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CYP1A and metallothionein expression in the hepatopancreas of *Merluccius merluccius* and *Mullus barbatus* from the Adriatic Sea

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Abstract: The enzyme CYP1A is an established biomarker of fish exposure to polycyclic aromatic hydrocarbons (PAHs) and polychlorinated biphenyls (PCBs). The metallothioneins (MT), a family of Cys-rich proteins, bind a wide range of metals and participate in their metabolism. The aim of the study was to examine the correlation between CYP1A and MT expression in commercially important fish species *Mullus barbatus* and *Merluccius merluccius* and contaminants (PAHs, PCBs, toxic metals) in seawater and sediment from three localities with different level of contamination in the Adriatic Sea in winter, *i.e.*, Platamuni, Valdanos and the port of Bar. The relative concentration of CYP1A was the highest in both fish species from Bar. Increased concentrations of PCBs in the seawater were observed only in Bar. A species-specific higher increase in the protein concentration of CYP1A was observed in *Mullus barbatus* compared to *Merluccius merluccius*. The levels of MT were the highest in *Merluccius merluccius* from Bar and in *Mullus barbatus* from Valdanos. The induction of MT correlated with the elevated concentrations of Cu and Pb determined by chemical analysis of the seawater from Bar and Valdanos, respectively. According to the chemical analysis of the seawater and the biological response of the fish, the Platamuni locality exhibited the lowest level of contamination.

Keywords: CYP1A; metallothionein; polycyclic aromatic hydrocarbons; polychlorinated biphenyls; metals; fish.

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INTRODUCTION

Biochemical markers or biomarkers represent early warning signals that reflect on adverse biological responses to anthropogenic environmental toxins.[1] In order to assess the exposure to or the effects of environmental pollutants on aquatic ecosystems, different biomarkers are examined, *i.e*. the biotransformation enzymes (phases I and II), oxidative stress parameters, biotransformation products, stress proteins, metallothioneins, immunological, reproductive and endocrine parameters, genotoxic and physiological, histological and morphological parameters.[1]

The phase I biotransformation enzymes, notably CYP1A, belong to a group of very sensitive biomarkers in fish. They are responsible for the biotransformation of xenobiotic compounds, such as polycyclic aromatic hydrocarbons (PAHs) and polychlorinated biphenyls (PCBs).[2–4] The induction of CYP1A occurs through ligand binding of PAHs and PCBs to a cytoplasmic receptor, known as the hydrocarbon receptor. Upon heterodimerization with the aryl hydrocarbon nuclear translocator, the complex translocates to the nucleus. This is followed by its specific binding to the xenobiotic-response element on the DNA upstream from the CYP1A gene promoter and up regulation of gene transcription, which leads to elevated mRNA and protein levels, and increased CYP1A catalytic activity.[5]

Metallothioneins (MTs) are a special group of stress proteins which are inducible by both essential and toxic metals. The MTs constitute a family of low-molecular-weight, cysteine-rich proteins that regulate the essential metals Cu and Zn.[6] For nonessential metals, MT assumes a sequestering function that protects against metal toxicity.[7] Induction of MT following exposure to toxic metals can serve as a defense mechanism and a biomarker of environmental exposure to chemical stressors, such as toxic metals. Another hallmark of MTs is their induction by multiple toxic metal species at the transcriptional level. Studies of the regulation of MT gene expression revealed that the induction by metals is a direct response to increases in the intracellular metal concentrations and is mediated through the action of metal-binding regulatory factors.[8]

The aim of the present study was to examine the correlation between the expression of biomarkers (CYP1A and MT) in fish and chemical contaminants (PAHs, PCBs and toxic metals) in seawater and sediment. Relative changes of CYP1A and MT concentrations in the hepatopancreas of Red mullet *Mullus barbatus* and European hake *Merluccius merluccius* were examined. The fish were caught in three different types of localities in the Adriatic Sea in winter: Platamuni, an open sea locality; Valdanos, a locality of low urban and industrial influence, and the port of Bar, a locality of intense industrial and anthropogenic activity. Both fish species are of considerable commercial importance.

