Issues in Toxicology

# Ecotoxicology and Genotoxicology

## Non-traditional Aquatic Models

Edited by Marcelo L. Larramendy

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## *Non-traditional Aquatic Models*

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CHAPTER 10

# *The Crab* Ucides cordatus *(Malacostraca, Decapoda, Brachyura) and Other Related Taxa as Environmental Sentinels for Assessment and Monitoring of Tropical Mangroves from South America*

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## 10.1 Anthropic History: Actions *vs.* Reaction

Since early times, man has been settling near to rivers and other freshwater sources, which are used as water supplies for their own maintenance, as well as for animal husbandry and agronomic purposes. Therefore, human densification has occurred worldwide on riverbanks, as well as in coastal regions where water flows, promoting relevant areas for many activities. Among them, there are the establishment of seaport, food stock/transportation, and others, that are responsible for city infrastructures. Actually, more than 50% of the human population lives less than 60 km from the sea,[1] in a narrow territorial strip. With the advent of the industrial revolution, this uncontrolled occupation caused harmful effects to the environment, firstly through the diversification of pollutants generated and second by the damages promoted by contact or inadequate disposal of slag resulting from anthropic activities. Among these, there are seaport facilities and the traffic of ships, generally associated with large industrial complexes, where products are stocked, many of them with strong pollutant power (*e.g.*, gas, liquids, oils, greases and other petrochemicals).

Several regions of the world stand out in terms of industrial development, often with no regard to the neutralization of the pollutants generated, which inevitably reach the adjacent environments through the action of the water cycle, and are finally dispersed, reaching the oceans. Among developing countries, the greatest concern as to the target levels of pollution is in China, where the diversity of the products generated and the myriad of xenobiotics from different origins have a large contamination potential.[2,3]

Industrialization led many European countries through alarming advents of pollution. An example occurred in the 1950s in London (UK), where remarkable industrial atmospheric pollution (smog = smoke + fog) provoked the death of at least 4000 people and a range of diseases.[4] More recently, a serious atmospheric accident occurred in the 1960s in Cubatão municipality (Southeast Brazil); a large industrial complex caused contamination of air, rivers, estuary and forests, increasing the incidence of pulmonary diseases, congenic malformations,[5] and high mortality of aquatic organisms, making them unfit for human consumption.[6]

## 10.2 Coastal Environments: Biodiversity and Conservation

Coastal environments are highly affected by pollutants, carried from terrestrial areas to rivers and estuarine systems, in densely occupied areas where anthropic activities occur with no monitoring. Many accidents have occurred and here we highlight some significant ones: oil spills, *e.g.*, ‘Deepwater Horizon’ drilling platform accident,[7] at a depth of 1500 m, occurred in April in the at Gulf of Mexico, where approximately 780 000 $m^3$ of crude oil was released to the sea over the course of three months; mine slag contamination, *e.g.*, the Fundão dam collapse by the mining company

Samarco (a subsidiary of Vale & BHP)[8] in the central region of Brazil in November 2015, leading to pollution of the Doce river and the death of millions of fishes in the coastal ecosystems, including the marine life of the Abrolhos archipelago; and chemical accidents, *e.g.*, a leak of 400 thousand liters of firefighting liquid foam generator, used to eradicate fire accidents, occurred with six fuel tanks at Ultracargo[9] in April 2015, and this was dispersed to the estuarine area of Santos-São Vicente, Southeast Brazil. Therefore, coastal areas are subjected to elevated environmental risk of anthropic origin, despite the existence of security plans that unfortunately are insufficient, inefficient and dysfunctional.

The release of these products promotes the death of organisms responsible for primary productivity (microalgae in aquatic ecosystems, and aquatic vegetation from wetlands *e.g.*, mangroves), to other trophic levels, such as filter feeders (*e.g.*, oysters and clams) up to benthonic organisms with different food habits, among them herbivores, omnivores, detritivores (*e.g.*, turtles, fishes, shrimps and crabs), and carnivores (*e.g.*, aquatic birds, porpoises, raccoons).

## 10.3 Mangrove Ecosystem: Importance and Threats

Mangroves are unique coastal ecosystems, found in shallow areas in intertidal zones present in estuarine systems, in an ecotone that involves terrestrial and aquatic environments, the last one being made up of marine and freshwater areas.[10,11] Mangroves are registered in tropical and subtropical regions of the world, and total 137 760 $km^2$, mainly near the equatorial zone.[12] In these areas, deposition of fine sediments occurs, mainly silt (0.05–0.002 mm) and clay (<0.002 mm), but very fine sand (0.1–0.05 mm) can also occur, resulting in banks of fluid mud.[13] The fine granulometric characteristics of mangrove sediments make this environment susceptible to contamination by oil residues, toxic metals, and organic/inorganic compounds.[14] Therefore, these areas act as efficient biogeochemical barriers, holding back these xenobiotics (mainly toxic metals), blocking their free circulation and making them unavailable to plants and animals of these areas.[15,16] This sedimentary matrix involves an association of organic matter and nutrients, varying with flooding area and tides, and promoting different scenarios of redox potential, availability and action of chemical elements over the course of the day.[16] This specific natural oscillation of physical and chemical parameters (*e.g.*, salinity, pH and oxygen content) acts on the diversity and density of micro/macro organisms that live in this environment, which show specific structures and physiological conditions to survive in these areas. Owing to the high productivity of mangroves, this ecosystem has an important role during nutrient cycling, affecting adjacent environments.[10,11] Therefore, mangrove areas attract animal species that use this environment for food, protection, and reproduction purposes.[17] The muddy sediment of mangroves is flooded by tides daily, and the interstitial water of the sediment is characterized by a significant change of the salinity[7] and low oxygen because of the high organic matter content.[18] These stressful environmental conditions allow the colonization of facultative halophyte plants called mangue,[10,11] adapted to live there, with suitable morphology for fixation in the sediment (rhizophores), oxygen extraction by aerial roots (pneumatophores) and physiological strategies to control salt load.[16] These characteristics attenuate the impacts of flood tides and are fundamental for the retention of high concentrations of organic and inorganic substances,[18] many of which are not essential to metabolic processes and are considered pollutants.

In this nutrition-rich environment, organic matter (particulate and dissolved) is available to microorganisms, where a diversity of zooplankton and juveniles of many invertebrates, such as molluscs, crustaceans and fishes, can survive.[11,17] The mangrove is composed of resident species (*e.g.* decapod crustaceans), semi-residents (occupying these areas in specific moments of life, *e.g.* during reproduction), regular visitors and opportunistic visitors.[17] In Brazil, a few species live permanently in mangroves, among them decapod crustaceans (Figure 10.1), found in diverse strata or in adjacent environments, as follows: (A) *uçá* crab *Ucides cordatus* (Linnaeus, 1763), a herbivorous crab that uses mangrove litter and actively digs mangrove sediments; (B) grapsid crab *Goniopsis cruentata* (Latreille, 1803), which lives among roots and holes in trees, and is omnivorous; (C) arboreal crab *Aratus pisonii* (H. Milne Edwards, 1837), which lives in canopies and branches of trees and is uniquely herbivorous, feeding on green leaves; (D) species of the genus *Uca* (Leach, 1814), here represented by *Uca maracoani* (Latreille, 1802), which are deposit feeders, using organic matter in the sediment for their survival, and digging galleries in the sediment; (E) *guaiamú* crab *Cardisoma guanhumi* (Latreille, 1828), generally occurring in sandy estuaries, associated with freshwater in transition with tropical forests, where the topography is more elevated and flooding tides are not frequent; and (F) ghost crab *Ocypode quadrata* (Fabricius, 1787), with omnivorous habit and that use macroinvertebrates present in the intertidal zone of sandy beaches, dig their galleries in supralittoral areas, and are generally associated with sandy dunes. These representatives of the infraorder Brachyura (brachyuran crabs) are important to many biological processes, where scavengers promote bioturbation of the sediment, increasing aeration, stratum mixture, and the content of organic matter and other nutrients content. Thus, they have been called ecosystem engineers.[19,20]

