the Gulf of Gdańsk (4 sampling stations) and off Lithuania – the Klajpėda area (3 sampling stations), in 2001 (June and October) and 2002 (April and October). The AChE activities [nmol min^{-1} mg protein^{-1}] were in the range: 15–38 (in blue mussels) and 94–315 (in flounder), and agreed well with those reported for flounder in other coastal Baltic areas, and other European seas. Sources of contaminants in the study area are rather localized in the Gulf of Gdańsk, (mouth of the Vistula due to runoff, ports, sewage discharges), while an accidental oil spill occurred off Lithuania, in the course of the study (November

* This work was financially supported by the EU FP5 Program, Biological Effects of Environmental Pollution – BEEP. Contract No EVK3-CT-2000-00025.

The complete text of the paper is available at http://www.iopan.gda.pl/oceanologia/

2001). Geographical and temporal AChE levels changes followed the contamination pattern. AChE activities and gradients in the study area are well documented and confirmed in this study. The study confirms the potential use of AChE as biomarker of organic pollution.

1. Introduction

A significant part of the anthropogenic contaminant load reaching the marine environment enters the food chain. Traditionally, in order to monitor the effect of pollution on biota, analyses of the contaminant concentration in the soft tissue of organisms have been carried out. Recently biomarkers, defined as biological effects of environmental pollution, were recognized as a useful tool for the assessment of the pollution impact on marine organisms. This is due to their sensitivity, relative ease of application, low cost, and specifity (Huggett et al. 1992, Walker & Livingstone 1992, WHO 1993).

For example, acetylcholinesterase (AChE) inhibition in bivalves and fish is used as a biomarker of the species' exposure to organophosphate and carbamate compounds (present in the environment as a result of their agricultural use as pesticides). The role of AChE in cholinergic transmission is to regulate nervous transmission by reducing the concentration of acetylcholine (ACh) in the junction through AChE-catalyzed hydrolysis of ACh. When AChE is inactivated by organophosphate or carbamate esters, the concentration of ACh in the junction remains high in comparison with unaffected organisms (Bocquené et al. 1990, Galgani & Bocquené 1990, 2000, Galgani et al. 1992, Escartin & Porte 1997, Narbonne et al. 1999).

In order to access the AChE as a measure of the influence of the relevant contaminants on biota, certain conditions should be met. The study area should be located in estuaries and coastal zones, where contamination is most pronounced due to run-off (Kirby et al. 2000). It is also important to investigate benthic biota species, which have contact with bottom sediments (Giam & Ray 1987). It is better to investigate species that do not migrate (Galgani et al. 1992). Although bivalvia fulfil the requirements, their AChE activities are insufficient to study contamination gradients. Therefore, benthic fish species are suggested as biomonitor organisms (Bocquené et al. 1993, Kirby et al. 2000).

The history of AChE related studies in the Baltic Sea off Poland is relatively short. In a number of short time studies, statistically significant differences between areas differing with the exposure to contaminants load have been reported (Kopecka & Pempkowiak 2002, 2003, Napierska & Podolska 2003). However, neither have extensive reports been published nor has a comparison of AChE levels with other areas in the Baltic been carried out.

In this paper the results of a two year-long study on the AChE activities in mussels (*Mytilus trossulus*), and fish (*Platichthys flesus*) collected off Poland and off Lithuania (southern Baltic coast) are reported. It is concluded that the measured AChE activities are comparable with other areas and follow the expected temporal and spatial gradients of contamination in the study area.

2. Study area

The sampling grounds were located in the Gulf of Gdańsk and off the Lithuanian coast (Fig. 1). The aerial distance between the two locations is about two hundred kilometers, so the climatic conditions should be comparable. It is suggested that in biomarker related studies, results from the same climatic zone only should be compared (Bocquené et al. 1993). Both areas are under the influence of freshwater outflow, a direct one from the Vistula river in the case of the Gulf of Gdańsk sampling stations, and an indirect one from the Nemunas via the Curonian lagoon, in the case of the Lithuanian sampling stations.

