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Effects of Sublethal Zn^{++} and Cd^{++} Concentrations on Filtration Rate, Absorption Efficiency and Scope for Growth in *Donax trunculus* (Bivalvia; Donacidae)

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Abstract Physiological parameters (filtration rate measured as clearance rate; absorption efficiency measured as Conover index) and energy balance [scope for growth (SFG)] where determined under Zn++ (essential metal) and Cd++ (nonessential metal) at different concentration levels and timing. The toxic effect observed on *Donax trunculus* was higher for Cd++ than for Zn++ in almost all the parameters studied. The SFG is proposed as an integrated environmental biomarker to be used like a tool with others from different organization levels to obtain a more comprehensive vision of the ecological consequences of pollutants.

Keywords *Donax trunculus* · Molluscs · Bivalves · Filtration rate · Heavy metals · Biomarkers · Scope for growth

Biomonitoring by biological responses or biomarkers is used for assessing contaminant presence in the environment as a complementary approach to chemical monitoring; being biomarkers an important key for environmental monitoring assessment programs (Connell et al. 1999; Moore et al. 2004; Neuberger-Cywiak 2005). The responses measured in living organisms and the information generated, together with physical and chemical environmental studies and ecological issues; are important tools applicable to management, environmental impact assessment, and to protection and conservation programs. Responses can be measured at different biological organization levels, being at the organism level where the disturbance can generate impairment growth, behavior alterations, and reproduction damage, among others. For example, pollutant presence in the environment and bioavailability in the organisms could promote birth rate reduction and/or somatic growth rate (production rate) reduction in the organism, with a consequent population growth rate decrease, affecting the studied population life cycle with all the future implications at ecosystem levels (Cormier and Danie 1994; Walker et al. 2001).

Widdows (1991) indicated that the animal's energy budget represents an integration of basic physiological responses (feeding, food absorption, respiration, excretion and production). The energy available for growth and reproduction can be useful in assessing the effects of biological pollution. When production is estimated by the difference between energy gains or intake (energy absorbed from the food) and energy losses or metabolic losses (energy expenditure via respiration and excretion), it is referred as the "scope for growth" (SFG) (Widdows 1985, 1991). This relevant physiological response is one of the four physiological stress indexes proposed by Widdows (1985). The SFG is a useful stress index integrating the whole organism's response to total environmental stimuli including both natural and anthropogenic stressors (Widdows 1985).

The bivalve *Donax* has been used as a sentinel organism for chemical contamination in sandy beach ecosystems and

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has been considered a pollution bioindicator. Different biomarkers are studied at diverse biological organization levels (Fishelson et al. 1999; Moukrim et al. 2004; Neuberger-Cywiak et al. 2005). The aim of this study was to determine the clearance rate, absorption efficiency (AE) and SFG relevance in the *Donax trunculus* physiological energy measurements on individuals exposed to Zn^{++} and Cd^{++} pollutant, with the purpose of implementing their use and the SFG as a complementary biomarker and as a tool for environmental studies.

Materials and Methods

Physiological measurements were performed on *D. trunculus* clams (24–30 mm shell length) collected from an unpolluted area in Akko (Israel) and kept at Bar Ilan's University laboratories (Neuberger-Cywiak et al. 2005). The effect of zinc and cadmium ions (introduced as $ZnCl_2$ and $CdCl_2$) on the feeding activity (estimated by the clearance rate); food AE (Conover ratio) and SFG were studied on static systems without water replacement, after the clams were exposed to each metal for 24 and 48 h periods at 0.1, 1.0 and 10 ppm ion concentration and control (Neuberger-Cywiak et al. 2005).

