Helgol Mar Res (1999) 53:209–218

ORIGINAL ARTICLE

D.L. Angel · U. Fiedler · N. Eden · N. Kress
D. Adelung · B. Herut

Catalase activity in macro- and microorganisms as an indicator of biotic stress in coastal waters of the eastern Mediterranean Sea

Received: 1 March 1999 / Received in revised form: 20 July 1999 / Accepted: 3 August 1999

Abstract In this study we examined the activity of catalase in the water column (mainly attributed to planktonic microorganisms) and the activity of catalase and superoxide dismutase (SOD), as well as lipid peroxidation in the midgut gland of the benthic bivalve *Donax trunculus* as possible indicators of biotic stress. The measurements were performed at stations situated at known contaminated and clean sites in the coastal waters and shores along the Israeli coast (eastern Mediterranean Sea). In the water column, we found that catalase activity was higher in polluted coastal waters than in nearby unpolluted or less-polluted stations. Moreover, there was diurnal periodicity in catalase activity rates which matched the diurnal changes in hydrogen peroxide levels in seawater. Consistent evidence of extracellular catalase activity was found in the seawater sampled. Catalase activity rates in the midgut gland of *D. trunculus* did not exhibit clear patterns with respect to site (polluted or clean) or season. However, SOD activity and lipid peroxidation measured in the same tissues were good indicators of organic pollution in the coastal waters examined and, among the three stations examined in Haifa Bay, Qiriat Haim was the most polluted.

Key words Catalase activity · Superoxide dismutase · Biotic stress · *Donax trunculus* · Eastern Mediterranean Sea

D.L. Angel (✉) · N. Eden
Israel Oceanographic and Limnological Research,
National Center of Mariculture, P.O. Box 1212, Eilat 88112, Israel
e-mail: angel@agri.huji.ac.il

U. Fiedler · D. Adelung
Institut für Meereskunde, Dusternbrooker Weg 20, 24105 Kiel, Germany

N. Kress · B. Herut
Israel Oceanographic and Limnological Research,
National Institute of Oceanography, P.O. Box 8030, Haifa 31080, Israel

Introduction

Hydrogen peroxide, H_2O_2 (HP), is a small, non-charged molecule that, unlike other reactive oxygen species (ROS), can readily diffuse across cytoplasmic membranes and cause considerable intracellular damage to living cells (Abele-Oeschger et al. 1994; Richardson 1994; Xenopoulos and Bird 1997). The damage includes formation of additional reactive molecules inside the cell, mainly hydroxyl radicals, which may inhibit collagen gelation, modify amino acid residues of proteins and react with cell components, thereby producing organic peroxides which can attack DNA (Fridovich 1978; Dean 1987; Malins and Haimanot 1990, 1991; Halliwell, 1994). The interaction of hydroxyl radicals with lipids leads to formation of lipid peroxides, which affects the functioning of cell membranes, membrane-bound enzymes and other macromolecules (Winston 1991). Furthermore, superoxide radicals and peroxides bind to DNA, thereby altering or even breaking the double helix structure (Richardson 1994).

Several studies have shown that there is enhanced photochemical production of hydrogen peroxide in surface waters that are enriched in chromophoric dissolved organic matter (CDOM) (e.g. Szymczak and Waite 1991; Moore et al. 1993). Herut et al. (1998) found higher HP production rates (HPPR) in polluted than in relatively clean coastal waters, but could not relate HPPR to the DOM content in the water samples from different stations. This was attributed to enzymatic breakdown of HP, mainly associated with microorganisms (Petasne and Zika 1997; Herut et al. 1998). Moffett and Zafiriou (1990) reported that decomposition of peroxide in Vineyard Sound, Massachusetts, USA was dominated by biological processes; 65–80% of the biological decay was due to catalase activity and 20–35% was due to peroxidase activity. When exposed to higher-than-normal levels of the hazardous peroxide molecule, living organisms protect themselves by inducing catalase activity (Morgan et al. 1986; Beyer and Fridovich 1987; Naclerio et al. 1995). Although ambient, in situ concentrations of H_2O_2

generally do not vary widely on a temporal basis, this is largely due to rapid and efficient biological breakdown of the peroxide molecule. The energetic consequence of altering normal cellular activities to accomodate enhanced synthesis of the catalase enzyme may be construed as biological stress.

Relatively few studies have examined catalase activity in seawater (e.g. Pamatmat 1988, 1990; Moffett and Zafiriou 1990; Petasne and Zika, 1997) and none have considered its potential use as an early indicator of anthropogenic pollution and marine ecosystem stress. On the other hand, several workers have found enhanced activity of catalase and superoxide dismutase (SOD) in marine fishes and macrofauna that were attributed to pollution (e.g. Livingstone et al. 1992; Eertman et al. 1993; Lemaire et al. 1993; Abele-Oeschger et al. 1994; Buchner et al. 1996; Pedrajas et al. 1996).

Benthic marine organisms are similar to their pelagic counterparts with regard to exogenous peroxides, and respond to elevated levels of H_2O_2 by inducing catalase and/or peroxidase activity (Abele et al. 1999). In addition, they also respond to peroxides that are produced endogenously. Superoxide radicals are regular by-products of normal aerobic respiration. These radicals are highly reactive, possibly even more dangerous than hydrogen peroxide and are rapidly transformed from superoxide to H_2O_2 by the enzyme SOD. The hydrogen peroxide formed by SOD is subsequently decomposed by catalase and peroxidases to prevent further possibility of intracellular damage (Brock et al. 1984).

In the presence of xenobiotics, such as heavy metals, polychlorinated biphenyls (PCBs) and polycyclic aromatic hydrocarbons (PAHs), endogenous production of superoxide radicals may be amplified (Hassan and Fridovich 1979; Porte et al. 1991). To counter the increase in superoxide radical levels, cells increase activities of SOD and catalase. Porte et al. (1991) sampled along an organic pollution gradient and observed elevated activities of SOD and catalase among bivalves in the more heavily polluted stations.

Thus, there is a clear difference between the response of the cell to *exogenous* peroxides (increased catalase and/or peroxidase activity) and to xenobiotics that lead to *endogenous* increase in both superoxide radicals (increased SOD activity) and hydrogen peroxide (increased catalase and/or peroxidase activity). By comparing catalase and SOD activity in the same organism, it may be possible to resolve whether there has been an enrichment in the levels of H_2O_2 in the seawater or whether the organism has been exposed to xenobiotics, as described above.

In this study we have compared catalase activity rates measured in clean and polluted waters in both the water column and benthic bivalves in order to test whether changes in enzyme activities can be used as indicators of biological stress associated with pollution. Increased catalase activity (above normal) is indicative of elevated levels of hydrogen peroxide in the surrounding seawater, often associated with anthropogenic pollution. Benthic bivalves may also serve as biological stress indicators of heavy metals and hydrocarbons since both SOD and catalase activity rates increase in the presence of xenobiotics. These enzyme activities were measured in the tissues of bivalves collected at clean and polluted sites in order to test the use of this bioassay in the field.