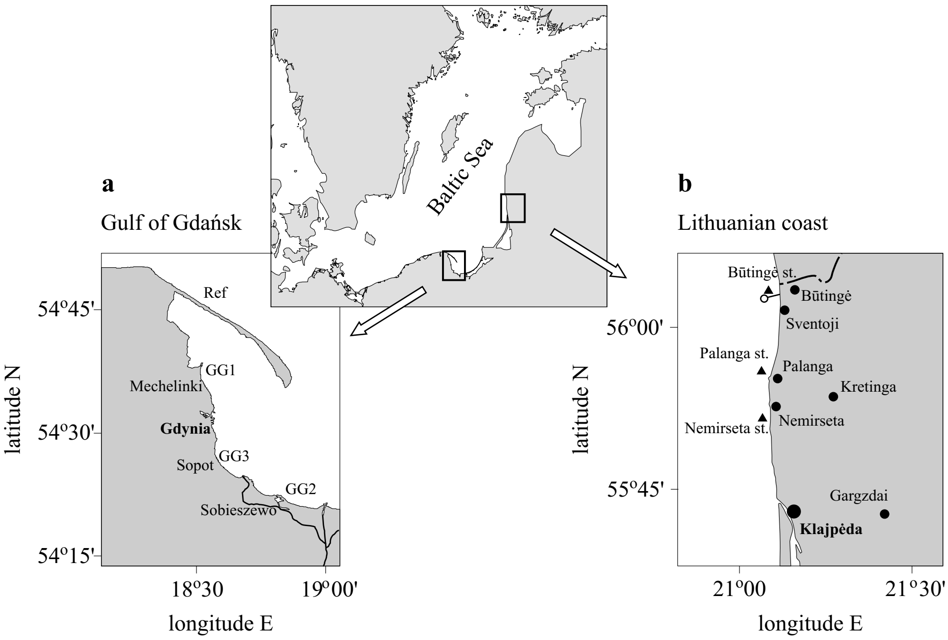

Fig. 1. Distribution of sampling stations in the study area: Gulf of Gdańsk (a), Klajpėda area (b)

Air and water temperature

The average long-term air temperature is close to 7°C in both locations, varying from −2.4°C (January–February) to 17°C (August). Water temperature in February is between 1 and 2°C (both locations), while in July it is between 17 and 18°C (Klajpėda stations), and between 19 and 18°C (the Gulf of Gdańsk stations).

Water salinity

Salinity levels in the coastal waters of Klajpėda (Nemirseta) – Būtingė area depend on the fresh water outflow from the Curonian lagoon (Fig. 1). The Klajpėda Strait is characterized by the intensive and continuous circulation of water masses. The annual water outflow from the Curonian lagoon to the Baltic Sea is approx. 28 km^3, which is equal to about 890 m^3 s^{-1}. It is estimated that 23 km^3 of it is fresh river water. The water from the lagoon flows northward on average 250 days per year, southward – 40 days, and for some 75 days sea water flows into Curonian lagoon. The greatest amount of fresh water reaches the Baltic Sea in spring and early summer. During spring floods, the lagoon water spreads as far as 20 km offshore at Klajpėda and reduces salinity in the surface layer of sea water 5–15 m thick. The average salinity levels range between 7 and 7.5 PSU (summer and winter). Near-bottom salinity levels are in the range between 7 and 8 PSU in the nearshore zone.

Salinity levels in the Gulf of Gdańsk depend on the intensity of the Vistula river run-off, and the direction of wind-induced currents. The Vistula discharges on average 35 km^3 freshwater per year. In the study area salinity ranges between 5 and 6 PSU in winter, and between 6 and 7 PSU in summer. Salinity at the station closest to the Vistula river mouth (GG2) may be as low as 4 PSU.

Chemical contaminants

Previous studies (Baršienė et al. 2003) indicate that the concentration of hydrocarbons (HC) in the surface (0–5 cm) layer of bottom sediments in the Lithuanian nearshore zone ranges from 0.5 to 9.8 μg g^{-1} dry weight (d.w.). The average concentration of HC in the nearshore sands is equal to 1.8 μg g^{-1} d.w. The concentration of HC in the bottom sediments of the Klajpėda Strait ranges between 1.19 and 2029.0 μg g^{-1} d.w., and is closely associated with the granular types of lithogenic sediments and the quantity of biogenic matter in the sediments.