The SFG and its final calculation were based on Widdows and Salked (1993). Clearance rate (CR), defined as the volume of water cleared of suspended particles per hour, was employed to determine the feeding rate which was measured in terms of algal cell depletion in the water column. Cultures of *Isochrysis galbana* were used in this study. Clams were exposed to the contaminant for 24 and 48 h as indicated by Neuberger-Cywiak et al. (2005). The CR was determined for nine individual organisms in each treatment (0.1, 1, 10 ppm of Zn^{++} and Cd^{++} independently and a control at 24 and 48 h, a total of 126 individuals) that where kept individually in 500 mL beakers filled with filtered sea water (0.22 μm) and aerated gently by bubbling. Initial algae concentration was about 35×10^4 cell mL^{-1} to quantify accurately the algae concentration by optical density-turbidity technique (Sorokin 1975; $\%T$ at 438 nm vs. number of cell per mL; $r^2 = 0.9957$; 4040 spectrophotometer LKB NovaspecII, Biochrom). The counting of algae was determined microscopically in lugol fixed samples observed in a Neubauer chamber. After 15 min, when bivalves opened their shell valves and resume pumping, algae were added, waiting 2 min to uniform the algae concentration in the container. This was considered the initial time (T_0) and the initial concentration (C_0). Prior experiences at the laboratory (unpublished data) indicate that at this C_0, no inhibition of CR and pseudofaeces production was observed at control conditions. Four readings at 30 min intervals over a 2 h period were performed. The CR was only determined in organisms that opened their valves or extended their siphons. To avoid circadian rhythm effect on the filtration rate (Newell 1979), the determinations were performed at the same daily timing. The CR of individual clams was calculated through the Coughlan (1969) equation. The maximum CR was considered on 1 h period as the greatest cell concentration decline observed, avoiding valve closure. After the CR was determined, the shell length and dry tissue weight of each clam was recorded. Body tissue was dissected carefully from the shell and dried to constant weight at 90°C. The CR was corrected to a "standard body size" (0.1 g dry weight).

The food AE was determined for ten organisms exposed at each treatment as CR, specified before. Faeces were collected with a pipette from the 10–250 mL-beaker containing individual clams at the different treatments filled with filtered sea water (0.2 μm) and food (*I. galbana*). Bivalves had been held for 5 h in these conditions allowing gut contents to be evacuated, then faeces samples were collected and filtered in pre-treated 4.5 cm GFC filter (washed with distilled water, burned −450°C for 4 h and pre-weighed), washed with 0.5 M ammonium formate to remove salts without inducing osmotic stress in the algae cells (Widdows and Salked 1993), placed the filters over 110°C for 24 h, weighed, and then burned in a furnace at 450°C for 6 h to obtain ash free dry weight of the sample and calculate the weight of organic material combusted. Two blank GFC filters were performed to correct weigh changes The calculations of AE were done as indicated by Conover (1966): $AE = (f - e) \times 100/(1 - e) \times f$; where AE = assimilation efficiency, f = organic fraction of food, and e = organic fraction of faeces.

For SFG calculation, the physiological measurement values such as respiration rate and excretion rates were obtained from previous studies (Neuberger-Cywiak et al. 2005). Following Widdows and Salked (1993) recommendations, the physiological responses were converted into energy equivalents ($J\ h^{-1}$) and used in the balanced energy equation in order to calculate the energy available for growth and reproduction. The energetic value of the algae was determined following biochemical analyses (Mizrahi and Achituv 1990) of carbohydrate, lipids and proteins and converted to energy values using the enthalpy of combustion values of 17 KJ/g glycogen; 39.5 KJ/g lipids; and 24 KJ/g proteins. The energetic value of the algae obtained in this study was 24.4 J/mg dw; being in the order of the energy content of particulate organic matter or algae food (Widdows and Salked 1993; Savina and Pouvreau 2004). Employing also the dry weight value of 19 pg/cell (Savina and Pouvreau 2004) and the depletion in the cell number in time (CR), the energy consumed or ingested (C) was calculated. The

energy absorbed from the food ($A = C \times AE$), energy respired (R), energy lost as excreta (U) and finally, SFG, was calculated following Widdows and Salked (1993) indications, where the physiological responses are converted into energy equivalents (J h^{-1}) and introduced in the SFG equation: $P = A - (R + U)$.

The statistical analysis was carried out using the Univariate General Lineal Model (GLM) procedure of the SPSS® package. The two-way analysis of variance (ANOVA) was conducted on CR and combinations of the independent variables time, concentrations and metals. A Dunnet's T3 Post Hoc multiple comparisons between the treatment and with the control was performed for heterocedastic data and Tukey's test in homoscedastic data (Zar 1999; SPSS® 2003).

Results and Discussion

The CR was differentially affected by metal and time exposure. It presented a decreasing tendency with increasing Zn^{++} (essential metal) and Cd^{++} (not essential) concentrations in organisms exposed to the pollutant for 24 and 48 h (Table 1).

