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# Freshwater Decapods and Pesticides: An Unavoidable Relation in the Modern World

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## 1. Introduction

### 1.1 Levels of organisation in biological systems and their relationships

Scales in nature can be difficult to define and understand because several ecological factors can interact. The study of different biological scales contributes to information that varies in its quality and significance for humans. Observations at the ecosystem scale are of great ecological significance but can be of low quality or provide little information about causes; at the other extreme, molecular studies that provide exact determinations of causes can have very little relevance to effects at a larger scale (Figure 1). In the middle of these extremes are observations that provide more or less significant and relevant information. Increasing the level of biological complexity in our observations can lead to an unexpected increase in the number of variables to be considered, requiring the consideration of n-adimensional conditions.

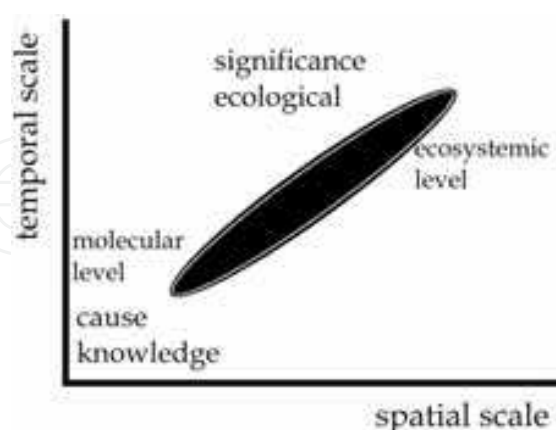


Fig. 1. Relationship between spatial and temporal scales with the quality information obtained

Each level of study is influenced by the level below it, and each level affects the level above it, which is mediated by interspecific relationships that influence ecosystem structure, and this can vary according to the heterogeneity of an ecosystem. When biological complexity

increases, it is important to consider that temporal and spatial dimensions are interconnected, e.g., molecular reactions occur in spaces smaller than one-hundredth of a millimetre and at reaction times of less than one second. At the same time, the effects of the predation of one species on the populations of other species may play out in spaces at the scale of kilometres and at timescales that can exceed a year. According to the heterogeneity of a system, its component species and the processes involved, variations in the time and space involved in a given process can be very important (Figure 1).

## 1.2 Fauna in aquatic systems

Year after year, the quality of aquatic environments is recognised as a priority for humanity, with particular emphasis on the quantity and quality of freshwater.

Among the faunal components of aquatic environments, decapods, an order of crustaceans, are an interesting group that possesses biological characteristics useful in assessing the quality of inland aquatic systems. In addition, some species of decapods may be used as food by humans and are part of the food chain of other species used by humans as food, mainly fish and birds.

Five decapod families occur in southern South America and east of the Andes. Some of these decapods are endemic at the family level, others at the genus level and still others at the species level. These families include prawns and shrimp (Palaemonidae and Sergestidae), crabs (Trichodactylidae), pseudocrabs (Aeglidae) and crayfish (Parastacidae) (Collins et al., 2007).

Some of these families live in burrows constructed of fine sediment (some Trichodactylidae and Parastacidae). Others live in the background using clasts, rocks or tree trunks for hide under this cover (Aeglidae). Some decapod families live among aquatic vegetation (some Trichodactylidae and Palaemonidae), while others live all or part of their lives in the water column (some Palaemonidae and Sergestidae). Thus, the habitats used by this group are very diverse, and different taxa have different relationships to the land environment. The densities of decapods can be very high at certain times of year and may exceed 500 animals per square meter (e.g., Palaemonidae). Their diets are varied and may include plant matter (e.g., aquatic plants and phytoplankton debris), microinvertebrates (e.g., protozoa, cladocerans, rotifers, and copepods), macroinvertebrates (Palaemonidae insect larvae, oligochaetes, molluscs) and vertebrates (fish). The trophic resource used by decapods is mainly composed of live animals, but dead animals are also commonly fed upon. Consumption intensities are very high, transferring energy and material from various bottom levels (e.g., oligochaetes, chironomid larvae, zooplankton, and vegetal remains) to the top trophic levels (e.g., fish, mammals, reptiles, birds) (Collins & Paggi, 1998; Collins 1999; Williner & Collins, 2002; Collins 2005; Collins et al., 2006).

Since the industrial revolution, the human population has been growing rapidly and has therefore required more intensive management of natural environments. This need for intensive management has included the use of more land for growing food, causing the conversion of forests, jungles, and grasslands, among other ecosystems, into farmland. Subsequently, different poisons (e.g., herbicides, insecticides, fungicides) have been employed with the aim of eliminating those plants and animals that could use the crop resources (cereal or other crops), which humans call "pests". The use of these chemicals grew during the last century in an unprecedented manner in both volume of use and in the

different formulations available. In addition, the creation of new compounds, the genetic modification of plants to withstand pesticides, and the improved effectiveness of the methods of pesticide application have had substantial economic support for research and development. Unfortunately, studies of the damage caused by these chemicals have not had similar financial support.