Despite the economic and ecological importance of mangroves, they are subjected to high and systematic anthropic pressure, with significant decline (approximately 1–2% per year), leading to the disappearance of this ecosystem in about 100 years.[21] Environmental contamination, as a result from disorderly and rapid urbanization and industrialization, has led to concerns from researchers and environmental analysts because the residues produced (*e.g.*, by estuarine dredging, dumping of liquid effluents, indiscriminate use of fertilizers and pesticides in cultivable fields), are cumulative, toxic and persistent, as seen with toxic metals.[22] Information about this situation reveals the urgent necessity for more basal studies and

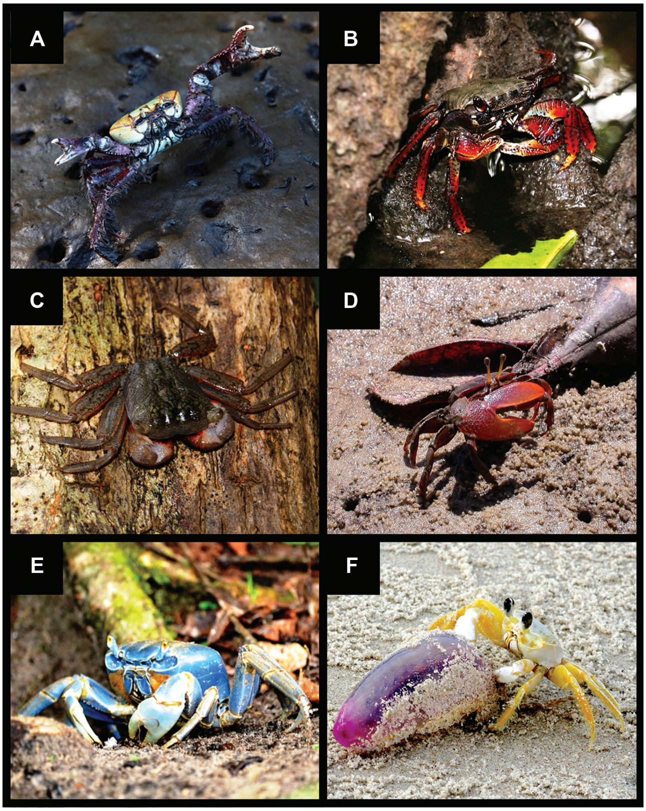


**Figure 10.1** Brachyuran species (crabs) and their occurrence on the Brazilian coast, with potential use for evaluation and monitoring of mangroves [A: *Ucides cordatus* (Linnaeus, 1763), photo by Delson Gomes; B: *Goniopsis cruentata* (Latreille, 1803), photo by Marcelo Pinheiro; C: *Aratus pisonii* (H. Milne-Edwards, 1837), photo by Marcelo Pinheiro; D: genus *Uca*, represented by *Uca maracoani* (Leach, 1814), photo by Luis Ernesto Bezerra], *restingas* [E: *Cardisoma guanhumi* (Latreille, 1828), photo by Carlos Cantareli], and sandy beaches [F: *Ocypode quadrata* (Fabricius, 1787), photo by Alexandre Almeida]. Graphic design by Gustavo Pinheiro.

monitoring programs to increase knowledge about mangrove contamination levels, and an integration with coastal and fishery management.[23] In this sense, application of models involving many areas, among them, morphology, physiology, geological processes, as well as those resulting from climatic changes of oceans water levels is highly desirable.[24]

## 10.4 Contaminants: Main Types, Origins and Effects on Biota

The liberation and dispersion of chemical compounds in aquatic ecosystems can occur through incorrect discharge. This can include disposal of untreated domestic sewage, industrial effluents, indiscriminate (or unnecessary) use of pesticides, and others that result from accidents with petroleum (or their organic derivatives), and atmospheric pollution from fossil fuel burning.[25] These compounds can lead to metabolic alterations, and strongly compromise vital physiological processes of the biota, with effects in respiration, reproduction and growth.[26]

In Brazil, the southeast coastal region of São Paulo state, comprises nine municipalities forming the Metropolitan Region of Baixada Santista (MRBS), and the most central portion (São Vicente, Cubatão and Santos) hosts one of the most industrialized regions of the Brazilian coast and the largest seaport of Latin America, with a human density of approximately 450 million people (see review in ref. 27). According to these authors, eight toxic metals, four organochlorine pesticides, 12 PAHs and one PCB contaminated this central coast of São Paulo state. These numbers are much higher than the southern coast, where the diversity of contaminants is 50% lower, with reduced presence of toxic metals (37.5%), similar amounts of pesticides and the absence of the other two pollutants. As a result of the higher human density on the central coast, resulting from the historic use of this area since the discovery of Brazil, pollutant amounts are higher than those cited in guidelines used by environmental agencies.[28] Among the identified substances present in water, sediment and local biota, there are dangerous toxic metals (*e.g.*, As, Cd, Pb, Cu, Cr, Hg, Ni, Zn, and others), PCBs widely used in plastic products and the paint and fluid industries, as well as PAHs, synthesized during incomplete burning of organic matter.[27,29,30]

Release of xenobiotic compounds in water can increase waterborne diseases, chemical contamination and accumulation/magnification effects of toxic metals.[31] As a consequence, decreased fishery stocks and reduced resource quality are obvious consequences of pollutant effects. Many of these substances present a toxicity period, persistence, mobility and bioaccumulation, leading to risks to higher trophic levels, especially to human health. As an example, accumulation of organochlorine, found in agricultural pesticides such as DDT and others organochlorine insecticides, favours bioaccumulation and biomagnification along the trophic chain.[32]

Bioaccumulation processes due to absorption and discharge of chemical substances through water and food ingestion have been a particular concern in estuarine regions, where high contamination indexes are frequently found (*e.g.*, Baixada Santista in Brazil). The bioaccumulation leads to biomagnification, a process where a substance is absorbed by aquatic organisms through the trophic chain, accumulating at higher trophic levels.[33] Bioaccumulation of xenobiotics depends on factors such as biological processes (feeding, physical/chemical composition and lifestyle) and environmental chemical compounds (pH, salinity, sediment composition, *etc.*).[34] Sediments reflect this variability owing to changes occurring in abiotic factors and promoting water quality alteration due to pollutant dispersion to the water column, followed by the distribution of potential toxic trace elements to biota and human populations.[29] It is important to highlight those studies using biomarkers and environmental impacts should take into account differences promoted by a range of abiotic parameters as a function of climatic seasons. Among them, local variations of abiotic parameters (*e.g.*, pH, salinity, and others) can cause distinct contamination levels in biota, influencing the health of these organisms.