At 24 h exposure to both metals, significant differences in the CR value were observed at 0.1 ppm, being the CR lower in organisms exposed to Cd^{++} ($\alpha' = 0.05$) (Fig. 1). CR response decreased about 60% for Cd^{++} and 25% for Zn^{++} (Table 1). At 1 ppm, no statistical significant differences were observed but the results shown a decrease tendency higher for Cd^{++} exposure than for Zn^{++} (62.96% and 77.4%, respectively) (Fig. 1, Table 1). At 10 ppm concentrations, the CR was less than 7% in both cases (Table 1). Organisms exposed to Zn^{++} for 24 h presented significant differences in CR values among the control and 10 ppm (Post hoc Dunnet's T3,$\alpha' = 0.05$) despite for Cd^{++} where significant differences were observed additionally between 10 and 0.1 ppm; and also between 10 and 1 ppm (Post hoc Dunnet's T3, $0.001 \leq \alpha \leq 0.01$).

At 48 h exposure to Cd^{++} and Zn^{++}, no significant differences in the CR were observed at 0.1 ppm (82.90% and 80.19%, respectively) At 1 ppm of contaminant, a tendency decrease in the CR was observed in organisms exposed to Cd^{++} rather than to Zn^{++} (45% and 40.74%, respectively). For both metals at 10 ppm metal concentration, the CR decreased below 9% (Fig. 1, Table 1). Organisms exposed to Zn^{++} for 48 h presented significant differences in CR values among 10 ppm and all the concentrations including the control (48 h Post hoc Dunnet's T3, $0.001 \leq \alpha \leq 0.01$). In organisms exposed to Cd^{++} for 48 h, addition significant difference was found between the control and 1 ppm Cd^{++} (Post hoc Dunnet's T3, $\alpha' = 0.01$).

Its important to point out that in our results a significant difference was observed in relation to time exposure for the CR at 0.1 ppm Cd^{++} exposure and 1 ppm Zn^{++} exposure ($\alpha' = 0.05$; Fig. 2)

The CR drop observed at 24 h can be related to the organism's first defense system: the closing of its siphons as a reaction to a stress substance and the avoidance of contact with it (Kraak et al. 1994; Neuberger-Cywiak et al. 2003). Second, ciliary activity of the gills can also be affected, and subsequently the CR. Studies performed on ciliary activity measured under metal exposure of gill tissue, showed an increase in dopamine content of the visceral ganglia of *M. edulis* followed by copper exposure, suggesting that the dopaminergic nervous systems may play a role in the ciliary activity (Smith 1985). Moreover, Sunila and Lindstrom (1985) studied the uncoupling by copper and cadmium exposures in the structure of the mussels interfilamentary junction, showing the break and sliding away of ciliary disks upon exposure to copper and cadmium. Also, the pronounced effect of Cd^{++} over Zn^{++} in the filtration rate, measured by the CR, can be supported by the differential inhibition gill Ca ATPasa in the order $Hg^{++}>Pb^{++}>Cu^{++}>Cd^{++}>Zn^{++}$ (Viarengo et al. 1993).

The AE, based on internal metabolic and cellular processes, is not affected by two of the sub-lethal concentrations of Zn^{++} at 24 and 48 h exposure; only at 10 ppm concentration a decrease was observed (Table 1). The effect of 10 ppm Zn^{++} on the AE was higher at 24 h (63% of the control value) rather than 48 h (88% of the control value). In contrast, for 24 h Cd^{++} exposure, a small decrease in the AE was observed with an increase in concentrations (0.1 and 1 ppm), but at 10 ppm this parameter was impossible to measure due to the high quantity of pseudofaeces in the sample (Table 1). Organisms exposed to 0.1 and 1 ppm Cd^{++} for 48 h presented a different response on the AE, being at 0.1 ppm, 60% of the control AE value and at 1 ppm, 50%. Also, the high quantity of pseudofaeces impaired the determination of the AE value at 10 ppm Cd^{++} for 24 and 48 h exposure (Table 1). The Conover (1966) ratio method represents the organic material AE coming from ingested food material, assuming that only the organic food compound is significantly affected by the digestive process and compares the proportion of organic matter in food and faeces.

