TECHNIQUES AND STRATEGIES
Crustacea and heavy metal accumulation

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Abstract: Some of the special features of crusta-ceans, particularly of reproduction strategies, may be highly important for the interpretation of data from bioindicator studies using these organisms, and for the development of ecotoxicological endpoints. In order to set discharge effluent and surfacewater quality standards, chemical criteria have originally been developed and applied to natural water bod-ies for centuries. The use of physiological, cellular and biochemical effects, so-called biomarkers, has become attractive and useful for assessing the effects of environmental stressors on the sub-lethal level of biological systems. Physiological effects and toxicity of trace metals strongly depend on their intracellular localization and binding to organelles and ligands. Mitochondria are key intracellular targets for metal toxicity, which are very sensitive to metal exposures. Cadmium accumulation in mitochondria may result in serious disturbances of tissue energy balance and eventually cell death. Temperature can also increase the concentration of heavy metals in water due to their desorption. All these factors together may make sublethal/ subtoxic environments become lethal to aquatic animals. Possibility of chronic effects related with the onset of global warming (climate change) needs to be investigated.

Key words: heavy metals, Crustacea, climate changes, accumulation, bioindicators

Introduction

Zooplankton of the Tatra lakes have been studied since the middle of the 19th century, which later enabled the recognition of lake acidification and the assessment of its effect on the plankton com-munity of lake ecosystems. Crustacean zooplankton in larger lakes consisted of a limited number of species, with *Acanthodiaptomus denticornis* and *Daphnia longispina* dominating lakes in the forest zone, and Arctodiaptomus alpinus, *Cyclops abyssorum, Daphnia longispina, Daphnia pulicaria,* and *Holopedium gib-berum* dominating lakes in the alpine zone. *Cerodaph-nia quadrangula, Daphnia obtusa, Daphnia pulex*, and *Mixodiaptomus tatricus* occured in lakes with high concentrations of dissolved organic matter and in

strongly acidified waters. Anthropogenic acidification has caused drastic changes in both the chemistry and biology of the Tatra lakes (Horička 2006).

Heavy metals, like elements which constitute an important class of pollutants, have received the attention of researchers all over the world, mainly due to their harmful effects on living beings. Heavy metals occur, in atmosphere, basicaly in particulate form. Hence, the transfer of air borne particles to land or water surfaces by dry, wet and occult deposition constitutes the first stage of accumulation of atmo-spheric heavy metals. Dry deposition involves four distinct processes: gravitational settling, impaction, turbulent transfer and transfer by Brownian motion. Acidification of water not only reduces heavy metal scavenging but also remobilizes metals from the surface sediment layers (Shrivastav 2001).

Crustaceans differ from other living arthropods in two ways. They have two pairs of antennae, whereas all other arthropods have one pair ore none. Members of the class Branchiopoda are primarily found in fresh water. Members of the order Cladocera are called water fleas, their bodies are covered by a large carapace, and they swim using their second antennae, which they repeatedly thrust downward to create a jerky, upward locomotion. Members of the class Copepoda include some of the most abundant crustaceans. The first antennae are modified for swimming, and the abdomen is free of appendages.

Crustaceans are frequently used as bioindicators and biomonitors in various aquatic systems. One rea-son is that they are a very successful group of animals, distributed in a number of different habitats includ-ing marine, terrestrial and freshwater environments (Rinderhagen *et al.* 2000). They are thus interesting candidates for comparative investigations. Some of the special features of crustaceans, particularly of reproduction strategies, may be highly important for the interpretation of data from bioindicator studies using these organisms, and for the development of ecotoxicological endpoints.

Chemical criteria indicate contamination but cannot show biological or environmental damage

In order to set discharge effluent and surfacewater quality standards, chemical criteria have originally been developed and applied to natural water bodies for centuries. Relying on chemical criteria alone for assessing the status of surface water integrity can, in many instances inaccurately portray the biologi-cal and ecological condition of aquatic ecosystems (Adams 2002). With a great variety of biological assess-ment tools now available, an improved understand-

ing of contaminant effects on ecosystem structure and function, and an increased ability to interpret biological data is possible (Yoder and Rankin 1998). The best investigated stress protein family, hsp70, is commonly used as a marker which effectively integrates overall adverse effects on protein integrity, hence measures proteotoxicity. Its induction by heavy metals in a variety of species has been shown in numerous studies (Schramm *et al.* 1999; Kammenga *et al.* 2000). In order to set discharge effluent and surfacewater quality standards, chemical criteria have originally been developed and applied to natural water bodies for centuries. The use of physiological, cellular and biochemical effects, so-called biomarkers, has become attractive and useful for assessing the effects of environmental stressors on the sub-lethal level of biological systems.

The role of solar UV radiation in the ecology of alpine lakes

Solar ultraviolet radiation (UVR, 290–400 nm) is a crucial environmental factor in alpine lakes because of the natural increase of the UVR flux with elevation and the high water transparency of these ecosystems. The ecological importance of UVR, however, has only recently been recognized (Sommaruga 2001). Unlike the pattern observed in most lowland lakes, variability of UV attenuation in alpine lakes is poorly explained by differences dissolved organic carbon (DOC) concentrations, and depends mainly on optical characteristics (absorption) of the chromophoric dissolved organic matter (CDOM). Alpine aquatic organisms have developed a number of strategies to minimize UV damage. The widespread synthesis or bioaccumulation of different compounds that directly or indirectly absorb UV energy is one such strategy. Although most benthic and planktonic primary producers and crustacean zooplankton are well adapted to high intensities of solar radiation, heterotrophic protists, bacteria, and viruses seem to be particularly sensitive to UVR. Understanding the overall impact of UVR on alpine lakes would need to consider synergistic and antagonistic processes resulting from the pronounced climatic warming, which have the potential to modify the UV underwater climate and consequently the stress on aquatic organisms. Organisms living in alpine lakes have developed, among other strategies to minimize damage when exposed to solar UVR, the synthesis of compounds that directly or indirectly absorb UV energy, like melanin, carotenoids, scytonemin, and mycosporine-like amino acids (MAAs). These compouds have been found in different taxa of marine organisms ranging from bacteria to fish 34,36, and more recently in phytoplancton, benthic cyanobacteria, and in the copepod *Cyclops abyssorum tatricus* from a transparent alpine lake (Sommaruga and Garcia-Pichel 1999, Sommaruga *et al.* 1999). For example melanization of the dorsal portion of the carapace and the antennae is typically observed in *Daphnia* sp. from alpine, and high latitude lakes (Hebert and Emery 1990). Copepods from alpine lakes can also accumulate high amounts of carotenoids that give them the intense red appearance typical for alpine forms (Brehm 1938, Tartarotti *et al.* 1994).