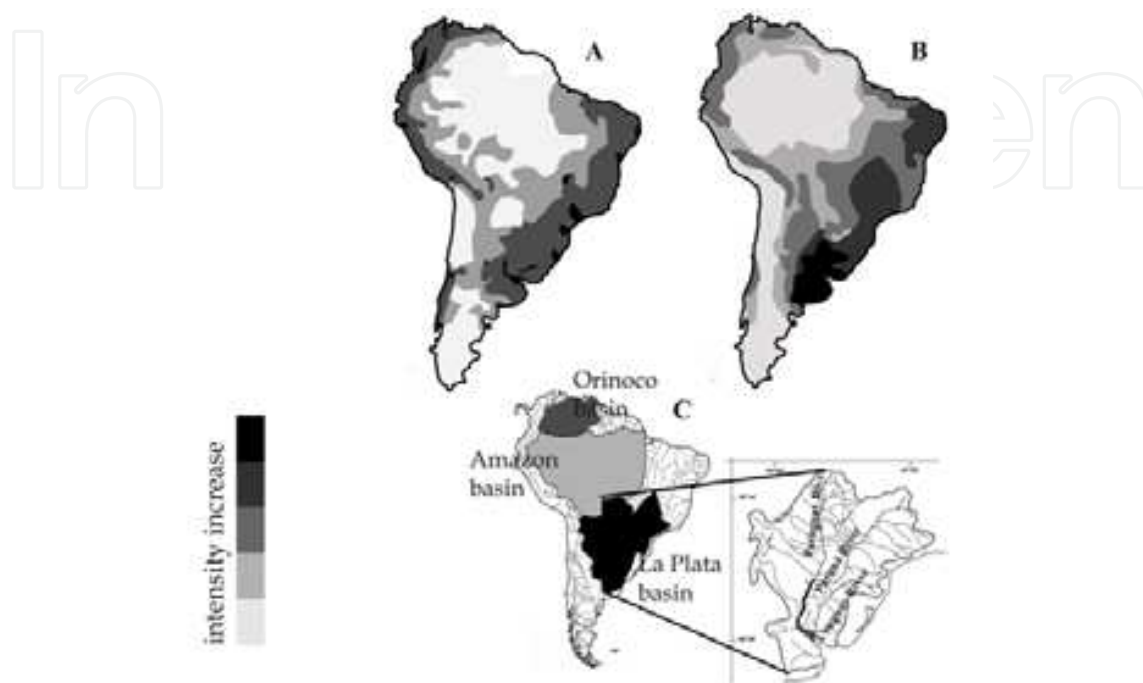


Fig. 2. Land use in South America indicating the surface of Orinoco, Amazon and La Plata basins, approximately. A) urban development and level (A) farm and industrial activities (B). Scale indicates differences in the intensities of the activities (modifies of Collins et al., in press).

Thus, the man invades and uses natural systems, intensively modifying them, and the fauna suffers extreme stress. Aquatic systems, although generally not targeted by direct application of pesticides, are impacted as a result of runoff after rain. This water, with all the elements that may be associated with it, goes into rivers or depressed areas. Groundwater may also be contaminated by percolation after rainfall. This mobility of elements occurs more intensively when the fields have no vegetative cover.

Moreover, the higher populations in cities have caused an increase in the urban area required to accommodate people and their families. This, together with increased biocide use and the increased area of impermeable surfaces in cities has meant that household chemicals and waste products are transported rapidly to aquatic systems during rains (José de Paggi et al., 2008).

Watersheds are continually being impacted, and care must be taken to ensure their quality control because these watersheds provide people with water to live. In South America, there are three major basins with water flows ranging from  $18.000 \text{ m}^3\text{s}^{-1}$  to approximately  $220.000 \text{ m}^3\text{s}^{-1}$  (Bonetto & Waiss, 1995; Lewis et al., 1995). These basins are the Amazon, Orinoco and La Plata. Of these three, the most densely populated watershed, with the greatest number of agricultural enterprises and the largest number of factories, is La Plata Basin (Figure 2) (Collins et al., in press).

### 1.3 Biocides

The variety of active ingredients used as biocides and their commercial formulations, solvents and coadjuvants or related chemicals is immense. All of them are used by application with agricultural aircraft, sprayers, hand-held units, or trucks that carry the spraying equipment, according to the extension land, application protocols, crop types and soil characteristics. Studies on native fauna are scarce, and only for very few taxa have the biological effects of biocides been studied. Studies on the interrelationships among the fauna components in relation to pesticide use have also been scarce. The actions of each biocide cause different biological responses, e.g., cypermethrin provokes an increase in metabolic activity and glyphosate a decrease (Collins et al., in press). The action of each pesticide is different, and the scarce information in their effects makes it very difficult to recognise the magnitude of the harm caused by these biocides on non-target species and on aquatic environments. The studies that have been conducted have focused on assays involving the active ingredient; however, it is not only the active ingredients that cause damage to the environment but also those compounds that are in the formulation and are considered inert. These compounds can increase the toxicity of the active ingredient, facilitating its ingress in biological systems, or may be toxic by themselves. It is therefore necessary for studies not to ignore commercial formulations, because they may include several compounds that can affect aquatic systems.