EXPERIMENTAL

Animals

Specimens of *Mullus barbatus* and *Merluccius merluccius* were collected by trawling at Platamuni, Valdanos and the port of Bar (Fig. 1). The investigations took place in winter (25th February, 2009). At least seven (and up to nine) individual fish of one species were pooled. The fish were killed immediately by spinosectomy according to standard animal care regulations. The hepatopancreas was quickly removed, washed in ice-cold 0.15 M NaCl and frozen in liquid nitrogen. Individuals of the same size were selected to ensure uniformity of samples.

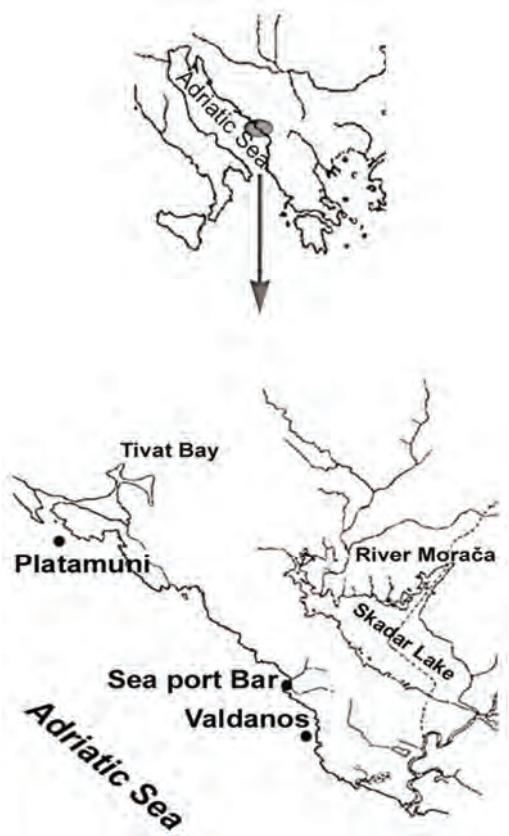

Fig. 1. A map of the Adriatic coast of Montenegro with the locations where the sea water samples were collected and the fish caught (Platamuni, Valdanos, port Bar).

Determination of PAHs and PCBs

The sampling of water and sediments was performed after trawling at the same place as where the fish were caught. PCBs from filtered seawater (1 L) were extracted using a Bakerbond spe™ C18, 6 ml, 500 mg column (JT Baker products).[9] Prior to extraction, the column was washed with ethyl acetate, dried under vacuum for 30 s and conditioned with 2×5 ml of methanol, followed by 2×5 mL of water. Samples were added to the column and drawn

through the column at 10 mL min^{-1}. After washing with water, the column was dried under vacuum for 30 min. Compounds from the column was eluted using 2×2.5 mL of ethyl acetate at a flow rate of 2 mL min^{-1}, followed by 2×2.5 mL of dichloromethane, before being concentrated to 1 mL at room temperature by evaporation under a gentle stream of nitrogen gas. PCBs from sediments were extracted using a Soxhlet apparatus employing an acetone/hexane mixture (51:49, v/v) for 8 h.[10] The dried and evaporated extract was transferred to a silica gel column (10 g, 100–200 mesh activated at 130 °C for 18 h before use), eluted using hexane and concentrated to 1 mL at room temperature by evaporation under a gentle stream of nitrogen gas.

The PCB concentrations were determined by gas chromatography (GC) with an ECD detector and linear programmable temperature vaporizer (PTV) injector.[11,12] A capillary column BPX5 30 m×0.25 mm×0.25 μm was used for all the determinations. The operating conditions were: detector temperature 310 °C, column temperature programmed to start at 80 °C for 2 min, first ramp 30 °C min^{-1} to 150 °C, second ramp 5 °C min^{-1} to 280 °C and holding for the next 5 min. The concentrations of PCBs in the samples were calculated using araclor standards. The absence of an individual peak is not reported as zero but as less than the detection limit.

The PAHs from filtered seawater (1 L) were extracted using a Bakerbond spe™ PAH Aqua, 6 mL column (JT Baker products).[13] The column was conditioned with cyclohexane and dichloromethane, dried for 15 s after each addition and again conditioned using a water/2--propanol mixture (92:8). The sample (1 L with the addition of 100 mL of 2-propanol) was applied to the column at 5 mL min^{-1} and dried using vacuum for 10 min. The PAHs were eluted using 2×3 mL and 1×2 mL of dichloromethane and then concentrated to 1 mL at room temperature by evaporation under a gentle stream of nitrogen gas.