The community structure of zooplancton in alpine lakes is relatively simple, with few dominant spieces. Thus, for example *Cyclops abyssorum tatricus* and *Arctodiaptomus alpinus* are the dominant copepods species found in alpine lakes of the Austrian Alps, while *Daphnia* sp. are in many lakes probably due to the introduction of Arctic char *Salvenilus alpinus*, and brown trout *Salmo trutta*.

Alpine lakes, though remote, are not always pristine environments as demonstrated by recent studies that report high concentrations of pollutants like polycyclic aromatic hydrocarbons, organochlorine compounds, and heavy metals in snow, water, sediments and fish. These pollutants result from regional and long-range atmospheric transports, and in some cases like in some organochlorines, their concentration can be enriched due to cold-condenstaion of volatile compounds.

Finally, the potential impact of UVR on these ecosystems needs to be assessed considering synergistic and antagonistic processess, which are expected to result from the pronounced climatic warming and the interaction with pollutants supplied by long-range atmospheric transport (Sommaruga 2001).

Tissue-specific accumulation of cadmium

Cadmium distribution was studied in different subcellular fractions of gill and hepatopancreas tissues of eastern oysters *Crassostrea virginica*. Oysters were exposed for up to 21 days to low sublethal Cd concentrations (25 g/l). Gill and hepatopancreas tissues were sampled and divided into organelle fractions and cytosol by differential centrifugation. Organelle content of different fractions was verified by activities of marker enzymes, citrate synthase and acid phosphatase for mitochondria and lysosomes, respectively. In both tissue types, there was a significant accumulation of cadmium in cytosol reaching 230–350 g/l protein. Among organelles, mitochondria were the main target for Cd bioaccumulation in gills (250–300 µg/l protein), whereas in hepatopancreas tissues, the highest cadmium accumulation occurred in lysosomes (90–94 µg/l protein). Although 75–83% of total cadmium burden was associated with the cytosol reflecting high volume fraction of this compartment, Cd concentrations in organelle fractions reached levels that could cause dysfunction of mitochondria and lysosomes. Organ- and organelle-specific patterns of cadmium bioaccumulation support our previous in vivo studies, which showed adverse effects of cadmium exposures on mitochondrial oxidation in gills and on the lysosomal system of hepatopancreas. This may have important implications for the development of biomarkers of effect for heavy metals and for understanding the mechanisms of toxic effects of metals. At high concentrations, cadmium is extremely toxic to aquatic organisms, but even low levels may adversely affect their physiology (Viarengo 1994; Stohs and Bagchi 1995; Sokolova 2004; Sokolova *et al.* 2004). Adverse effects on physiological and cellular processes are observed at low, environmentally realistic concentrations (Auffret *et al.* 2002; Sokolova 2004; Sokolova *et al.* 2004, Sokolova *et al.* 2005a,b). Other key intracellular compartments involved in Cd accumulation are lysosomes and the cytoplasm. Lysosomes are an important organelle in which metals are sequestered in mollusks, especially in lysosomal-rich hepatopancreas tissues (Marigomez *et al.* 2002). Accumulated metals can be incorporated into lysosome-derived insoluble granules and either

stored intracellularly or excreted from the cell and thus detoxified (Marigomez et al. 2002). Therefore, localization of the accumulated cadmium into lysosomal fractions can function as a detoxification mechanism alleviating potential toxic effects on oyster physiology. In the cytoplasm, metallothioneins (MT), which are low molecular weight, cysteine-rich metalbinding proteins, constitute another important detoxification mechanism that can serve to minimize the availability of metal ions to cytosolic components (Roesijadi 1994). Recent studies have suggested that MTs may also be involved in transfer of metals to lysosomes thus serving as a link between the cytosolic and lysosomal systems of metal sequestration. However, toxicity is observed when lysosomal or MT detoxification systems are overwhelmed, which causes destabilization of lysosomal membranes, reduced ATPase function, and interactions of free metal ions with essential enzyme systems (Regoli 1992, Roesijadi 1994, Klaassen et al. 1999, Ringwood et al. 2002).

Clearly, more studies are required to investigate the dynamics of cadmium accumulation in different organelles, which is a key in understanding of the mechanisms of toxicity of this metal.

In general, these results suggest that the actual concentrations of cadmium associated with key intracellular organelles could be more important than % of the total load accumulated in each fraction. The percent of cadmium load associated with different intracellular compartments may reflect the relative volume of these compartments rather than physiologically relevant concentrations of cadmium. Indeed, in this study cytosolic cadmium accounted for 75–83% of the total tissue cadmium load, and less than 10% of total cadmium was associated with each of the organelle fractions. In summary, this study showed that exposure of oysters to low sublethal amounts of cadmium resulted in accumulation of high levels of this metal in organelle fractions as well as in cytoplasm. In gill tissues, mitochondria were the primary target organelle for bioaccumulation, which agrees with the results of our previous studies showing that mitochondrial dysfunction is an early toxic event in gills of Cd-exposed animals (Sokolova 2004; Sokolova et al. 2005a,b). In hepatopancreas, lysosomes were the primary target for bioaccumulation, supporting results of our previous work showing high sensitivity of hepatopancreas lysosomes to metals (Ringwood et al. 2002). It is likely that this pattern of predominantly mitochondrial effects in gills and lysosomal effects in hepatopancreas tissues will be observed with other metals or pollutants in other species.