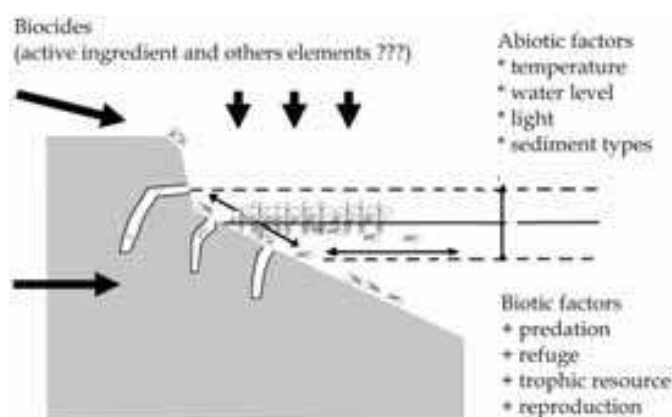


Fig. 3. Structure of typical area more affected by biocides through of sprayer with airplane, runoff after rain or groundwater potentially contaminated

## 2. Biocides in organisms: internal actions

### 2.1 Uptake at the tissue and cellular level

The toxicants direct absorption from the water by the integument and gills of crustaceans and/ or through the ingestion of contaminated food via the gastrointestinal tract can cause serious toxicity to normal biological functions at the tissue, cellular, and molecular levels. However, the permeability of biological barriers and the rate of transport of chemicals into an organism are affected by the metabolic activity of the animal and, indirectly, by factors influencing this activity (water temperature, pH, hardness, the presence of other chemicals). The metabolic activity of the animal is influenced by its body size, growth rate, physical activity, and physiological state (juvenile or mature, moulting, feeding) (Zitko, 1980).

As the mechanisms of the toxic action of many pesticides usually occur on the surface of or inside the cells (Fent, 2004), the movement of these xenobiotics across membranes depends



on the chemical nature of the pesticide involved. Matsumura (1977) summarised the specific properties that influence uptake into aquatic organisms: lipid and water solubility, chemical stability against degradative action by biological systems (biotransformation), and the molecular weight of the chemical. These physicochemical properties determine the affinities of toxic compounds for the materials comprising the arthropod cuticle and plasma membrane of the cell (Hartley & Graham-Bryce, 1980).

Because lipids constitute a substantial part of the plasma membrane, lipid solubility is a very significant factor determining the rate of penetration of many toxic compounds (such as organochlorine pesticides) by passive diffusion through the non-polar portion of the membranes. Lipid solubility is usually characterised by the octanol/ water partition coefficient ( $K_{ow}$ ). In other cases, both facilitated diffusion and active transport are required for the passage of toxic into the cell through channel proteins and via their association with carrier proteins, respectively (Newman & Unger, 2003). The passage through a protein channel occurs down a concentration gradient that may be subject to saturation kinetics, and it is influenced by the size of the molecule, which determines a lower permeability of the membrane with increasing molecular size (Zitko, 1980). Moreover, the uptake of several pesticide compounds requires an active process with an expenditure of metabolic energy in living tissue. Through these pathways, toxicants enter cells and cause alterations in the physicochemical properties of the cytoplasm and the pH of the medium, destruction of the membranes of the organelles, disruption of the normal functioning of the cell proteins, and inhibition of the actions of the enzymes (Sohna et al., 2004; Collins, 2010).

Because in multicellular organisms the distribution of toxicants occurs in more than one compartment, within the crustacean body, haemolymph circulation may be involved in the transport of these chemicals to their sites of action and even more so if it is an open system that flows around the organs. In other arthropods, such as insects, Brooks (1974) reported that phosphoric acid penetrates the cuticle more rapidly than organochlorine insecticides, and having passed this barrier, the toxicant enters the haemolymph and may be transported to all parts of the organism in solution, if water soluble, or bound to proteins or dissolved in lipid particles, if lipophilic. The relatively hydrophilic molecules are much more likely to remain in this circulatory fluid than small, hydrophobic molecules, which are rapidly distributed in several organs and stored in lipid tissue (Hartley & Graham-Bryce, 1980).

## 2.2 Toxicity and biotransformation

The adverse effects of toxic products on crustaceans depend on its concentration and affinity, activity (intrinsic toxicity, which is function of molecular structure) and chemical biotransformations (James, 1987) and the acclimation responses of the individual (Klerks, 1999). For biocides, such as organophosphates and carbamate anticholinesterases (anti-ChEs), intrinsic toxicity can be judged by measuring the inhibition of cholinesterase and propagation of action potentials on synaptic transmission (see biomarkers section).

While some organic compounds are sufficiently water-soluble (hydrophilic) for excretion and can be eliminated rapidly, many lipophilic components cannot be directly excreted and would accumulate if not processed to more polar derivatives. Because the unaltered toxicant and any of its transformation products (metabolites) may be excreted, excretion represents a possible protective mechanism against the toxicant (Newman & Unger, 2003). Usually, organic pesticides are subject to modifications through enzyme-catalysed biotransformations leading to *detoxification* or *activation* (Figure 4). Chemical

biotransformation in animals occurs via Phase I (functionalisation) and Phase II (conjugation) reactions, which are more readily excreted than the parent compound (Brooks, 1974; Oesch & Arand, 1999).

1. **Phase I reactions.** In this phase, several enzymes introduce a polar reactive group to the molecule, making it more water soluble while also increasing the possibility of further metabolism by Phase II enzymes. Two major groups of enzymes involved in Phase I metabolism include oxidoreductases and hydrolases that are located in the endoplasmic reticulum of the cell in many organs and tissues (James, 1987).

1. The oxidoreductases include the quantitatively most important superfamily of xenobiotic-metabolising enzymes, the cytochrome P450-dependent monooxygenases (CYP), flavin-containing monooxygenases (FMO), monoamine oxidases (MAO), and cyclooxygenases (COX), all of which introduce oxygen into or remove electrons from their substrates, with a few exceptions.
2. The dehydrogenases and reductases, such as alcohol dehydrogenases, aldehyde dehydrogenases, and carbonyl reductases, add or remove hydrogen atoms to or from the target molecule. The hydrolases comprise families of enzymes specialised in the hydrolysis of esters, amides, epoxides, or glucuronides (Oesch & Arand, 2005).

