Proteome modifications of blue mussel (Mytilus edulis L.) gills as an effect of water pollution

Hélène Manduzio, Pascal Cosette, Linda Gricourt, Thierry Jouenne, Christof Lenz, Odd-Ketil Andersen, Francois Leboulenger, Béatrice Rocher

SHORT COMMUNICATION

Proteome modifications of blue mussel (*Mytilus edulis* L.) gills as an effect of water pollution

Hélène Manduzio[1], Pascal Cosette[2], Linda Gricourt[1], Thierry Jouenne[2], Christof Lenz[3], Odd-Ketil Andersen[4], François Leboulenger[1] and Béatrice Rocher[1]

[1] Laboratory of Ecotoxicology (LEMA), UPRES-EA 3222, IFRMP 23, University of Le Havre, Le Havre Cedex, France
[2] UMR 6522 CNRS, IFRMP 23, University of Rouen, Boulevard M. de Broglie, Mont-Saint-Aignan Cedex, France
[3] Applied Biosystems, Darmstadt, Germany
[4] RF-Akvamiljø, Mekjarvik 12, Randaberg, Norway

The discharge of chemicals such as oil associated or not with derived products constitutes a real threat for the environment. We report here the differential expression of the blue mussel (*Mytilus edulis*) gill proteins corresponding to two contaminated environmental conditions: crude oil and offshore produced water. In order to evaluate and understand contaminants, effects and adaptive response of these organisms, we identified proteins using MS. The latter can be grouped into three main classes: proteins involved in the cellular structure, in metabolism, and in defence proteins.

Received: November 9, 2004
Revised: March 23, 2005
Accepted: April 18, 2005

Keywords:
Blue mussel / Polyaromatic hydrocarbons / Proteome maps / Water ecosystems

To evaluate the ecological incidence of the various entropic pressures stemming from different human activities, a comprehensive view of the xenobiotic actions and the understanding of associated molecular responses of the exposed organisms represent two important objectives of ecotoxicology. One contemporary source of such perturbations consists in the use of fossil fuels and related by-products, particularly in the offshore produced water [1]. This water generated at time of oil extraction contains polycyclic aromatic hydrocarbons (PAHs), metals, and chemical additives such as alkylphenols. After separation from oil, the produced water is released directly in the marine environment. Some recent studies linked environmental disruptions (*e.g.*, presence of dissolved chemicals) to adverse health effects, such as decreased survival of *Haliotis rufescens* larvae [2] or alteration of the fauna near diffusers [3]. These studies pointed out the presence of oil residues in water. Polyaromatic hydrocarbons were identified as the main components involved in the environmental contamination mainly because of their carcinogenic and mutagenic properties [4, 5]. Hence, considering the large release of these compounds into the environment, it becomes crucial to evaluate their toxicity.

In this study, we proposed to identify proteins involved in the response of bivalves to contaminants, *e.g.*, alterations due to the toxicity of xenobiotics or adaptive response of the organisms in the blue mussel *Mytilus edulis*. These molluscs are of particular interest for biomonitoring (*i.e.*, [6, 7]) because they are filter feeders with a high capacity of bioaccumulation and live in direct contact with substrate. In addition, they are widespread and easy to collect. Gills are respiratory and feeding structures and are able to absorb pollutants such as metals and especially PAHs directly from seawater [8–10] and to accumulate them and other contaminants [11]. The incidence of pollutants on gills of molluscs has already been shown. The perturbations observed are of

Correspondence: Dr. Hélène Manduzio, Laboratory of Ecotoxicology (LEMA), UPRES-EA 3222, IFRMP 23, UFR of Sciences and Techniques, University of Le Havre, 25 rue Philippe Lebon–BP 540, 76058 Le Havre Cedex, France
E-mail: helene.manduzio@univ-lehavre.fr
Fax: +33-232-74-4314

Abbreviation: PAHs, polycyclic aromatic hydrocarbons

different kinds. Thus, changes in the shape and architecture of gills have been observed after exposure to metals with progressive necrosis of epithelial cells, increased exfoliation, and mucus secretion following exposure to metals such as cadmium [12–14] and mercury [15, 16]. Alterations of the ciliary activity of gills have been described too [17–19].