Synergism between environmental stressors

Environmental stress often results in a reduction of net energy balance due to a reduction in assimilation of energy and/or its conservation in the form of ATP and other high-energy phosphates ('supplyside effects'), increases in basal metabolic demands ('demandside effects') or a combination of both (Koehn and Bayne 1989; Baird et al. 1990). The resulting energy deficit can have adverse effects on survival and performance of organisms and on the long-term persistence of their populations in the stressful environment. To date, case studies analyzing stressinduced changes of energy budgets have mostly focused on single environmental stressors (Widdows 1978, Dorigan and Harrison 1987, Koehn and Bayne 1989, Lee et al. 2006), whereas effects of multiple stressors are not well understood. Because multiple stresses often have non-additive effects on physiology, analysis of environmentally relevant combinations of stressors is important to obtain a realistic picture of the impact of stress on animal bioenergetics in nature. Trace metals (such as cadmium) and temperature are common stressors in estuaries, and their importance is increasing due to global climate change. In order to understand the role of metabolic regulation in environmental stress tolerance, a comprehensive analysis of demand-side effects (i.e. changes in energy demands for basal maintenance) and supply-side effects (i.e. metabolic capacity to provide ATP to cover the energy demand) of environmental stressors is required. Cherkasov et al. (2006) were studied the effects of temperature (12, 20 and 28°C) and exposure to a trace metal, cadmium (50 g/l), on the cellular energy budget of a model marine poikilotherm, *Crassostrea virginica* (eastern oysters), using oxygen demand for ATP turnover, protein synthesis, mitochondrial proton leak and non-mitochondrial respiration in isolated gill and hepatopancreas cells as demand-side endpoints and mitochondrial oxidation capacity, abundance and fractional volume as supply-side endpoints. Cadmium exposure and high acclimation temperatures resulted in a strong increase of oxygen demand in gill and hepatopancreas cells of oysters. Cd induced increases in cellular energy demand were significant at 12 and 20°C but not at 28°C, possibly indicating a metabolic capacity limitation at the highest temperature. Elevated cellular demand in cells from Cd exposed oysters was associated with a 2–6-fold increase in protein synthesis and, at cold acclimation temperatures, with a 1.5-fold elevated mitochondrial proton leak. Cellular aerobic capacity, as indicated by mitochondrial oxidation capacity, abundance and volume, did not increase in parallel to compensate for the elevated energy demand. Mitochondrial oxidation capacity was reduced in 28°C-acclimated oysters, and mitochondrial abundance decreased in Cd-exposed oysters, with a stronger decrease (by 20–24%) in warm-acclimated oysters compared with cold-acclimated ones (by 8–13%). These data provide a mechanistic basis for synergism between temperature and cadmium stress on metabolism of marine poikilotherms. Exposure to combined temperature and cadmium stress may result in a strong energy deficiency due to the elevated energy demand on one hand and a reduced mitochondrial capacity to cover this demand on the other hand, which may have important implications for surviving seasonally and/or globally elevated temperatures in polluted estuaries.

Recent studies (Sokolova 2004, Cherkasov et al. 2006) showed that elevated temperatures strongly enhance the adverse effects of Cd on mitochondrial ATP synthesis and coupling in a model marine poikilotherm, *Crassostrea virginica,* suggesting synergism between these two environmental stressors). Earlier studies have also shown that elevated temperatures and exposures to metals may result in elevated standard metabolic rates (SMR) in poikilotherms (Barber et al. 1990, Rowe et al. 1998, Hopkins et al. 1999, Rowe et al. 2001). This suggests that poikilotherms exposed to elevated temperature and toxic metals

may face a dilemma of elevated energy demand combined with reduced aerobic capacity to produce ATP. A comprehensive analysis of the combined effects of environmental temperature and Cd exposure on energy demand and aerobic capa-city for energy supply will provide key information for mechanistic understanding of metabolic effects of these stressors and will further our knowledge of the role of bioenergetics in stress tolerance.

Crustaceans as Bioindicators

Crustaceans are frequently used as bioindicators and biomonitors in various aquatic systems. One reason is that they are a very successful group of animals, distributed in a number of different habitats including marine, terrestrial and freshwater environments (Rinderhagen *et al.* 2000). They are thus interesting candidates for comparative investigations. Some of the special features of crustaceans, particularly of reproduction strategies, may be highly important for the interpretation of data from bioindicator studies using these organisms, and for the development of ecotoxicological endpoints. The focus of this presentation is on the utilisation of crustaceans, mainly freshwater species, as bioindicators or biomonitors. The two terms are not always clearly distinguished. The term bioindicator defines a collective of organisms from the field (in a statistical sense) which give information about the environmental state, with effect variables being their mere presence or absence, their life history status or population dynamics (e.g. regarding age structure, abundance, genetic structure or condition index and the term biomonitor defines an organism which can be used to establish geographical and temporal variations in the bioavailability of contaminants by measuring the accumulated concentrations of chemicals in the whole body or in specific tissues (Rainbow 1995). Thus, results of standard toxicity tests are not within the scope of this section. Some recent developments of such tests are critically reviewed in (Persoone 1998).

Some of special biological features of crustaceans, particularly of reproduction strategies, may be highly important for the interpretation of data from bioindicator studies using these organisms. Basically, crustaceans are arthropods that are equipped with mandibles, and have two pairs of antennae and respire by means of gills. The body of crustaceans is segmented. It consists of the head (cephalon), various numbers of segments (thorax and abdomen) and the end of the body (telson), but the physique of crustacean species may vary widely (Stachowitsch 1992).

Considerable amounts of calcium minerals are deposited in the chitin-protein cuticle of numerous crustaceans, and make the shell hard and durable. Calcium is mainly taken up from the environment (e.g. from water or food). It can also be stored in the haemolymph, in the hepatopancreas and in some crustacean groups in special organs. In these cases, the stored calcium often is excreted (e.g. in Carcinus to 90%). There exists a strong relationship between the metabolism of calcium and that of other essential and nonessential metals. The respiration occurs mostly via gills. The respiration mechanisms make crustaceans susceptible to water-borne chemicals. The circulatory system of the crustaceans is open,

and the development of the arterial blood vessel system varies greatly among the different groups of crustaceans. Oxygen carriers in crustaceans are either the ferruginous (iron-containing) haemoglobin or the cupriferous (coppercontaining) haemocyanin. Special adaptations have been evolved to provide crustaceans with the essential element copper; among these molecules, the metallothioneins serve a dual purpose, being also involved in the detoxification of potentially toxic metals like cadmium (Ritterhoff and Zauke 1998, Roesijadi 1994).

The primary organs for excretion and osmotic regulation in crustaceans are the antennal glands and maxillary glands. The primary urine is formed in the end sac by means of membrane filtration. A reabsorption of ions occurs in the distal sections. Normally, the antennal gland functions in the early larval stages; later it regresses and the maxillary gland is formed, which is responsible for the excretion in adult animals. Other organs are involved in excretion, including the body surface, the gut, the epithelia of the hepatopancreas and, above all, the gills. The gills are important exchange organs and are able to take up ions from the water. Therefore, often a special epithelium with osmotic regulatory activity exists in the gills. In limnetic crustaceans, the body surface is less permeable to water and ions than in marine species. A more detailed description of osmotic regulation in crustaceans is given by Pequeux 1995). Most crustaceans are dioecious. However, within the different groups of crustaceans both hermaphroditism and parthenogenesis (e.g. in most Cladocera) can be found.