Invertebrates play an important role influencing many biological functions in wetland ecosystems,[35] especially as food for birds and mammals. Generally, the diversity and richness of species decreases with local stressors (*e.g.*, wetland hydrology, vegetation complexity, and water quality). Considering aquatic biota, a more intensive effect occurs in freshwater as a function of the strong effect of pollutants from terrestrial and reclaimed lands generated by agronomical contamination sources (*e.g.*, organochlorine and organophosphate pesticides, petroleum products and other xenobiotics), which accumulate in water bodies, lagoons, rivers, estuaries and the ocean. It is clear that a higher concentration of contaminants in smaller water bodies causes a stronger impact compared to in the marine environment. In this sense, an evaluation of the conservation status of 255 species of Brazilian decapod crustaceans, conducted from 2010 to 2014 by the Chico Mendes Institute for Biodiversity Conservation (ICMBio), from the Brazilian Environmental Ministry (MMA), revealed that of 11% of threatened species ($N = 28$), 93% originated from freshwater, 4% from estuaries, and only 3% from marine origin.[36]

As previously mentioned, abiotic and biotic factors contribute to changes in the concentrations of some pollutants, and their complexation to other toxic molecules. An example is the presence of environmental mercury and its transformation to methylmercury, a known neurotoxin that is produced by anaerobic bacteria, with significant health risk to humans.[37,38] This is of relevance because most bioassays are specific to only one type of contaminant and, because of the chemical complexation that occurs with a set of contaminants, the results obtained with biomarkers are not clear enough to explain the contamination registered in a particular area.

The dynamics of bioaccumulation differ among contaminants, with a response variation according to the organisms and organs of the same individual.[39] Little is known about the physiological and biochemical modifications that affect organisms[40] or the changes in animals' homeostasis in chronically polluted areas. Some populations acquire tolerance mechanisms under moderate pollutant levels, developing mechanisms of excretion and other processes to deal with chemical compounds.[41] These mechanisms grant the animals in affected areas the ability to minimize and/or repair the effects of environmental pollution, especially through a detoxification ability, compartmentalization of metals in certain organs, higher excretion rates and even escape behavior.[39]

Crabs, in particular, are able to store toxic metals in intracellular organelles when these metals are above physiologically tolerable limits through detoxification processes that render the metals inactive.[42] High concentrations of metals can induce the cellular production of intracellular proteins, such as metallothioneins.[43] Toxic metals may also be captured by lysosomes,[42] or even compartmentalized into granules in detoxification organs, such as the hepatopancreas.[44] Gills, on the other hand, as the first organ affected by pollutants, represent a selective organ that is in contact with both the internal and external environments, acting as a temporary store for accumulated metals.[45]

The damage involves a cascade of events promoted by stressors[46] where bioindicator responses act at several levels of biological organization to show sublethal stress effects, serving as early warning signals in a causal relationship between stressors and effects, later manifested at higher levels of biological organization. Therefore, stress responses measured at a biochemical or physiological level can represent possible (and irreparable) future damage to the population and community levels (Figure 10.2). Therefore, biomarkers reflecting the health status at lower organizational levels show an immediate response to stress and have a high toxicological relevance, while biomarkers that reflect health conditions at higher organizational levels respond slowly to stress and have decreased toxicological relevance, but are more relevant ecologically.

## 10.5 Environmental Monitoring Based on Biomarkers

Faced with a growing panorama of industrial and reckless occupation of important ecosystems on our planet, there is an acceleration and intensification of environmental degradation, with risks and consequences requiring continued vigilance. Biota exposed to pollutants react to the exposure, and the response produced can be identified and measured using biomarkers.[34] The use of these biological parameters reflects the behaviour and the interactions between toxic agents and the biological systems, and can be used as powerful tools for environmental monitoring. Their responses can be used as a signal of toxic effects to the organism, involving perturbations of biochemical and molecular nature inside the cells, later leading to effects at higher levels of organization.[46]

Biomarkers can be used in a variety of studies and types of chemical exposition. The general idea of their application is the possibility of precocious detection of environmental perturbations that can contribute to the decline of populations or whole communities, through sublethal effects, not those responsible for an immediate animal death, but rather for alterations of biological processes, as in endocrine and reproductive systems, for example.[34] In general biomarkers can be classified into three types:

(1) Exposure: covering the detection and measurement of an exogenous substance or its metabolite or the product of an interaction between a xenobiotic agent and some target molecule or cell that is measured in a compartment within an organism.

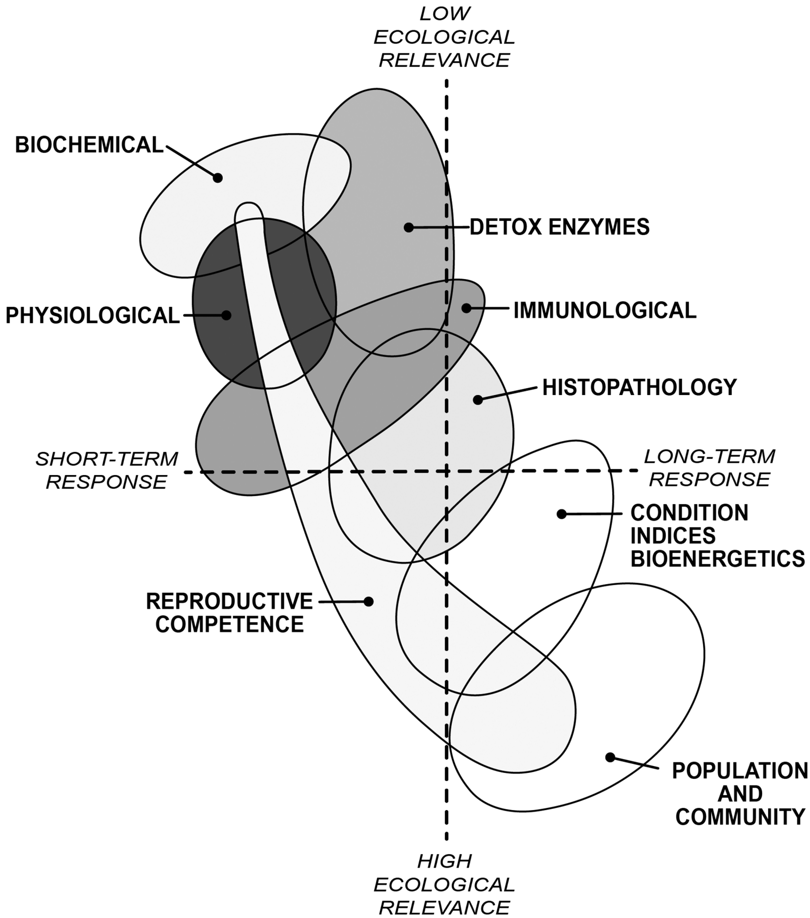


**Figure 10.2** Levels of biological responses to pollutant stress in aquatic organisms, representing the continuum of these responses along time gradients and according to toxicological and ecological relevance.[46]
Reproduced from *Mar. Environ. Res.*, **28**, S. M. Adams, K. L. Shepard, M. S. Greeley Jr, B. D. Jimenez, M. G. Ryon, L. R. Shugart, J. F. McCarthy. The use of bioindicators for assessing the effects of pollutant stress on fish, 459–464, Copyright 1990, with permission from Elsevier. Redrawn by Gustavo Pinheiro.