The predominant functions of Phase I reactions are the conversion of polar, lipophilic compounds into more polar, more hydrophilic compounds and the introduction or liberation of functional groups that can be used for conjugation in the subsequent Phase II of xenobiotic metabolism.

2. **Phase II reactions.** Phase II enzymes often conjugate the polar groups produced by Phase I enzymes to introduce more bulky hydrophilic substituents, such as sugars, sulphates, or amino acids, into the molecule. This conjugation substantially increases the water solubility of a chemical, making it more easily excreted. The conjugation of the xenobiotic metabolism is carried out by transferases.

1. Electrophilic substrates are taken over by the glutathione S-transferases (GSH S-transferase).
2. Nucleophilic substrates (i.e., those with hydroxyl, sulfhydryl, amino, or carboxyl groups) are metabolised by UDP-glucuronosyltransferases (UGT), sulfotransferases (SULT), acetyltransferases (AT), acyl-CoA amino acid N-acyltransferases, and methyltransferases.

Phase II involves reactions such as glycosylation, sulfation, mercapturic acid formation, amino acid conjugation, and acetylation. Carboxylic acid groups in xenobiotics can be conjugated with amino acids prior to excretion (Tang et al., 2005). Metabolites formed by conjugation reactions are usually less toxic than the unconjugated compound, although there are notable exceptions to this rule (James, 1987). In addition, the metabolic events that increase the water solubility of a chemical usually cause a significant reduction in its biological half-life by making it more readily excreted (Brooks, 1974).

However, the patterns of activity of key enzymes involved in the detoxification of pesticides can be modified by the same toxic effect of xenobiotics. An elevation in glutathione S-transferase (GSH S-transferase) levels in the hepatopancreas and gills was reported for freshwater prawns (*Macrobrachium malcolmsonii*) and crabs (*Paratelphusa hydrodromus*) exposed to endosulfan, reflecting the formation of glutathione (GSH) and endosulfan complexes as a means of detoxification/ elimination (Yadwad, 1989; Saravana Bhavan &

Geraldine, 2001). Conjugation of xenobiotics with reduced glutathione (GSH), catalysed by glutathione S-transferase (GSH S-transferase), is an important physiological process in the elimination of toxic substances from the body. These authors suggest that the activation of such a mechanism probably confers cytoprotection against endosulfan-induced cellular stress.

There are some toxicants in which biotransformation through either Phase I or Phase II can produce a highly reactive chemical, for example, the organophosphorus compounds. Although many of the insecticides in other chemical classes are toxic in their original parent forms, this is not true for many of the organophosphorus insecticides, especially those of the phosphorothioate configuration (such as parathion, chlorpyrifos, and diazinon), characterised by a P=S group. The insecticides possessing a P=S group are usually not very potent anti-ChEs, and they require bioactivation of their P=O metabolites, called oxons, to display appreciable anti-ChE potency (Tang et al., 2005). This bioactivation (reaction of desulfuration) is mediated by cytochrome P450-dependent monooxygenases through an attack on the sulphur by oxygen to create an unstable phosphooxythiiran intermediate (a three-membered ring composed of P, O, and S) that subsequently decomposes to the oxon (P=O) metabolite plus an active form of S (S<sup>2</sup>). In addition, the S is reactive in the tissues and is capable of damaging some proteins, including the cytochrome P450-dependent monooxygenases.

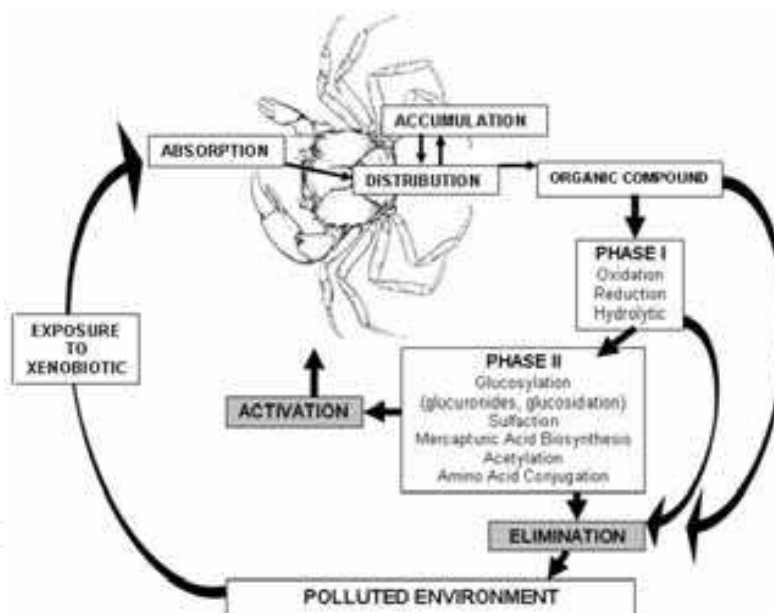


Fig. 4. Different process that can occur in decapods when the animals are affected by some biocide.

Other modifications to the toxic action of xenobiotics in crustaceans may occur via the phenomenon of physiological acclimation. In this case, an individual organism that becomes exposed to a specific contaminant may be less severely affected by this contaminant if it had been previously exposed to it. This effect is generally the result of the induction of a detoxification mechanism, as cytochrome P450, in response to the initial exposure (Tang & Garside, 1987; Stuhlbacher et al., 1992). Klerks (1999) observed (in the shrimp, *Palaemonetes pugio*) that acclimation results in an increased resistance at only a limited range of concentrations, with generally no change in resistance at lower pre-exposure levels and a



decreased resistance at higher pre-exposure concentrations that are stressful or result in a significant increase in contaminant body burdens. Such resistance occurs for some contaminants but not for others, and a lack of acclimation to complex mixtures occurs because positive responses to one contaminant are offset by negative responses to another contaminant. According to this observation, this can be explained by the fact that the energetic costs resulting from exposure to one contaminant (either for damage-repair functions or for detoxification processes, such as the production of P450 oxygenases) would compete with the energetic requirements associated with exposure to the other contaminant.