The data obtained also suggest that the distribution of metals in the surface layer of sediments of the investigated areas is governed by mechanical differentiation of the sedimentary matter, which depends on the sedimentation regime and degree of pollution in the area. This is also indicated by the observation that the same lithological types of bottom sediments in different areas contain different amounts of metals, but the general tendency of increased metal concentrations in finer fractions remains. In the Klajpėda (Nemirseta) – Būtingė area metal concentrations in sediments are comparatively low. This is explained by the predominance of sandy sediments. In the zones of local pollution near towns, industrial regions and river mouths, increased values of heavy metals are found. Such a situation can be observed at the Būtingė site, where the surface of bottom sediments is especially badly polluted. Among the heavy metals, Cu, Pb, Ni, Zn and Hg are prevalent in the bottom sediment. Their maximum concentrations were recorded in the accumulation areas of aleurite mud.

According to recent studies (Sapota 2000, Pazdro 2004), concentrations of persistent organic pollutants (POPs) in the analyzed sediment samples collected from the Gulf of Gdańsk followed the order PAHs>PCBs>DDT>lindane. Persistent organic pollutants in the areas investigated here occurred in low concentrations when compared to other marine, coastal areas in industrialized regions (Mediterrean Sea, Adriatic Sea, North Sea) (Bouloubassi & Saliot 1991, van Zoest & van Eck 1993, Fava et al. 2003). A positive correlation between sediment POP concentrations and sediment organic matter concentrations as well as the fine-grained fraction content were observed. The highest content of POPs were observed not in the vicinity of the pollution sources, but in the Gdańsk Deep – an area of intensive net sediment accumulation. This indicates the importance of POP transport processes for distribution in sediments. Both atmospheric fluxes and near-bottom fluxes should be taken into account in this respect (Pazdro 2004).

3. Material and methods

3.1. Sampling strategy

Specimens from one species of fish (*Platichthys flesus*) were used as biomonitoring organisms of coastal pollution in this study. The distribution of sampling stations where they were collected is shown in Fig. 1. In addition, blue mussels (*Mytilus trossulus*) were collected in the Gulf of Gdańsk from the same sampling stations as the fish.

Off Poland the sampling grounds are distributed inside the Gulf of Gdańsk, except for a reference station located on the 'open sea' side of the Hel Peninsula. Inside the bay, three sampling grounds were selected:

- off Mechelinki – an area affected by the waste water treatment plant (WWTP),

- off Sopot – a recreational and tourist area,
- off Sobieszewo – close to the mouth of the Vistula river (Fig. 1a).

Three sampling grounds were selected off Lithuania, in the Klajpėda area, along a suspected pollution gradient:

- Palanga (least contaminated) – considered to be a reference site,
- Nemirseta – under the influence of the water flowing out of the Curonian Lagoon,
- Būtingė (most contaminated) – under the influence of municipal sewage discharge, an oil refinery, and an oil terminal (Fig. 1b).

In the course of this study, in November 2001, an oil spill occurred in the Klajpėda area, affecting the sea coast and offshore areas in the vicinity of Palanga. The accident, one of the largest in the Baltic area, was likely to affect biota in the region.

The specimens were collected in 2001 (June and October) and 2002 (April and October). The flounder (20 females and 3–10 males, body length 20–30 cm) were collected by local fishermen using flounder nets ($\phi_{\text{mesh}} = 60$ mm), transferred to the laboratory, and dissected on the same day. In the laboratory the flounder condition, length, sex, weight (whole and empty fish, liver, gonads and spleen) and degree of parasitism were recorded.

Muscle tissues were dissected from flounder (Galgani & Bocquené 1990; Kirby et al. 2000), while gills from the mussels were utilized (Mora et al. 1999a, b). The sectioned organs were immediately transferred to liquid nitrogen and then kept in a deep freeze at $-80°$C.

3.2. Extraction – fraction S9

100–200 mg wet weight aliquots of the desired tissue samples were homogenized in 0.02 M phosphate buffer (pH = 7.0; 0.1% Triton X-100) in the ratio of 1/4 (weight/volume) using an electric homogenizer (homogenization was performed twice, each time for 20 s, in glass vessels kept in ice). Then the homogenate was centrifuged at 10 000 g at a temperature of 4°C for 20 min. The supernatant (fraction S9) was stored at –80°C before the biochemical measurements. The procedure used was that of Ellman et al. (1961), modified for microplate readings by Bocquené & Galgani (1998).

3.3. Protein determination

The method adopted from Bradford (1976) was used for protein quantitative determination using BSA (bovine serum albumin) as the protein standard. All protein measurements were carried out in a microplate reader 'Genios' (TECAN). For each microplate well the following solutions were added: 10 μl of diluted S9 extract (dilution factor with MilliQ water applied to the samples were: for fish – 1/50; for mussels – 1/10), 90 μl MilliQ water and 280 μl Bradford reagent (diluted 1/5). Absorbance was read at $\lambda = 595$ nm and the protein concentration was calculated from the calibration curve constructed from measurements of the following BSA standard solutions (0; 2.63; 5.26; 7.89; 10.52; 15.78 μg BSA ml^{-1}).