The absorbed energy intake, related with the feeding process and the AE, is used for basal metabolic rate, detoxification energy cost, synthesis metabolic cost, production (measured as its effect on SFG) and excreta, providing the input energy necessary for anabolism (growth) and catabolism (respiration and excretion) (Widdows and Salked 1993; Apeti et al. 2005). In normal conditions, bivalves keep their valves slightly open and their siphons extended most of the time for respiration and

Table 1 Effect of Zn and Cd ions at different concentrations on clearance rate (CR%, % related to control), absorption efficiency (AE, %), energy consumed (*C*, J 0.1 g^{-1} h^{-1}); energy respired (*R*, J 0.1 g^{-1} h^{-1}); energy excreted (*E*, J 0.1 g^{-1} h^{-1}) and scope for growth (SFG, J 0.1 g^{-1} h^{-1}) on *D. trunculus* exposed to each metal during 24 and 48 h

ppm Zn^{++}	CR (%)	AE	*C*	*R* [a]	*E* [a]	SFG
24h						
0	100	88.43	24.052 ± 13.806	3.597 ± 1.133	0.0893 ± 0.027	17.583
0.1	85.00	89.32	20.441 ± 11.577	2.424 ± 0.964	0.127 ± 0.690	15.706
1	22.60	89.11	5.442 ± 4.682	0.871 ± 0.981	0.113 ± 0.555	3.866
10	4.32	55.93	1.036 ± 0.846	0.478 ± 0.329	0.0230 ± 0.099	0.079
48h						
0	100	83.11	21.650 ± 6.030	4.047 ± 1.113	0.144 ± 0.662	13.803
0.1	80.19	84.21	17.3655 ± 3.645	2.738 ± 1.726	0.125 ± 0.257	11.761
1	59.26	82.64	12.838 ± 6.013	1.323 ± 0.767	0.184 ± 0.390	9.103
10	2.56	73.55	0.5547 ± 0.483	0.659 ± 0.407	0.0440 ± 0.081	−0.296
Cd^{++}						
24 h						
0	100	87.43	24.0525 ± 13.806	3.5971 ± 1.133	0.0534 ± 0.152	17.379
0.1	42.32	80.51	9.9355 ± 2.194	1.7385 ± 0.800	0.0577 ± 0.199	6.203
1	37.04	78.15	8.9161 ± 2.631	1.2947 ± 0.565	0.0697 ± 0.140	5.604
10	6.17	[b]	1.4860 ± 1.002	0.4784 ± 0.329	0.0116 ± 0.048	[b]
48h						
0	100	83.43	21.6507 ± 6.030	4.0470 ± 1.101	0.1219 ± 0.283	13.894
0.1	82.90	49.87	17.9530 ± 9.434	1.4128 ± 0.726	0.1222 ± 0.385	7.418
1	45.00	41.08	9.7454 ± 2.453	0.6290 ± 0.382	0.1004 ± 0.159	3.274
10	8.56	[b]	1.8489 ± 1.555	0.1363 ± 0.121	0.0177 ± 0.071	[b]

Values are mean ± SD

[a] Reference: Neuberger-Cywiak et al. (2005)

[b] High quantity of pseudofaeces on the sample, not determined

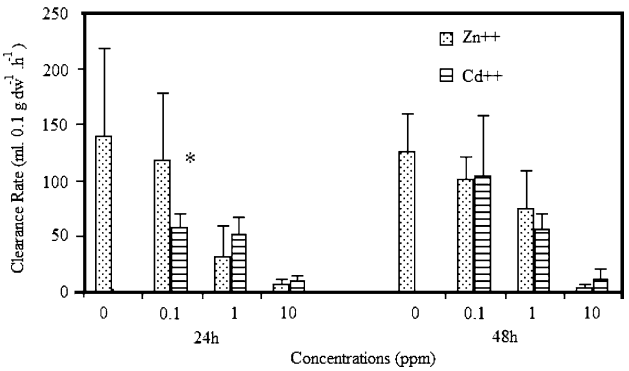

Fig. 1 Effect of Zn^{++} and Cd^{++} (10, 1, 0.1 ppm and control 0 ppm) on clearance rate (mL 0.1 g tissue dry wt^{-1} h^{-1}) in *D. trunculus* after 24 and 48 h exposure to metal. Each *bar* represents the mean value ± SD. *Asterisks* means statistically different between both bars. $\alpha' = 0.05$ (n = 9)