In order to identify new molecular biomarkers, we compared the gill protein patterns obtained by 2-DE of the blue mussels exposed to environmental relevant concentrations of North Sea oil (Norway) used alone or in combination with PAHs and alkylphenols.

Adult blue mussels *M. edulis* (7–9 cm shell length) were collected in a clean area off the West Norwegian coast and maintained in flow through marine water in the Akvamiljø, RF-Rogaland Research (Stavanger, Norway) facilities. Proteomes were obtained from gills of mussels continuously exposed for 21 days to 0.5 ppm North Sea oil from Statfjord (Norway), or to a mixture of 0.5 ppm North Sea oil, 0.1 ppm alkylphenols (mix of 8 alkylphenols equivalent to that produced water offshore), and 0.1 ppm extra PAHs (mix of 11 PAHs equivalent of main forms present in the North Sea crude oil) and compared to proteins signatures of control performed on unexposed animals. This situation was thus highly representative of the conditions encountered in the mussel exposed environment. During the experiment, the mussels were fed every day. After 21 days, the gills of six animals for each experimental condition were removed, frozen in liquid nitrogen, and stored at −80°C until analysis.

The gills were mechanically homogenized in cold lysis buffer containing urea (9 M), CHAPS (2% v/v), 2-mercaptoethanol (2% v/v), PMSF (8 mM), pharmalytes pH 3–10 (0.8% w/v), and 50 mM Tris buffer pH 7.5. Cellular debris were then removed by centrifugation (9000 × *g* for 15 min, 4°C), supernatants were collected and their protein content was determined according to the method of Bradford [20] with BSA as standard.

A 750 μg of total proteins were added to an IEF buffer containing 0.4% w/v of bromophenol Blue (Sigma) for each run. The first-dimension gel separation was carried out on nonlinear wide-range IPG Immobiline Dry Strips NL (18 cm, pH 3–10; Amersham Pharmacia Biotech, Uppsala, Sweden) with a horizontal electrophoresis apparatus (Multiphor; Amersham Pharmacia Biotech). The second dimension was obtained after SDS-PAGE with a 12% w/v polyacrylamide resolving gel using a Protean plus Dodeca Cell (BioRad). After migration, proteins were visualized by colloidal blue staining. The pH gradient was determined in function of distance covered at 20°C and 8 M urea as defined by the manufacturer (Amersham Pharmacia Biotech). Molecular masses were estimated on the basis of comigrating broad-range standards (BioRad) in the second dimension.

Gels were scanned to TIFF images using an Image Scanner (Amersham Pharmacia Biotech). For each experimental condition the six gels were matched together to form a composite image consisting of spots found on four of the six component gels by using the Image Master 2-DE analysis software ■ **provide company name**■. The different composite gels were then matched together for spot quantification. Only proteins exhibiting a volume ≥20 were considered and taken into account for comparative analysis. The differences were characterized by an ANOVA on the volume values of each spot. Protein spots were considered to display significant quantitative differences if they fulfilled the following criteria: statistical significance by ANOVA ($p \leq 0.01$), an average volume ≥20 ($n = 6$), and present in four out of six gels for one of exposure conditions.

The conditions of electrophoresis and analysis allowed to discriminate 700 protein spots on control gels from gills of nonexposed mussels (Fig. 1). To give an idea of the homogeneity between gels, 62% of the detected spots exhibit an SD of the estimated volume ≤20%. Fifty-six spots were differently expressed in gills after exposure of mussels to crude oil (Table 1). Among them 29 were overexpressed compared with the control gel, 27 were newly detected. Moreover, seven spots exhibited a decreased expression whereas 20 polypeptides disappeared (noted d in Table 1).