3.4. AChE measurements

The method for measurements of AChE activity in the microplate reader, adapted from Bocquené & 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 replicates	3 replicates
0.02M Phosphate 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 test)	10 μl	10 μl	10 μl

This was followed by absorption measurements at $\lambda = 405$ nm in 4 kinetic intervals (0, 1, 2, 3 min). The following equation was used to calculate the AChE activities:

$$\text{AChE}_{ac} = \frac{\Delta\text{OD}_{405}\ \text{min}^{-1}}{\text{protein [mg]}}$$

ΔOD – optical density increase.

4. Results and discussion

The samples derived from individual fish displayed an AChE activity in the range from 94 to 315 nmol min^{-1} mg protein^{-1}, while the mussel gill activity ranged from 15 to 38 nmol min^{-1} mg protein^{-1}. The obvious, species dependence on AChE levels, already reported for other areas (Bocquené et al. 1990, Bocquené & Galgani 1998) is evident.

The lowest mean values of AChE activity in *M. trossulus* were observed near the Mechelinki GG1 station (15–20 nmol min^{-1} mg protein^{-1}) – Fig. 2.

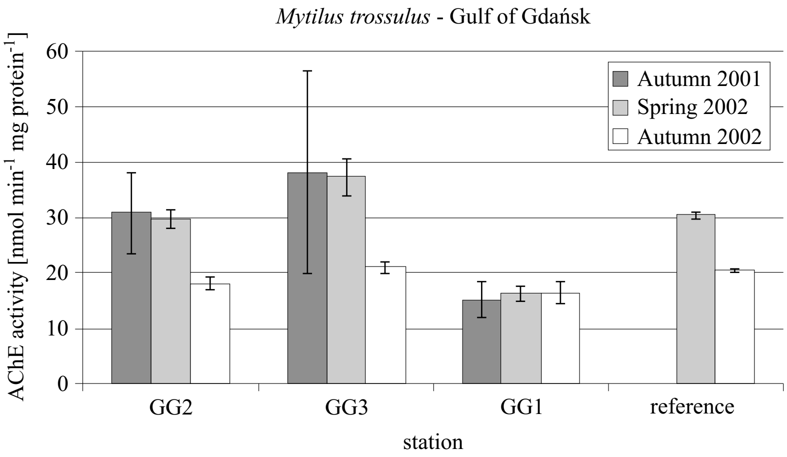

