Measurements of biochemical markers of pollution in mussels Mytilus galloprovincialis from coastal areas of the Saronikos Gulf (Greece)

TSANGARIS C. Hellenic Centre for Marine Research, Institute of Oceanography, P.O. Box 712, P.C. 19013, Anavyssos, Attiki

STROGYLOUDI E. Hellenic Centre for Marine Research, Institute of Oceanography, P.O. Box 712, P.C. 19013, Anavyssos, Attiki

PAPATHANASSIOU E. Hellenic Centre for Marine Research, Institute of Oceanography, P.O. Box 712, P.C. 19013, Anavyssos, Attiki

https://doi.org/10.12681/mms.223

To cite this article:

TSANGARIS, C., STROGYLOUDI, E., & PAPATHANASSIOU, E. (2004). Measurements of biochemical markers of pollution in mussels Mytilus galloprovincialis from coastal areas of the Saronikos Gulf (Greece). Mediterranean Marine Science, 5(1), 175-186. doi:https://doi.org/10.12681/mms.223
Measurements of biochemical markers of pollution in mussels *Mytilus galloprovincialis* from coastal areas of the Saronikos Gulf (Greece)

C. TSANGARIS, E. STROGYLOUDI and E. PAPATHANASSIOU

Hellenic Center for Marine Research
Institute of Oceanography, P.O. 712, 190 13
Anavissos, Attiki, Greece
e-mail: ctsangar@ncmr.gr, estro@ncmr.gr, vpapath@ncmr.gr

**Abstract**

Alterations in a number of biochemical parameters in marine organisms represent specific markers of exposure to particular classes of contaminants. They are used as tools for the detection and monitoring of pollution. In this study, two biochemical markers of pollution, metallothionein (MT) content and acetylcholinesterase (AChE) activity were measured in indigenous and transplanted mussels *Mytilus galloprovincialis* from coastal areas within the Saronikos Gulf subject to high anthropogenic activities. Bi-annual measurements of the two biomarkers in indigenous mussel populations for two years revealed no significant differences among stations representing a pollution gradient. Limited differences in MT levels were only found between mussel populations transplanted at lesser and more impacted stations. Both biomarkers showed a variation with respect to the season of sampling, whilst during the second year of measurements a concomitant increase in metallothionein content with a decrease in acetylcholinesterase activity was noted. Our results indicate that the applied biochemical markers in indigenous mussel populations do not reflect the type of pollution in the Saronikos Gulf to a degree that can be used for pollution monitoring in the area.

**Keywords:** Biomarker; Metallothionein; Acetylcholinesterase; *Mytilus galloprovincialis*; Saronikos Gulf.

**Introduction**

Pollution responses at various levels of biological organisation are widely used in environmental monitoring since by definition pollution implies a hazard to living resources. When relating to individual organisms, responses used for the detection and evaluation of pollution, are often referred to as biomarkers. Biomarker measurements revealing exposure to and/or biological effects of chemical contaminants have been increasingly used for pollution assessment during the last decades (LIVINGSTONE *et al.*, 1995, STAGG, 1998, CAJARAVILLE *et al.*, 2000, GALLOWAY *et al.*, 2002). Very few biomarkers have been applied in Greek marine areas (COTOU *et al.*, 2001,
Most pollution monitoring programmes in Greece focus on biological indicators i.e. pollution effects at the population and community levels and/or chemical analysis in water, sediment and biota (GEORGAKOPOULOU-GREGORIADOU et al., 1997, NCMR, 1997, NCMR, 2001, NCMR, 2002). In the Saronikos Gulf, pollution monitoring has been performed within the framework of the MED POL programme (NCMR, 1997a) but the use of biomarker measurements in the area is scarce (COTOU et al., 2001, COTOU et al., 2002, KALPAXIS et al., 2003). In this study, two biochemical markers, metallothionein (MT) levels and acetylcholinesterase (AChE) activity, were measured in Mytilus galloprovincialis indigenous or transplanted to several sites in the Saronikos Gulf subject to high anthropogenic activities. MT content is suggested as a biomarker of heavy metal exposure (VIARENGO et al., 1999). AChE activity is mainly considered as a biomarker of exposure to organophosphate and carbamate pesticides (BOCUQUÉNE & GALGANI, 1991) and also responds to exposure to other type of contaminants including heavy metals (NAJIMI et al., 1997, HAMZA-CHAFFAI et al., 1998), undetermined compounds of complex mixtures of pollutants, detergents and surfactants (PAYNE et al., 1996, GUILHERMINO et al., 1998). The aim of this study is to investigate the response of MT content and AChE activity to contaminant levels in the Saronikos Gulf in order to evaluate their use as tools for the detection and monitoring of pollution in the area.