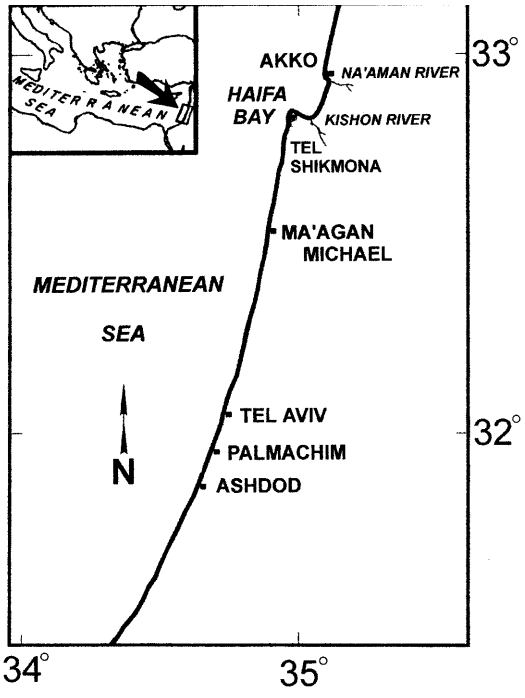

Fig. 1 Map. Sampling locations on the Israeli coast. Arrow in insert indicates geographical location of Israel in the eastern Mediterranean

Materials and methods

Study sites

Three main areas were sampled along the Mediterranean coast of Israel: Haifa Bay (HB), Ma'agan Michael (MM) and Palmachim (see Fig. 1). Haifa Bay is adjacent to a dense population center and an industrial area containing, among others, petrochemical and chemical industries. The bay is exposed to two main contamination sources, one at its northern part where anthropogenic mercury is introduced from the outfall of a chlor-alkali plant (Herut et al. 1993, 1996, 1997), and one at the southern part of the bay, the Kishon River. The Kishon River, regarded as the most polluted river in Israel, is the main source of anthropogenic pollutants (organic compounds, heavy metals, nutrients) to Haifa Bay (Hornung et al. 1989; Cohen et al. 1993; Herut et al. 1993, 1996, 1999). Ma'agan Michael and the mouth of the Taninim river is considered a clean area (Herut et al. 1998b). The Palmachim area receives excess activated sewage sludge (about 14000 m^3/day) through a 5-km-long marine outfall at 38-m water depth. The activated sludge is composed of 95% water and 5% solids that may contain, in addition to the stable biomass, pathogenic bacteria, inorganic nutrients, heavy metals and synthetic organic compounds (Kress and Hornung 1996).

In this study, microplankton was sampled along a pollution gradient in Haifa Bay (five stations) and the Palmachim region (four stations); see Fig. 1. In HB, station A (polluted) was located at the mouth of the Kishon River, station B at ~10-m water depth,

Table 1 Pool size, mean shell length and standard deviation (*SD*) of composite smples of *Donax trunculus*

Date	Station	Number of pools	Mean number of specimens/pool	Mean shell length (mm)	SD
Jan 1997	Frutarom	10	3	23.2	2.83
	Q. Yam	10	3	22.53	2.57
	Q. Haim	10	3	24.33	2.39
	M. Michael	10	3	23.4	2.08
Jul 1997	Frutarom	10	4	26.23	2.69
	Q. Yam	10	4	25.78	1.67
	Q. Haim	10	4	26.95	2.05
	M. Michael	10	3	27.4	2.93
Jan 1998	Frutarom	10	4.1	21.41	4.59
	Q. Yam	10	5.6	17.61	2.35
	Q. Haim	10	4	20.58	1.41
	M. Michael	10	3	28.67	3.16
Incubation experiments		5 per incubation level	3	21.05	1.27

station C at ~14-m water depth and stations D and D' (relatively clean) in the northern part of the bay at ~17-m water depth. In Palmachim, station "0", the polluted site, is located at the sewage sludge outlet, while stations 9, 15 and 20 were 500 m east, 1000 m south and 1000 m west, respectively, of the pollution point source.

Bivalves were collected in HB (three stations) and at Ma'agan Michael. The sampling locations in HB were next to the chlor-alkali plant (FR), at a recreational beach in the central part of the bay (Qiriat Yam, QY) and at a beach near the Kishon River estuary (Qiriat Haim, QH).

Sampling

Surface seawater samples (1-m depth) were collected in Haifa Bay (May 1997) and in the Palmachim area (September 1997) with Niskin bottles mounted on a General Oceanics Rosette. The bivalve, *Donax trunculus* was collected from the shallow water (0.5–1.5 m) sandy sediments, with the aid of a custom-made fork-like device, in January 1997, July 1997 and January 1998. Sampling was always conducted between 6 and 10 a.m. Bivalves were transferred to the laboratory within 1–2 h after collection; shell length was measured and the animals were dissected immediately. For hydrogen peroxide incubation experiments (see below), *D. trunculus* specimens were collected in Haifa Bay (station FR) and maintained in aquaria at ambient conditions for 24–48 h prior to experimentation. Three to five specimens had to be pooled to obtain enough tissue from midgut glands for the enzymatic analyses (see Table 1). Midgut glands were analyzed for SOD and catalase. Lipid peroxidation was measured from bivalves sampled in July 1997.

Analytical methods

Catalase activity in the water column

The oxygen release method was used to examine enzymatic hydrogen peroxide decomposition by quantifying the oxygen produced after hydrogen peroxide was injected into a seawater sample, assuming all conversion of peroxide to oxygen was due to catalase (Pamatmat 1988). This approach measures the activity of catalase alone since peroxidases do not release molecular oxygen from the hydrogen peroxide molecule. In order to measure catalase activity in seawater by oxygen production, a sensitive oxygen electrode (Yellow Springs Instrument, YSI 5331), signal amplifier and recorder system (a modification of the system described in Dubinsky et al. 1987) were employed. Briefly, the method involves placing 15 ml of seawater (best results were achieved when fresh seawater samples were used) into a sealed chamber equipped with a magnetic stir bar and an oxygen electrode connected to an amplifier and a recorder in order to measure the rate of oxygen release. When a stable baseline of oxygen values was obtained, 200 µl of hydrogen peroxide (5%) was injected into the sealed chamber and the subsequent increase in dissolved oxygen was recorded and interpreted as the catalase activity rate.