(2) Effect: including measurable biochemical, physiological or other alterations within tissues or body fluids of an organism that can be recognized as being associated with an established or possible health impairment or disease.
(3) Susceptibility: indicating the inherent or acquired ability of an organism to respond to the challenge of exposure to a specific xenobiotic substance, including genetic factors and changes in receptors which alter the susceptibility of an organism to that exposure.[34,39]

Since the perturbations occurred by exposure to contaminants are evidenced by changes in different biochemical processes (biotransformation and excretion), cellular damages can occur, with special attention to protocols involving DNA and organelles damages. The action of xenobiotics on DNA is called 'genotoxic disease syndrome', where DNA damage can promote a strong effect at the individual and population levels, and genotoxic effects in marine organisms exposed to organic compounds, such as aromatic hydrocarbons.[47–49]

Environmental monitoring *in situ* is widely used for some crustacean species, such as Micronucleus Assay (MN‰), Comet Assay (CO) and the Neutral Red Retention Time (NRRT), all indicating the cellular, genetic and physiological integrity of the organism.

Micronuclei are structures formed from snippets of chromosomes that are not incorporated into the nucleus of the daughter cells during cellular division, with subsequent encapsulation of these DNA snippets, promoting a depletion of genic expression, endangering the production of proteins by cells, and causing DNA damage.[50] Thus, the generation of micronucleus cells in high frequency when compared with basal values in the mollusc *Mytillus galloprovincialis* (>4 MN‰),[51] and persistence in the cytoplasm are indicative of extended interaction with pollutants that are compromising DNA integrity. The micronucleus assay has been used for a long time in environmental monitoring to evaluate genotoxicity, as well as in toxicity tests (acute and chronic), owing to use equipment requirements and low cost. A single hemolymph drop (in the case of arthropods, such as crustaceans) can produce thousands of valid cells for analysis without any special treatment, except for fixation (Carnoy solution), coloration (Giemsa solution), and micronucleated cell counting under a microscope, in relation to 1000 cells analysed. The test does not need a trained cytogeneticist, and hence the protocol is considered an excellent tool to be applied in management projects of conservation units (CUs).

The Comet assay (single cell gel electrophoresis) is a technique that is able to detect DNA damage in single cells,[52] and has wide use in biomonitoring of genotoxic agents.[53] The advantages of this assay include its simplicity, quick performance and high sensibility to many types of DNA damage,[54] confirmed by alteration of the damaged DNA during its migration in electrophoresis gel. Cells in eukaryote organisms have DNA of a few centimetres in length, which needs to be strongly condensed inside the cell nuclei. Damaged DNA is less condensed and, occasionally, structural breaks appear,[55] with differential migration in the gel slide as a function of the size of the snippets. Cells with non-fragmented DNA will have a preserved nucleus during electrophoretic migration, maintaining its circular form, whereas DNA with minor damage tends to migrate more quickly. When the DNA is very damaged, many snippets with distinct sizes are formed with DNA migration at different velocities, generating a typical figure of a Comet tail.[56] This is a more sensitive method to detect genomic microlesions, allowing individual identification of damage in most eukaryote cells,[57] and is used as an ecological indicator for diagnosis and monitoring of coastal areas.[58,59] Success has been obtained with this assay during environmental monitoring using vertebrates such as fishes, amphibians and mammals, as well as invertebrates such as decapod crustaceans.[60–62]

The integrity of the lysosome membrane (neutral red retention time, NRRT) is an *in vitro* test that has been used as a chemical stress biomarker for the detection of xenobiotics' harmful effects in organelles. This test is low cost, efficient and easily replicated, based on the observation of the retention time of the neutral red dye by the lysosome membrane, evaluating its selective permeability.[63] The first animals that NRRT was used in were in bivalves, where their hemocytes were applied for detection of alterations in the lysosome membrane as a function of pollutant exposure, causing autophagy and cellular degeneration processes.[63] Evidence suggests that NRRT is a biomarker for a large range of chemical stressors owing to its high sensitivity to a set of contaminants and it is thus recommended in environmental monitoring.[64] Animals subject to constant stress in their environment show damage in their cellular membrane through the action of free radicals, affecting lysosome functions, such as recycling of other cellular organelles, cellular constituents and particles from the external environment. Therefore, a reduction of the lysosomal membrane stability is considered a general indicator of the physiological condition of the individuals,[65] affecting cellular nutrition, immunological defence processes, and reproductive efficiency of marine invertebrates,[66] and can predict future damages to higher levels of biological organization.[64,67]

Finally, it is important to highlight that some biomarkers are specific for a set of pollutants. As an example, we can mention the protein metallothionein (MTs), a biomarker specially synthetized by aquatic organisms (*e.g.*, crustaceans) and used exclusively to bind some essential and non-essential toxic metals (*e.g.*, arsenic, cadmium, copper, mercury, selenium and zinc),[68] but not all metals. Other physiological (*e.g.*, NRRT) and genetic biomarkers (*e.g.*, CO and MN‰) are more representative for environmental monitoring because they respond to a range of environmental pollutants. This indicates the greater importance of some biomarkers when compared to others; some are more relevant for monitoring environmental quality.

## 10.6 Sampling Design: Spatial Distribution, Replicates, and Other Parameters

Studies conducted using biomarkers for environmental monitoring need a careful sampling design to obtain reliable data to be used in coastal management within a study area. When the objective is to determine the response of biota to specific pollutants, it is necessary to carry out an initial screening test to avoid expenditure of financial resources because some methods are very expensive to apply (*e.g.*, concentration of toxic metals, PAH levels, and others). A bibliographic consultation can provide details of common contaminants in a selected area to be studied, as well as knowledge about industrial facilities present in the area, all of them providing information that is important to reveal the set of xenobiotics that can influence the local biota, specially sessile and/or resident species.

During the screening phase, other aspects to be considered are information about the total size of the area to be studied, as well as the spatial distribution of the species, which are important measures to obtain an optimum response. A comparison between estuarine systems also involves concerns with the selection of subareas where the organisms occur, which should have similar characteristics. Considering different ecosystems pertaining to estuarine systems (*e.g.*, mangrove forests, estuarine channel), selected subareas should have similar abiotic (*e.g.*, granulometry of the sediment and flooding level) or biotic factors (*e.g.*, arboreal composition and/or dominance of mangrove forest). However, some factors are considered determinant in some cases owing to the higher variation in estuarine systems (*e.g.*, salinity and water pH), where different physiological processes can affect the biota subjected to a specific pollutant (*e.g.*, toxic metals), as mentioned earlier. Therefore, a good sampling design leads to a good spatial area representation, with a selection of similar subareas and, whenever possible, separating haloclines with freshwater, brackish water and seawater characteristics (Figure 10.3). According to the goal of the study (*e.g.*, a specific river, a region inside an estuary or an entire estuarine system) it is necessary to establish a reduced number of subareas as a function of size and objectives (B, C and D) or a wider number of subareas to be sampled that represent an entire mangrove peninsula region (A) or estuarine system (E).