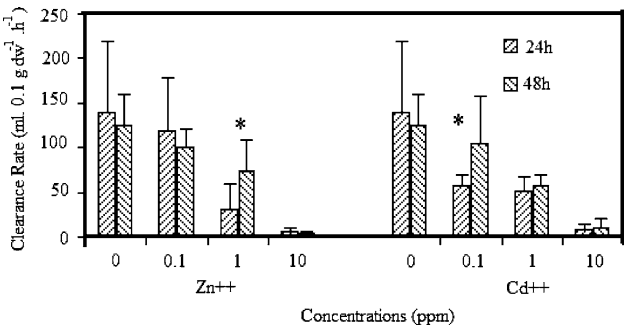

Fig. 2 Effect of time exposure to Zn^{++} and Cd^{++} (10, 1, 0.1 ppm and control 0 ppm) on clearance rate (mL 0.1 g tissue dry wt^{-1} h^{-1}) in *D. trunculus*, after 24 and 48 h exposure. Each *bar* represents the mean value ± SD *Asterisks* means statistically different between both bars. $\alpha' = 0.05$ (n = 9)

feeding, and close and retract their siphons under stress for an extended period of time. In the presence of contaminants or other stressors such as Cd^{++} and Zn^{++} used in this study, it has been shown that almost all bivalves closed their valves and retracted their siphons; hence the filtration rate (measured in this experiment as CR) fell to zero (Micallef and Tyler 1990; Kraak et al. 1994). Neuberger-Cywiak et al.

(2003, 2005) pointed out that this behavior was observed in *D. trunculus*, suggesting that in this way, the organism avoided contact with the stressor, being one of the first strategies to keep it away. But this behavior also causes secondary effects, like reducing the energy input as a direct consequence of the filtration rate observed in this study, and also in the respiration rate and ammonia excretion (Neuberger-Cywiak et al. 2005). The energy consumed by an organism is closely related to the AE necessary to obtain the energy input or energy absorbed from the food, which is equal to energy consumed × AE. This is an important value in the SFG calculations. We observed a decrease in the CR with an increase in the essential metal Zn^{++}, but the AE was not affected at 0.1 and 1 ppm, treating to compensate the energy needs. Only at 10 ppm Zn^{++} a decrease in the AE was observed being this concentration value at the inferior limit of LC_{50} at 48 h (Neuberger-Cywiak et al. 2003). In contrast, organism's exposures to the nonessential metal Cd^{++} showed a decrease in the CR and also the AE was reduced drastically at 48 h exposures at 0.1 and 1 ppm. Under these conditions, the energy absorbed from the food, used in metabolic process, is reduced as a possible result of internal impairments and behavioral patterns. The assumption that metabolic homeostasis was disrupted under these conditions was corroborated by Neuberger-Cywiak et al. (2003, 2005). Metal toxicity appears to be related to the amount of free metal which interacts with cell structures (oxidative stress) and/or enzymes, thus affecting metabolic pathways (Narbonne and Michel 1993). Baillieul et al. (2005) found in *Daphnia magma* studies that cadmium stress decreases energy assimilation and also disturbs energy metabolism. When integrating the changes in CR and AE in exposed organisms under laboratory controlled conditions with the cellular mechanisms observed in the field, it's important to point out that in natural conditions, the AE can be related with the amount of food available (much more complex in composition), and can also fluctuate with time and interact with environmental factors. Results obtained must be used with caution always relating them with the physiological activities and comparing them to control references data (Axiak 1991).

Bayne (1989) showed the biological effects of pollution under the mussel watch approach, indicating the relevance of biomarker studies at different levels of functional complexity. Biochemical and cellular responses are the first effects of potential toxic compounds taken up by organisms and physiological responses are those observed in the whole organism as a direct response to them. The pronounced reduction in SFG observed in organisms exposures to Cd^{++} (0.1 and 1 ppm) and Zn^{++} (1 and 10 ppm) (Table 1) indicates severe stress, suggesting that these individuals are consuming bodily reserves for survival. At 10 ppm Cd ion, the determination of SFG was not viable as discussed before. Our results show that AE plays an important role in the SFG by the energy absorbed from food. Only a partial decrease in the SFG can be attributed to a decrease in CR or energy intake (Smolders et al. 2002; Baillieul et al. 2005). The SFG, an integrated index, presents the metabolic responses of the total energy balance. At 0.1 ppm Zn^{++} concentration, a very small effect on the SFG was observed in relation to the control but for Cd^{++} at the same concentration and time, a sharper decrease was observed (Table 1). A small recovery in the SFG was detected in organisms exposed to 1 ppm Zn^{++} in time that was not observed with Cd^{++}. It is well documented that Zn^{++} is an essential trace metal in bivalve physiology (Walker et al. 2001).