The PAH concentrations were determined by GC with a FID detector and a linear PTV injector.[14] A capillary column DB5-MS 30 m×0.25 mm×0.25 μm was used for all the determinations. The operating conditions were: detector temperature 310 °C, column temperature programmed to start at 40 °C for 1 min, first ramp 15 °C min^{-1} to 150 °C, second ramp 5 °C min^{-1} to 290 °C and holding for the next 11 min. The concentrations of PAHs in the samples were calculated using PAH mix 13 (Supelco) standards. The absence of individual peaks is not reported as zero but as less than the detection limit.

Determination of metals

Water samples were taken in polyethylene bottles of 1.5 L volume, preserved with 5 cm^3 concentrated nitric acid during the sampling. After transport, the samples were kept at room temperature until analysis. Before analysis of the investigated toxic metals, to enable its determination, their concentration in samples were increased. For metal content determination in samples of seawater from the Montenegrin coast, the extraction method by ammonium pyrrolidine dithiocarbamate/methyl isobutyl ketone (APDC/MIBK) was used, *i.e.*, method of separation of complex metals with the organic solvent MIBK, previously examined in 1 % solution of APDC, at pH 3 and analyzed with atomic absorption spectrometry (AAS).[15,16]

Isolation of the hepatopanceas fractions

The microsomal fraction of the hepatopancreas was prepared following the procedure of Krauss *et al.*[17] The tissues were excised and homogenized (1 g liver mL^{-1}) in STM buffer: 0.25 M sucrose, 50 mM Tris-HCl, pH 7.4, 4 mM MgCl2, 1 mM PMSF) and centrifuged at 10000 x g at 4 °C for 25 min. The obtained post mitochondrial supernatant was then centrifuged at 150000 x g, 4 °C for 60 min. The obtained microsomal pellets were resuspended in STM buffer and used for analysis of CYP1A, while the supernatant was used for analysis of MT.

SDS-polyacrylamide gel electrophoresis and immunoblot analysis

For the SDS-polyacrylamide gel electrophoresis (SDS-PAGE), 20 μg of microsomal proteins were loaded onto 4 % stacking/12 % separating slab gels as described by Laemmli.[18] The gels were stained using Coomassie Brilliant Blue R-250. The proteins separated by SDS--PAGE were electroblotted onto PVDF membranes (Hybond-P, Amersham Pharmacia Biotech). Immunoblot analysis was performed according to Towbin *et al.*[19] using polyclonal antibodies to fish CYP1A and MT (CP226 and KH-1, respectively; Biosense Laboratories, Norway). The immunoreactive bands were identified by an enhanced chemiluminescence (ECL) detection system (Santa Cruz Biotechnology) according to the manufacturer's instructions. Protein concentrations were determined according to Lowry *et al.*[20] The bands were visualized and quantified with TotalLab (Phoretix) electrophoresis software (version 1.10).

RESULTS AND DISCUSSION

The effects of exposure to PAHs and PCBs on CYP1A expression

Chemical analysis of the environment and animal tissues provides information concerning the presence of specific xenobiotic compounds. However, this data on its own is not particularly indicative of the concentrations to which the animals were exposed and cannot serve as bioaccumulation markers for exposure assessment.[2] In addition, the application of biomarkers to complement traditional chemical methods of detecting pollution can reveal the presence of contaminants that were not initially suspected. CYP1A is an established biomarker of exposure to PAHs and PCBs.[2] In order to detect CYP1A, equal quantities of microsomal proteins were separated by SDS-PAGE and subjected to immunoblot analysis using polyclonal antibody to CYP1A protein (Fig. 2). CYP1A was detected in both fish species at all examined localities. The highest degree of induction was observed in both fish species that were caught in the port Bar (Fig. 2 and Table I). The results presented in Fig. 2 indicate a species-specific response of CYP1A. They show that the protein concentration of CYP1A was about 5-fold higher in the hepatopancreas of *Mullus barbatus* than in *Merluccius merluccius* (Table I).