The rate of oxygen release is a function of the substrate (hydrogen peroxide) concentration and the enzyme activity. In our standard method, we injected a constant amount of peroxide into the reaction chamber. Therefore, changes in oxygen release rate are related to differences in the catalase activity rate. The YSI 5331 oxygen electrode was sensitive to either hydrogen peroxide or oxygen and required a "recovery" time of at least 15 min between samples in order to function properly. At sea, we were not able to measure catalase activity in replicate samples due to the fact that subsequent samples could only be processed once every 20 or 25 min and stations were often reached 15 min after sampling at the previous site (see Table 2). Nonetheless, repeated laboratory measurements of subsamples of seawater taken from a homogeneous source indicated that there was generally a high degree of precision (coefficient of variation ranged from 2 to 14%) between subsequently measured samples.

Catalase activities were measured in both bulk and 0.2-µm filtered seawater samples. Extracellular catalase activities were measured by gently (<125 mm Hg) filtering freshly collected water samples via 0.2-µm membrane filters and measuring catalase activity rates in the filtrate.

Activity of superoxide dismutase, catalase and lipid peroxidation in D. trunculus

Superoxide dismutase activity was determined following Marklund and Marklund (1974). Sample tissue (midgut glands) was homogenized in ice-cooled 0.05 M tris-EDTA-succinate buffer (pH 8.2) followed by centrifugation (6000–14000 rpm for 10 min). The enzyme activity was determined photometrically at 420 nm using a Shimadzu 1202 spectrophotometer equipped with a Kinetik-2 program card. SOD activity was related to the protein concentration in the homogenate. Protein concentration in tissue homogenates was determined following Bradford (1976).

Catalase activity was measured following Aebi (1985), as modified by Jahn (1997). Midgut glands were homogenized in ice-cooled 0.05 M phosphate buffer (pH 7.0) with 1% Triton X-100. Samples were then frozen in liquid nitrogen and stored until analysis at –80°C (Ribera et al. 1991). Livingstone et al. (1992) described catalase of *Mytilus edulis* as a stable enzyme and did not note a decrease in activity after frozen storage. Prior to analysis, samples were thawed and then centrifuged for 10 min (6000–14000 rpm). Catalase activity was determined by the decay rate of hydrogen peroxide (monitored at 240 nm using a Shimadzu 1202 spectrophotometer connected to a PC) that was added to the

Table 2 Catalase activity rates in bulk and filtered seawater, hydrogen peroxide concentrations, hydrogen peroxide production rates (*HPPR*), chlorophyll *a* levels and dissolved organic matter (*DOM*) measurements (*QS* quinine sulfate; see Herut et al. 1998a) at stations in Haifa Bay (*HB*) and near Palmachim (*P*). *n.d.* not determined, *nM* nanomol/l

Date	Time	Station	Distance from pollution source (m)	Catalase activity		HPPR (nM×m²/W×h)	H_2O_2 (nM)	chlorophyll *a* (µg/l)	DOM (QS units)
				Bulk (nmol O_2/min)	Filtered (nmol O_2/min)				
16/10/96	10:00	HB-A	0	3.48	1.22	n.d.	13	4.8	n.d.
	10:20	HB-B	1200	1.97	3.31	n.d.	32	2.5	n.d.
	10:45	HB-C	3000	1.11	1.74	n.d.	19	0.83	n.d.
	11:15	HB-D	5600	1.04	2.47	n.d.	23	0.54	n.d.
	16:30	HB-D'	9500	1.74	n.d.	n.d.	7	0.42	n.d.
20/9/97	04:30	P-9	500	5.56	n.d.	n.d.	15	n.d.	6.12
	07:45	P-20	1000 (west)	2.27	1.02	n.d.	21	0.38	5.04
	09:30	P-15	1000 (south)	3.27	0.79	2.4	72	0.62	6.12
	14:30	P-0	0	9.20	0.77	15.2	64	0.68	6.02

tissue extract. We used custom-made software for data acquisition. Catalase activity was related to the protein concentration in the tissue homogenate.

Lipid peroxidation analysis was based on the lipoperoxidase assay kit 437636 (CALBIOCHEM). This assay measures the concentration of two common by-products of lipoperoxidation; malonyldialdehyde (MDA) and 4-hydroxy-nonenal (4-HNE) which react with n-methyl-2-phenylindolene, forming a stable compound that can be measured photometrically. Midgut gland samples were homogenized with ice-cooled 20 mM tris-HCl-buffer (pH 7.4), shock-frozen in liquid nitrogen and stored until analysis at –80°C. Prior to analysis, samples were thawed and centrifuged for 10 min (6000–14000 rpm) and the supernatant was taken for the assay. Concentration of peroxidation products was related to the protein concentration in the tissue homogenate.

Hydrogen peroxide incubation experiments.

Following initial acclimation in aquaria, *D. trunculus* were exposed to a set of hydrogen peroxide concentrations (final concentrations in seawater: 0, 0.5, 2.5, 5 µM) for 24 h and subsequently processed, as described above, for lipid peroxidation and catalase activity determinations.

Results

Catalase activity in the water column

The catalase activities along a transect, from the polluted Kishon river estuary (station A) across Haifa Bay, are given in Table 2. Catalase activities in bulk seawater samples ranged from 1 to 3.5 nmol O_2/min. There was a sharp drop in activity from station A to D, corresponding to the distance from the source of pollution, yet a slight increase at station D' which was closer to shore than station D. Filtered seawater prepared from samples taken at stations B through D yielded catalase activity rates that were greater than those in the bulk samples. These samples were inadvertently prepared using a strong vacuum (>625 mmHg) instead of gentle vacuum filtration (<125 mmHg), probably rupturing many of the cells captured on the filters and leading to release of intracellular catalase to the filtered seawater sample and the unusually high catalase activity rates measured filtered samples. There was a positive linear correlation (r=0.87, P<0.05; n=5) between bulk catalase activity and chlorophyll *a* concentrations along this transect.

An additional transect was conducted near Palmachim (Table 2). Catalase activities were considerably higher in these waters (2.3–9.2 nmol O_2/min) in comparison to those measured in Haifa Bay, yet the same pattern was observed. High catalase activities were recorded at the polluted station (0) and adjacent to this (9) and activities were lower at the clean stations (15 and 20). We did not find a linear correlation between catalase activity rates and either concentration of dissolved organic matter or chlorophyll *a* in the Palmachim samples.

Catalase activity was found in 0.2 µm filtered seawater samples indicating extracellular catalase activity. When samples were gently filtered (<125 mmHg) via 0.2-µm filters, catalase activity was always lower than the activity in bulk samples, but the ratio between these was variable (Table 2).

Antioxidant enzyme activity and lipid peroxidation in *Donax trunculus*

SOD activities in *D. trunculus* midgut glands ranged from 2 to 22 U/mg. Among the four stations sampled, a seasonal trend of SOD activity was visible at station QY (Fig. 2). SOD activity was generally higher in July 1997 than in January 1998. Overall, samples taken at QH had the highest mean SOD activity, whereas bivalves sampled at the other three stations had lower activity rates.