Table 1. ■provide caption■

Table 1A

Spot	Variation (/Te)	PM, kDa/p*I*	Spot	Variation (/Te)	PM, kDa/p*I*
94	+222%	50/5.88	14	−41%	23.7/5.24
28	+ 96%	16.9/5.16	17	−37%	38.4/5.76
			21	−42%	28.8/.49
			23	−33%	26.4/6.37
			31	−36%	12.9/5.78
			53	−56%	31.7/4.85
			59	−75%	11.1/4.46

Table 1B

Spot	Variation (/Te)	PM, kDa/p*I*	Spot	Variation (/Te)	PM, kDa/p*I*
18	a	69.3/6.24	7	d	48.8/7.94
179	a	35.3/5.37	11	d	34.9/.82
184	a	58.1/6.15	33	d	11.5/8.04
194	a	41.6/5.98	34	d	9.5/4.41
195	a	41.8/6.24	35	d	9.3/5.37
196	a	41.9/7.64	40	d	65.8/7.37
198	a	33.9/5.51	52	d	50.8/4.96
199	a	31.5/5.56	54	d	48.2/5.05
203	a	25.9/6.43			
204	a	26.1/7.36			
206	a	23.0/6.36			
207	a	21.4/5.94			
208	a	23.7/4.58			
211	a	13.6/4.57			
212	a	13.9/6.33			
216	a	28.8/6.96			
218	a	12.9/4.55			

VCH Auftr.-Nr. 31626 Vers.: 6.06
S:/3D/vch/_proteomics/18_2005/pr_1328.3d
Proteomics
1. Papierlauf Seite 2 – ge –
Freitag, den 30. 09. 05, 11 Uhr 39

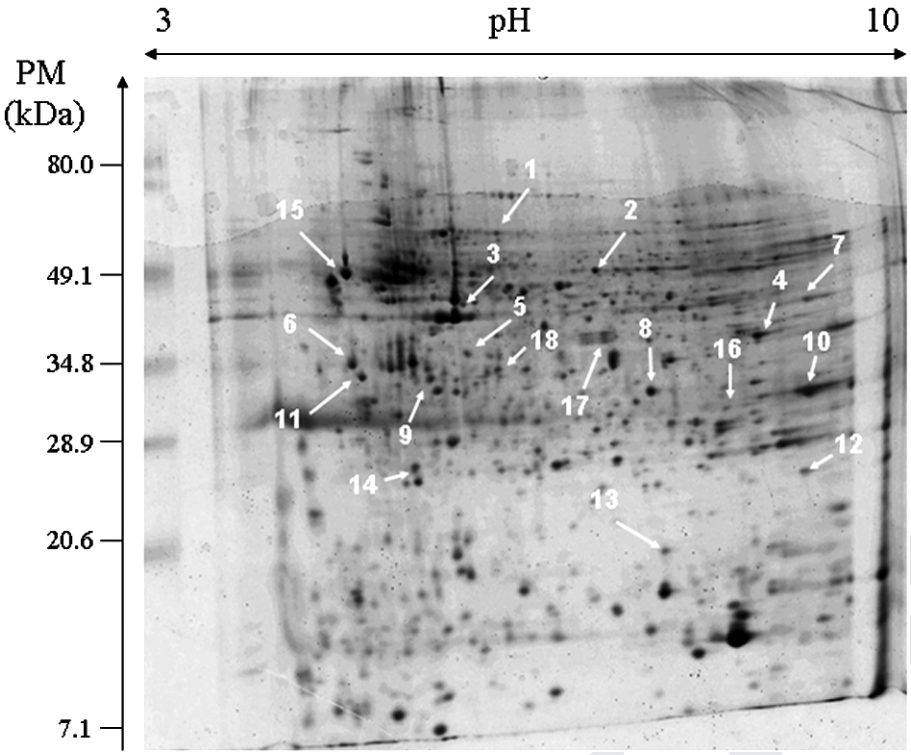

Figure 1. ■ ■ ■

The comparison of gels obtained from mussels exposed to produced water with control gels showed that 76 spots were differentially expressed: 40 were overexpressed (39 appeared) and 36 were under-expressed (32 disappeared) (Table 1). Some spot alterations observed in mussels exposed to crude oil were also observed after exposure to produced water, *i.e.*, spots 179, 204, and 54. However, the variations of protein expression levels were higher in the first experiment (until four-fold of the control value) than in the second one (<70% of the control value).