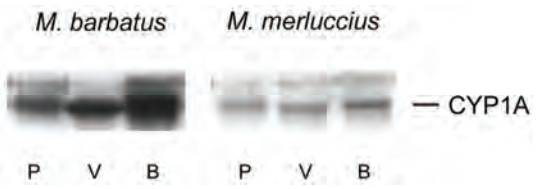

Fig. 2. Immunoblot analysis with anti--CYP1A antibody. P – Platamuni; V – Valdanos; B – Bar. Twenty μg of proteins were subjected to 12 % SDS--PAGE, electroblotted to membranes and immunoblotting was performed with a polyclonal antibody for CYP1A. The antigen–antibody complex formation was detected using an ECL detection system.

Virgin *et al*. showed that there are large differences in CYPlA mRNA induction between species.[21] Thus, the Atlantic tomcod exhibited significant induction of CYPlA mRNA after exposure to chemicals (97-fold) and changes in the en-

vironment (34-fold), whereas in the smooth flounder, a considerably lower level of CYP1A induction was observed in response to chemical exposure (14-fold) but no change in expression after exposure to adjustments in the environment.[21] In hog chokers and striped bass, very low levels of CYP1A gene induction were detected. The degree of CYPlA mRNA inductibility above the basal level differs significantly among fish taxa, most likely as a result of differences in the regulation of gene expression. Based on their findings, the authors concluded that careful selection of sentinel species should be exercised prior to the use of CYPlA mRNA induction data in environmental monitoring programs.

TABLE I. Quantification by immunoblot analysis with anti-CYP1A antibody. Antigen–antibody complexes (changes of the relative concentrations of CYP1A), were analyzed by densitometry using Total Lab (Phoretix) electrophoresis software

Location	Fish	
	Mullus barbatus	*Merluccius merlucius*
Platamuni	446.3±15.1	88.2±2.8
Valdanos	603.7±21.5	117.1±4.2
Bar	824.1±33.4	139.2±4.9

Due to their mutagenic and carcinogenic properties, measurement of PAH concentrations are included in most monitoring programs.[22] PCBs are produced for diverse industrial applications and although their use has been banned since the 1970s, due to their resistance to breakdown and tendency for bioaccumulation, PCBs continue to cycle through the environment.[1]

Analysis of the seawater did not reveal the presence of PAHs (Table II). The limit of detection of PAHs using a GC column is 10 ng L^{-1} and the presence of PAHs at lower concentrations cannot be excluded. PCBs were observed in the seawater only in Bar, where the concentrations of PCB-28, PCB-52 and PCB-153 were 20, 15 and 15 ng L^{-1}, respectively (Table III). According to Nagpal,[23] the recommended maximal concentration of total PCB is 0.1 ng L^{-1}. Thus, the concentrations of PCBs measured in the port of Bar could be taken as representing contamination of the seawater. Since the minimal concentration of PCBs that exerts a negative impact on marine organisms, as reported in the Environmental Quality Standards for the Mediterranean Sea in Israel,[24] is 42 ng L^{-1}, the concentrations detected in Bar are at an acceptable level. The presence of PCBs in seawater is in correlation with the highest level of CYP1A induction in the hepatopancreas from *Mullus barbatus* and *Merluccius merluccius* in Bar. As the limit of detection of PCBs using a GC column is 10 ng l^{-1} (which is above the recommended maximum concentration described by Nagpa[l23]), the presence of other PCBs at other localities cannot be ruled out. The concentrations of PCBs in sediments are presented in Table III. The results revealed the presence of pcb101 in Valdanos (2.31 ng g^{-1}) and in samples PCB-101, PCB-138, PCB-153 and PCB-180

at Bar at concentrations 2.85, 3.05, 3.55 and 3.65 ng g^{-1}, respectively. Considering that the recommended maximum concentration of total PCBs is 2 μg per 100 g in sediments (Nagpal[23]), the detected PCBs in the sediment are under the recommended maximum. However, the presence of PCBs in sediment could induce the sensitive biomarker CYP1A in the hepatopancreas.