## 2.3 Biomarkers

To evaluate effects of pollutants on animal populations, communities and ecosystems, various methods have been developed, ranging from the (sub)cellular to the ecosystem level of biological responses. However, the predictive ability of measurements at higher levels of biological organisation is limited because ecologically important effects (e.g., death or impaired organismal function) have already occurred before they can be detected at population and community levels. In recent decades, biomarkers at suborganismal levels of organisation (biochemical components or processes, physiological functions, and histological structures) have been considered to be viable measures of responses to stressors (Hansen, 2003). These indicators of stress responses are useful in assessing the short-term well-being or long-term health status of an animal (Paterson & Spanoghe, 1997).

Metabolic changes observed in crustaceans exposed to pesticide pollution create widespread disturbances in general physiological processes, such as enzymatic activities, oxygen consumption, and changing energetic requirements. Some of the standardised types of biomarkers are those linked to disturbance to osmoregulation and water balance/ ion-homeostasis, cholinesterase inhibition activity, protein stress, oxidative stress, and endocrine disruption.

### 2.3.1 Haematological parameters

Alterations in the haemolymph protein, haemocyanin, osmolality, ion compositions, total haemocyte counts, differential haemocyte counts, total free amino acid, nucleic acids (concentrations of DNA and RNA), phenoloxidase (PO) activity, and superoxide anion ( $O_2^-$ ) may occur in crustaceans as a result of toxicant expositions. Yeh et al. (2005) reported a significant depression in haemolymph osmolality that mainly resulted from a decrease in the haemolymph chloride concentration ( $Cl^-$ ) in the prawn, *Macrobrachium rosenbergii*, after 8 days of exposure to sublethal concentrations of trichlorfon. However, a decrease in haemolymph  $pO_2$  was found among these prawns, which may be related to decreased ventilation and impeded respiratory gas exchange, leading to respiratory disturbances via the inhibition of respiratory mechanisms and damage to respiratory organ epithelial cells. Similarly, a decrease in the pH and  $HCO_3^-$  of the haemolymph induced an increase in the  $pCO_2$  level, benefiting the excretion of  $CO_2$  in the haemolymph and resulting in a decrease in  $TCO_2$ , suggesting that trichlorfon disturbs the extracellular acid–base balance of prawns.

In crustaceans, gill lamellae and epipodites are involved in osmoregulation, and the histopathological changes in these structures (haemocytic congestion, gill lamellae necrosis, and the accumulation of particles surrounding the gill lamellae) were observed with lethal concentrations of fenitrothion (Lignot et al., 1997). According to these authors, the presence of particles surrounding the gill lamellae may have been a consequence of a lack of

ventilation in the branchial cavity due to the inhibitory action of the pesticide on the nervous system. In contrast, Saravana Bhavan & Geraldine (2001) observed in the prawn, *M. malcolmsonii*, an increase in the content of total free amino acid in the haemolymph as a result of protein degradation. In addition, the accumulation of soluble protein suggests that this was necessary to serve as a compensatory pool to restore enzymes lost to tissue necrosis and to provide prawns with the energy required to cope with the stress of exposure to endosulfan.

### 2.3.2 Cholinesterase (ChE) activity (inhibition)

Cholinesterases are serine hydrolase enzymes and degrade the neurotransmitters in cholinergic synapses. The toxicity of some pesticides, such as organophosphates and carbamate insecticides, is mainly caused by the inhibition of ChE activity of vertebrates and invertebrates. This inhibition leads to the accumulation of acetylcholine in the synaptic terminals and therefore to a change in the normal transmission of the nervous impulse. This interference may result in neurological manifestations, such as irritability, restlessness, muscular twitching, and convulsions, that may end in the respiratory failure and death of the animal (WHO, 1986). Consequently, most studies describe the use of ChE levels as a biomarker of exposure and/ or the effect of several pesticide compounds in aquatic species. However, distinct enzyme isoforms with different sensitivities towards anticholinergic contaminants may exist, depending on the species. These isoforms are usually divided into two broad classes: acetylcholinesterases (AChE) and butyrylcholinesterases (BChE), which are distinguished primarily based on substrate specificity (Sultatos, 2005).

In crustaceans, published studies have also shown mixed results with regard to substrate preference. Fulton & Key (2001) reported that AChE in *Palaemonetes pugio* hydrolyses acetylcholine iodide (ACTH) and acetyl-b-methylthiocholine iodide (AMTH) much faster than other choline esters (such as propionylcholine) and is inactive on butyrylcholine. In contrast, BChE not only hydrolyses butyrylcholine but may also hydrolyse acetylcholine. The two enzyme isoforms may also be distinguished by their susceptibility to selective inhibitors; 1,5-bis-(4-allyldimethyl-aminoniumphenyl)-pentan-3-one dibromide (BW284c51) and tetraisopropyl pyrophosphoramidate (*iso*-OMPA) are selective inhibitors for AChE and BChE, respectively (Sultatos, 2005).