Another important fact is that the subareas should represent true replicates, with the capture of a minimum (and adequate) number of specimens in each one. As an example, in studies conducted in São Paulo State, we used a minimum of five individuals per subarea to perform CO and MN‰ assays, generally with three subareas representing the whole studied area ($N = 15$). For the NRRT assay, a greater number of individuals is required (10 individuals per subarea; $N = 30$ per area). However, the number of subareas depends on the total size of the area to be studied. Following this procedure, the reliability of the data obtained is increased, avoiding pseudoreplication, which is not statistically independent.[69,70]

## 10.7 Case Study of Mangrove Crab *Ucides cordatus* and Other Semi-terrestrial Brachyuran Crabs

The mangrove crab *Ucides cordatus* (Linnaeus, 1763), called the *uçá* crab, is a semi-terrestrial brachyuran species endemic in mangroves, with economic relevance in coastal areas of the occidental Atlantic coast of the south American continent, generating employment and income to riverside communities.[71] This species has a geographic distribution from Florida state

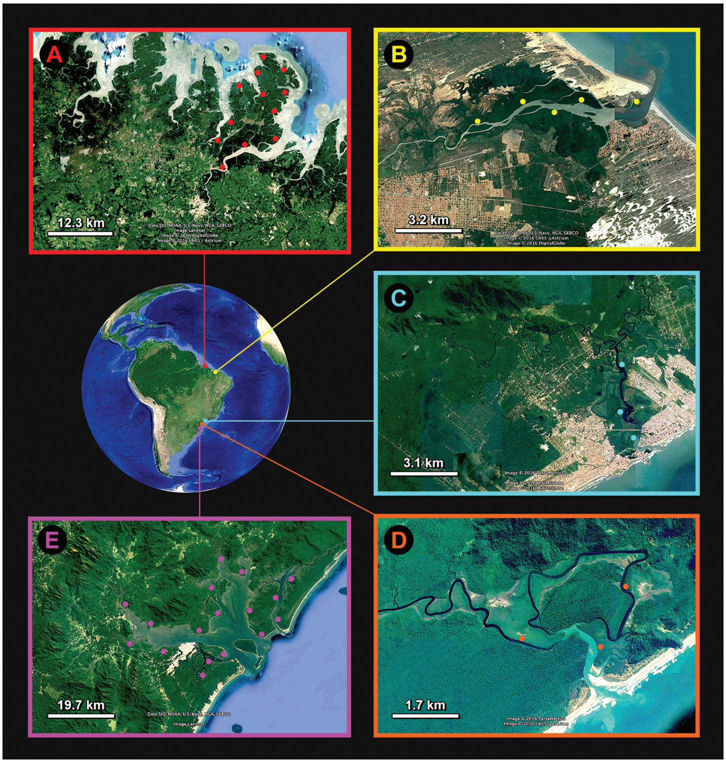


**Figure 10.3** Examples of some Brazilian estuarine systems with indication of sub-area sampling in relation to size, more important in greater than in smaller areas. A: Bragantina peninsula in Pará state (near Bragança city), very large and jagged, with expressive size and an integral mangrove vegetation cover ($N = 12$). B: Delta of the Parnaíba river (near Parnaíba city), in Piauí state, with a medium size ($N = 5$). C: Itanhaém river (in Itanhaém city), in São Paulo state, with reduced mangrove fragments and reduced size, with two small tributary rivers (left and right in the upper position), where the salinity wedge does not penetrate ($N = 3$). D: Estuarine system of Una river at Juréia-Itatins Ecologic Station (22.5 km from Peruíbe city), with a pristine mangrove but with a reduced size ($N = 3$). E: Paranaguá Estuarine system, with a large and expressive size and a complex structure, formed by Paranaguá Bay, two small estuarine cities (Antonina at W, and Guaraqueçaba at N) and conservation units at Superagui National Park, requiring more sampling ($N = 17$). In the last example, the sampling design followed the complexity of the environment with different anthropic pressures. Photos from Google Earth and (A) from Data SIO, NOAA, U.S. Navy, NGA, GEBCO, Image Landsat. Image copyright 2016 DigitalGlobe, image copyright 2016 CNES/Astrium. Photo (B) from Data SIO, NOAA, U.S. Navy, NGA, GEBCO, image copyright 2016 CNES/Astrium, image copyright 2016 DigitalGlobe. Photo (C) image copyright 2016 TerraMetrics, image copyright 2016 DigitalGlobe. Photo (D) image copyright 2016 TerraMetrics, image copyright 2016 CNES/Astrium. Photo (E) from Data SIO, NOAA, U.S. Navy, NGA, GEBCO, Image Landsat. Graphic design by Gustavo Pinheiro.

(USA) to the south of Brazil (Laguna city).[72] The crab reaches a large size in the adult phase,[71] building burrows in the muddy sediment, mainly in the intertidal mangrove zone, feeding on senescent leaves and propagules of the arboreal species in this ecosystem,[73] representing an ecological relevance in recycling nutrients in this environment.[71,74] Due to their economical importance, there is an intense capture above the growth capacity, which affects population renewal, mainly in estuaries of the North Brazilian region.[75] The capture is artisanal and occurs through the introduction of the arm of crab catchers or using fishing tackle, some of them traditional (*e.g.*, catch hook) and others prohibited by Brazilian law (*e.g.*, a trap called a *redinha*).[71,75] *U. cordatus* has a slow growth rate and it reaches its maximum size by 10 years, with maturity age at 3 years old and commercial size at 4 years old for males (CW, carapace width $\geq$60 mm).[76] Capture of females is prohibited by law during the reproductive season (December to May), while males cannot be captured during the *andada* phenomenon, when individuals of this species actively walk on mangrove sediment during reproduction.[75,77] Owing to these biological limitations, this species was included in Annex II of the Normative Instruction n°5/2003,[78] where it is categorized as underexplored or threatened by overexploitation, with other species of invertebrates and vertebrates used for fishing. Since 2011, the National Plan of Management indicated the need for monitoring populations of three species of the infraorder Brachyura with commercial importance (*U. cordatus, Cardisoma guanhumi* and *Callinectes sapidus*).[79] The National Plan Management reinforced the importance of maintaining the quality of the estuarine environment, with the establishment of a Monitoring Program to evaluate water and sediment quality, as well as the meat of these crustaceans, aiming to elaborate recovery plans.[79]

Populations of *uçá* crab have been subjected to a range of anthropic environmental pressures. Therefore, this globally distributed ecosystem of mangroves is continually affected by mining activities, effluent discharge, deforestation, grounding, and improper occupation by aquaculture tanks, among others.[11] In this reality, all mangrove species have been influenced by the synergic action of a range of pressures; among them are those caused by pollutants, which are damaging at many biological levels.[80] Metal contamination and the bioaccumulation that occur in local fauna is based on contact with different environmental matrices (water and sediment) and by trophic pathway.[42] In this sense, *U. cordatus* is highlighted as a bioindicator species due to many characteristics that are considered relevant in monitoring studies *in situ*, based on its capacity of dealing with metals and xenobiotics by different pathways,[30] such as:

(1) Trophic position: *uçá* crab use as food the leaf litter composed of senescent leaves and propagules of arboreal species in mangroves (*e.g., Rhizophora mangle, Laguncularia racemosa* and *Avicennia* spp. in Brazil).[73] The transference of toxic metals and other pollutants from the sediment to vegetation is a result of metabolic processes of each

resident vegetal species, having a special role in water and sediment depuration of high quantities of these pollutants.[81] The decomposition process is very common in mangroves as an effect of decomposers (fungi and bacteria) on senescent leaves and other organisms, and contributes to reduce dissolved oxygen in the sediment, directly affecting the mobility of toxic metals. This organic matter (particulate or dissolved) is transported by tides and accumulates toxic metals, promoting contamination of the trophic chain in adjacent coastal ecosystems.[82] This accumulated biomass transfers metals to the sediment, and also to higher trophic levels by nutrient recycling promoted by mangrove crabs, increasing bioavailability, for example, through filtering and through decomposing organisms. Studies about the relationship between consumption of *R. mangle* leaves and assimilation of metals by *U. cordatus* have been conducted in Brazilian regions (north and southeast), proving that the *uçá* crab accumulate metals in dangerous quantities for human consumption.[30,83]

(2) Bioturbation of sediment: occurs when this species digs the muddy sediment and incorporates organic matter and nutrients.[73,84] Due to the mineralogical characteristics of this sediment, being anoxic with fine granulometry, it is a typical reducer, acting as a true 'sink' of heavy metals, petroleum, and other residues (organics and inorganics).[14,85] However, in natural conditions, without the influence of pollutant sources, these sediment characteristics ensure the reduction of potential deleterious effects caused by metals to biota, impeding the remobilization and availability of these pollutants.[14] When inside burrows, *U. cordatus* maintains close contact with the sediment, the water in the burrow, and contaminants present in ingested leaf litter;[73,74] as a consequence this species is subjected to three sources of contamination.

(3) Biological limitations: due to slow growth rate and long life cycle, expressive abundance, wide distribution and a low mobility during their life.[76] The minimum capture size of 60 mm carapace width by law, and a minimum age of 3 years old, is a relevant standardization to allow comparisons. Another important characteristic of decapod crustaceans is the selection of intermoult exemplars to use in biomarker evaluations because in the pre- and post-moult stages there are many metabolic demands, compromising the dynamics of absorption of pollutants and their defence system.[58] The larval development of *U. cordatus* comprises six instars of a zoea stage and one stage of megalopa, that last one moulting to juvenile. During the first juvenile stage the animals have reduced size, being attracted by conspecific odours that are left by adults in the sediment, aiming to attract individuals for recruitment in mangroves.[86–88] Therefore, polluted mangrove areas can affect the attractiveness of the *uçá* crab and recruitment, leading to a reduction of population density.[27] Otherwise, this species can be used as a testimonial of mangrove

health, which has been proved in ecotoxicological studies that indicate the reliability of the contamination status of mangrove ecosystem using the *uçá* crab as a bioindicator. Thus, in Brazil *U. cordatus* has been used as an important bioindicator of environmental quality and is strongly responsive to many pollutants (*e.g.*, oils, polycyclic aromatic hydrocarbons, toxic metals, and others).[30,89] Many studies have identified the accumulation of some pollutants in greater or lesser amounts in environmental matrices (*e.g.*, sediment, water and food), as well as accumulation in different tissues of the crab. In the case of decapod crustaceans, such as *U. cordatus*, some contaminants such as toxic metals can accumulate in the muscles (meat), gills, midgut gland (hepatopancreas),[30,89–91] including the carapace (*e.g.*, Pb), and even in eggs carried in the female abdomen.[92]

On the São Paulo state coast, Brazil (see Figure 10.4), there are highly contaminated mangrove areas, contrasting with pristine areas inside conservation areas managed by governmental institutions (*e.g.*, Juréia-Itatins Ecologic Station, supervised by the Florestal Foundation, an environmetal agency). In general, the north coast of São Paulo has a few mangrove fragments, with greater abundance occurring on the central and south coasts of this state. On the central coast of São Paulo state the first Brazilian colony (São Vicente) was established in 1532, and historically it is the most anthropic region, where the Santos port and Cubatão industrial complex facilities were established, both with a high population, contrasting with the south coast of this state, which have preserved mangroves with no human influence (317 600 and 2606 habitants km$^{-2}$, respectively).[93] Studies using two biomarkers (micronucleus and neutral red, see Figures 10.5 and 10.6, respectively) were conducted in *U. cordatus*, indicating that mangroves of the south coast of São Paulo state have a better conservation status, considered for categorization purposes[27] as areas with a Probable No Impact (PNI), where these biomarkers were <3 MN‰ and >120 minutes NRRT. Juréia and Cananéia are examples of these mangrove areas, both localized in the large Environmental Protected Area (EPA) of Cananéia, Iguape and Peruíbe. Considering the same categorization purpose, Cubatão and São Vicente, both pertaining to the Estuarine Complex of Santos-São Vicente, were considered as Probable High Impact (PHI) to the health of biota in that area, based on the *U. cordatus* response, with values >5 MN‰ and <60 minutes for NRRT.[27] Iguape was categorized as a Probable Low Impact (PLI) area, where intermediate values were found for each biomarker. Another study captured a specimen of *U. cordatus* with cheliped malformation in São Vicente (in the east of SAV1 subarea, see Figure 10.4), presenting a large quantity of micronuclei ($11.5 \pm 2$ MN‰), explained by a set of industrial contaminants from Cubatão and close to two public dumps (called *Alemoa* and *Sambaiatuba*).[94] The last influence was caused by a high concentration of leachate produced by these dump sites that carries great concentrations of many xenobiotics, mainly toxic metals.[95]