Bioaccumulation in experiments and field studies Crustaceans, particularly amphipods and isopods, are often used in bioaccumulation experiments and in field studies, as the large number of papers on this topic attest. Amyot *et al.* 1996 used *G. fasciatus* to investigate the distribution of some metals in various organs of these animals. This distribution was strongly dependent on the metal under consideration. For Cd and Cu, highest levels were found in the hepatopancreas, while for Cr, Fe, Mn and Ni concentrations were highest in the gut (one order of magnitude higher than in the other organs). Zn showed rather invariable concentrations throughout the body. An important fraction of Pb was associated with the exoskeleton. Depuration did not alter the mean body concentration of Cd, Cu and Zn, whereas Pb significantly decreased. Furthermore, the gut content had a significant effect on concentrations of Cr, Fe, Mn, Ni and Pb. The preceding examples show that bioaccumulation is a very complex process, influenced by several environmental factors. Another kind of sub-lethal response to contamination, viz. the effect of pollutants on moulting and regeneration in crustaceans, is described for several species (Weis *et al.* 1992). Moulting and regeneration are controlled by the neuroendocrine system, on which the toxicants act. Heavy metals commonly retard the regeneration of limbs and affect the moult cycle by causing delay in ecdysis.

Behavioural changes have been shown to be a very sensitive, non-destructive and rapid sublethal response to a wide range of pollutants. They basically represent an integrated response of an individual organism, although some behavioural changes such as precopula disruption (see above)

may lead to changes at the community or population level. Poulton and Pascoe (1990) described a behavioural bioassay to evaluate environmental stress using the freshwater amphipod *Gammarus pulex*. Here the disruption of precopulatory pairing is taken as a signal of the presence of pollutants or parasites. Koivisto *et al.* 1997 addressed the question whether or not cadmium pollution changes trophic interactions in experimental foodwebs composed of phytoplankton, small-bodied zooplankton (*Chydorus spharicus, Cyclops* sp. *and rotifers), Daphnia magna* and *Notonecta* sp. as zooplanktivorous predator. Thus the study of Koivisto *et al.* 1997 links ecotoxicology to the theoretical concept of the trophic cascade. Their results indicated that Cd (ranging from 10-20 µg/l) negatively affected phytoplankton and zooplankton abundance, whereas *Notonecta* reduced the *Daphnia* population and hence indirectly increased phytoplankton biomass in the dominant odd-linked food web. Cadmium and *Notonecta* predation had an interaction effect on phytoplankton but not on zooplankton.

Evolution of cadmium resistance in Daphnia magna

Artificial selection for increased resistance to the acute toxic effects of cadmium was performed, and after eight generations, the average median effective concentration increased from 61 to 180 mg/l. No differences in life span, offspring production, time to first brood, number of offspring in the first brood, or intrinsic rate of population increase (r) were observed between the cadmium - adapted population and the controls under ideal conditions or under conditions of temperature or feeding ration stress, but cadmium – adapted daphnids were smaller (Ward and Robinson 2005). Control and cadmium-adapted populations were equally sensitive to copper and malathion, but the cadmium-adapted population was less sensitive to lead and more sensitive to phenol. Analysis of amplified fragment-length polymorphisms indicated a significant decrease in genetic diversity in the cadmium-adapted population. Although the evolved cadmium resistance would allow adapted populations to exist in areas where cadmium concentrations would be toxic to unadapted populations, the decreased genetic diversity, smaller size, and increased sensitivity to at least one other toxicant could reduce the probability of long-term survival even in the absence of future cadmium exposure.

The work of Klerks and Levinton (1989) may have been the first clear observation of the evolution of toxicant resistance by aquatic animals. Their studies showed that the observed metal resistance was heritable and that selection leading to resistance occurred within one to four generations. Selection for resistance can increase survival in contaminated environments; however, the associated changes in allele frequencies within the population also could decrease overall fitness (Baird 1990, Barber 1990). Evolved tolerance is especially important for ecological risk assessment, where the survival of populations in contaminated areas generally is viewed as a positive ecological event. However, decreased tolerance of secondary stress and long-term costs of reduced genetic variability could offset the advantages of evolved resistance (Guttman 1994). Because they are cyclic parthenogens, alternating cycles of sexual and

asexual reproduction, daphnids were chosen for the present study (Deng 1996). This unusual reproductive strategy allows sexual reproduction to create a diverse gene pool on which selection can act and then permits development of adapted clones by parthenogenic reproduction. Cadmium was chosen because among studied toxicants, different populations of daphnids collected throughout Europe have exhibited the greatest range of sensitivity to cadmium (Baird *et al.* 1991, Barata *et al.* 2002). Once established, the adapted daphnid population was used to investigate specific genetic and physiological differences from the control populations. Potential consequences of adaptation were evaluated by measuring differences in life-history traits that contribute to the population growth rate and the ability of the population to handle secondary stress.

The ability to evolve metal tolerance might not be universal, but the daphnid model of evolved toxicant resistance is applicable to all species that possess a differential resistance or detoxification mechanism on which selection can act.

However, an even larger loss of genetic variation was observed in the cadmium-adapted population, which became increasingly resistant to the acute toxic effects of cadmium through selection for that trait. The number of polymorphic bands and gene diversity were reduced to a level that cannot be explained exclusively by the genetic drift observed in the control or reduced-control populations over the eight generations.

Toxicity might be related to a single major gene or to a very few genes, and that gene might be toxicant specific or toxicant-class specific or be able to control a more general response (Barata *et al.* 2002). However, genetic control of toxicant resistance also might be a polygenic or genome-wide response, and selection for increased resistance to one toxicant might cause trade-offs that affect resistance to a second toxicant in a positive, negative, or neutral way. This absence of metabolic cost also has been observed for cadmium tolerance in natural daphnid populations (Barata et al. 2002). One explanation for these observations might be an inducible detoxification system that functions only in the presence of the metal. It has been suggested that the deployment of a metabolically expensive stress-resisting process only in the presence of a toxicant would allow the adapted and unadapted populations to have equivalent metabolic rates in a clean environment and still allow use of the process in the presence of the toxicant, where an increased metabolic rate would be more than offset by an increased probability of survival (Calow and Sibly 1990). Cadmium-adapted adult daphnids were, on average, smaller than control daphnids. Although reduced size was not associated with reduced fecundity in the adapted population (a positive correlation between size and offspring production is common in daphnids), reduced size alone might be ecologically significant and capable of affecting fitness. It also could indicate a trade-off between growth and detoxification ability (selection for cadmium resistance and size also may be independent). Exposure of daphnids to continuous, low-level concentrations of nikel for seven generations resulted in reduced length, and during chronic toxicity tests with daphnids, a similar relationship between body length and cadmium sensitivity was observed (van Leeuwen *et al.* 1985) suggesting a linkage of these characters. In those experiments,

smaller size was observed in daphnids that had been exposed to sublethal metal concentrations during their lives, and reduced size might have resulted from reduced nutrition (cadmium has been shown to reduce feeding rates in fish). In these experiments, smaller size occurred in daphnids that were never exposed to cadmium but, rather, were the offspring of daphnids that had been exposed (however, we cannot exclude the possibility that cadmium was passed from parent to offspring).