Organophosphates are generally irreversible inhibitors because the dephosphorylation rate of the bound enzyme proceeds at an insignificant rate. Therefore, the inhibitory effects of organophosphate exposure may be long lasting, with recovery depending on new enzyme synthesis (Habig & Di Giulio, 1991). Several studies with prawn, crab, and lobster species have shown that AChE inhibition in the animals still occurred days after exposure had ended (Reddy & Rao, 1988; McHenery et al., 1991; Abdullah et al., 1994; Key & Fulton, 2002). A slow time course for recovery of depressed AChE levels may cause exposed organisms to be susceptible to other anthropogenic or natural hazards or to exhibit behaviours not conducive to maintaining the population.

### 2.3.3 Stress proteins

The most abundant and widely studied group of stress proteins is the hsp70 (heat shock protein 70) protein family. The cellular functions of these proteins include the stabilisation of unfolded protein precursors before assembly, translocation of proteins into organelles, rearrangement of protein oligomers, dissolution of protein aggregates, and refolding or

degradation of denatured proteins (Feige & Polla, 1995). Induction of stress protein synthesis by pesticides is reported to be highly tissue-specific in aquatic animals. Among the tissues analysed (gill, skeletal muscle and hepatopancreas) by Selvakumar et al. (2005) in *Macrobrachium malcolmsonii*, induction of hsp70 synthesis was recorded only in the gill tissue of prawns that had been exposed to sublethal concentrations of endosulfan. In contrast, exposure of prawns to sublethal concentrations of carbaryl failed to elicit hsp70 synthesis in any of the three tissues analysed.

#### 2.3.4 Oxidative stress

Under normal conditions, equilibrium exists between the amounts of free radicals generated and antioxidants available to quench or scavenge them, thereby protecting the organism against the deleterious effects of pollutants. However, oxidative stress occurs when the critical balance between oxidants and antioxidants is disrupted as a result of the depletion of antioxidants or excessive accumulation of the reactive oxygen species (ROS), or both, leading to damage to macromolecular components (Scandalios, 2005). Many xenobiotics, such as pesticides, may cause oxidative stress, leading to the generation of ROS and alterations in antioxidants or free oxygen radicals scavenging enzyme systems in aquatic animals (Dettbarn et al., 2005). However, the cells of crustaceans possess a variety of chemical and enzymatic mechanisms to protect them from oxidative damage. These mechanisms include an enzymatic antioxidant defence system comprising enzymes such as superoxide dismutase (SOD), glutathione peroxidase (GPx), catalase (CAT), glutathione S-transferase (GSH S-transferase) and non-enzymatic antioxidants like glutathione (GSH), ascorbic acid (vitamin C) and  $\alpha$ -tocopherol (vitamin E), which are capable of neutralising or scavenging the reactive oxygen species (Vijayavel & Balasubramanian, 2009). These authors showed that the toxicity of fenvalerate to the prawn, *Penaeus monodon*, led to a significant induction of lipid peroxidation and GSH S-transferase activity in the hepatopancreas, muscle and gills. On the contrary, the activities of SOD, CAT, glutathione peroxidase, vitamin C, vitamin E and GSH were reduced in prawns exposed to sublethal concentrations of fenvalerate.

#### 2.3.5 Neuroendocrine systems

Toxicity induced by a pesticide is the result of interaction of the compound or one of its metabolites with the biochemical events involved in the homeostatic control of a physiological process (Newman & Unger, 2003). Physiological processes are mostly coordinated by hormones. Therefore, the effects of organic compounds on functions regulated by hormones in crustaceans could be used as biomarkers of environmental pollutants.

According to Rodríguez et al. (2007), endocrine disruption can take place at different physiological levels: 1) altering (inhibiting or stimulating) the secretion of hormones; this possible effect is related to mechanisms that control both the release of hormones from endocrine cells and the synthesis of these hormones; 2) interfering with hormone-receptor interaction; in this sense, endocrine-disrupting compounds (EDCs) can act as agonists or antagonists by directly binding to a hormone receptor. Indirectly, however, an EDC could interfere via several mechanisms at any step of the transductional pathway of a hormone, therefore altering its final effect; 3) modifying the metabolism of circulating hormones, that

is, by increasing or decreasing their excretion rates and/ or biotransformation in the liver, hepatopancreas or other organs.

Neurosecretory structures (X-organ–sinus gland) in the eyestalk are the most important components of the neuroendocrine system of the stalk-eyed crustaceans. The main hormones secreted by the sinus gland are the following: MIH (moult-inhibiting hormone), GIH (gonad-inhibiting hormone), MOIH (mandibular-organ-inhibiting hormone), CHH (crustacean hyperglycaemic hormone), several colour change hormones (controlling pigment migration) and NDH (neurodepressing hormone). Some of these hormones have a second endocrine gland as their target (MIH, GIH, MOIH), while the others have somatic tissues as targets. MIH, GIH, MOIH and CHH belong to a single family of peptides (Fingerman et al., 1998; Chang, 2001). These neuropeptides, synthesised in the XO (X-organ), a cluster of neuron perikarya located in the medulla terminalis of the eyestalk, are transported to and stored in the axon terminals, forming a neurohaemal organ named SG (sinus gland) and released by exocytosis into the haemolymph (Lorenzon, 2005).