Hawkins et al. (1986) suggested that in mussels, 30% of its normal energy demand is due to the turnover of its proteins. So, any process that increases the requirement of protein synthesis will reduce the energy available for growth and reproduction, raising the energy consumption use and detecting a decline in the SFG. Cellular damage, detoxification mechanisms and protein synthesis (metalloproteins, stress proteins), generally consume energy and other resources reducing its chance of death, but are therefore denied to production, so, the organism trades off a loss of production for a reduction in mortality rate (Walker et al. 2001). Smolders et al. (2004) proposed that the reduction in energy uptake, as a result of toxicant-induced stress in *D. magma*, could be linked to a reduction in available energy reserves at the cellular level of biological organization. This relationship could possibly link energy-based sub-organismal effects with those emerging at higher levels of biological organization.

From our results we can conclude that the use as biomarkers of physiological parameters like CR, AE, R, U alone, provides a small picture of the real spectrum. They do not integrate the organism compensatory metabolic mechanisms involved influenced, among others, by behavioral responses, and do not correspond to a representative ecological feature. The determination of the SFG index, a non-specific biomarker for environmental pollution, offers an integrated measurement of the health and "well being" of the organisms, it is clearly effective in detecting sub-lethal impact of a pollutant (Bayne 1985; Narbonne and Michel 1993). The lack of energy for growth and reproduction represented in a decrease in the SFG on a significant number of organisms, will affect population fitness, survival in the area, chances of survival in the ecosystem and further alterations at the community level and the ecosystem could be observed. In conclusion, SFG is proposed as an environmental biomarker to be used with others biomarkers from different organization levels to obtain a more comprehensive vision of the ecological consequences of pollutants.

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References

Apeti DA, Johnson E, Robinson L (2005) A model for bioaccumulation of metals in *Crassostrea virginica* from Apalachicola Bay, Florida. Am J Environ Sci 1:239–248

Axiak V (1991) Sublethal toxicity test: physiological responses. In: Abel PD, Axiak V (eds) Ecotoxicology and marine environment. Ellis Horwood, Avon, pp 132–146

Baillieul M, Smolders R, Blust R (2005) The effect of environmental stress on absolute and mass-specific scope for growth in *Daphnia magma* Strauss. Comp Biochem Physiol C Toxicol Pharmacol 140(3–4):364–373

Bayne BL (1985) Cellular and physiological measures of pollution effects. Mar Pollut Bull 16:127–129

Bayne BL (1989) Measuring the biological effect of pollution: the mussel watch approach. Wat Sci Tech, vol 21, Brighton, pp 1089–1100

Connell D, Lam P, Richardson B, Wu R (1999) Introduction to ecotoxicology. Blackwell, London

Conover RJ (1966) Assimilation of organic matter by zooplankton. Limnol Oceanogr 11:338–354

Cormier SM, Danie FB (1994) Biomarkers: taking the science forward. Environ Toxicol Chem 13:1011–1012

Coughlan J (1969) The estimation of filtration rate from the clearance of suspensions. Mar Biol 2:356–358

Fishelson L, Bresler V, Manelis R, Zuk-Rimon Z, Dotan A, Hornung H, Yawetz A (1999) Toxicological aspects associated with the ecology of *Donax trunculus* (Bivalvia-Mollusca) in polluted environment. Sci Total Environ 226:121–131

Hawkins AJS, Bayne BL, Day AJ (1986) Protein turnover, physiological energetics an heterozygosity in the blue mussel, *Mytilus edulis*: the basis of variable age-specific growth. Proc R Sci Lond 229:161–176

Kraak MHS, Toussaint M, Levy D, Davids C (1994) Short-term effects of metals on the filtration rate of the zebra mussel *Dreissena polymorpha*. Environ Pollut 84:139–143