Lipid peroxidation was measured in *D. trunculus* midgut glands collected during July 1997 and ranged from 0.3 to almost 10 µmol (MDA+4 HNE)/mg protein (Fig. 3). Lipid peroxidation levels were low at both FR and QH and high at both QY and MM, and there was a significant difference (P<0.01) between the two pairs of stations. The lipid peroxidation pattern is inverse to that of SOD activity during July 1997.

Catalase activity rates generally ranged between 0.05 and 0.15 U/mg protein although some outlier values exceeded this range. The pattern of catalase activity in the

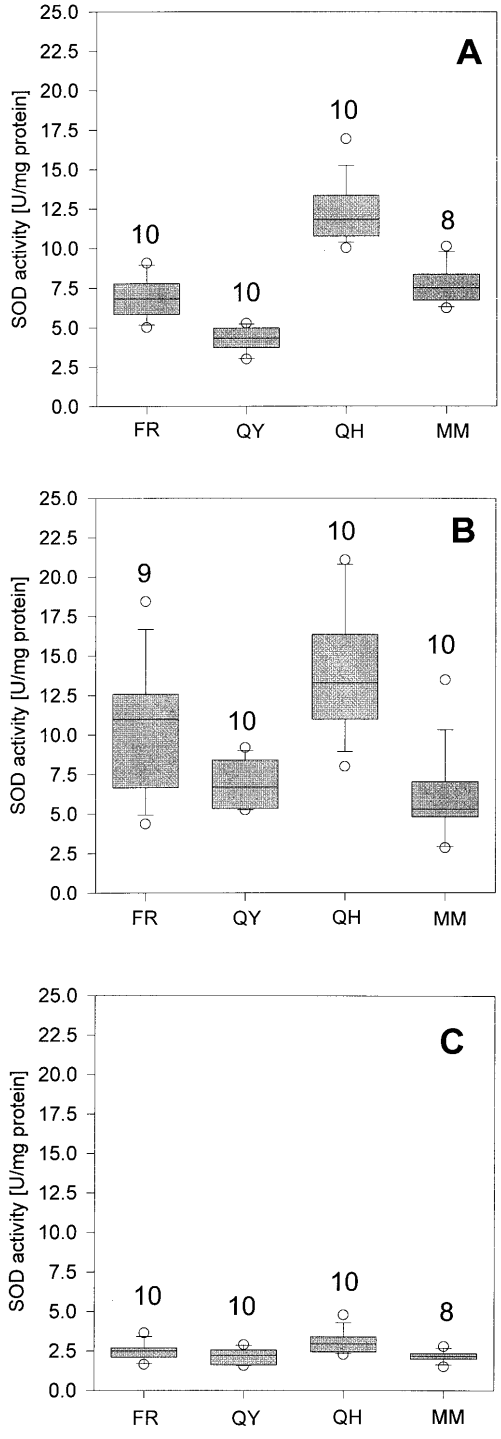

Fig. 2 Superoxide dismutase (SOD) activity in midgut glands of *Donax trunculus* in **A** January 1997, **B** July 1997 and **C** January 1998. Plots show medians, 25th-75th percentile (within *box*), 10th–90th percentile (*error bars*), and outlying points (*circles*); *numbers above boxes* indicate number of analyzed pools. Significant differences: January 1997 QH<FR=MM<QY (P<0.01); July 1997 QH<QY, MM (P<0.01); January 1998 QH<MM (P<0.01)

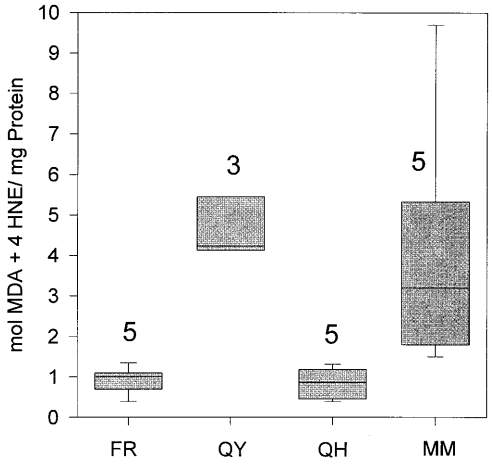

Fig. 3 Lipid peroxidation (as MDA+4HNE concentration) in midgut glands of *Donax trunculus* in July 1997. Plots show medians, 25th–75th percentile (within *box*) and 10th–90th percentile (*error bars*); *numbers above boxes* indicate number of analyzed pools

bivalves differed from the pattern of SOD activity (Fig. 4). Low catalase activities were recorded in July 1997, in contrast to the high SOD activities measured at this time in the same bivalves (Fig. 2). Overall, there was no evident seasonality in the catalase activity. In January 1997 MM bivalves had significantly higher activity than the other stations (P<0.01), whereas in January 1998 QH samples showed significantly higher activity (P<0.01). Catalase activities at FR were not significantly higher at any of the sampling dates than at MM or the other two HB stations, QH and QY. The catalase samples taken at station QH in January 1997 were accidentally lost.

In the laboratory incubation experiments, lipid peroxidation levels dropped sharply with increasing hydrogen peroxide concentration (Fig. 5A). Lipid peroxidation levels in *D. trunculus* exposed to 5 µM H_2O_2 were significantly lower (P<0.01) than those of specimens exposed to 0.5 µM H_2O_2. Conversely, catalase activity was stimulated by increasing hydrogen peroxide concentrations (Fig. 5B) and specimens exposed to 5 µM H_2O_2 had significantly higher catalase activity (P<0.001) than the control group.

Discussion

Catalase activity in the water column

We found a clear decrease in catalase activity rates with distance from point sources of organic pollution such as the mouth of the Kishon river and the sewage sludge outlet near Palmachim (Table 2). Similar patterns were observed in the Gulf of Aqaba, Red Sea and in the Baltic Sea (Angel 1997). Petasne and Zika (1997) measured hydrogen peroxide in coastal and oligotrophic offshore waters near south Florida, USA, and reported higher peroxide decay rates in the organically enriched coastal wa-