**Materials and Methods**

**Field sites**

Six stations in different areas of the Saronikos Gulf were selected (Fig 1). ‘Piraeus’ within Piraeus harbour and ‘Skaramagas’, a shipyard in the eastern part of Elefsis bay, were considered as the most impacted stations. ‘Megara’ situated in the western part of Elefsis bay and ‘Ag. Kosmas’, ‘Aigina’ and ‘Anavissos’ along the coast of the eastern part of the Saronikos Gulf were regarded as less impacted stations. Industrial activities are mainly located along the eastern part of Elefsis bay and around Piraeus harbour. The wastewater treatment plant of the city of Athens is also situated in this area. Enriched levels of Cd, Cu, Zn, Pb, Cr and Fe are found in sediments in Piraeus harbour and Elefsis bay, decreasing towards the western part of Elefsis bay (VOUTSINOU–TALIADOURI et al., 1989) where Megara station is located. The most recent data on Hg levels in sediments from the Saronikos Gulf ranged from 0.13 to 0.27 µg.g⁻¹ in Elefsis bay, 0.04 to 0.14 µg.g⁻¹ in west Saronikos and 0.06 to 0.07 µg.g⁻¹ in east Saronikos (STATHOPOULOU et al., 2001). Mean values of aliphatic hydrocarbons and polycyclic aromatic hydrocarbons in sediments were found to be 531 µg.g⁻¹ and 3037 ng.g⁻¹ respectively in Elefsis Bay (SKLIVAGOU et al., 2001), 53 µg.g⁻¹ and 166 ng.g⁻¹ in the western Saronikos Gulf (NCMR, 1997b) and 97 µg.g⁻¹ and 1464 ng.g⁻¹ in Ag. Kosmas located in the eastern Saronikos Gulf (KARAGEORGIS & HATZIANESTIS, 2003). In addition significant TBT contamination has been recorded in sediments from marinas in the Saronikos Gulf with very high levels of 10 µg Sn.g⁻¹ in Piraeus harbour (TSELENTIS et al., 1999).

**Sampling**

Mussels *Mytilus galloprovincialis* of similar shell length (41-61mm) were sampled in the spring and autumn over a two-year period (May 2001-October 2002). Indigenous mussels were collected from ‘Piraeus’ and ‘Skaramagas’. At ‘Megara’ mussels were collected from an aquaculture farm. At ‘Aigina’, ‘Ag. Kosmas’ and ‘Anavissos’, where there were no indigenous populations available, mussels from ‘Megara’ were transplanted for one month and placed in

http://epublishing.ekt.gr | e-Publisher: EKT | Downloaded at 18/05/2020 22:09:11 |
plastic cages at 2 to 3m below the surface. Additionally, for a comparison between indigenous and transplanted populations, mussels were transplanted as above to the ‘Skaramagas’ station whereas at the ‘Megara’ station a portion of the cultured mussels was transplanted within the immediate area. Just after collection, mussels were transferred to the laboratory where gills and digestive glands were rapidly dissected out and stored at -70°C for biochemical measurements. Whole mussel tissues were stored at -20°C for heavy metal analysis.

**Acetylcholinesterase (AChE) activity**

Gill tissues (1 g) from 2-5 individuals were pooled and homogenised using a Potter-Elvehjem homogeniser in 1:2 (w:v) 0.1M Tris-HCl buffer containing 0.1% TRITON X 100, pH 7. Homogenates were centrifuged at 10,000g for 20min. All preparation procedures were carried out at 4°C. AChE activity was assayed by the method of Ellman (ELLMAN et al., 1961) adapted to microplate reading by BOCQUENÉ and GALGANI (1992). The method is based on the increase in yellow colour produced due to the reaction of thiocholine with DTNB (dithio-bis-nitrobenzoic acid) during acetylthiocholine hydrolysis by AChE. 320μl of 0.1M Tris-HCl buffer containing 0.1% TRITON X 100, pH 7, 20μl of 0.01M DTNB and 30 μl of sample supernatant were added in each well of the microplate and the reaction initiated by addition of 10 μl of 0.1 M acetylthiocholine substrate. The enzyme kinetic was measured every 15s for 2 min at 414 nm. The assay was carried out at 25°C. Total protein content in the homogenate supernatants was measured by the Bradford method (1976) adapted for microplate reading. 280 μl of Bradford reagent and 100 μl of sample were added to each microplate well and absorbance read at 595nm. Protein concentration was calculated using a Bovine Serum Albumin (BSA) standard curve. Specific enzyme activity was expressed as U/mg protein. One unit (U) of AChE activity is the...
amount of enzyme, which causes a variation of 0.001 in optical density per minute.

**Metallothionein (MT) content**

MT concentration was determined according to VIARENGO et al., (1997). The method is based on the estimation of the sulphhydryl content of MT proteins by spectrophotometric determination of the -SH groups using Ellman’s reagent. Pooled samples of digestive gland tissues (1g) from 2-5 individuals were prepared. MT concentration was calculated utilizing reduced glutathione (GSH) as a reference standard and expressed as mg MT/g wet weight tissue.

**Heavy metal analysis**

Whole mussel tissues were dissected out, freeze-dried, and digested with concentrated HNO₃ under pressure in a CEM MDS 2100 microwave device. Metal analysis was performed by Atomic Absorption Spectrophotometry (AAS). Cu, Zn and Hg concentrations were measured with a Varian Spectra AA20Plus Spectrophotometer. Hg concentrations were determined by the cold vapour technique. Cd analysis was performed with an AAS Perkin Elmer 4100 equipped with a HGA 700. Heavy metal concentrations were expressed as µg.g⁻¹ dry weight tissue. Analytical quality control was achieved using certified reference material provided by the National Research Council of Canada.