Fig. 2. Levels of AChE activity in *Mytilus trossulus* from the Gulf of Gdańsk

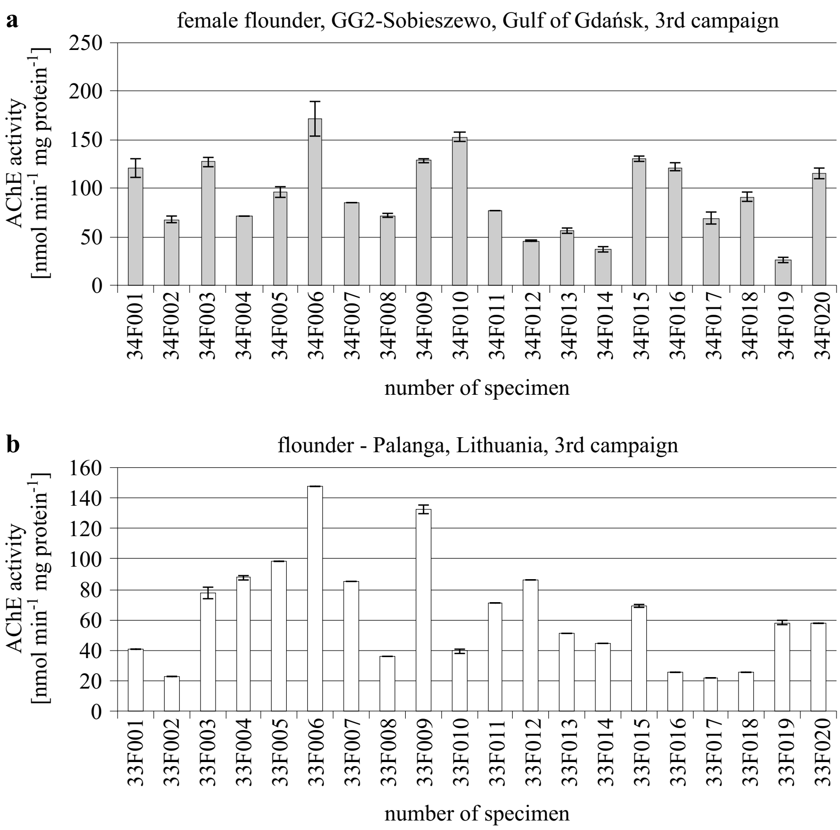

Fig. 3. Results of AChE activity measured in individual fish from; the Gulf of Gdańsk, Sobieszewo sampling ground, March 2002 (a); the Klajpėda area, Palanga sampling ground, March 2002 (b)

This area is regarded as a polluted one (Sapota 2000, Potrykus et al. 2003, Pazdro 2004), owing to the treated sewage dicharges. The highest values of AChE activity in *M. trossulus* (c. 38 nmol min^{-1} mg protein^{-1}) were measured at the sampling site near Sopot (GG3), indicating a more favourable environment there.

AChE activities in samples of flounder muscles are presented in Fig. 3 (individual fish, one station, one sampling campaign), Fig. 4 (averages for individual sampling sites), and Fig. 5 (averages for subsequent sampling campaigns).

Very high individual variability of the AChE activities in the muscle tissue of individual fish was a characteristic feature of the data sets

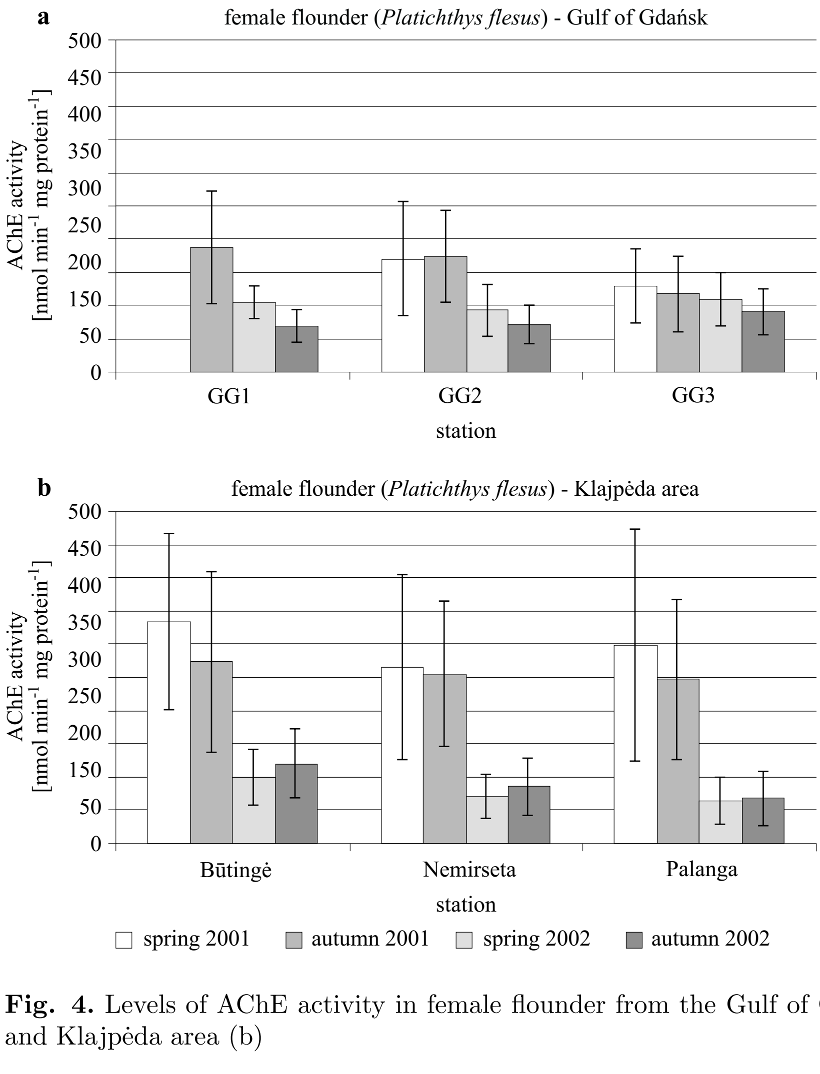

Fig. 4. Levels of AChE activity in female flounder from the Gulf of Gdańsk (a), and Klajpėda area (b)