TABLE II. Concentrations of PAHs (ng L^{-1}) in seawater collected from Platamuni, Valdanos and the port of Bar

Compound	Platamuni	Valdanos	Bar
Acenaphthylene	<10	<10	<10
Fluorene	<10	<10	<10
Phenantrene	<10	<10	<10
Anthracene	<10	<10	<10
Pyrene	<10	<10	<10
Benz(A)anthracene	<10	<10	<10
Chrysene	<10	<10	<10
Benzo(B)fluoranthene	<10	<10	<10
Benzo(K)fluoranthene	<10	<10	<10
Benzo(A)pyrene	<10	<10	<10
Benzoperylene	<10	<10	<10
Indeno(1.2.3.cd)pyrene	<10	<10	<10
Dibenzo(A)anthracene	<10	<10	<10

Table III. Concentrations of PCBs in seawater and sediment collected from Platamuni, Valdanos and the port of Bar

Compound	Location		
	Platamuni	Valdanos	Bar
PCBs in seawater, ng L^{-1}			
2,4,4'-trichlorobiphenyl (PCB-28)	<10	<10	20
2,2',5,5'-tetrachlorobiphenyl (PCB-52)	<10	<10	15
2,2',4,5,5'-pentachlorobiphenyl (PCB-101)	<10	<10	<10
2,2',3,4,4',5'-heksachlorobiphenyl (PCB-138)	<10	<10	<10
2,2',4,4',5,5'-heksachlorobiphenyl (PCB-153)	<10	<10	15
2,2',3,4,4',5,5'-heptachlorobiphenyl (PCB-180)	<10	<10	<10
PCBs in sediment, ng/g			
2,4,4'-trichlorobiphenyl (PCB-28)	<10	<10	<10
2,2',5,5'-tetrachlorobiphenyl (PCB-52)	<10	<10	<10
2,2',4,5,5'-pentachlorobiphenyl (PCB-101)	<10	2.31	2.85
2,2',3,4,4',5'-heksachlorobiphenyl (PCB-138)	<10	<10	3.05
2,2',4,4',5,5'-heksachlorobiphenyl (PCB-153)	<10	<10	3.55
2,2',3,4,4',5,5'-heptachlorobiphenyl (PCB-180)	<10	<10	3.65

The effect of exposure to toxic metals on MT expression

Considering that MTs play a role in the metabolism of essential metals, they possess basal levels of expression. The occurrence of higher doses of both essen-

tial and toxic metals provokes the induction of MTs.[6,7] The same quantity of the supernatant fractions were separated by SDS-PAGE and subjected to immunoblot analysis using polyclonal antibodies to MT (Fig. 3 and Table IV). These experiments revealed that MT was present in all samples; however, MT induction was observed in *Merluccius merluccius* from Bar (Fig. 3) and in *Mullus barbatus* from Valdanos (Fig. 3).

Fig. 3. Immunoblot analysis with anti-metallothionein antibody. P – Platamuni; V – Valdanos; B – Bar. Twenty μg of proteins were subjected to 12 % SDS-PAGE, electroblotted to membranes and immunoblotting was performed with a polyclonal antibody for metallothionein. The antigen–antibody complex formation was detected using an ECL detection system.

TABLE IV. Quantification of immunoblot analysis with anti-metallothionein antibody. Antigen–antibody complexes (changes of the relative concentrations of anti-metallothionein), were analyzed by densitometry using Total Lab (Phoretix) electrophoresis software

Location	Fish	
	Mullus barbatus	*Merluccius merlucius*
Platamuni	16.5±0.6	89.7±2.5
Valdanos	40.9±1.3	107.4±3.5
Bar	26.9±0.9	132.1±4.1

Analyses of metals in seawater obtained from the localities of Platamuni, Valdanos and the port in Bar during winter are shown in Table V. In view of the acceptable average concentrations according to the Environmental Quality Standards for the Mediterranean Sea in Israel[24] for the examined metals, it can be concluded that the concentration of Pb in Valdanos and of Cu in Bar were significantly increased. Namely, the recommended average concentration for both Pb and Cu is 0.005 mg L^{-1}. The measured concentrations for Pb (Valdanos) and Cu (Bar) were 0.022 and 0.009 mg L^{-1}, respectively. The minimal concentrations of Pb and Cu that have a negative impact on marine organisms are 0.005 and 0.003 mg L^{-1}, respectively.