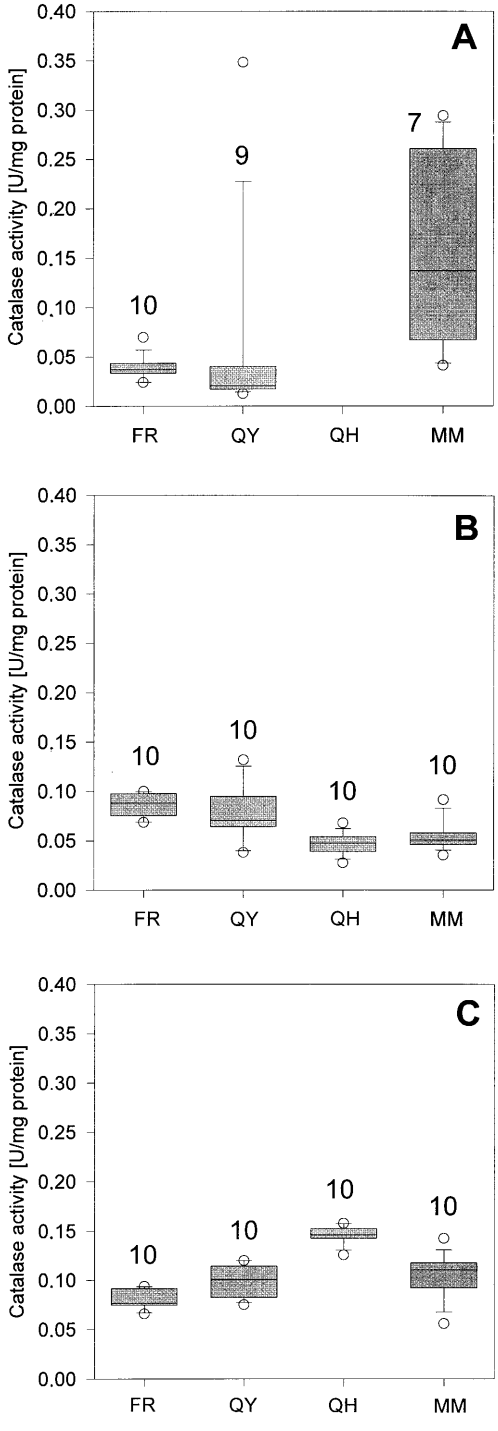

Fig. 4 Catalase activity in midgut glands of *Donax trunculus* in **A** January 1997, **B** July 1997 and **C** January 1998. Plots show the medians, 25th–75th percentile (within *box*), 10th-90th percentile (*error bars*) and outlying points (*circles*); *numbers above boxes* indicate the number of analyzed pools

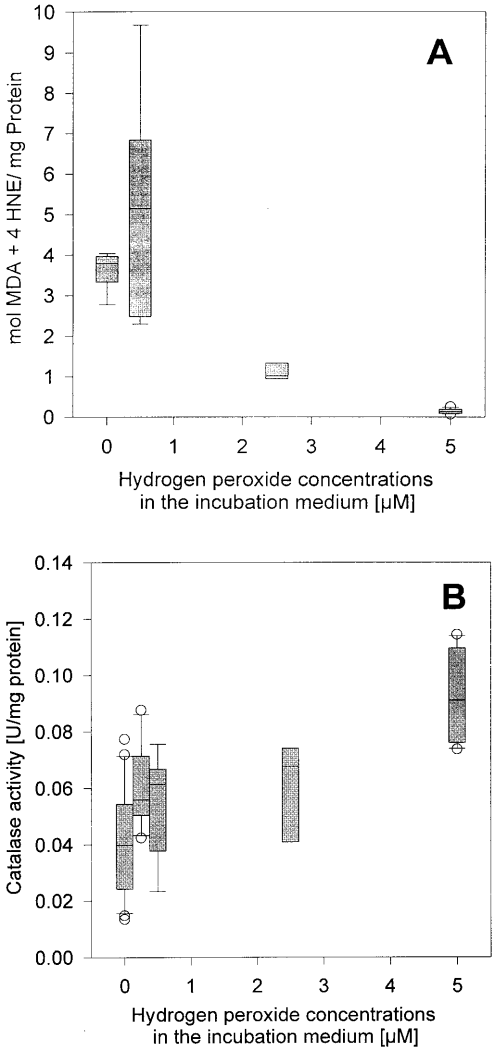

Fig. 5 Analysis of midgut glands of *Donax trunculus* after 24-h hydrogen peroxide incubation experiments: **A** lipid peroxidation (as MDA+4HNE concentration) and **B** catalase activity. Plots show medians, 25th–75th percentile (within *box*), 10th–90th percentile (*error bars*) and outlying points (*circles*); *numbers above boxes* indicate number of analyzed pools

ters. Moreover, Petasne and Zika (1997) recorded a positive linear correlation between DOM and H_2O_2 decay rates. We did not find a correlation between catalase activity rates and DOM (Angel 1997). The absence of correlation may be the result of heterogeneity in the small samples (15 ml) tested for catalase activity (see Methods, above). It is also possible that the different findings are related to differences in methodology since Petasne and Zika (1997) measured DOM by optical absorbance at 300 nm and H_2O_2 decay rates by direct measurements of hydrogen peroxide concentration, whereas we measured DOM by the fluorescence method (Moore et al. 1993) and catalase activity as described above.

In Haifa Bay, catalase activity was higher at station D' (closer to shore) than at station D (Table 2). Whereas both stations D and D' were considered clean, it is possible that the increased catalase activity at station D' was due to the northward long-shore flow of Kishon River water as described in Kress and Herut (1998). Hydrogen peroxide was measured in the same water samples (Table 2); however, we did not find a linear correlation between catalase activity rates and hydrogen peroxide. As proposed by Herut et al. (1998a), the presence of heavy met-

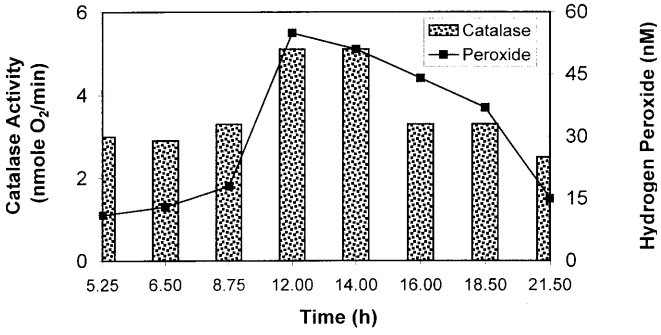

Fig. 6 Diurnal pattern of hydrogen peroxide (adapted from Herut et al. 1998a) and catalase activity (adapted from Angel 1996) in seawater taken from the North Beach of Eilat, Gulf of Aqaba, September 1996

als and other peroxide-scavengers in polluted waters may inhibit H_2O_2 production in these waters, despite high DOM levels, but these variables may not affect catalase activity in the same manner.