Whether the reduced size was caused directly by the cadmium or was simply a coincidental linkage of genes controlling size and metal resistance, the ecological consequences of reduced size could be very important, because reduced body size is more important than age or nutritional status in determining swimming speed in daphnids.

The evolution of cadmium tolerance demonstrated by this laboratory experiments resulted in a daphnid population that was better adapted to live in a cadmium-contaminated environment. However, this adaptation may have come at a high price: reduced size (and, therefore, slower swimming speed), increased sensitivity to at least one toxicant (phenol), and reduced genetic variability. Any one of these changes in the daphnid population could potentially decrease the long-term survival probability, but taken together, they likely represent an increased risk of population extinction. Because this adapted population was as fit as the control population in the absence of cadmium, its continued existence would seem to be as likely as that of the control population in the absence of cadmium and more likely during episodes of cadmium (or lead) exposure. However, the reduced genetic diversity, reduced size, and greater sensitivity to other toxicants, which would persist in future generations, could significantly compromise the survival probability of the adapted population when faced with a novel selection pressure, a predator, or a chemical with the same mechanism of toxicity as phenol.

Effect of temperature on the toxicity of heavy metals to aquatic invertebrates. (MAQ Khan)

The slow onset of global warming and increased inputs of effluent discharges into bodies of water have created concerns, in addition to other things, about the nature and extent of thermal effects on aquatic organisms, especially the poikilotherms. The metabolism of these aquatic animals (invertebrates and lower vertebrates) fluctuates with the ambient temperature.

An increase in temperature can increase their rate of metabolism (respiration/oxygen consumption), which demands more oxygen extraction and transport.

However, the concentration of dissolved oxygen decreases with temperature. This can increase the demand for oxygen extraction and utilization.

However, at higher temperature metals are desorbed from surfaces and this can increase their concentration in water.

It is posible to hypothesize that in bodies of water contaminated with heavy metals increase in temperature and acidity, can make stressed animals become susceptible to otherwise sublethal concentrations of metals.

Accordingly, freshwater invertebrates namely zebra mussels *Dreissena polymorpha* (Mollusca: Pelecypoda), crayfish *Orconectes immunis* (Crustacea: Decapoda), and water flea *Daphnia magna* (Crustacea: Ostracoda) were tested as models to investigate the effects of temperature on the toxicity of heavy metals using copper, cadmium, zinc and lead.

Temperature can also increase the concentration of heavy metals in water due to their desorption. All these factors together may make sublethal/subtoxic environments become lethal to aquatic animals.

Possibility of chronic effects related with the onset of global warming (climate change) needs to be investigated.

Proteomic and heavy metals

Whether aquatic organisms are adapted to the heavy-metal pollutants or not, such environmental stress causes changes in physiological responses (Lee *et al.* 2006). In this study, the aquatic midge, *Chironomus riparius*, was used to find changes of expression of proteins in relation to cadmium exposure. Dose-response relationships between cadmium concentrations and mortality of 3rd instar midge larvae were observed and the protein levels were compared using PD-Quest after 2-DE. Comparing the intensity of protein spots, 21 proteins decreased and 18 proteins increased in response to cadmium treatment. With increased proteins, three enzymes such as S-adenosylmethionine decarboxylase, O-methyltransferase, and aspartokinase were involved in the glutathione biosynthesis and a key enzyme regulating fatty acid biosynthesis, oleyl-acyl carrier protein thioesterase was also identified.

According to the functional classification of decreased levels of proteins, they were involved in energy production, protein fate, nucleotide biosynthesis, cell division, transport and binding, signal transduction, and fatty acid and phospholipid metabolism in the cell. In addition, phenol hydroxylase, thioesterase, zinc metalloprotease, and aspartate kinase were newly expressed after cadmium exposure at the concentration of the LC_{10} value. Therefore, these proteins seem to be potential biomarkers for cadmium exposure.

Temperature dependent effects of cadmium on Daphnia magna: accumulation versus sensitivity.

Standard toxicity tests are performed at one constant, optimal temperature (usualy 20° C), while in the field variable and suboptimal temperatures may occur. Lack of knowledge on the interactions between chemicals and temperature hampers the extrapolation of laboratory toxicity data to ecosystems. To study temperature dependency of the intrinsic sensitivity of the daphnids to cadmium, the DEBtox model was used to estimate internal threshold concentrations (ITCs) and killing rates from the toxicity and accumulation data. The results revealed that increasing temperature lowered the ITC and increased the killing rate and the uptake rate of the metal. Enhanced sensitivity of *D. magna* was shown to be the primary factor for temperature-dependent toxicity. Since temperature has such a major impact on toxicity, a temperature correction may be necessary when translating toxicity data from the laboratory to the field.

Daphnia - established model species in toxicology. This freshwater crustacean is commonly used for environmental monitoring of pollutants around the globe and plays an important role in establish-