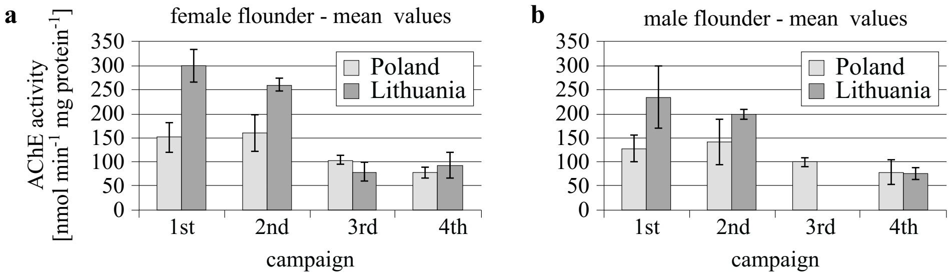

Fig. 5. Overall average activities of AChE in flounder collected in the Gulf of Gdańsk (Poland) and in the Klaipeda area (Lithuania): female flounder (a), male flounder (b)

obtained from both sampling areas (Fig. 3). This, most likely, indicates the substantial influence of chemical contamination on organisms combined with the variable resistance of individual fish (Kirby et al. 2000). It is also likely to reflect the influence of localized sources of contamination in the area under study, already mentioned when the sampling strategy was outlined. Substantial exchange of fish between the areas must be assumed, too.

Average AChE activities in fish [nmol min^{-1} mg protein^{-1}] were in the range from 105 to 187 in the Gulf of Gdańsk, and from 65 to 380 off Lithuania (Fig. 4). The lower AChE activities in the Gulf of Gdańsk as compared to the Klajpėda area can be attributed to either one or both of the following reasons: a lower water temperature during sampling in the Gulf of Gdańsk, and/or a lower level of contamination by the relevant pollutants in the Klajpėda area. Sampling in the Gulf of Gdańsk took place earlier in spring, and later in autumn, at lower water temperatures. The lowest mean values in flounder from the Gulf of Gdańsk were found in spring 2002 at all sampling sites. At that time a very low water temperature was recorded: this could have influenced the levels of AChE activity (Hogan 1970, Bocquené & Galgani 1998). Bocquené et al. (1990), Bocquené & Galgani (1998) reported the temperature dependence of AChE activities in fish, with lowering activities paralleling the temperature decrease. A 2%/°C increase of AChE activity in brain tissue in the temperature range from 2°C to 10°C, was reported by Hogan (1970).

On average AChE activities in male flounder were smaller than those in the female flounder (105 vs 176 – the Gulf of Gdańsk, 102 vs 150 – off Lithuania). However, no statistically significant differences were found when all the results were pooled (Fig. 5). This feature has already been observed by Galgani et al. (1992), who reported no sex influence on AChE levels, and Kirby et al. (2000), who confirmed the finding.

One interesting feature of the data set is the large scatter of individual results as characterized by the standard deviations of the means (Figs 4 and 5). The relative standard deviations of average values are in the range from 0.25 to 0.67, indicating a mixed stock of fish at the sampling grounds. Such a phenomenon may be regarded as an indication of localized pollution (Kirby et al. 2000), and was discussed above.

Strong geographical variation of the measured biomarker activities is noticeable at the Gulf of Gdańsk sites (Fig. 4a). The lowest values are observed at the Mechelinki GG1 station. The water off Mechelinki is strongly influenced by treated sewage outflow. Fish contamination with persistent organic pollutants was reported earlier at the same location (Sapota 2000). Since it is suggested that AChE activities may also depend on metals and hydrocarbons (Payne et al. 1996), the contamination of the site by these chemicals may contribute to the findings. The site with the highest AChE levels – GG3 Sopot – is located off a recreational and tourist area.