There is diurnal periodicity in hydrogen peroxide levels in seawater, as has been shown by several workers (e.g. Zika et al. 1985; Szymczak and Waite, 1991; Herut et al. 1998a). In laboratory cultures, it has been shown that catalase activity may be induced by exposure of bacteria to hydrogen peroxide (Morgan et al. 1986; Beyer and Fridovich 1987; Naclerio et al. 1995). It is likely, therefore, that the daily increases and decreases in peroxide are countered by respective changes in catalase production and activity. In a related study conducted in the Gulf of Aqaba (Red Sea) at a coastal site (Angel 1996), there was a positive linear correlation between diurnal dynamics of H_2O_2 levels and catalase activity rates (Fig. 6; r^2=0.726; P<0.05; n=8). Although we did not carry out simultaneous diurnal measurements of peroxide concentrations and catalase activity rates during the cruises in the Mediterranean Sea, it is likely that this correlation applies to the Mediterranean as well. Indeed, the diurnal pattern of hydrogen peroxide concentrations, as measured from shore at Tel-Shikmona (Mediterranean Sea; Herut et al. 1998a), was very similar to that recorded in the Gulf of Aqaba.

The diurnal range of catalase activity rates measured in the Gulf of Aqaba study (Angel 1996) was 2.5–5.1 nmol O_2/min (Δ=2.6 nmol O_2/min; Fig. 6). In comparison, the difference between the polluted and the clean stations in Palmachim and in Haifa Bay was 6.9 and 2.5 nmol O_2/min, respectively. We can use these differences in catalase activity as an indication of stress (or adaptive response) to water-column microbiota considering the natural diurnal range of catalase activities. The difference between station 0 and 15 at Palmachim (Δ=6.9 nmol O_2/min) was considerably greater than 2.6 nmol O_2/min and we therefore assume there was biological stress at the polluted station. In comparison, the difference between the polluted (A) and the relatively clean (D or D') Haifa Bay stations was 1.8–2.5 nmol O_2/min; less than the natural diurnal range and we might conclude that there is not a substantial difference between these stations. This may be because the polluted station A was sampled early in the morning, as compared to station D and D' which were sampled at noon (Table 2). Therefore, it is possible that in this case, the daily cycle in catalase activity masked differences due to pollution. These findings underline the need to sample water at the same time of day, or find a normalizing parameter for comparative purposes, not only with regard to hydrogen peroxide but also for catalase activity measurements.

Extracellular catalase activity

A number of laboratory studies have found extracellular release of catalase, e.g. in *Bacillus subtilis* (Naclerio et al. 1995) and in *Aspergillus niger* (Fiedurek and Gromada 1997). Beyer and Fridovich (1987), Bol and Yasbin (1990) and Naclerio et al. (1995) have shown that catalase production and extracellular release may be induced by exposure of the cells to elevated levels of hydrogen peroxide. Thus, it is possible that we will find enhanced extracellular catalase activity in polluted marine environments where hydrogen peroxide levels are higher than those found at nearby control sites.

Our observations regarding extracellular (<0.2 µm) catalase activity in most of the samples examined are supported by those of other workers. In coastal waters of the USA and Germany, Pamatmat (1990) recorded variable levels of extracellular catalase activity. Likewise, Petasne and Zika (1997) found that filtered seawater samples had lower, yet measureable, peroxide decay rates than non-filtered samples. On the other hand, when Moffett and Zafiriou (1990) placed filtered (0.2 µm) seawater samples in the dark, they did not find any decomposition during a 48-h incubation period and concluded that there was no catalase activity in particle-free seawater.

Antioxidant enzyme activity and lipid peroxidation in *Donax trunculus*

Abele et al. (1998) found seasonality in SOD and catalase activity in several invertebrates from North Sea mud flats; SOD activity was higher in summer than in winter. Although we do not have multi-annual data at hand, our results suggest that a similar seasonal trend may also exist in the eastern Mediterranean *D. trunculus* (Fig. 1). *Donax trunculus* were sampled in the same month during subsequent years; however, SOD activity was considerably lower in January 1998 than in January 1997 (Fig. 2). There were no significant differences in seawater temperature, bivalve age structure or other obvious factors that could affect SOD activity when comparing January 1997 and 1998; the reason for such different results remains unclear.

In addition to the seasonal factor, other external variables may affect antioxidant enzyme activities. Elevated

levels of xenobiotics (e.g. PAHs and PCBs) and of various metals (e.g. copper and cadmium) may lead to increased production of reactive oxygen species (ROS) inside the cell (Doyotte et al. 1997) and increased activity of ROS scavenging enzymes such as SOD (e.g. Porte et al. 1991; Sole et al. 1995; Winston et al. 1996; Krishnakumar et al. 1997; Otto 1997; Reddy 1997). *Donax trunculus* exhibited highest SOD activity at QH. This station is located downstream of the Kishon estuary, which is the main source of xenobiotics such as PAHs and petroleum-derived compounds to Haifa Bay (Cohen et al. 1993; Kress and Herut 1998). In comparison, all other stations can be regarded as less polluted.

Despite the proximity of some of the sampling stations to pollution sources, there were no significant differences in the levels of Cu, Cd, Mn, Fe or Zn (Herut et al. in press) in the tissues of bivalves sampled at the Haifa Bay stations. The only exception was FR where *D. trunculus* had higher levels of Hg. Moreover, cadmium and zinc were enriched in the specimens from all the stations in HB as compared to MM.

In addition to SOD activity, ROS damage was assessed by measuring lipid peroxidation in the bivalve tissues. We found that low lipid peroxidation values corresponded to high SOD activity, yet there was not a significant correlation between these two variables. We suggest that low lipid peroxidation is the result of effective ROS scavenging (e.g. by SOD), while higher values reflect either low activity of the antioxidative defense or a flux of ROS that exceeds the ROS scavenging capacity of the cell. Effective ROS scavenging has been demonstrated in laboratory experiments with *D. trunculus* wherein lipid peroxidation levels dropped almost to baseline values with increasing hydrogen peroxide concentrations in the medium (Fig. 5A). Even at the highest hydrogen peroxide concentration tested (5 μM), the antioxidative system (catalase) coped with elevated peroxide levels by increasing its activity (Fig. 5B).

Reddy (1997) found that enhanced ROS production in the gills of the crab *Scylla serrata* caused both SOD stimulation and lipid peroxidation. On the other hand, Doyotte et al. (1997) exposed a freshwater bivalve to copper and found a slight increase in lipid peroxidation, whereas SOD activity remained constant. On the basis of our results, we suggest that lipid peroxidation in benthic marine bivalves may be an additional sensitive indicator for ROS and oxidative stress.

In addition to SOD and lipid peroxidation, catalase activity was measured in the bivalve tissue to assess whether bivalves located at polluted sites showed evidence of oxidative stress. Moreover, we attempted to determine whether catalase activity was correlated with SOD activity in order to evaluate the source of the xenobiotic: endogenous vs exogenous. We did not find a correlation between SOD and catalase activity rates at the stations sampled, indicating that SOD did not necessarily stimulate catalase by intracellular production of hydrogen peroxide. Thus, increased catalase activity rates were attributed to elevated levels of exogenous hydrogen peroxide (i.e. H_2O_2 in the surrounding seawater) at the respective stations. This is also supported by laboratory experiments which demonstrated that catalase activity was stimulated by increasing hydrogen peroxide concentrations (Fig. 4B).