As a first attempt, proteins of interest were characterized by PMF. Briefly, gel pieces excised from the polyacrylamide gel were washed with CH_3CN/H_2O mixtures and dried. These plugs were rehydrated with 10 µL of buffered trypsin solution (Promega, Charbonnières, France; 10 ng/µL) and placed at 37°C for digestion overnight. After extraction, peptide samples were dried in a SpeedVac centrifuge and then resuspended in 5 µL of a water/ACN/TFA mixture (50:50:0.1). MALDI-TOF analyses were performed with a Voyager DE Pro (Applied Biosystems). The peptide fingerprints were matched to the Genepept database using the MS-FIT software (http:\prospector.ucsf.edu). For some proteins, the degree of sequence conservation with respect to proteins present in databases was sufficiently high to yield an acceptable number of matched peptides (*N*) and/or sequence coverage (%). For instance, in the case of spot 3 (42.2 kDa, 5.48 p*I*), seven peptides matched with actin for which the previously described parameters were high enough to conclude to the identity of this spot. In contrast, an important number of spots gave MALDI-TOF mass spectra of reasonable intensities but were unable to produce any significant score in peptide mapping, this most likely correlated with an increase of polymorphic variations for these proteins.

We thus used automated nanoLC/MS/MS analysis to determine sequence fragments. The previously described digest was resuspended in 20 µL 0.2% formic acid/5% ACN and 5 µL of the sample was injected onto an LC Packings Ultimate nanoLC system. Peptides were enriched and desalted on an RP-C18 trap column, and separated on a 75 µm ID × 15 cm C18 Pep-Map column. A 45 min linear gradient (10–35% ACN in 0.2% formic acid) at a flow rate of 200 nL/min was used. The eluent was analyzed on a Q TRAP System equipped with a nanospray source (Applied Biosystem). The LC/MS/MS data were evaluated using two different methods. First, MS/MS peak lists were extracted and submitted to MASCOT software searching of protein database. Second, the data were analyzed using ProBLAST Software (Applied Biosystems) [21]. Because the MS BLAST algorithm allows for substitutions in peptide sequence, it generally gave complementary results allowing unambiguous protein identification. Thus, 15% of the HSP70 protein sequence was accessible after MASCOT search and 21% of the same sequence was covered with ProBLAST. It should be also noted that other clearly attributed fragmentation patterns were obtained and remain till now unsuccessful in matching proteins in databases. This statement is far more frustrating since it concerns most of the proteins that have a markedly different expression. This also suggests that mus-

VCH Auftr.-Nr. 31626 Vers.: 6.06
S:/3D/vch/_proteomics/18_2005/pr_1328.3d
Proteomics
1. Papierlauf Seite 3 – ge –
Freitag, den 30. 09. 05, 11 Uhr 39

sels are very distant in the point of view of evolution from other organisms for which genomic or proteomic databases are accessible.

Together, these methods allowed to identify 19 proteins presented in Table 2. Among the proteins differently expressed by animals exposed to crude oil, five were identified. Four of them were down-regulated: actin (spot 14), tropomyosin (spot 11), fructose-bisphosphate aldolase (spot 7), and malate dehydrogenase (spot 17), respectively. Among the up-regulated proteins, the heavy metal binding protein (spot 18) was identified. The presence of heavy metal binding protein in exposed animals was in accordance with a study of Roesijadi

Table 2. ■provide caption■

Table 2A

Spot	Variation (/Te)	PM, kDa/p*I*	Spot	Variation (Te)	PM, kDa/p*I*
206	+58%	20.5/5.58	15	−38%	70.2/6.33
			30	−51%	60.6/5.08
			57	−33%	54.1/5.56
			153	−68%	31.3/5.39

Spot	Variation (/Te)	PM, kDa/p*I*	Spot	Variation (/Te)	PM, kDa/p*I*
18	a	37.9/5.42	7	d	44.2/7.56
38	a	65.8/5.41	11	d	34.5/5.13
57	a	24.9/5.42	34	d	11.0/5.70
60	a	11.5/5.35	35	d	9.7/5.27
179	a	71.5/6.22	54	d	48.5/5.03
199	a	32.2/5.33	62	d	83.8/6.45
204	a	27.5/7.60	97	d	21.7/5.94
208	a	25.1/4.54	98	d	47.8/7.69