**Flesh Condition Index**

Flesh Condition index (FC) was calculated according to LOBEL & WRIGHT (1982) as dry whole tissue weight (mg) versus dry shell weight (gr) per individual.

**Statistical analysis**

Results are shown as means ± SD. Logarithmic transformation of data was performed to obtain homogeneity of variance. Significant differences between means were determined by one-way analysis of variance (ANOVA) followed by the Tukey HSD multiple comparison test. Correlation was tested by Pearson’s correlation coefficient (r). Statistical analysis was carried out using SPSS software. Significance level was set at p<0.05.

**Results**

MT content and AChE activity in indigenous and transplanted mussels sampled at stations along the coast of the Saronikos Gulf are shown in Figure 2. Bi-annual measurements of the two biomarkers in indigenous mussels for two years revealed no significant differences among stations with the exception of a decreased AChE value in ‘Megara’ in spring 2002. In transplanted mussels, no differences in AChE activities were found among stations while MT levels showed a significant increase in Skaramagas in spring 2001 and in Anavissos in autumn 2001.

When measurements in indigenous and transplanted mussel populations were compared, significant differences were found in MT content. MT levels were significantly increased in transplanted mussels compared to indigenous populations both at Skaramagas and Megara in spring 2001 (Table 1). Furthermore, pooled data from all stations indicated higher MT levels in transplanted populations in spring 2001 (Fig 3).

Both MT content and AChE activity revealed a variation with respect to the season of sampling (Fig 2). Significantly higher MT levels in autumn than in spring were found in indigenous mussels in Megara and Skaramagas during 2002. In contrast, significantly higher MT levels were detected in spring than in the autumn in transplanted mussels in Megara and Ag. Kosmas during 2001. AChE activities in indigenous mussels were generally higher in spring than in autumn with the exception of Megara during 2002 where activities were significantly higher in autumn. In addition both MT levels and AChE activities showed a variation with respect to the year of sampling with a general increase in MT levels and
decrease in AChE activities in 2002 compared to 2001.

Heavy metal concentrations in whole tissues of indigenous and transplanted mussels from the different stations in the Saronikos Gulf are shown in Table 2. Mean values ranged from 2.36 to 31.5 µg.g⁻¹ dw for Cu, from 0.24 to 1.24 µg.g⁻¹ dw for Cd, from 80 to 285 µg.g⁻¹ dw for Zn and from 0.07 to 0.12 µg.g⁻¹ dw for Hg. In indigenous mussels, the highest Cu and Zn concentrations were found in Piraeus followed by Skaramagas and the highest Cd

Fig. 2: MT content (µg/g ww tissue) in the digestive gland and AChE activity (U/mg protein) in the gills of indigenous and transplanted mussels *Mytilus galloprovincialis* at different stations in the Saronikos Gulf over a two-year period (2001-2002). Significant differences between stations represented by different small letters (p<0.05), significant differences between seasons displayed by different capital letters (p<0.05).

Table 1
MT content in the digestive gland and AChE specific activity in the gills of indigenous and transplanted mussels at Megara and Skaramagas during 2001 (mean±SD).

| Date      | Station | MT content (µg/g ww tissue) | AChE activity (U/mg protein) |
|-----------|---------|-----------------------------|-----------------------------|
|           |         | Indigenous mussels          | Transplanted mussels        |
| Spring 2001 Megara | 79.9±16.7 a | 129±8 b                     | 1089.9±304.2               | 763.5±174.2           |
| Spring 2001 Skaramagas | 92.1±19.5 a | 143±33 b                    | 851.4±264.9                | nd                    |
| Autumn 2001 Megara | 85.6±7.6 | 61.3±15.6                    | 458.9±125.4                | nd                    |

nd: not determined
Significant differences between populations represented by different letters (t-test, p<0.05).
levels were recorded in Skaramagas that were both considered to be the most impacted stations. No differences in Hg concentrations among stations were recorded. In transplanted mussels the highest Cu values were also found in ‘Skaramagas’ while no significant differences in Zn and Cd concentrations were detected among stations with the exception of ‘Anavissos’. In ‘Anavissos’, Cd, Zn and also Cu levels were elevated compared to ‘Megara’ and ‘Ag. Kosmas’, in contrast to the expected pollution gradient. In general, no differences in heavy metal concentrations were found between indigenous and transplanted mussels with the exception of increased Cd concentrations in transplanted mussels at ‘Megara’ in spring 2001. Heavy metal concentrations varied with respect to season of sampling, showing significantly higher values in autumn than in spring in Megara and ‘Skaramagas’ during 2002 with the exception of Cu in ‘Skaramagas’ where levels were constantly high.