The low catalase activity at FR, as compared to MM, may have been due to the absence of high peroxide levels at this station, as proposed by Herut et al. (1998a). They argued that despite substantial levels of dissolved organic matter in the waters at polluted sites, such as FR, peroxide production may be inhibited by the presence of heavy metals and other peroxide scavengers, as suggested by Moffet and Zika (1987) and Szymczak and Waite (1988). Accordingly, at low levels of peroxides, there is no need for the bivalves to increase catalase activity. Another possibility is that the low catalase activity at the polluted stations is due to reduction or inhibition of the catalase activity on account of poisoning by xenobiotics.

Conclusions

1. We have generally found that catalase activity in the water column is higher in polluted coastal waters than in nearby unpolluted or less-polluted stations.
2. There is diurnal periodicity in catalase activity rates which matches the diurnal changes in hydrogen peroxide levels in seawater. Thus, for comparative purposes, it is necessary to sample seawater that has experienced the same sunlight levels when measuring catalase activity rates.
3. We have found consistent evidence of extracellular catalase activity in the coastal waters of the eastern Mediterranean Sea. Additional work is needed to determine whether there is any relationship between the level of extracellular catalase activity and biological stress.
4. SOD activity and lipid peroxidation, as measured in midgut glands of *Donax trunculus*, are sensitive indicators of organic pollution in shallow coastal waters. Among the three stations examined in Haifa Bay, Qiriat Haim was the most polluted, on the basis of SOD activity.
5. Catalase activity rates in the midgut gland of *D. trunculus* did not exhibit clear patterns with respect to site (polluted vs clean) or season. Laboratory incubation experiments demonstrated that catalase activity increased with hydrogen peroxide concentration. On the basis of our field results, we do not recommend using catalase as a single indicator for pollution in benthic bivalves.

Acknowledgements We acknowledge BMBF-MOST for funding of the joint German–Israeli binational research project MARS 1/3, the crew of R.V. *Shikmona* for their assistance in field sampling, Yaron Gertner for his assistance in the field, Wolfgang Hukriede for writing software for data acquisition, Anton Post for lending his oximeter, two anonymous reviewers and the editors of this special issue of *Helgoland Marine Research* for their helpful comments.

References

Abele D, Burlando B, Viarengo A, Poertner HO (1999) Exposure to elevated temperatures and hydrogen peroxide elicits oxidative stress and antioxidative response in the Antarctic intertidal limpet *Nacella concinna*. Comp Biochem Physiol B (in press)

Abele D, Grosspietsch H, Poertner HO (1999) Temporal fluctuations and spatial gradients of environmental PO_2, temperature, H_2O_2 and H_2S in its intertidal habitat trigger enzymatic antioxidant protection in the capitellid worm *Heteromastus filiformis*. Mar Ecol Prog Ser 163:179–191

Abele-Oeschger D, Oeschger R, Theede H (1994) Biochemical adaptations of *Nereis diversicolor* (Polychaeta) to temporarily increased hydrogen peroxide levels in intertidal sandflats. Mar Ecol Prog Ser 106:101–110

Aebi H (1985) Catalase. In: Bergemeyer HU (ed) Methods in enzymatic analysis, vol II. Academic Press, New York, pp 673–683

Angel DL (1996) Annual report on the activities at the National Center for Mariculture during 1996 (in Hebrew). Israel Oceanographic and Limnological Research Annu Rep E15/96, pp 99–111

Angel DL (1997) Annual report on the activities at the National Center for Mariculture during 1997 (in Hebrew). Israel Oceanographic and Limnological Research Annu Rep E15/97, pp 150–152

Beyer WF Jr, Fridovich I (1987) Catalases with and without heme. In: Simic MG, Taylor KA, Ward JF, Von Sonntag C (eds). Plenum Press, New York, pp 651–661

Bol DK, Yasbin RE (1990) Characterization of an inducible oxidative stress system in *Bacillus subtilis*. J Bacteriol 172:3503–3506

Bradford MM (1976) A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal Biochem 72:248–254

Brock TD, Smith DW, Madigan MT (1984) Biology of microorganisms, 4th edn. Prentice Hall, Englewood Cliff, 263 pp

Buchner T, Abele-Oeschger D, Theede H (1996) Aspects of antioxidant status in the polychaete *Arenicola marina*: tissue and subcellular distribution, and reaction to environmental hydrogen peroxide and elevated temperatures. Mar Ecol Prog Ser 143:141–150

Cohen Y, Kress N, Hornung H (1993) Organic and trace metal pollution in the sediments of the Kishon River (Israel) and possible influence on the marine environment. Water Sci Tech 27:439–447

Dean RT (1987) Free radicals, membrane damage and cell-mediated cytolysis. Br J Cancer 55:39–45

Doyotte A, Cossu C, Jacquin MC, Babut M, Vasseur P (1997) Antioxidant enzymes, glutathione and lipid peroxidation as relevant biomarkers of experimental or field exposure in the gills and the digestive gland of the freshwater bivalve *Unio tumidus*. Aquat Toxicol 39:93–110

Dubinsky Z, Falkowski GP, Post AF, van Hes UM (1987) A system for measuring phytoplankton photosynthesis in a defined light field with an oxygen electrode. J Plank Res 9: 607–612

Eertman RHM, Wagenvoort AJ, Hummel H, Smaal AC (1993) "Survival in air" of the blue mussel *Mytilus edulis* L. as a sensitive response to pollution-induced environmental stress. J Exp Mar Biol Ecol 170:179–195

Fiedurek J, Gromada A (1997) Selection of biochemical mutants of *Aspergillus niger* with enhanced catalase production. Appl Microbiol Biotech 47.313–316

Fridovich I (1978) The biology of oxygen radicals. Science 201: 875–880

Halliwell B (1994) How to characterize an antioxidant: an update. Biochem Soc Symp 61:73–101

Hassan HM, Fridovich I (1979) Intracellular production of superoxide radicals and H_2O_2 by redox active compounds. Arch Biochem Biophys 196:385–395

Herut B, Hornung H, Krom MD, Kress N, Cohen Y (1993) Trace metals in shallow sediments from the Mediterranean coastal region of Israel. Mar Pollut Bull 26:675–682

Herut B, Hornung H, Kress N, Cohen Y (1996) Environmental relaxation in response to reduced contaminant input: the case study of mercury pollution in Haifa Bay, Israel. Mar Pollut Bull 32:366–373

Herut B, Hornung H, Kress N (1997) Long-term record mercury decline in Haifa Bay (Israel) shallow sediments. Fresenius Environ Bull 6:48–53

Herut B, Shoham-Frider E, Kress N, Fiedler U, Angel DL (1998a) Hydrogen peroxide production rates in clean and polluted coastal marine waters of the Mediterranean, Red and Baltic Seas. Mar Pollut Bull 36:994–1003