Table 2B. ■ editor: check■

Spot	Protein (*organism*)	Matched peptides (% coverage)	PM, kDa/p*I*	Determination
1	Heat shock protein 70 (*Dicentrarchus labrax*)	18 (21)	69.5/5.40	MS/■ **check change throughout**■MS
2	Glutamyl tRNA synthetase (*Rhodobacter capsulatus*)	5 (9)	50.7/5.79	MS/MS
3	Actin (*Fugu rubripes*)	8 (77)	42.2/5.48	MALDI
4	Replication factor c (*Mus musculus*)	7 (62)	37.1/7.63	MALDI
5	Probable arginase (*Arabidopsis thaliana*)	5 (83)	36.3/5.51	MALDI
6	Cyclin D1 (*Gallus gallus*)	3 (7)	33.5/5.35	MS/MS
7	Fructose-biphosphate aldolase (*Caenorhabditis elegans*)	4 (12)	48.8/7.94	MALDI
8	Aminopeptidase (*Homo sapiens*)	7 (57)	32.8/6.07	MALDI
9	Cyclin D1 (*Gallus gallus*)	5 (62)	30.7/5.48	MALDI
10	Probable protease inhibitor (*Homo sapiens*)	6 (42)	29.3/8.29	MALDI
11	Tropomyosin (*Theragra chalcogramma*)	14 (35)	34.9/4.82	MS/MS
12	Glutathione S-transferase (*Rattus norvegicus*)	4 (57)	23.5/7.66	MALDI
13	Thioredoxine peroxydase (*Homo sapiens*)	2 (11)	15.0/6.57	MS/MS
14	Actin (*Debaryomyces hansenii*)	3 (5)	23.7/5.24	MS/MS
15	ATP synthase β Chain (*Rickettsia prowazekii*)	6 (42)	55.2/4.60	MALDI
16	Mitochondrial porin (*Bos taurus*)	10 (19)	26.8/7.25	MS/MS
17	Malate dehydrogenase (*Tetratrichimonas gallinarum*)	7 (16)	38.3/5.76	MS/MS
18	Heavy metal binding protein (*M. edulis*)	13 (22)	35.3/5.37	MS/MS
19	Heavy metal binding protein (*M. edulis*)	4 (12)	36.0/4.90	MS/MS

VCH Auftr.-Nr. 31626 Vers.: 6.06
S:/3D/vch/_proteomics/18_2005/pr_1328.3d
Proteomics
1. Papierlauf Seite 4 – ge –
Freitag, den 30. 09. 05, 11 Uhr 39

[22] that described in SDS-PAGE pattern of proteins of *M. edulis*, low molecular mass proteins (between 10 and 20 kDa) that were able to chelate metals. In our case, the induction of expression of one of these proteins could be explained by the presence of metals (vanadium, molybdenum, and nickel) in oil.

The down-regulation of actin and tropomyosin points out the decline of the condition state of animals probably due to an oxidative stress. Actin is one of the most abundant proteins in cells. As tropomyosin, it is a fundamental component of cytoskeleton in muscular and nonmuscular cells. In the haemocytes of *Mytilus galloprovincialis*, the perturbations of the actin filaments have been correlated with exposure to copper Cu(II) [23]. The decrease of expression of actin and tropomyosin is in accordance with the previous data that show the attack of the cytoskeleton proteins after exposure to oil. In the rat, exposure to JP-8 jet fuel represses the expression of the isoform 4 of tropomyosin but increases the expression of the isoform 1 [24]. These changes in the cytoskeleton are coherent with the alteration of the general architecture of gill filaments reported before [12–16]. In mussel *M. galloprovincialis*, the exposure to B(a)P decreases the number of actin filaments. The authors linked this effect to the production of ROS [25]. In fact, many studies correlated the alteration of cytoskeleton proteins as actin in relation with the production of ROS. The metabolism of PAHs and more particularly of B(a)P results in the formation of diols, quinines, and phenols [26]. The metabolization of quinonic compounds leads to the formation of oxygenated radicals [27]. The ROS could act directly or indirectly. The oxidative stress mediates an alteration of Ca^{2+} homeostasis, and consequently induces perturbations of actin organizations [25, 28, 29]. High concentrations of Ca^{2+} activate proteases which might hydrolyze the actin filaments and the proteins fixing them to the cellular membranes. However, anoxic conditions which do not implicate an increase of Ca^{2+} concentrations could modify membrane properties [30]. This hypothesis is coherent with the decrease of the expression of the fructose-bisphosphate aldolase (48.8 kDa, 7.94 p*I*) and malate dehydrogenase (38.35 kDa, 5.76 p*I*), two enzymes involved in the energetic catabolism. In fact, malate dehydrogenase catalyzes the interconversion of malate to oxaloacetate. Aldolase catalyzes the aldol condensation in glycolysis. The decrease of their expression might indicate an alteration of the aerobic metabolism. The decline of malate dehydrogenase activity has been observed in the muscle of fish treated with the pesticide endosulfan [31]. In the same way, Osenberg *et al.* [3] have shown alterations of general condition and tissue production in mussels *Mytilus californianus* and *M. edulis* sampled close to a diffuser and consequently exposed to produced water.