| Station    | Date          | Cu (µg/g dw) Indigenous mussels | Transplanted mussels | Zn (µg/g dw) Indigenous mussels | Transplanted mussels | Cd (µg/g dw) Indigenous mussels | Transplanted mussels | Hg (µg/g dw) Indigenous mussels |
|------------|---------------|---------------------------------|----------------------|---------------------------------|----------------------|---------------------------------|----------------------|---------------------------------|
| Ag.Kosmas  | Spring 2001   | 4.27±0.12                        | 221±33               | 1.03±0.13                       | -                    | 0.99±0.10                       | -                    | 0.91±0.05                       |
|            | Autumn 2001   | 3.79±0.35                        | 188±12               | 0.97±0.10                       | -                    | 0.92±0.06                       | -                    | 0.91±0.05                       |
| Aigina     | Spring 2001   | 2.36±0.65                        | 176±17               | 0.69±0.27                       | -                    | 0.67±0.25                       | -                    | 0.65±0.24                       |
| Anavissos  | Autumn 2001   | 4.95±0.55                        | 197±18               | 1.21±0.08                       | -                    | 0.92±0.06                       | -                    | 0.91±0.05                       |
| Megara     | Spring 2001   | 5.37±2.48                        | 199±43               | 0.75±0.02                       | -                    | 0.66±0.03                       | -                    | 0.68±0.03                       |
|            | Autumn 2001   | 3.16±0.30                        | 120±18               | 0.66±0.03                       | -                    | 0.68±0.03                       | -                    | 0.68±0.03                       |
|            | Spring 2002   | 4.21±0.55                        | 126±18               | 0.24±0.03                       | -                    | 0.24±0.03                       | -                    | 0.24±0.03                       |
|            | Autumn 2002   | 6.38±0.48                        | 151±34               | 0.36±0.07                       | -                    | 0.36±0.07                       | -                    | 0.36±0.07                       |
| Skaramagas | Spring 2001   | 11.22±1.42                       | 197±50               | 1.24±0.16                       | -                    | 1.07±0.12                       | -                    | 1.06±0.12                       |
|            | Autumn 2001   | 9.48±0.22                        | 190±56               | 1.20±0.07                       | -                    | 0.97±0.06                       | -                    | 0.96±0.06                       |
|            | Spring 2002   | 9.28±0.94                        | 153±22               | 0.26±0.04                       | -                    | 0.26±0.04                       | -                    | 0.26±0.04                       |
|            | Autumn 2002   | 9.98±0.17                        | 244±31               | 0.41±0.08                       | -                    | 0.41±0.08                       | -                    | 0.41±0.08                       |
| Piraeus    | Spring 2002   | 31.5±1.6                         | 146±22               | 0.25±0.06                       | -                    | 0.25±0.06                       | -                    | 0.25±0.06                       |
|            | Autumn 2002   | 17.1±0.7                         | 285±59               | 0.61±0.15                       | -                    | 0.61±0.15                       | -                    | 0.61±0.15                       |

Table 2
Heavy metal concentrations in whole tissues of mussels Mytilus galloprovincialis at different stations in the Saronikos Gulf during 2001 and 2002 (mean±SD).

Discussion
MT content is considered as a biomarker of exposure to heavy metal pollution and field studies using mussels have shown increases of MT content in areas contaminated with heavy metals (VIARENGO et al., 1999). In the present study, MT contents in indigenous mussels from several stations were recorded in Skaramagas that were both considered to be the most impacted stations. No differences in Hg concentrations among stations were recorded. In transplanted mussels the highest Cu values were also found in ‘Skaramagas’ while no significant differences in Zn and Cd concentrations were detected among stations with the exception of ‘Anavissos’. In ‘Anavissos’, Cd, Zn and also Cu levels were elevated compared to ‘Megara’ and ‘Ag. Kosmas’, in contrast to the expected pollution gradient. In general, no differences in heavy metal concentrations were found between indigenous and transplanted mussels with the exception of increased Cd concentrations in transplanted mussels at ‘Megara’ in spring 2001. Heavy metal concentrations varied with respect to season of sampling, showing significantly higher values in autumn than in spring in Megara and ‘Skaramagas’ during 2002 with the exception of Cu in ‘Skaramagas’ where levels were constantly high.

Correlations between biomarkers and heavy metal concentrations were not significant (p>0.05), while a significant correlation was found between AChE activity and MT content (r=-0.436, p<0.05).