The observed presence of Cu and Pb in seawater is in correlation with MT induction in the hepatopanceas of *Merluccius merluccius* from Bar and in *Mullus barbatus* from Valdanos. In contrast to the species-specific induction of CYP1A, 3–5-fold higher MT protein concentrations were observed in the hepatopancreas of *Merluccius merluccius* compared to *Mullus barbatus* (Fig. 3 and Table IV). Species-specific MT induction in fish was reported by Lam *et al.*[25] When carp was exposed to sub-lethal doses of Cu, Zn and Cd, MT mRNA in the gill and

liver was not induced while exposure of tilapia caused a significant induction of MT mRNA in the same tissues.[25] These results imply that the MT level in carp is not a reliable biomarker for monitoring metal pollution; the MT level in tilapia is a better biomarker. In view of these results, it can be concluded that MT induction in the hepatopancreas of *Meluccius merluccius* is a more reliable biomarker than in *Mullus barbatus*.

TABLE V. Concentrations of metals (mg L^{-1}) in seawater collected from Platamuni, Valdanos and the port of Bar

Location	Depth, m	Pb	Zn	Cd	Cu	Co	Ni
Platamuni	0	0.0	0.0	0.0	0.0	0.0	0.0
Platamuni	80 (bottom)	0.0	0.014	0.0	0.001	0.0	0.0
Valdanos	0	0.002	0.017	0.00012	0.005	0.0	0.0
Valdanos	20 (bottom)	0.022	0.009	0.0	0.0	0.0	0.0
Bar	0	0.0	0.0	0.0	0.0	0.0	0.0
Bar	10 (bottom)	0.003	0.010	0.00008	0.009	0.0	0.0

Whereas heavy pollution causes the sudden death of large numbers of fish, exposure to sub-lethal levels of pollutants has to be estimated by measurements of specific biochemical, physiological or histological responses of fish.[26] Together with chemical analysis, biomarker responses represent an additional approach in the study of the biological impact of environmental contaminants. The observed changes at the molecular level show that the pollutants had entered the organisms, been distributed between the tissues and elicited toxic effects at critical targets. This biochemical response is only the first signal of exposure to contaminants and is usually reversible, contrary to the changes manifested at higher levels of organization of an organism, the population, community and ecosystem.[1]

CONCLUSION

Increased expression of CYP1A and metallothioneins, biomarkers of fish exposure to PAHs/PCBs and toxic metals, respectively, correlated with increased levels of environmental contaminants observed by chemical analysis in Valdanos and Bar in winter. Fish from the Platamuni locality, an open sea area with very low anthropogenic and industrial influences, exhibited the lowest level of contamination and displayed significantly lower levels of biomarker responses.

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ИЗВОД

ЕКСПРЕСИЈА CYP1A И МЕТАЛОТИОНЕИНА У ХЕПАТОПАНКРЕАСУ ОСЛИЋА И ТРЉЕ ИЗ ЈАДРАНСКОГ МОРА

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CYP1A представља добро окарактерисан биомаркер код риба при излагању полицикличним ароматичним угљоводоницима (ПАХ) и полицикличним бифехолима (ПЦБ). Металотионеини (МТ) претстављају фамилију протеина који везују метале и учествују у њиховој метаболизму, транспорту и регулацији. Циљ овог рада је био да се испитају корелације између промена у нивоу CYP1A и МТ у хепатопанкреасу две комерцијално важне рибе: *Mullus barbatus* (трља) и *Merluccius merluccius* (ослић) и контаминанатата: ПАХ, ПЦБ и токсичних метала) на три локалитета у Јадрнском мору (Платамуни, Валданос и лука Бар) у зиму. CYP1A је у највећем степену индукован на локалитету Бар у обе испитиване врсте, што је у корелацији са присуством повећане количине ПЦБ у морској води у Бару. Ниво МТ је највећи код ослића изловљеног у Бару, а код трље у Валданосу. То је у корелацији са измереним повећаним концентрацијама бакра у Бару, а олова у Валданосу. На основу изучаваних параметара, Платамуни су локалитет са најмањим степеном контаминације.