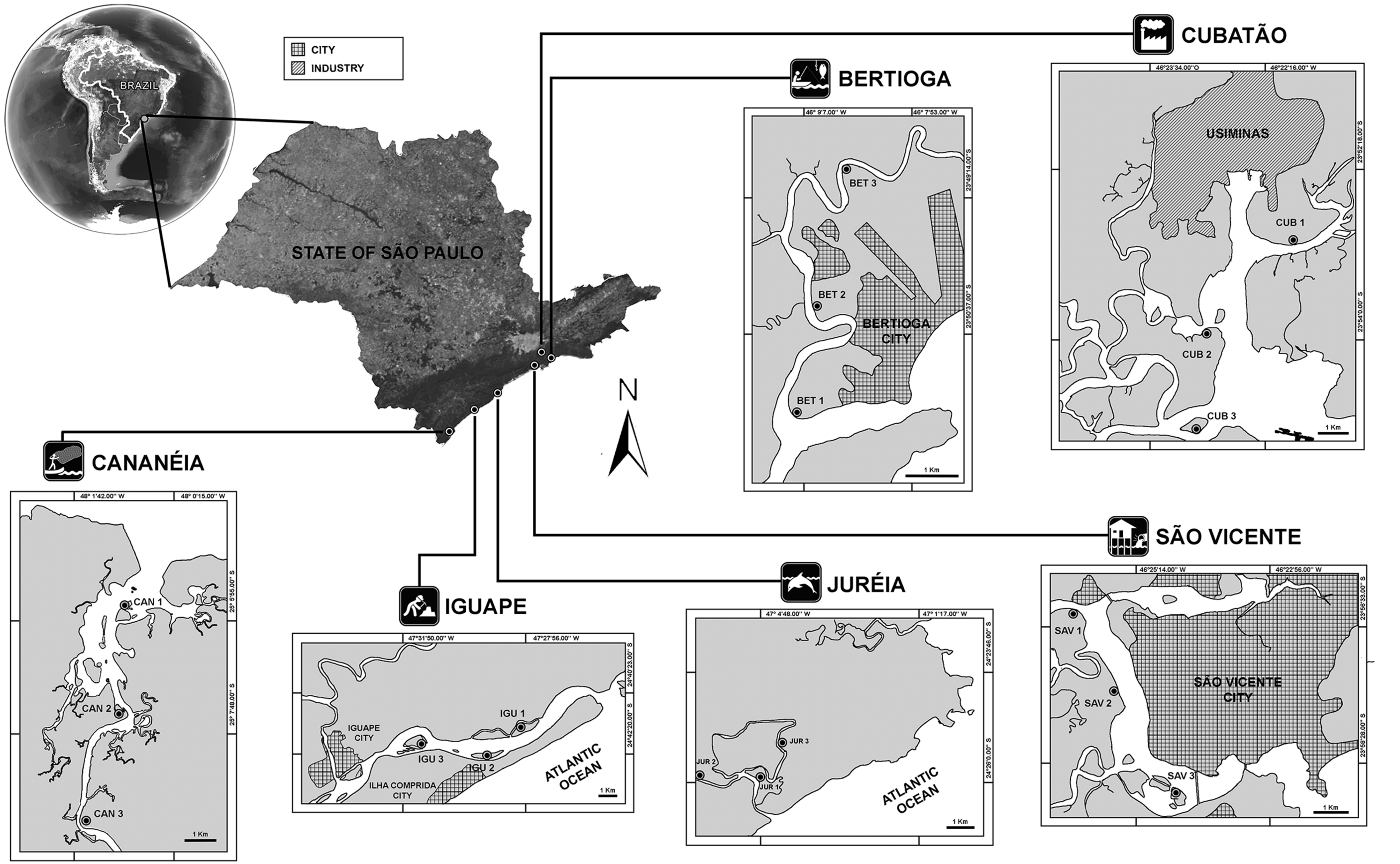


**Figure 10.4** Location of six mangrove areas sampled in the state of São Paulo (Brazil), represented by 18 subareas located in the central and southern coasts.
Figure created using data from L. F. A. Duarte, C. A. Souza, C. R. Nobre, C. D. S. Pereira, M. A. A. Pinheiro. Multi-level biological responses in *Ucides cordatus* (Linnaeus, 1763) (Brachyura, Ucididae) as indicators of conservation status in mangrove areas from the western Atlantic, *Ecotoxicol. Environ. Saf.*, 2016, **133**, 176–187.[27] Graphic design by Gustavo Pinheiro.

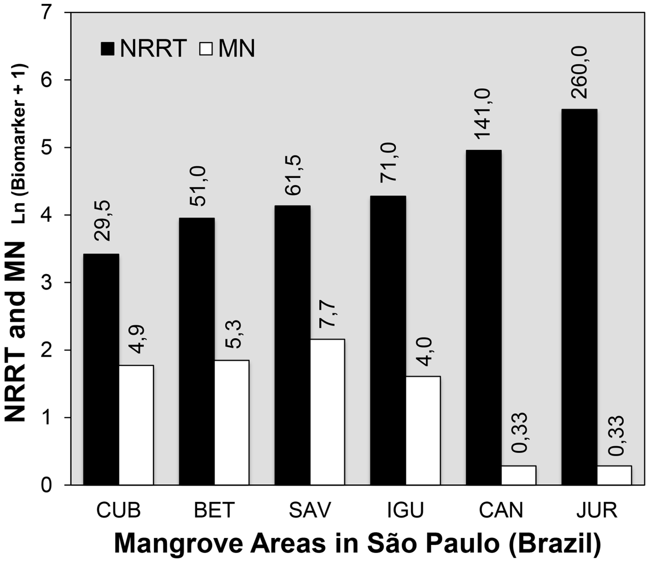


**Figure 10.5** Mean values of Neutral Red Retention Time (NRRT, in minutes) and Micronucleus assay (MN‰, micronucleus cells in 1000 analysed) obtained from the crab *Ucides cordatus* (Linnaeus, 1763) from six mangrove areas sampled in the state of São Paulo (Brazil), represented in a logarithmic scale. Values were from three subareas, each one with replicates for NRRT ($n = 10$ per subarea and 30 per area) and MN‰ ($n = 5$ per subarea and 15 per area). Data were obtained during the conduction of the Project Uçá (Phase III – FAPESP 2009/14725-1).[27,115] Graphic design by Gustavo Pinheiro. Where: BET, Bertioga; CAN, Cananéia; CUB, Cubatão; IGU, Iguape; JUR, Ecologic Station of Juréia-Itatins; and SAV, São Vicente.

*Ucides cordatus* has been used for physiological studies related to toxic metal transport using both gills and hepatopancreas cells as tissue targets.[96–98] In general, both Cu and Cd are transported through cell membrane transporters that involve carriers associated with calcium transport.[97,98] Posterior gills, which are responsible for ion transport, compared to the respiratory role of anterior gills, transport and accumulate more toxic metals compared to anterior gills.[98] These should be target organs for study of pollution effects, together with the hepatopancreas, which is known in these crabs as a detoxifying organ, similar to the pancreas and liver in vertebrates. Studies with discarded drugs containing iron (Fe) showed that this metal is transported in *U. cordatus* hepatopancreatic cells, rendering these crabs as good models to study the deleterious effects of discarded metal-rich wastes.[99]

*Ucides cordatus* responds well to biomarkers such as the neutral red retention test and the micronucleus assay.[27,89,100] Additional stress biomarkers, such as metallothionein (MT),[96] a protein found in the cell cytoplasm of a range of animals, respond specifically to pollutants known as

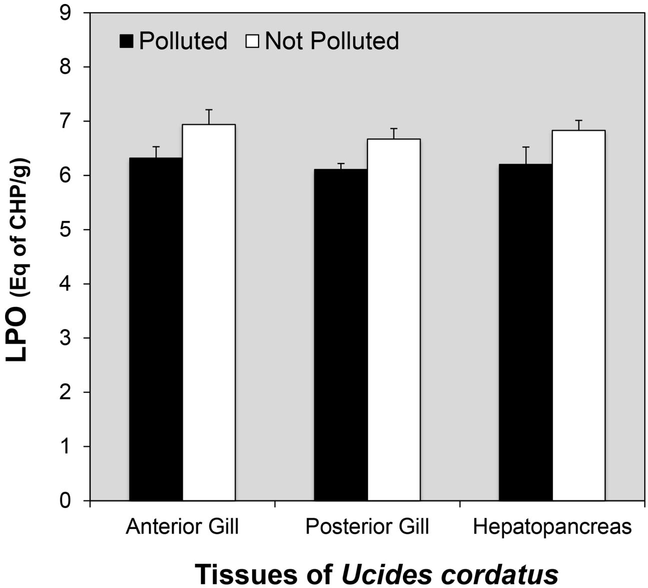


**Figure 10.6** Mean values of lipid peroxidation (LPO, in Eq CHP$\times$g$^{-1}$) using three tissues (anterior gill, posterior gill and hepatopancreas) of the crab *Ucides cordatus* (Linnaeus, 1763) in a polluted mangrove (Itanhaém river) and a non-polluted one (Ecologic Station of Juréia-Itatins), measured from four individuals in triplicate.
Graphic design by Gustavo Pinheiro. Data obtained from M. G. Sá, F. P. Zanotto. Characterization of copper transport in gill cells of a mangrove crab *Ucides cordatus, Aquat. Toxicol.*, 2013, **144–145**, 275–283.[96]