ing regulatory criteria by government agencies (e.g., U.S. EPA, Environment Canada, Organization for Economic Cooperation and Development, Environment Agency of Japan). Consequently, daphniids represent 8% of all experimental data for aquatic animals within the toxicological databases. As such, their incorporation within the new field of toxicological genomics is limited only by the advancement of genomic resources. Since the development of these technologies requires the input and feedback of a large research community that extends far beyond the boundaries of any one discipline, the Daphnia Genomics Consortium (DGC) was formed in 2001 to provide the organizational framework to coordinate efforts at developing the Daphnia genomic toolbox; facilitate collaborative research; and develop bioinformatics strategies for organizing the rapidly growing database. Species of the freshwater crustacean genus Daphnia have been the focus of steady research by naturalists and experimental biologist for centuries (Korovchinsky 1997). They inhabit a remarkably diverse array of environments throughout the world, ranging from permanent lakes to temporary ponds, oligotrophic to eutrophic, hypersaline to freshwater, and extending into the UV-rich settings of coastal dune ponds and high-alpine lakes. These radically different waters have been colonized on multiple occasions with a characteristic pattern of convergence of adaptive traits linked to specific habitats (Colbourne *et al.* 1997). Daphnia possess unique characteristics that offer experimental genetic strategies necessary to translate knowledge about their population structure and ecology to the study of general theories that cross both biological scales and disciplines (de Bernardi and Peters 1987). Several of these attributes revolve around their complex life-cycle. The Daphnia system is poised to become a leading research model for understanding environmental influences on gene regulation, and subsequent stressor induced acclimation and adaptation. One reason for this emerging utility derives from their life-cycle, which during sexual reproduction produces embryos that diapause (e.g., delayed development). Diapausing embryos, which encase in ephippia, are resistant to harsh environmental conditions (e.g., desiccation, freezing) and represent a "bank of genetic diversity from which an existing population can draw new genotypes (Mort 1991)". Experimentally, resting egg banks provide access to past populations as these can be hatched from sediments several decades old (Hairston *et al.* 1995, Cáceres 1998, Kerfoot *et al.* 1999), allowing the past products of evolution to be resurrected and evaluated against their current descendants in a controlled setting. The resting-egg bank of Daphnia has been used to study the influence of natural stressors (i.e., cyanobacteria, predation) on the distribution of stressor induced phenotypes in populations and through time (Hairston *et al.* 2001, Cousyn *et al.* 2001). These studies have demonstrated rapid adaptive change (i.e., acquisition and loss of phenotypes) in presence of stress. Current studies have extended these applications to investigate the influence of metal pollution on the structure of Daphnia communities (Pollard *et al.* 2003), examining pollution and subsequent recovery. In cases where the egg banks of interest are no longer viable, the DNA is still accessible to genetic probes for centuries (Limburg and Weider 2002).

Traditional toxicity assays, although informative as to the levels of a chemical that may be lethal to a population, do not provide information on the mechanism by which a chemical has its lethal effect. In addition, many chemicals are found at sublethal levels in the environment affecting populations by altering the general physiology, reproductive capacity or the ability of an organism to fight disease. Examining changes in gene expression provides a means to identify biochemical pathways that are altered in an organism after even a low-level chemical exposure. Genomics can provide a level of detail absent in general mortality studies, indicating mode of action, differences between low and high dose effects, providing complex early biomarkers or a "canary" to indicate exposure or potential effects of an exposure (Klaper and Thomas 2004). Environmental Protection Agency (Dix *et al.* 2006, Gallagher *et al.* 2006). This potential is illustrated by a pilot project using Daphnia Chemicals with similar modes of action provide similar expression patterns (e.g., heavy metals, Andrew *et al.* 2003), but even within a chemical class exposures can be distinguished based upon gene expression patterns (Hamadeh *et al.* 2002).

In general, these resources are expected to provide reliable diagnostic tools that determine whether a defined ecosystem (or individual) is exposed to toxic agents (Andrew *et al.* 2003), whether substances are harmful (Hayes and Bradfield 2005) and whether a population is susceptible to certain chemicals (Pedra *et al.* 2004).

References

Adams, S.M. 2002: Biological indicators of aquatic ecosystem stress. American Fisheris Society, Bethesda MD, USA.

Amyot, M., Pinelalloul, B., Campbell, P.G.C. and Desy, J.C. 1996: Total metal burdens in the freshwater amphipod *Gammarus fasciatus:* Contribution of various body parts and influence of gut contents. *Freshwater Biology,* **35**: 363-373.

Andrew, A., Warren, A.J., Barchowsky, A., Temple, K.A., Klei, L., Soucy, N.V., O'Hara, K.A. and Hamilton, J.W. 2003: Genomic and proteomic profiling of responses to toxic metals in human lung cells. *Environmental Health Perspectives,* **111**: 825-838.

Auffret, M., Mujdzic, N., Corporeau, C. and Moraga, D. 2002: Xenobiotic-induced immunomodulation in the European Flatoyster, *Ostrea edulis. Mar. Environ. Res.,* **54**: 585–589.

Baird, D.J., Barber, I. and Calow, P. 1990: Clonal variation in general responses of *Daphnia magna* Straus to toxic stress. I. Chronic life-history effects. *Funct. Ecol.,* **4**: 399-407.

Baird, D.J., Barber I,. Bradley, M., Soares, A. and Calow, P. 1991: A comparative study of genotype sensitivity to acute toxic stress using clones of Daphnia magna Straus. *Ecotoxicol. Environ. Saf,* **21**: 257–265.

Barata, C., Baird, D.J. and Markich, S.J. 1998: Influence of genetic and environmental factors on the tolerance of *Daphnia magna Straus* to essential and nonessential metals. *Aquat. Toxicol,* **42**: 115-137.

Barata, C., Baird, D.J. and Soares A.M.V.M. 2002: Determining genetic variability in the distribution of sensitivities to toxic stress among and within field populations of *Daphnia magna. Environ. Sci. Technol*, **36**: 3045–3049.

Barber, I., Baird, D.J. and Calow, P. 1990: Clonal variation in general response of *Daphnia magna,* Straus to toxic stress. II. Physiological effects. *Funct. Ecol.* **4**: 409–414.

Bergmeyer, H.U. 1985: Methods of Enzymatic Analysis.

VCH Verlagsgesellschaft, Weinheim.

Brehm, V. 1938: Die Rotfarbung von Hochge- birgsorganismen. *Biol. Rev. and antagonistic processes, which are expected to result*, **13**: 307–318.

Cáceres, C. E. 1998: Interspecific variation in the abundance, production, and emergence of Daphnia diapausing eggs. *Ecology,* **79**: 1699-1710.

Cherkasov, A.S., Biswas, P.K., Ridings, D.M, Ringwood, A.H. and Sokolova, I.M, 2006: Effects of acclimation temperature and cadmium exposure on cellular energy budgets in the marine mollusk *Crassostrea virginica*: linking cellular and mitochondrial responses. *The Journal of Experimental Biology,* **209**: 1274-1284.

Colbourne, J.K., Hebert, P.D.N. and Taylor, D.J. 1997: Evolutionary origins of phenotypic diversity in Daphnia. In *Molecular Evolution and Adaptive Radiation*, (ed. K.S.T. Givnish), pp. 163-188. London: Cambridge University Press, UK.

Calow, P. and Sibly, R.M. 1990: A physiological basis of population processes: Ecotoxicological implications. *Funct Ecol,* **4**: 283–288.