MT content is considered as a biomarker of exposure to heavy metal pollution and field studies using mussels have shown increases of MT content in areas contaminated with heavy metals (VIARENGO et al., 1999). In the present study, mean MT concentrations in indigenous mussels from several stations were recorded in Skaramagas that were both considered to be the most impacted stations. No differences in Hg concentrations among stations were recorded. In transplanted mussels the highest Cu values were also found in ‘Skaramagas’ while no significant differences in Zn and Cd concentrations were detected among stations with the exception of ‘Anavissos’. In ‘Anavissos’, Cd, Zn and also Cu levels were elevated compared to ‘Megara’ and ‘Ag. Kosmas’, in contrast to the expected pollution gradient. In general, no differences in heavy metal concentrations were found between indigenous and transplanted mussels with the exception of increased Cd concentrations in transplanted mussels at ‘Megara’ in spring 2001. Heavy metal concentrations varied with respect to season of sampling, showing significantly higher values in autumn than in spring in Megara and ‘Skaramagas’ during 2002 with the exception of Cu in ‘Skaramagas’ where levels were constantly high.
mussel populations from coastal areas of the Saronikos Gulf varied from 53 to 153 mg/g and were similar to values obtained from a previous study in the framework of the MED POL programme in the same area (HCMR, 2003) and to values found in wild mussels collected from other Mediterranean and East Atlantic coastal areas (BEIBIANNO & MACHADO, 1997, VIARENGO et al., 1997, STIEN et al., 1998, PETROVIC et al., 2001, DOUMOUHTSIDOU et al., 2004). Heavy metal concentrations in indigenous mussels from the Saronikos Gulf were generally low and similar to those found in non-polluted coastal areas (ROMEO et al., 2003, BESADA et al., 2002, BEIRAS et al., 2003) apart from few increased Cu values in ‘Piraeus’ although overall variation was according to the expected pollution gradient. However, significant increases in MT content parallel to the higher Cu, Cd and Zn tissue levels found in indigenous mussels from stations regarded as most impacted were not recorded. DOUMOUTSIDOU et al. (2004) also reported that increases in MT content in mussel populations from Northern Greece did not follow tissue heavy metal concentrations although they found MT variations among stations displaying a pollution gradient. Our results are similar to PETROVIĆ et al., (2001) that failed to reveal increases of MT levels in mussels from polluted sites of the North-Eastern Adriatic and attributed their results to a generally low concentration of heavy metals.
metals in the mussel tissues that could not induce MT biosynthesis. Furthermore GÉRET et al. (2002) found no increase in MT levels in the digestive gland of M. edulis exposed to Cd or Cu at tissue metal concentrations comparable to those measured in the present study. The only differences observed in MT content among mussel populations of this study are possibly related to transplantation, as MT responses differed between indigenous and transplanted mussels. BOLOGNESI et al., (2004) found differences in genotoxicity biomarker responses between wild and caged mussels and suggested that caged mussels showed a higher level of DNA damage as a result of very recent exposure to genotoxic agents. Similarly the higher MT levels in transplanted compared to indigenous mussels of the present study may be a result of recent heavy metal exposure. In addition, the only cases where increased MT content was found concurrently with increased Cu, Cd and Zn tissue levels occurred in transplanted mussels (‘Skaramagas’ in spring 2001 and ‘Anavissos’ in autumn 2001). These results tend to show that the use of transplanted mussels may be a better approach for the application of MT as a biomarker of heavy metal exposure as proposed by VIARENGO et al. (1999), and point out the need for further research.

AChE inhibition is considered as a biomarker of organophosphate and carbamate exposure (BOCQUENÉ & GALGANI, 1991) and decreased AChE activities of mussels have been reported in relation to presence of organophosphate and carbamate pesticides (ESCARTIN & PORTE, 1997). Additionally, several field studies suggest that other environmental contaminants including heavy metals can affect AChE (AMIARD-TRIQUET et al., 1998, NARBONNE et al., 1993). AChE activities measured in the gills of mussels from coastal areas of the Saronikos Gulf ranged from 273 to 1090 U/mg protein independent of the pollution gradient. These levels were generally in the high range of those reported in mussels from Northern Greek coasts (DAILIANIS et al., 2003) and other Mediterranean regions (BOCQUENÉ et al., 1993, ESCARTIN & PORTE, 1997). The limited differences observed in AChE activity among stations were inconsistent and not in accordance with the expected pollution gradient. DAILIANIS et al., (2003) also reported differences in AChE activities of mussels among stations in the Thermaikos Gulf (Northern Greece) that were not consistent with respect to season of sampling and in a few cases were unexpectedly higher in stations regarded as more polluted, although in several cases they found a decrease in AChE activity at the most polluted stations. The lack of AChE response to the pollution gradient in the Saronikos Gulf may be related to the absence of highly elevated levels of contaminants capable to inhibit AChE.

Since biomarker responses are influenced by the physiological status of the bioindicator organisms, the flesh condition index was used as an indication of the physiological status of the different mussel populations of this study. Cultured and transplanted mussel populations at Megara showed a higher condition index compared to indigenous and transplanted populations at all other stations. Generally, mussel populations showed decreased body mass in areas regarded as most contaminated and in areas where they are not naturally present. These findings may be related to stress responses due to increased contaminant levels and/or variations in environmental conditions such as food availability as also reported by NESTO et al., (2004) in mussels from the Venice Lagoon, Italy. In addition, differences in the reproductive status may have occurred among indigenous mussel populations while no significant differences in temperature were observed among stations within the same sampling season. Such variations in the physiological condition of mussels may have masked the net response to pollutant exposure.