toxic metals, and for specific ones like Cu, Cd and Zn.[101,102] Another stress biomarker of environmental pollution is lipid peroxidation (LPO), which is also associated with the presence of metals[103–105] and oxidative processes in general, caused by a myriad of other pollutants. Several studies with invertebrates have shown that with rising pollution levels there is an increase in the antioxidant systems, including lipid peroxidation.[104] The crab *U. cordatus* from polluted and unpolluted regions in Brazil was responsive to LPO and there was a good correlation between LPO and pollution levels (Figure 10.6), but not when metallothionein levels were evaluated, which were not directly related to metal pollution for *U. cordatus*.[96] These results suggest that *U. cordatus* from chronically polluted regions do not increase metallothionein production as a defence mechanism. Therefore, in crabs from chronically polluted regions, it is possible that the main mechanism of detoxification occurs through the formation of vacuoles and/or accumulation of metals in cellular organelles, leaving the metals in a non-toxic state.[96] Such mechanism has already been seen in *U. cordatus*, with respect to Zn accumulation in the hepatopancreas.[45] Moreover, there are tissue-related variations in MT levels and the relative influence of contamination

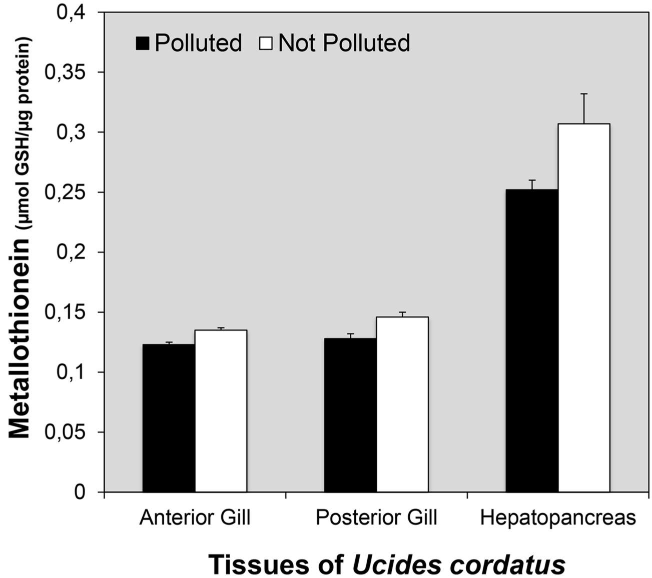


**Figure 10.7** Mean values of metallothioneins (MT, μmol GSH μg of protein$^{-1}$) using three tissues (anterior gill, posterior gill and hepatopancreas) of the crab *Ucides cordatus* (Linnaeus, 1763) in a polluted mangrove (Itanhaém river) and a non-polluted one (Ecologic Station of Juréia-Itatins), measured from four individuals in triplicates.
Graphic design by Gustavo Pinheiro. Data obtained from M. G. Sá, F. P. Zanotto. Characterization of copper transport in gill cells of a mangrove crab *Ucides cordatus, Aquat. Toxicol.*, 2013, **144–145**, 275–283.[96]

factors limits the possibility of using MT levels as a reliable biomarker of metal exposure (Figure 10.7).[96]

Although crabs in general are able to metabolize and get rid of some of the toxic metals loaded into cells through vacuole disposal,[42] and possibly through the shedding of the exoskeleton, they are, as stated above, good and abundant models to indicate environmental contamination, mainly for polluted mangrove areas where they live, in addition to the established filter-feeding models, such as mussels.

Often a unique bioassay is not sufficient to reveal the effect of pollutants in a studied area. As an example, *U. cordatus* were subjected to two biomarkers assays (MN‰, micronucleus in hemocytes; and PM, pyrene metabolites in urine) related to oil-derived PAHs in mangrove sediments; no significant results were found for the MN‰ assay but excellent results were obtained for the PM assay, showing that *U. cordatus* is an excellent bioindicator of mangrove quality related to the concentration of individual or total PAHs.[61,106] In such cases, physiological analyses combined with biomarkers evaluation (*e.g.*, NRRT) can be very effective for quantification of environmental contamination and to establish their contamination category.[27]

Another aspect to be considered is the number of environmental matrices (*e.g.*, water, sediment, and contaminated food items) that each crab contacts in their habitat. Some crabs are exclusively aquatic (*e.g.*, Portunidae species, frequently called swimming crabs), while other species inhabit terrestrial environments (Figure 10.1), and have more matrices to interact with, as well as more pollutants sources to get contaminated by. These semi-terrestrial species have similar characteristics when compared with *U. cordatus*, and can provide good testimonials of environmental quality, but sometimes they interact to greater or lesser degrees with environmental matrices. Examples include the Occidental Atlantic mangroves of South America, where the species *A. pisonii* (Figure 10.1C) interacts with water and green leaves, while the red mangrove crab *G. cruentata* (Figure 10.1B) explores and interacts with more mangrove matrices (*e.g.*, water, sediment and plants/animals used as food items).[107] However, studies involving these species are still incipient, but reveal a promising application using the micronucleus assay and enzymatic activity. Therefore, the use of these species allows a comparison between polluted and pristine areas, and can provide valuable information about the status of diverse environmental matrices, including different trophic levels.[58,107] In addition, *U. cordatus* (Figure 10.1A) has an ecological similarity when compared with *C. guanhumi* (Figure 10.1E), considering the large contact with many environmental matrices in mangrove and *restinga* areas, respectively. However, *guaiamú* crabs (*Cardisoma guanhumi*) build their galleries several hundred meters offshore, particularly along estuaries and river banks, composed of sand, associated with adjacent coastal forests (*e.g.*, Atlantic forest), 5 km from rivers.[108,109] Owing to its occupation of coastal habitats, *C. guanhumi* has been studied from a genotoxic point of view in the northeast region of Brazil, where it is abundant and used as food by man, and the results obtained revealed its importance as a sentinel species as well as the future use in diagnosis and environmental monitoring.[110]

The ghost crab *Ocypode quadrata* (Figure 10.1F) has also been considered an important indicator of anthropic impacts, with population levels varying according to different recreational uses in sandy beaches, mainly related to density.[111–113] Therefore, this species could be used as a model to represent changes in these environments, with potential applications in studies involving biomarkers, although its longevity is only around 3 years,[114] comprising 30% of the entire lifespan of *U. cordatus*.[76]

Among all crab species studied, *U. cordatus* can be considered as a good bioindicator of mangrove quality. Results obtained from genetic and physiological biomarkers are comparable with local contamination, especially NRRT results, which indicate a reliable response to pollutant effects based on a set of contaminants in mangrove areas. In conclusion, these crab species are very useful bioindicators of environmental contamination owing to their abundance and easy capture, and because some of them show a relatively long life, an uncommon feature in macroinvertebrates.

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