Off the Lithuanian coast the differences between stations are neither obvious nor statistically significant (Fig. 4b). This may indicate comparable levels of contamination, despite the different positioning of the sampling stations in relation to sources of pollution. A large drop in AChE activities between 2001 and 2002 is evident, though. This was accompanied by physiological changes in the biota of the area (Baršienė et al. 2003). In Nov. 2001, right after the sampling campaign of autumn 2001, a substantial spill of oil from a tanker occurred at the Palanga site (Baršienė et al. 2003). Although no contaminants, usually associated with AChE activity in fish, were reported, the overall physiological state of the biota had changed (Baršienė et al. 2003). The AChE activity shift can, therefore, be attributed to this cause. Interestingly, measurements conducted in autumn, 2002, half a year after the spill had occurred, indicated continued low AChE levels, although it seems that recovery had already started and the AChE activity had increased in the Būtingė area, the most distant one from the scene of the accident.

5. Conclusions

The AChE activities measured in the study area are in the range reported for flounder in other coastal Baltic areas (BEEP 2004) and other European seas (Bocquené et al. 1993, Kirby et al. 2000). Levels of AChE activity are thought to be a useful indicator of biological responses in organisms (mussels and fish) to pollution by specific organic pollutants – organophosphates and carbamates (Bocquené & Galgani 1998). In the Gulf of Gdańsk sources of contaminants are rather localized (the mouth of the Vistula due to runoff, ports, sewage discharges), causing well-developed gradients of organic pollutants in biota (Sapota 2000), while an accidental oil spill occurred off Lithuania in the course of the study (Baršienė et al. 2003). Geographical (Gulf of Gdańsk) and temporal (Klajpėda region) AChE level changes follow the contamination patterns mentioned. AChE gradients are well documented and confirmed in this study. The large individual variability is thought to be caused by contamination gradients, and by the individual variability of fish. The study confirms the potential use of AChE as biomarker of organic pollution.

References

Baršienė J., Lehtonen K., Vuorinen P. K., Lang T., Pempkowiak J., Šyvokienė J., Dedonyte V., Rybakovas A., Vuontisjärvi H., Kopecka J., 2003, *Biomarker responses in the mussel (Mytilus edulis) and the flounder (Platichthys flesus) in the Klaipeda-Butinge area (Baltic Sea)*, ICES Ann. Sci. Conf., Theme Session on Biological Effects Monitoring in the Baltic Sea (M), ICES CM 2003/M:11.

BEEP (Biological Effects of Environmental Pollution), 2004, *Biomonitoring in the Baltic Sea*, WP2 Final Rep. (March 2004).

Bocquené G., Galgani F., 1998, *Biological effects of contaminants: Cholinesterases inhibition by organophosphate and carbamate compounds*, ICES Tech. Mar. Environ. Sci., 22, 1–13.

Bocquené G., Galgani F., Burgeot T., Le Dean L., Truquet P., 1993, *Acetylcholinesterase levels in marine organisms along French coasts*, Mar. Pollut. Bull., 26 (2), 101–106.

Bocquené G., Galgani F., Truquet P., 1990, *Characterization and assay conditions for use of AChE activity from several marine species in pollution monitoring*, Mar. Environ. Res., 30, 75–89.

Bouloubassi I., Saliot A., 1991, *Sources and transport of hydrocarbons in the Rhone Delta Sediments (North-Western Mediterranean)*, Fresen. Z. Anal. Chem., 339, 765–771.

Bradford M., 1976, *A rapid method quantification of protein utilizing the principle dyebinding*, Anal. Biochem., 72, 248–264.

Ellman G. L., Courtney K. D., Andres V., Featherstone R. M., 1961, *A new and rapid colorimetric determination of acetylcholinesterase activity*, Biochem. Pharmacol., 7, 88–95.

Escartin E., Porte C., 1997, *The use of cholinesterase and carboxylesterase activities from Mytilus galloprovincialis in pollution monitoring*, Environ. Toxicol. Chem., 16 (10), 2090–2095.

Fava F., Gentilucci S., Zanaroli G., 2003, *Anaerobic biodegradation of weathered polychlorinated biphenyls (PCBs) in contaminated sediments of Porto Maghera (Venice Lagoon, Italy)*, Chemosphere, 53 (2), 101–109.

Galgani F., Bocquené G., 1990, *In vitro inhibition of acetylcholinesterase from four marine species by organophosphates and carbamates*, Bull. Environ. Contam. Toxicol., 45, 243–249.

Galgani F., Bocquené G., 2000, *Molecular biomarkers of exposure of marine organisms to organophosphorus pesticides and carbamates*, [in:] *Use of biomarkers for environmental quality assessment*, L. Lagadic, T. Caquet, J. C. Amiard & F. Ramade (eds.), Sci. Publ., Inc., Enfield USA, Plymouth UK, 113–134.