Nevertheless, a significant correlation was found between AChE activity and MT content (r=-0.436, p<0.05), both parameters showed
a variation with respect to the season of sampling and during the second year of measurements a concomitant increase in MT content with a decrease in AChE activity was noted. Decreased MT content and higher AChE activities were recorded in spring although not consistently for both years at all stations as also reported by DOUMOUHTSIDOU et al., (2004) and BELIAEFF & BOCQUENÉ (2004). Seasonal variation in biomarkers can be attributed to a variety of abiotic and biotic factors such as temperature, food supply and reproductive status (BAUDRIMONT et al., 1997, SHEEHAN & POWER, 1999) that also affect the accumulation of contaminants (PHILLIPS, 1976). In the present study temperature differences between spring (16-17°C) and autumn (23-24°C) that can influence both MT and AChE (SERAFIM et al., 2002, BOCQUENÉ et al., 1993) may be among the factors related to the observed variation between seasons. On the other hand, the reason of variation in both biomarkers from one year to another is unclear and could not be attributed to differences in temperature, which was limited between years (0.5-1.0 °C) or variations in Cu, Cd and Zn levels in mussels. These temporal variations in biomarkers can be related to true variations in other contaminant levels or natural variability of biomarkers such as between-individual variations and seasonal changes related to physiological cycles of the organisms (BELIAEFF & BOCQUENÉ, 2004).

Although biomarker responses overall did not reflect the expected pollution gradient in the Saronikos Gulf our results are in accordance with those reported by BELIAEFF & BOCQUENÉ (2004) on biomarker responses in marine organisms from other Mediterranean areas. Results of the present study indicate that the applied biochemical markers in indigenous mussel populations do not reflect the type of pollution in the Saronikos Gulf to a degree that can be used for pollution monitoring in the area. However, further research is needed on the use of transplanted mussel populations for the application of MT in the area.

Acknowledgements

This work was funded by the UE project BEEP Biological Effects of Environmental Pollution in Marine Coastal Ecosystems Contract No EVK3-CT2000-00025. We are grateful to the anonymous referees for their helpful comments on this manuscript.

References

AMIARD-TRIQUET, C., ALTMAN, S., AMIARD, J. C., BALLAN DUFRANCAIS, C., BAUMARD, P., BUDZINSKI, H., CROUZET, C., GARRIGUES, P., HIS, E., JEANTET, A. Y., MENASRIA, R., MORA P., MOUNEYRAC, C., NARBONNE, J. F. & PAVILLON, J. F., 1998. Fate and effects of micropollutantes in the Gironde estuary, France: a multidisciplinary approach. Hydrobiologia, 373/374: 259-279.

BAUDRIMONT, M., LEMAIRE-GONY, S., RIBEYRE, F., METIVAUD, J. & BOUBOU, A., 1997. Seasonal variations of metallothionein concentrations in the asiatic clam (Corbicula fluminea). Comparative Biochemistry Physiology, 118C:361-367.

BEIRAS, R., BELLAS, J., FERNÁNDEZ, N., LORENZO, J. I. & COBELO-GARCÍA A., 2003. Assessment of coastal marine pollution in Galicia (NW Iberian Peninsula); metal concentrations in seawater, sediments and mussels (Mytilus galloprovincialis) versus embryo–larval bioassays using Paracentrotus lividus and Ciona intestinalis. Marine Environmental Research, 56: 531-553.

BELIAEFF, B. & BOCQUENÉG., 2004. Exploratory data analysis of the Mediterranean component of the BEEP programme. Marine Environmental Research, 58:239-244.

BESADA, V., FUMEGA, J. & VAAMONDE, A., 2002. Temporal trends of Cd, Cu, Hg, Pb and Zn in mussel (Mytilus galloprovincialis) from the Spanish

Medit. Mar. Sci., 5/1, 2004, 175-186
North-Atlantic coast 1991-1999. The Science of the Total Environment, 288: 239-253.

BOCQUENÉ, G. & GALGANI, F., 1992. Assessment of biological water quality using acetylcholinesterase (AChE) inhibition measurement. Report of the FAO/IOC/UNEP training workshop on the techniques for monitoring biological effects of pollutants in marine organisms, p205.

BOCQUENÉ, G., GALGANI, F., BURGEOT, T., LE DEAN, L. & TRUQUET, P., 1993. Acetylcholinesterase levels in marine organisms along French coasts. Marine Pollution Bulletin 26: 101-106.

BOCQUENÉ, G. & GALGANI F., 1991. L’acétylcholinesterase chez les organismes marins, outil de surveillance des effets des pesticides organophosphorés et carbamates. Océanis 17: 439-448.

Bolognesi, C., FenziLLI, G., Lasagna, C., Perrone, E. & Roggeri, P., 2004. Genotoxicity biomarkers in Mytilus galloprovincialis: wild versus caged mussels. Mutation Research, 552:153-162.

Bradford, M., 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Analytical Biochemistry 72: 248-264.

Cajaraville, M.P., Bebbiano, M. J., Blasco, J., Porte, C., Sarasquete, C. & ViarengO, A., 2000. The use of biomarkers to assess the impact of pollution in coastal environments of the Iberian Peninsula: A practical approach. The Science of the Total Environment 247: 295-311.

Cotou, E., Vagia, C., Rapti, Th. & Roussis, V., 2001. Metallothionein levels in Callista chione and Venus verrucosa from two areas of Eastern Mediterranean (Greece). Z. Naturforsch 56c: 848-852.