The protein expression signatures obtained after exposure to crude oil and produced water offshore have been compared. For this comparison, the coordinates of the proteins are expressed in an interval of confidence of 5% around the measured values. Marked total alteration of the protein pattern was observed in gills of mussels exposed to produced water (22%) as compared with that observed in the presence of crude oil (14%). This increase might be due to an additional effect of alkyl phenols and the difference in the response might reflect a better availability of hydrocarbons for the animals. The addition of surfactants as alkyl phenols in produced water allows probably to increase partitioning between water and hydrocarbons. Consequently, these compounds could be accumulated by mussels. However, alkylphenols could act directly, inducing for instance a hormonal disturbance [32, 33]. Recently, Aarab *et al.* [34] showed that some components of North Sea oil were oestrogen mimics. However, a direct effect of alkylphenols cannot be excluded.

In conclusion, the specificity of the organism response can be showed by the alterations of the whole proteomic map. In this context, the 2-DE approach might be used for the biomonitoring in coastal ecosystems. Additionally, in an evolved proposal, protein chips could be designed to detect the presence of proteins from mussels lysates that only appear in exposed conditions. The identification of some proteins specifically regulated by the exposure to pollutants allows to reach a global view of the xenobiotic action and might conduct to identify the molecular mechanisms that are involved in the animal response.

H.M. was the recipient of a thesis grant from the Région Haute-Normandie. This work was funded by the French Ministry for Research and Technology (EA 3222), the council of the Région Haute-Normandie through the Seine Aval scientific program, the Agence de l'Eau Seine Normandie (AESN) and Electricité de France (EDF). We thank Professor C. Lange and Dr. M. Hubert (UMR 6044-CNRS) for their help in protein identification.

References

[1] Stephenson, M. T., in: Ray, J. P., Engelhardt, F. R. (Eds.), *Produced Water: Technological/Environmental Issues and Solutions*, Plenum, New York 1992, pp. 1–11.

[2] Raimondi, P. T., Schmitt, R. J., in: Ray, J. P., Engelhardt, F. R. (Eds.), *Produced Water: Technological/Environmental Issues and Solutions*, Plenum, New York 1992, pp. 415–430.

[3] Osenberg, C. W., Schmitt, R. J., Holbrook, S. J., Canestro, D., in: Ray, J. P., Engelhardt, F. R. (Eds.), *Produced Water: Technological/Environmental Issues and Solutions*, Plenum, New York 1992, pp. 387–402.

[4] Varanasi, U., *Metabolism of Polycyclic Aromatic Hydrocarbons in the Aquatic Environment*, CRC Press, Boca Raton, FL 1989.

[5] Aas, E., Baussant, T., Balk, L., Liewenborg, B., Andersen, O. K., *Aquat. Toxicol.* 2000, *51*, 241–258.

[6] Goldberg, E. D., Bowen, V. T., Farrington, J. W., Harvey, G., Martin, J., Parker, P. L., Risebourg, R., Robertson, W., *Marine Pollut. Bull.* 1975, *5*, 101–126.

VCH Auftr.-Nr. 31626 Vers.: 6.06
S:/3D/vch/_proteomics/18_2005/pr_1328.3d
Proteomics
1. Papierlauf Seite 5 – ge –
Freitag, den 30. 09. 05, 11 Uhr 39

[7] Tanabe, S., Prudente, M. S., Kan-atireklap, S., Subramanian, A., *Ocean Coastal Management* 2000, *43*, 819–839.

[8] Burgess, R. M., McKinney, R. A., *Environ. Pollut.* 1999, *104*, 373–382.