Galgani F., Bocquené G., Cadiou Y., 1992, *Evidence of variation in cholinesterase activity in fish along a pollution gradient in the North Sea*, Mar. Ecol. Prog. Ser., 91, 77–82.

Giam C. S., Ray L. E. (eds.), 1987, *Pollutant studies in marine animals*, CRC Press, Boca Raton, Fla, 187 pp.

Hogan J. W., 1970, *Water temperature as a source of variation in specific activity of brain acetylcholinesterase of bluegills*, Bull. Environ. Contam. Toxicol., 5 (4), 347–353.

Huggett R. J., Kimerle R. A., Mehrle Jr P. M., Bergman H. L. (eds.), 1992, *Biomarkers: biochemical, physiological and histological markers of anthropogenic stress*, SETAC Spec. Publ. Ser., Lewis Publ., Boca Raton, 5–17.

Kirby M. F., Morris S., Hurst M., Kirby S. J., Neall P., Tylor T., Fagg A., 2000, *The use of cholinesterase activity in flounder (Platichthys flesus) muscle tissue as a biomarker of neurotoxic contamination in the UK estuaries*, Mar. Pollut. Bull., 40 (9), 780–791.

Kopecka J., Pempkowiak J., 2002, Poster presentation, SETAC 2002 Conf. 'AChE as biomarker of mussels and fish contamination with chemicals in the Gulf of Gdańsk', Vienna 2002, Abstracts book No 223.

Kopecka J., Pempkowiak J., 2003, Poster presentation, SETAC 2003 Conf. 'The feasibility of AChE, GST and CAT as biomarker of bivalvia contamination with chemicals in the Gulf of Gdańsk', Hamburg 2003, Abstracts book No 135.

Mora P., Fournier D., Narbonne J. F., 1999a, *Cholinesterases from the marine mussels (Mytilus galloprovincialis) Lmk. and Mytilus edulis L. and from the freshwater bivalve Corbicula fluminea Müller*, Comp. Biochem. Physiol. (Part C), 122, 353–361.

Mora P., Michel X., Narbonne J. F., 1999b, *Cholinesterase activity as a potential biomarker in two bivalves*, Environ. Toxicol. Phar., 7, 253–260.

Napierska D., Podolska M., 2003, Poster presentation, BSC 2003 Congr. 'Preliminary results of AChE, GST and EROD measurements in flounder *Platichthys flesus* and eelpout *Zoarces viviparus*, from the southern Baltic Sea', Helsinki 2003, Abstracts book No 196.

Narbonne J. F., Daubeze M., Clerandau C., Garrigues P., 1999, *Scale of classification based on biochemical markers in mussels: application to pollution monitoring in European coasts*, Biomarkers, 4 (6), 415–424.

Payne J. F., Mathieu A., Melvin W., Fancey L. L., 1996, *Acetylcholinesterase, an old biomarker with a new future? Field trials in association with two urban rivers and a paper mill in Newfoundland*, Mar. Pollut. Bull., 32 (2), 225–231.

Pazdro K., 2004, *Persistent organic pollutants in sediments in the Gulf of Gdańsk*, Ann. Environm. Prot., 4, 51–62.

Potrykus J., Albalat A., Pempkowiak J., Porte C., 2003, *Content and pattern of organic pollutants (PAHs, PCBs and DDT) in blue mussels (Mytilus trossulus) from the southern Baltic Sea*, Oceanologia, 45 (2), 337–355.

Sapota G., 2000, *Bioakumulacja węglowodorów chlorowanych w sieci troficznej Zatoki Gdańskiej*, Praca doktorska (Ph. D. thesis), Uniw. Gd., Gdańsk, 137 pp.

van Zoest R., van Eck G. T., 1993, *Historical input and behaviour of hexachlorobenzene, polichlorinated biphenyls and polycyclic aromatic hydrocarbons in two dated sediment cores from the Scheldt estuary, SW, Netherlands*, Mar. Chem., 44 (1), 95–103.

Walker C. H., Livingstone D. R., 1992, *Persistent pollutants in marine ecosystems*, SETAC Spec. Publ. Ser., Pergamon Press, New York, 3–35.

WHO (World Health Organization), 1993, *Biomarkers and risk assessment: concepts and principles*, Int. Prog. Chem. Safety, WHO, Vammole, 92 pp.