Cotou, E., Papathanassiou, E. & Tsangaris, C., 2002. Assessing the quality of marine coastal environments: comparison of scope for growth and Microtox® bioassay results of pollution gradient areas in eastern Mediterranean (Greece). Environmental Pollution 119: 141-149.

Dailianis, S., Doumouhtsidou, G.P., Raftopoulou, E., Kaloyanni, M. & Dimitriadis, V.K., 2003. Evaluation of the neutral red retention assay, micronucleus test, acetylcholinesterase activity and a signal transduction molecule (camp) in tissues of Mytilus galloprovincialis (L.), in pollution monitoring. Marine Environmental Research 56: 443-470.

Doumouhtsidou, G. P., Dailianis, S., Kaloyanni, M. & Dimitriadis, V. K., 2004. Lysosomal membrane stability and metallothionein content in <ytilus galloprovincialis (L.), as biomarkers Combination with trace metal concentrations. Marine Pollution Bulletin, 48:572-586.

Doumouhtsidou, G. P. & Dimitriadis, V. K., 2001. Lysosomal and lipid alterations in the digestive gland of mussels Mytilus galloprovincialis (L.) as biomarkers of environmental stress. Environmental Pollution 115: 123-137.

Ellman, L.G., Courtney, K. D., Andres, V. Jr. & FeaTherstone, R. M., 1961. A new and rapid colorimetric determination of acetylcholinesterase activity. Biochemical Pharmacology 7: 88-95.

Escartín, E & Porte, C., 1997. The use of cholinesterase and carboxylesterase activities from Mytilus galloprovincialis in pollution monitoring. Environmental Toxicology and Chemistry 16: 2090-2095.

Galloway, T.S., Sanger, R.C., Smith, K. L., Fillmann, G., Readman, J. W., Ford T. E. & Depledge, M. H., 2002. Rapid assessment of marine pollution using multiple biomarkers and chemical immunoassays. Environmental Science and Technology, 36: 2219-2226.

Georgakopoulou-Gregoriadou, E., Voutsinou-Taliadouri, F., Gotsis-Skretas, O. & Psyllidou-Giouranovits, R., 1997. Water quality assessment through organochlorine and biological analyses in a semi-enclosed embayment (Pagassitikos gulf, Hellas). Fresenius Environmental Bulletin 61:54-159.

Gerét, F., Jouan, A., Turpin, V., Bebbiano, M. J. & Cosson, R. P., 2002. Influence of metal exposure on metallothionein synthesis and lipid peroxidation in two bivalve mollusks: the oyster (Crassostrea gigas) and the mussel (Mytilus edulis). Aquatic Living Resources 15: 61-66.

Gulhermino, L, Barros, P., Silva, M. C. & Soares, A. M. V. M., 1998. Should the use of inhibition of cholinesterases as a specific biomarker for organophosphate and carbamate pesticides be questioned? Biomarkers 3: 157-163.
HAMZA-CHAFFAI, A., ROMEO, M., GNASSIA-BARELLI, M. & EL ABED, A., 1998. Effect of copper and lindane on some biomarkers measured in the clam Ruditapes decussates. Bulletin of Environmental Contamination and Toxicology 61: 397-404.

HCMR, 2003. Monitoring of the effects of pollution along the Saronikos Gulf. Final Report, Athens.

KALPAXIS, D. L. & AMARANTOS I., 2003. Regulation of translation initiation in mussels (Mytilus galloprovincialis, Lam.) following contamination stress. Journal of Toxicology and Environmental Health, Part A, 66:481-494.

KALPAXIS, D. L., THEOS, C., XAPLANTERI, M. A., DONOS., G. P., CATSKI, V. A. & LEOTSINIDIS, M., 2004. Biomonitoring of Gulf of Patras, N. Peloponnesus, Greece. Application of a biomarker suite including evaluation of translation efficiency in Mytilus galloprovincialis cells. Environmental Research 94:211-220.

KARAGEORGIS, A. P. & HATZIANESTIS, I., 2003. Surface sediment chemistry in the Olympic Games 2004 Sailing Center (Saronikos Gulf). Mediterranean Marine Science Vol. 4/1: 5-22.

LIVINGSTONE, D. R., LEMAIRE, P., MATTHEWS, A., PETERS, L. D., PORTE, C., FITZPATRICK, P. J., FORLIN, L., NASCI, C., FOSSATO, V., WOOTON, N. & GOLDFARB, P., 1995. Assessment of the impact of organic pollutants on Goby (Zosterisessor ophiocephalus) and mussel (Mytilusgalloprovincialis) from the Lagoon of Venice, Italy. Marine Environmental Research 39: 235-240.

LOBEL, P.B. & WRIGHT, M.P., 1982. Relationship between body zinc concentration and allometric growth measurements in the mussel Mytilus edulis. Marine Biology 66, 145-150.

NAJIMI, S., BOUHAIM, A., DAUBEZE, M., ZEKHINI, A., PELLERIN, J., NARBONNE, J. F. & MOUKRIM, A. 1997. Use of Acetylcholinesterase in Perna perna and Mytilus galloprovincialis as a biomarker of pollution in Agadir Marine Bay (South of Morocco).Bulletin of Environmental Contamination and Toxicology 58: 901-908.