Herut B, Hornung H, Kress N, Cohen Y (1998b) Monitoring of heavy metals along the Mediterranean coast of Israel in 1997 (in Hebrew). Israel Oceanographic and Limnological Research Rep H18/98

Herut B, Tibor G, Yacobi YZ, Kress N (1999) Synoptic measurements of chlorophyll-a and suspended particulate matter in a transitional zone from polluted to clean seawater utilizing airborne remote sensing and ground measurements, Haifa Bay (SE Mediterranean). Mar Pollut Bull 38:762–772

Herut B, Kress N, Shefer E, Hornung H (1999) Trace element levels in mollusks from clean and polluted coastal marine sites in the Mediterranean, Red and North Seas. Helgol Mar Res 53: 154–162

Hornung H, Krom MD, Cohen Y (1989) Trace metal distribution in sediments and benthic fauna of Haifa Bay, Israel. Estuarine Coastal Shelf Sci 29:43–56

Jahn A (1997) Oekophysiologische Untersuchungen an *Macoma balthica* (Bivalvia) und *Cyprideis torosa* (Ostracoda) oder Anpassungen an den Umweltfaktor Schwefelwasserstoff. Ber IfM 289

Kress N, Herut B (1998) Hypernutriphication in the oligotrophic eastern Mediterranean. A study in Haifa Bay, Israel. Estuarine Coastal Shelf Sci 46:645–656

Kress N, Hornung H (1996) Disposal of sewage sludge at the continental shelf of Israel. Fresenius Environ Bull 5:141–147

Krishnakumar PK, Casillas E, Varanasi U (1997) Cytochemical responses in the digestive tissue of *Mytilus edulis* complex exposed to microencapsulated PAHs or PCBs. Comp Biochem Physiol 118C:11–18

Lemaire P, Viarengo A, Canesi L, Livingstone DR (1993) Pro-oxidant and antioxidant processes in gas gland and other tissues of cod (*Gadus morhua*). J Comp Physiol 163:477–486

Livingstone DR, Lips F, Martinez PG, Pipe RK (1992) Antioxidant enzymes in the digestive gland of the common mussel *Mytilus edulis*. Mar Biol 112:265–276

Malins DC, Haimanot R (1990) The etiology of cancer: hydroxyl radical induced DNA lesions in histologically normal livers of fish from a population with liver tumors. Aquat Toxicol 20:123–130

Malins DC, Haimanot R (1991) 4,6-Diamino-5-formamido-pyrimidine, 8-hydroxyguanine and 8-hydroxyadenine in DNA from neoplastic liver of English sole exposed to carcinogens. Biochem Biophys Res Commun 173:614–619

Marklund S, Marklund G (1974) Involvement of the superoxide anion radical in the autoxidation of pyrogallol and a convenient assay for superoxide dismutase. Eur J Biochem 47: 469–479

Moffett JW, Zafiriou OC (1990) An investigation of hydrogen peroxide chemistry in surface waters of Vineyard Sound with $H_2{}^{18}O_2$ and $^{18}O_2$. Limnol Oceanogr 35:1221–1229

Moore CA, Farmer CT, Zika RG (1993) Influence of the Orinoco River on hydrogen peroxide distribution and production in the eastern Caribbean. J Geophys Res 98:2289–2298

Morgan RW, Christman MF, Jacobson FS, Storz G, Ames BN (1986) Hydrogen-peroxide inducible proteins in *Salmonella typhimurium* overlap with heat shock and other stress proteins. Proc Natl Acad Sci USA 83:8059–8063

Naclerio G, Baccigalupi L, Caruso C, de Felice M, Ricca E (1995) *Bacillus subtilis* vegetative catalase is an extracellulaar enzyme. Appl Environ Microbiol 61:4471–4473

Otto D (1997) Polychlorinated biphenyl (PCB) alterations of antioxidant defenses and xenobiotic metabolism in teleost fish: role of tissue glutathione metabolism in xenobiotic detoxication pathways. Diss Abst Int Pt B Sci Eng 58:2998

Pamatmat MM (1988) Catalase activity and the hydrogen peroxide cycle. Arch Hydrobiol Beih 31:107–114

Pamatmat MM (1990) Catalase activity: a variable affecting H_2O_2 distribution in Kiel Bight. Helgol Meeresunters 32:261–275

Pedrajas JR, Lopez-Barea J, Peinado J (1996) Dieldrin induces peroxisomal enzymes in fish (*Sparus aurata*) liver. Comp Biochem Physiol 115C:125–131

Petasne RG, Zika RG (1997) Hydrogen peroxide lifetimes in south Florida coastal and offshore waters. Mar Chem 56:215–225

Porte C, Sol M, Albaigs J, Livingstone DR (1991) Responses of mixed-function oxygenase and antioxidase enzyme system of *Mytilus* sp. to organic pollution. Comp Biochem Physiol 100C:183–186

Reddy PS (1997) Modulations in antioxidant enzymes in the gill and hepatopancreas of the edible crab *Scylla serrata* during exposure to cadmium and copper. Fresenius Environ Bull 6:589–597

Ribera D, Narbonne JF, Michel X, Livingstone DR, O'Hara S (1991) Responses of antioxidants and lipid peroxidation in mussels to oxidative damage exposure. Comp Biochem Physiol 100C:177–181

Richardson S (1994) The war on radicals. Discover 15:27–28

Sole M, Porte C, Albaiges J (1995) Seasonal variation in the mixed-function oxygenase system and antioxidant enzymes of the mussel *Mytilus galloprovincialis*. Environ Toxicol Chem 14:157–164

Szymczak R, Waite TD (1988) Generation and decay of hydrogen peroxide in estuarine waters. Aust J Mar Freshwater Res 39: 289–299

Szymczak R, Waite TD (1991) Photochemical activity in waters of the Great Barrier Reef. Estuarine Coast Shelf Sci 33:605–622

Winston GW (1991) Oxidants and antioxidants in aquatic animals Comp Biochem Physiol 100C:173–176

Winston GW, Moore MN, Kirchin MA, Soverchia C (1996) Production of reactive oxygen species by hemocytes from the marine mussel, *Mytilus edulis*: lysosomal localization and effect of xenobiotics. Comp Biochem Physiol 113C:221–229

Xenopoulos MA, Bird DF (1997) Effect of acute exposure to hydrogen peroxide on the production of phytoplankton and bacterioplankton in a mesohumic lake. Photochem Photobiol 63.471–478

Zika RG, Moffett JW, Petasne RG, Cooper WJ, Saltzman ES (1985) Spatial and temporal variations of hydrogen peroxide in Gulf of Mexico waters. Geochim Cosmochim Acta 49:1173–1184

Communicated by H.v. Westernhagen