Cousyn, C., De Meester, L., Colbourne, J. K., Brendonck, L., Verschuren, D. and Volckaert, F. 2001: Rapid, local adaptation of zooplankton behavior to changes in predation pressure in the absence of neutral genetic changes. *Proceedings of the National Academy of Sciences of the United States of America,* **98**: 6256-6260.

de Bernardi, R. and Peters, R.H. 1987: Why Daphnia. In „*Daphnia" Memorie dell' Istituto Italioano di Idrobiologia Dr. Marco De Marchi,* Vol. **45** (ed. R. Peters). Verbania Pallanza Consiglio Nazionale Delle Ricerche Instituto Italiano Di Idrobiologia.

Deng, H.-W. 1996: Environmental and genetic control of sexual reproduction in *Daphnia. Heredity,* **76**: 449–458.

Dix, D., Gallagher, K., Benson, W.H., Groskinsky, B.L., McClintock, J.T., Dearfield, K.L. and Farland, W.H. 2006: A framework for the use of genomics data at the EPA. *Nature Biotechnology,* **24**: 1108-1111.

Dorigan, J. V. and Harrison, F. L. (ed.) 1987: Physiological Responses of Marine Organisms to Environmental Stresses. US Department of Energy, Washington, DC.

Gallagher, K., Benson, W.H., Brody, M., Fairbrother, A., Hasan, J., Klaper, R., Lattier, D., Lundquist, S., McCarroll, N., Miller, G., Preston, J., Sayre, P., Smith, B., Street, A., Troast, R., Vu, B., Reiter, L., Farland, W. and Dearfield, K. 2006: Genomics: Applications, challenges and opportunities for the U.S. Environmental Protection Agency. *Human and Ecological Risk Assessment,* **12**: 572-590.

Guttman, S.I. 1994: Population genetic structure and ecotoxicology. *Environ. Health. Perspect.,* **102** (**Suppl. 12**): 97–100.

Hairston, N., Van Brunt, R. A., Kearns, C.M. and Engstrom, D.R. 1995: Age and survivorship of diapausing eggs in a sediment egg bank. *Ecology,* **76**: 1706-1711.

Hairston, N. G., Holtmeier, C. L., Lampert, W., Weider, L. J., Post, D. M., Fischer, J. M., Cáceres, C. E., Fox, J. A. and Gaedke, U. 2001: Natural selection for grazer resistance to toxic cyanobacteria: Evolution of phenotypic plasticity? *Evolution,* **55**: 2203-2214.

Hamadeh, H., Bushel, P.R, Jayadev, S., Martin, K., DiSorbo, O., Sieber, S., Bennett, L., Tennant, R., Stoll, T., Barrett, J.C., Blanchard, K., Paules, R.S. and Afshari, C.A. 2002: Gene expression analysis reveals chemical-specific profiles. *Toxicological Sciences,* **67**: 232-240.

Hayes, K. and Bradfield, R. 2005: Advances in toxicogenomics. *Chemical Research in Toxicology,* **18**: 403-414.

Hebert, P.D.N. and Emery, C.J. 1990: The adaptive significance of cuticular to test the solar UV-B impact on microplankton in a high-altitude pigmentation in Daphnia, *Funct. Ecol.,* **4**: 703–710.

Hopkins, W.A., Rowe, C.L. and Congdon, J.D. 1999: Elevated trace element concentrations and standard metabolic rate in banded water snakes (*Nerodia fasciata*) exposed to coal combustion wastes. Environ. *Toxicol. Chem.,* **18**: 1258-1263.

Horička, Z., Stuchlík, E., Hudec, I., Černý, M. and Fott, J. 2006: Acidification nd the structure of crustacean

zooplankton in mountain lakes: The Tatra Mountains (Slovakia, Poland). *Biologia,* **61:** 121-130.

Kammenga, J.E., Dallinger, R., Donker, M.H., Kohler, H.R., Simonsen, V., Triebskorn, R., et al. 2000: Biomarkers interrestrial invertebrates: Potential and limitations for ecotoxicological soil risk assessment. *Reviews of Environmental Contamination and Toxicology,* **164**: 93–147.

Kerfoot, W.C., Robbins, J.A. and Weider, L.J. 1999: A new approach to historical reconstruction: Combining descriptive and experimental paleolimnology. *Limnology and Oceanography,* **44**: 1232-1247.

Klaassen, C.D., Liu, J. and Choudhuri, S., 1999: Metallothionein: an intracellular protein to protect against cadmium

Klaper, R. and Thomas, M.A. 2004: At the crossroads of genomics and ecology: the promise of a canary on a chip. *BioScience,* **54**: 403-412.

Klerks, P.L. and Levinton, J.S. 1989: Effects of heavy metals in a polluted aquatic ecosystem. In *Rapid evolution of metal resistance in a benthic oligochaete inhabiting a metal-polluted site* (eds. S.A. Levin, M.A. Harwell, J.R. Kelly, P.L. Klerks, J.S. Levinton). *Biol. Bull.,* **176:** 135–141.

Koehn, R.K. and Bayne, B.L. 1989: Towards a physiological and genetical understanding of the energetics of the stress response. *Biol. J. Linn. Soc.,* **37:** 169-171.

Koivisto, S., Arner, M. and Kautsky, N. 1997: Does cadmium pollution change trophic interactions in rockpool food webs? *Environmental Toxicology and Chemistry*, **16**: 1330-1336.

Korovchinsky, N.M. 1997: On the history of studies on cladoceran taxonomy and morphology, with emphasis on early work and causes of insufficient knowledge of the diversity of the group. *Hydrobiologia,* **360**: 1-11.

Lee, S.E., Yoo, D., Son, K., Cho, K. 2006: Proteomic evaluation of cadmium toxicity on the midge *Chironomus riparius. Proteomics,* **6**(**3**): 945-957.

Limburg, P.A. and Weider, L.J. 2002: ‚Ancient' DNA in the resting egg bank of a microcrustacean can serve as palaeolimnological database. *Proc. of the Royal Society of London Series Biological Science,s* **269**: 281-287.

Mort, M. A. 1991: Bridging the gap between ecology and genetics: the case of freshwater zooplankton. *Trends in Ecology & Evolution,* **6**: 41-45.

Pedra, J., McIntyre, L., Scharf, M., and Pittendrigh, B. 2004: Genome-wide transcription profile of field and laborator-selected dichlorodiphenyltrichloroethane (DDT)-resistant Drosophila. *Proceedings of the National Academy of Sciences of the United States of America,* **101**: 7034-7039.

Persoone, G. 1998: Development and validation of toxkit microbiotests with invertebrates in particular crustaceans, In *Microscale Testing in Aquatic Toxicology* (eds. P.G. Wells, K. Lee and C. Blaise), pp. 437-449. CRC Press, Boca.