Micallef S, Tyler PA (1990) Effect of mercury and selenium on the gill function of *Mytilus edulis*. Mar Pollut Bull 21:288–292

Mizrahi L, Achituv Y (1990) Seasonal changes in body weight and biochemical components of Mediterranean *Chtamalus stellatus* (Poli) and *Euraphia depressa* (Poli). J Exp Mar Biol Ecol 137:185–193

Moore MN, Depledge MH, Redman JW, Paul Leonard DR (2004) An integrated biomarker-based strategy for ecotoxicological evaluation of risk in environmental management. Mut Res 552:247–268

Moukrim A, El Hamidi F, Lagbouri A, Kaaya A, Zekhnini A, Bouhaimi A, Narbonne JF (2004) Study of *Donax trunculus* as a sentinel species for environmental monitoring of sandy beaches on Moroccan coasts. Bull Environ Contam Toxicol 73:674–681

Narbonne JF, Michel X (1993) Use of biomarkers in the assessment of contamination in marine ecosystems, fundamental approach and application. MAP Technical Reports Series, vol 71. United Nations Environmental Programme, Athens, pp 1–20

Neuberger-Cywiak L (2005) La ecotoxicología como herramienta en las evaluaciones de riesgo ecológico y en las evaluaciones de impacto ambiental. In: CICTA, 6th Iberian and 3rd Iberoamerican congress of environmental contamination and toxicology, Cádiz

Neuberger-Cywiak L, Achituv Y, García EM (2003) Effects of zinc and cadmium on the burrowing behavior, LC_{50} and LT_{50} on *Donax trunculus* Linnaeus (Bivalvia: Donacidae). Bull Environ Contam Toxicol 70:713–722

Neuberger-Cywiak L, Achituv Y, Garcia E (2005) Sublethal effects of Zn++ and Cd++ on respiration rate, ammonia excretion, and O:N ratio of *Donax trunculus* (Bivalvia; Donacidae). Bull Environ Contam Toxicol 75:505–514

Newell RC (1979) Biology of intertidal animals. Marine Ecological Surveys LTD, Kent

Savina M, Pouvreau S (2004) A comparative ecophysiological study of two infaunal filter-feeding bivalves: *Paphia rhomboids* and *Glycymeris glycymeris*. Aquaculture 239:289–306

Smith JR (1985) Copper exposure and ciliary function in gill tissue on *Mytilus californianus*. Bull Environ Contam Toxicol 35:556–566

Smolders R, Bervoets L, Blust R (2002) Transplanted zebra mussels (*Dreissena polymorpha*) as active biomonitors in an effluent-dominated river. Environ Toxicol Chem 21:1889–1896

Sorokin C (1975) Dry weight, packed cell volume and optical density. In: Stein JR (ed) Handbook of phycological methods: culture methods and growth. Cambrige Univertsity Press, New York, pp 321–343

SPSS® (2003) 12.0 Command Syntax Reference. SPSS Inc., Chicago

Sunila I, Lindstrom R (1985) The structure of the interfilamentary junction of the mussels (*Mytilus edulis* L.) gill and its uncoupling by copper and cadmium exposures. Comp Biochem Phisiol 81C:267–272

Viarengo A, Mancinelli G, Pertica M, Fabri R, Orunesu M (1993) Effect of heavy metals On the Ca^{++}-ATPasa activity present in gill plasma-membrane of mussels (*Mytilus galloprovonciallis* Lam.). Comp Biochem Physiol 106C:655–660

Walker CH, Hopkin SP, Civil RM, Peakall DB (2001) Principles of exotoxicology. Taylor & Francis, London, p 309

Widdows J (1985) Physiological responses to pollution. Mar Pollut Bull 16:129–134

Widdows J (1991) Role of physiological energetics in ecotoxicology and environmental pollution monitoring. MAP Technical Reports Series, vol 69. United Nations Environmental Programme, Athens, pp 41–60

Widdows J, Salked P (1993) Practical procedures for the measurement of scope for growth. MAP Technical Reports Series, vol 71. United Nations Environmental Programme, Athens, pp 147–172

Zar JH (1999) Biostatistical analysis. 4th edn. Prentice Hall, Inc., New Jersey