The CHH have been shown to regulate carbohydrate metabolism in the shore crab, *Carcinus maenas*; the kumuran prawn, *Penaeus japonicus*; the lobster, *Homarus americanus*; the freshwater crab, *Oziotelphusa senex senex*; and the fiddler crab, *Uca triangularis* (Kegel et al., 1989; Lorenzon 2005; Purna Chandra Nagaraju et al., 2005). The neurotransmitter, 5-HT (serotonin), plays a fundamental role in hormone (CHH) modulation, and at the same time, pollutants can alter their level and function. Therefore, 5-HT has been known to have a potent hyperglycaemic effect with increases in the glucose haemolymphatic concentration resulting mainly from the stimulation of glycogen breakdown in the hepatopancreas (Fingerman et al., 1998). Hyperglycaemia is a typical response of several crustacean species to chemical stressors, including some pesticides, hydrocarbons and heavy metals. However, several reports have shown that an increased haemolymphatic level of glucose alone does not necessarily prove that there was a disruptive effect on the endocrine system. Because CHH is released to raise glycaemia as an adaptive response to several stimuli (such as emersion, starvation, critical temperatures and others), this hormone has been proposed as functioning as a crustacean stress hormone (Chang, 2001).

## 2.4 Histological effects

Crustaceans are considered as carrying a simple and primitive immune system (Fig. 5). The hepatopancreas is known as the detoxification site and also as a sensitive organ to stress, as it quickly responds to exposure to noxious compounds.

The hepatopancreas is essentially composed of branched tubules and of 4 types of epithelial cells: embryonic cells (E-cells), fibrillenzellen cells (F-cells), restzellen cells (R-cells) and blasenzellen cells (B-cells). E-cells are the only ones showing mitotic activity, being important in dead cell replacement. R-cells have absorptive functions supported by the presence of lipid droplets in the cytoplasm. These cells are involved in the delivery of nutrients to other organs via the haemolymph; the nutrient reserves are mobilised through R-cells to provide energy to the rest of the body. In addition, R-cells are interpreted as sites of intracellular waste deposition characterised by autophagosomes and residual bodies. These cells detoxify heavy metals and other lipophilic compounds by their accumulation in a soluble form in the cytoplasm, followed by excretion. F-cells are where protein synthesis and enzyme production occurs (Sousa et al., 2005).



Exposure to pesticides causes an imbalance in epithelial cells. Among the effects found, biocides cause an increase in R- and F-cells and an inhibition in E-cells. An R-cell increase in response to noxious compounds may be related to two different strategies. More R-cells may increase the detoxification rate because a higher number of cells increases detoxification. However, noxious compounds cause effects not only in the hepatopancreas but also in gills, gonads, and other organs. As other body parts require energy to recover from deleterious effects, the R-cell number increases for transporting energetic resources, i.e., lipids. When submitted to pesticides, F-cells increase for the production of more enzymes as a way of deactivating toxic compounds. R- and F-cells increase because both cellular types play roles in detoxification, and each one develops a different action for the same purpose. A decrease in E-cells becomes important if we consider that pesticides cause necrosis and increase cellular apoptosis. These cells replace dead cells with new ones, trying to mitigate cell loss. Exposure to pesticides also causes haemocytic infiltration in the interstitial sinus, abnormal lumen of the tubules, separation of necrotic cells from basal laminae, thickened basal laminae, necrotic tubules containing tissue debris, melanisation and coagulation in the thickened basal laminae and walling off of the tubules by haemocytes around the thickened basal laminae. All these effects combined may cause deficiencies in hepatopancreas function, with in turn may cause death (Saravana Bhavan & Geraldine, 2000, Bianchini & Monserrat 2007, Collins 2010).

In gills, one of the most important intake sites, biocide exposure also causes several histological damages, which in turn may cause functional deficiencies. Haemocytic infiltration in the haemocoelic space, swelling of the gill lamellae, lifting of lamellar epithelium, fusion of lamellae, abnormalities in the histoarchitecture, necrosis and other malformations are some the effects produced by pesticides in freshwater prawns and crabs. Gills are related to the transport of respiratory gases, their obvious function, and also with ammonia excretion, as the majority of waste nitrogenous compound excretion occurs through the gill epithelium. Gill damage may also cause difficulties in oxygen intake, eventually asphyxia, and disrupt osmoregulatory function. A decrease in oxygen consumption may cause progressive internal hypoxia, with several effects such as metabolism shifts and locomotive difficulties. Crustaceans generally maintain an aerobic metabolism as a way of obtaining energy from food reserves. Aerobic metabolism, through the Krebs cycle, provides more energy than anaerobic metabolism. However, this kind of cellular “respiration” requires enough oxygen to be developed. When the amount of oxygen needed for the maintenance of aerobic metabolism is not achieved, crustaceans obtain energy by glycolysis, an anaerobic metabolism of carbohydrates. This type of metabolism, although it allows individuals to obtain energy for vital actions, has two serious effects: lactic acid release and an underutilisation of the energy accumulated. While in aerobic metabolism, animals obtain 36 mol of ATP from 1 mol of glucose, in anaerobic metabolism, they obtain only 2 mol of ATP from 1 mol of glucose, with the production of 2 mol of lactic acid (Schmidt-Nielsen, 1997). In animals with sporadic hypoxia, lactic acid is used as a substrate for further oxidation, completing the Krebs cycle and gaining the full energy value of the original carbohydrate substrate. However, in animals with oxygen intake decreased by histological damage, hypoxia may be not temporary; if they continue to be exposed to the aggressor agent, gills are not able to reconstitute themselves, or recuperation time is not quick enough to supply the oxygen demand. The continuous internal hypoxia may provoke a constant release of lactic acid as metabolic waste, with the consequent acid imbalance. This



imbalance and its histological effects may eventually cause the death of the affected individual because of acidosis or progressive asphyxia (Vonk, 1960, Schmidt Nielsen, 1997).