NARBONNE, J. F., GARRIGUES, P., GALGANI, F. & LAFAURIE, M., 1993. The application of biochemical markers in field evaluation: A comparative study in marine organisms collected on the north coast of the Mediterranean Sea. Marine Environmental Research 35: 229-230.

NCMR, 1997a. Pollution research and monitoring programme in the Saronikos Gulf: Technical Report, Athens, p146.

NCMR, 1997b. Short and long-term effects of oil spillage in the Western Saronikos Gulf ecosystem. Final Report, May 1997, Athens, Hatzianestis I. (Ed).

NCMR, 2001. Pollution research and monitoring programme in the Aegean and Ionian Seas: Technical Report, Athens, p57.

NCMR, 2002. Pollution research and monitoring programme in Rhodes island. MED POL Programme: Technical report, p37.

NESTO, N., BERTOLDO, M., NASCI, C. & DA, ROS, L., 2004. Spatial and temporal variation of biomarkers in mussels (Mytilusgalloprovincialis) from the Lagoon of Venice, Italy. Marine Environmental Research, 58:287-291.

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. Marine Pollution Bulletin 32: 225-231.

PETROVIC, S., OZRETIC, B., KRAJNOVIC-OZRETIC, M., & BOBINAC, D., 2001. Lysosomal membrane stability and metallothioneins in digestive gland of mussels (Mytilusgalloprovincialis Lam.) as biomarkers in a field study. Marine Pollution Bulletin 42: 1373-1378.

PHILLIPS, D.J.H., 1976. The common mussel Mytilus edulis as an indicator of pollution by Zinc, Cadmium, Lead and Copper. I Effects of environmental variables on uptake of metals. Marine Biology. 38: 59-69.

ROMEO, M., HOARAU, P., GARELLO, G., GNASSIA-BARELLI, M. & GIRARD, J.P., 2003. Mussel transplantation and biomarkers as useful tools for assessing water quality in the NW Mediterranean. Environmental Pollution, 122: 369-378.

SERAFAIM, M. A., COMPANY, R. M., BEBIANNO, M.J. & LANGSTON, W. J., 2002. Effect of temperature and size on metallothionein synthesis in the gill of Mytilusgalloprovincialis exposed to cadmium. Marine Environmental Research 54: 361-365.
SKLIVAGOU, E., VARNAVAS, S. P. & HATZIANESTIS, I., 2001. Aliphatic and Polycyclic Aromatic Hydrocarbons in Surface Sediments from the Elefsis Bay, Greece (Eastern Mediterranean). *Toxicological and Environmental Chemistry*, 79 (3/4):195-210.

SHEEHAN, D. & POWER, A., 1999. Effects of seasonality on xenobiotic and antioxidant defense mechanisms of bivalve molluscs. *Comparative Biochemistry Physiology*, 123C: 193-199.

STAGG, R. M., 1998. The development of an international programme for monitoring the biological effects of contaminants in the OSPAR convention area. *Marine Environmental Research* 46: 307-313.

STATHOPOULOU, E., DASSENAKIS, M. & SKOULLOS, M., 2001. Levels of mercury concentration in sediments of the Saronikos Gulf. Proceedings 7th International Conference on Environmental Science and Technology, 3-6 September, Syros, Greece, p.854-860.

STIEN, X., PERCIC, P., GNASSIA-BARELLI, M., ROMEO, M., & LAFaurie M., 1998. Evaluation of biomarkers in caged fishes and mussels to assess the quality of waters in a bay of the NW Mediterranean Sea. *Environmental Pollution* 99: 339-345.

TSANGARIS, C., COTOU, E. & PAPATHANASSIOU, E., 2002. Multiple biomarker assessment for marine pollution: A case study to distinguish the type of pollutants in Amvrakikos Gulf (Greece). *Marine Environmental Research* 54: p845.

TSELENTIS, B. S., MAROULAKOU, M., LASCOURREGES, J. F., SZPUNAR, J., SMITH, V. & DONARD O. F. X., 1999. Organotins in sediments and tissues from Greek coastal areas: Preliminary results. *Marine Pollution Bulletin* 38:146-153.

VIARENGO, A., PONZANO, E., DONDERO, F. & FABBRI R., 1997. A Simple Spectrophotometric Method for Metallothionein Evaluation in Marine Organisms: Application to Mediterranean and Antarctic Molluscs. *Marine Environmental Research*, 44: 69-84.

VIARENGO, A., BURLANDO, B., DONDERO, F., MARRO, A. & FABBRI, R., 1999. Metallothionein as a tool in biomonitoring programmes. *Biomarkers* 4:455-466.

VOUTSINOU-TALIADOURI, F., SATSIMATZIDIS, J. & IATRIDIS B. 1989. Impact of Athens sewage and industrial discharges on the metal content of sediments from Piraeus harbour and Eleusis Bay. *Rev. Int. Oceanogr. Méd.*, 93-94:31-45.