[9] Randall, D. J., Connell, D. W., Yang, R., Wu, S. S., *Chemosphere* 1998, *37*, 1263–1270.

[10] Baumard, P., Budzinski, H., Garrigues, P., Narbonne, J. F., Burgeot, T., Michel, X., Bellocq, J., *Mar. Environ. Res.* 1999, *47*, 415–439.

[11] Axiak, C., George, J. J., Moore, M. N., *Mar. Biol.* 1988, *97*, 225–230.

[12] Sunila, I., *J. Invertebr. Pathol.* 1986, *47*, 125–142.

[13] Sunila, I., *J. Invertebr. Pathol.* 1988, *52*, 137–141.

[14] Sunila, I., *Mar. Environ. Res.* 1988, *24*, 277–280.

[15] Gregory, M. A., George, R. C., Marshall, D. J., Anandraj, A., McClurg, T. P., *Mar. Pollut. Bull.* 1999, *39*, 116–121.

[16] Gregory, M. A., Marshall, D. J., George, R. C., Anandraj, A., McClurg, T. P., *Mar. Pollut. Bull.* 2002, *45*, 114–125.

[17] Senius, K. E. O., *J. Thermal Biol.* 1977, *2*, 233–238.

[18] Pivovarova, N. B., Lagerspetz, K. Y. H., Skulskii, I. A., *Comput. Biochem. Physiol., Part C* 1992, *103*, 27–30.

[19] Lagerspetz, K. Y. H., Tiiska, A., Senius, K. E. O., *Comput. Biochem Physiol., Part C* 1993, *105*, 393–395.

[20] Bradford, M. M., *Anal. Biochem.* 1976, *72*, 248–254.

[21] Lenz, C., Main, L., Cosette, P., Siroy, A., Dé, E., Hunter, C., *Applied Biosystems Proteomics Technology Note* 2003.

[22] Roesijadi, G., *Mar. Biol.* 1982, *66*, 151–157.

[23] Fagotti, A., Di Rosan, I., Simoncelli, F., Pipe, R. K., Panara, F., Pascolini, R., *Dev. Comp. Immunol.* 1996, *20*, 383–391.

[24] Witzmann, F. A., Bauer, M. D., Fieno, A. M., Grant, R. A., Keough, T. W., Lacey, M. P., Sun, Y. *et al.*, *Electrophoresis* 2000, *21*, 976–984.

[25] Gomez-Mendikute, A., Etxeberria, A., Olabarrieta, I., Cajaraville, M. P., *Mar. Environ. Res.* 2002, *54*, 431–436.

[26] Michel, X. R., Beasse, C., Narbonne, J.-F., *Archives Environ. Contamin. Toxicol.* 1995, *28*, 215–222.

[27] Mason, R. P., *Environ. Health Perspect.* 1990, *87*, 237–243.

[28] Dalle-Donne, I., Rossi, R., Milzani, A., Di Simplicio, P., Colombo, R., *Free Radic. Biol. Med.* 2001, *31*, 1624–1632.

[29] Dalle-Donne, I., Rossi, R., Giustarini, D., Gagliano, N., Lusini, L., Milzani, A., Di Simplicio, P., Colombo, R., *Free Radic. Biol. Med.* 2001, *31*, 1075–1083.

[30] Lemasters, J. J., DiGuiseppi, J., Nieminen, A. L., Herman, B., *Nature* 1987, *325*, 78–81.

[31] Mishra, R., Shukla, S. P., *Ecotoxicol. Environ. Safety* 2003, *56*, 425–433.

[32] Renner, R., *Environ. Sci. Technol.* 1997, *31*, 316A–320A.

[33] Ying, G.-G., Williams, B., Kookana, R., *Environ. Internat.* 2002, *28*, 215–226.

[34] Aarab, N., Minier, C., Lemaire, S., Unruh, E., Hansen, P.-D., Larsen, B. K., Andersen, O.-K., Narbonne, J.-F., *Mar. Environ. Res.* 2004, *58*, 437–441.

VCH Auftr.-Nr. 31626 Vers.: 6.06
S:/3D/vch/_proteomics/18_2005/pr_1328.3d
Proteomics
1. Papierlauf Seite 6 – ge –
Freitag, den 30. 09. 05, 11 Uhr 39