Pequeux, A. 1995: Osmotic Regulation in Crusta ceans. *Journal of Crustacean Biology,* **15:** 1-60.

Pollard, H.G., Colbourne, J.K. and Keller, W. 2003: Reconstruction of centuries-old Daphnia communities in a lake recovering from acidification and metal contamination. *Ambio,* **32**: 214-218.

Poulton, M. and Pascoe, D. 1990: Disruption of precopula in *Gammarus pulex* (L.) Development of a behavioural bioassay for evaluating pollutant and parasite induced stress. *Chemosphere,* **20**: 403-415.

Rainbow, P.S. 1995: Biomonitoring of heavy metal availability in the marine environment. *Marine Pollution Bulletin,* **31**: 183-192.

Regoli, F., 1992. Lysosomal responses as a sensitive stress index in biomonitoring heavy metal pollution. *Mar. Ecol. Prog. Ser.,* **84**: 63–69.

Rinderhagen, M., Ritterhoff, J. and Zauke, G.-P. 2000: Crustaceans as Bioindicators *Environmental Toxicology and Chemistry,* **24**(**9**): 2341–2349.

Ringwood, A.H., Hoguet, J., Keppler, C.J. 2002. Seasonal variation in lysosomal destabilization in oysters, Crassostrea virginica. *Mar. Environ. Res.,* **54**: 793–797.

Ritterhoff, J. and Zauke, G.P. 1998: Potential role of metal-binding proteins in cadmium detoxification in *Themisto*

libellula (Mandt) and *Themisto abyssorum* (Boeck) from the Greenland sea. *Marine Environmental Research,* **45**: 179-191.

Roesijadi, G. 1994: Metallothionein induction as a measure of response to metal exposure in aquatic animals. *Environmental Health Perspectives*, **102:** 91-95.

Rowe, C.L., Kinney, O.M., Nagle, R.D. and Congdon, J.D. 1998: Elevated maintenance costs in an anuran (*Rana catesbeiana*) exposed to a mixture of trace elements during the embryonic and early larval periods. *Physiol. Zool.,* **71:** 27-35.

Rowe, C.L., Hopkins, W., A., Zehnder, C. and Congdon, J.D. 2001: Metabolic costs incurred by crayfish (*Procambarus acutus*) in a trace element-polluted habitat: further evidence of similar responses among diverse taxonomic groups. *Comp. Biochem. Physiol.,* **129**: 275-283.

Schramm, M., Behrens, A., Braunbeck, T., Eckwert, H., Kohler, H.-R., Konradt, J., et al. 1999: Cellular, histological and biochemical biomarkers. In *Biomonitoring of Polluted Water, Environmental Research Forum 98* (ed. A. Gerhardt), pp. 33-64. Trans Tech Publications, Utikon-Zurich.

Sokolova, I.M. 2004: Cadmium effects on mitochondrial function are enhanced by elevated temperatures in a marine poikilotherm, *Crassostrea virginica* Gmelin (Bivalvia: Ostreidae). *J. Exp. Biol.,* **207**: 2639-2648.

Sokolova, I.M., Evans, S. and Hughes, F.M., 2004: 2 Cadmium-induced apoptosis in oyster hemocytes involves disturbance of cellular energy balance but no mitochondrial permeability transition. *J. Exp. Biol.* **207**: 3369–3380.

Sokolova, I.M., Sokolov, E.P. and Ponnappa, K.M. 2005a: Cadmium exposure affects mitochondrial bioenergetics and gene expression of key mitochondrial proteins in the eastern oyster *Crassostrea virginica* Gmelin (Bivalvia: Ostreidae). *Aquat. Toxicol.* **73**: 242-255.

Sokolova, I.M., Ringwood, A.H. and Johnson, C. 2005b: Tissue-specific accumulation of cadmium in subcellular compartmens of eastern oysters *Crassostrea virginica* Gmelin (Bivalvia: Ostreidae). *Aquatic Toxicology,* **74**: 218-228.

Sommaruga, R. 2001: The role of solar UV radiation in the ecology of alpine lakes. J*ournal of Photochemistry and Photobiology B: Biology,* **62**: 35–42.

Sommaruga, R., Garcia-Pichel, R. 1999: UV-absorbing mycosporine-like mountain lake, *Arch. Hydrobiol.,* **144***:* 255–269.

Sommaruga, R., Obernosterer, I., Herndl, G., Psenner, R. 1999: *Inhibitorykella gracilipes* (Calanoida, Copepoda) under ambient levels of solar effect of solar radiation on thymidine and leucine incorporation by UV radiation in two high mountain lakes. *J. Plankton Res.*, **21**: 4178-4184.

Shrivastav, R. 2001: Atmospheric Heavy Metals Pollution. *Resonance,* **6**: 62-68.

Stachowitsch, M. 1992. The Invertebrates: An Illustrated Glossary. Wiley-Liss, New York.

Stohs, S.J. and Bagchi, D., 1995: Oxidative mechanisms in the toxicity of metal ions. Free Radic. *Biol. Med.* **18**: 321–336.

Tartarotti, B., Cabrera, S., Psenner, R. and Sommaruga, R. 1994: Survivorship of *Cyclops abyssorum tatricus* (Cyclopoida, Copepoda). *Algol. Stud.,* **75**: 249–264.

van Leeuwen, C.J., Luttmer, W.J. and Griffioen, P.S. 1985: The use of cohorts and populations in cadmium toxicity studies with *Daphnia magna*: A cadmium example. *Ecotoxicol. Environ. Saf.,* **9**: 26–39.

Viarengo, A. 1994: Heavy metal cytotoxicity in marine organisms: effects on Ca^{2+} homeostasis and possible alteration of signal transduction pathway. *Adv. Comp. Environ. Physiol.,* **20**: 85–109.

Ward, T.J. and Robinson, W.E. 2005: Evolution of cadmium resistance in Daphnia magna Environ. *Toxicol. Chem.,* **24**: 2345-2350.

Widdows, J. 1978: Physiological indices of stress in *Mytilus edulis. J. Mar. Biol. Assoc. UK,* **58**: 125-142.

Weis, J.S., Cristini, A. and Ranga-Rao, K. 1992: Effects of pollutants on molting and regeneration in crustacea. *American Zoologist,* **32**: 495-500.

Yoder, C.O., Rankin, E.T. 1998: The role of biological indicators in a state water quality management process. *Environmental Monitoring and Assessment,* **51**: 61–88.