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REFERENCES

1. R. Van der Oost, J. Beyer, N. P. E. Vermeuulen, *Environ. Toxicol. Pharmacol*. **13** (2003) 57
2. R. Van der Oost, E. Vindimian, P. J. V. D. Brink, K. Stumalay, M. Heida, N. P. E. Vermeulen, *Aquat. Toxicol*. **39** (1997) 453
3. M. Mihailović, J. Arambašić, D. Bogojević, S. Dinić, N. Grdović, I. Grigorov, S. Ivanović-Matić, S. Labus-Blagojević, V. Martinović, M. Petrović, A. Uskoković, M. Vidaković, G. Poznanović, *Arch. Biol. Sci*. **58** (2006) 165
4. M. Mihailović, D. Bogojević, S. Dinić, N. Grdović, I. Grigorov, S. Ivanović-Matić, S. Labus-Blagojević, V. Martinović, M. Petrović, A. Uskoković, M. Vidaković, G. Poznanović, *Bull. Environ. Contam. Toxicol.* **77** (2006) 559
5. T. D. Bucheli, K. Fent, *Crit. Rev. Environ. Sci. Technol.* **25** (1995) 201
6. G. A. Bonwick, P. R. Fielden, D. H. Davies, *Comp. Biochem. Physiol*. **99C** (1991) 119
7. N. Muto, H. W. Ren, G. S. Hwang, S. Tominaga, N. Itoh, K. Tanaka, *Comp. Biochem. Physiol*. **122** (1999) 75
8. G. M. Dethloff, H. C. Bailey, K. J. Maier, *Arch. Environ. Contam. Toxicol*. **40** (2001) 371
9. J. Dachs, J. M. Bayona, *Chemosphere* **35** (1997) 1669
10. *EPA Method 3540C: Soxhlet extraction*, 1996, http://www.epa.gov/osw/hazard/testmethods/sw846/pdfs/3540c.pdf (last accessed: August, 2010)
11. N. Kannan, G. Petrick, R. Bruhn, D. E. Schulz-Bull, *Chemosphere* **37** (1998) 2385
12. *EPA Method 8082: Polychlorinated biphenyls (PCBs) by gas chromatography*, 1996, http://www.epa.gov/osw/hazard/testmethods/sw846/pdfs/8082a.pdf (last accessed: August, 2010)
13. P. W. Crozier, J. B. PlomLey, L. Matchuk, *Analyst* **126** (2001) 1974

14. H. B. Lee, G. Dookhran, A. S. Y. Chau, *Analyst* **112** (1987) 31
15. L. Rasmussen, *Anal. Chim. Acta* **125** (1981) 117
16. R. E. Sturgeon, S. S. Berman, J. A. H. Desaulniers, A. P. Mykytluk, J. W. McLaren, D. S. Rusel, *Anal. Chem.* **52** (1980) 15
17. G. J. Krauss, K. Grancharov, D. Genchev, R. Walther, N. Spassovska, G. Karamanov, H. Reinbothe, E. Golovinsky, *Biomed. Biochim. Acta* **42** (1983) 1045
18. U. K. Laemmli, *Nature* **227** (1970) 680
19. H. Towbin, T. Staehelin, J. Gordon, *Proc. Natl. Acad. Sci. USA* **76** (1979) 4350
20. O. H. Lowry, W. J. Rosebrough, A. L. Farr, R. J. Randall, *J. Biol. Chem.* **193** (1951) 265
21. I. Virgin, B. Konkle, M. Pedersen, C. Grumwald, J. Williams, S. C. Courtenay, *Estuaries* **19** (1996) 216
22. J. P. Meador, J. E. Stein, W. L. Reichert, U. Varanasi, *Rev. Environ. Contam. Toxicol.* **143** (1995) 79
23. N. K. Nagpal, *Water Quality Criteria for Polychlorinated Biphenyls*, Water Quality Branch, Water Management Division, Ministry of Environment, Lands, and Parks, Victoria, BC, 1992, http://www.env.gov.bc.ca/wat/wq/BCguidelines/approv_wq_guide/ /approved.html#toc (last accessed: August, 2010)
24. *Environmental Quality Standards for the Mediterranean Sea in Israel*, Marine and Coastal Environment Division, Ministry of the Environment, 2002, http:// //www.sviva.gov.il/ Enviroment/Static/Binaries/index_pirsumim/p0124_eng_1.pdf (last accessed: August, 2010)
25. K. L. Lam, P. W. Ko, J. K.-Y. Wong, K. M. Ghan, *Marine Environ. Res.* **46** (1998) 563
26. J. A. Mondon, S. Duda, B. F. Nowak, *Aquat. Toxicol.* **54** (2001) 231.