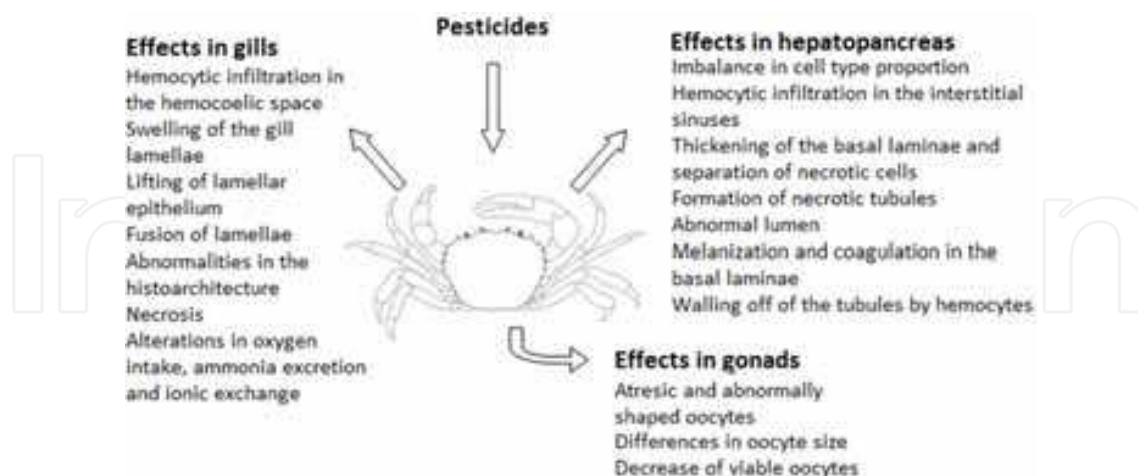


Fig. 5. Histological pesticide effects in crustacean organs and its results. Crab image modified from Collins et al. (2004).

Another effect of hypoxia is a decrease in locomotion. Crustaceans regulate their oxygen consumption within a range of dissolved oxygen concentrations. The minimal dissolved oxygen concentration within this range is called the critical oxygen concentration, below which crustaceans are not able to regulate their oxygen consumption. Given this situation of hypoxia, many crustaceans reduce their movements as a way of reducing the oxygen consumed by muscles, using the available oxygen instead of using it for metabolism (Zou et al., 1992, Zou & Stueben, 2006). The same response occurs if an animal has a deficiency in oxygen uptake, as in both situations the oxygen concentration in haemolymph is unsaturated. The oxygen deficiency causes a decrease in locomotive activities, with several effects. Animals are unable to escape from the contaminated area, exacerbating the effects of the contaminants. At the same time, a decrease in movement, as mentioned above makes animals more susceptible to predators.

In addition to their respiratory function, gills play an important role in nitrogen compound excretion. Crustaceans are ammoniotelic animals, i.e., their nitrogenous metabolic end products are mainly excreted in the form of ammonia. The antennal gland plays the key role in body water and divalent cation regulation, but it plays a minor role in ammonia excretion, as in some cases less than 2% of the total ammonia is excreted in the urine via the antennal gland system (Parry, 1960; Cameron & Batterton, 2004).

The high lipid solubility of ammonia makes it more diffusible through phospholipid bilayers. The mechanism supporting ammonia excretion in crustaceans is the simple diffusion of the non-ionic  $\text{NH}_3$  along a concentration gradient and the partial excretion of the ionised form  $\text{NH}_4^+$ , whose release through diffusion is facilitated because of its hydrophobicity (Weihrauch et al., 1999, 2004).

Several aquatic crab species possess an excretion system based on the ionised form of ammonia,  $\text{NH}_4^+$ , a water soluble compound which effluxes through the gill epithelium. Freshwater crabs have tighter gill epithelia than their marine relatives, developed to avoid ionic efflux and tolerate a hyposmotic environment. This epithelium is much less permeable by  $\text{NH}_4^+$ , and freshwater crabs release their nitrogen compounds mainly as ammonia (Weihrauch et al., 1999).

Concentrations of  $\text{NH}_3$  in the environment are kept low as a result of bacterial nitrification of ammonia to nitrite and nitrate, followed by the absorption of autotrophic organisms. This kind of environment favours ammonia excretion as a passive process driven by diffusion along a gradient. This process applies only to pelagic animals, generally prawns, colonising the water column, where the dilution and nitrification processes of aquatic biota keep ammonia concentrations really low. Benthic animals, such as crabs and crayfishes, are often faced with higher ambient concentrations of ammonia, present especially in anoxic, deep, stagnant water. Some species take refuge by hiding in riparian rocks and vegetation. Other species bury themselves in mud or, in the case of burrowing species, build extensive caves, in some cases more than 1 metre deep, with aerial or aquatic entry holes. They live in the bottom, where they find protection from predators and where the water is stagnant for hours. The very low water exchange rate, along with the fact that animals produce and excrete metabolic ammonia, increases the ammonia concentration and difficult simple diffusion.

The process that these crustaceans use to eliminate ammonia is active excretion. Ammonia excretion rates are correlated with  $\text{Na}^+$  absorption (Pressley et al., 1981, Harris et al., 2001).  $\text{NH}_4^+$  substitutes for  $\text{K}^+$  in the activation of the ouabain-sensitive  $\text{Na}^+/\text{K}^+$ -ATPase, which is located in the basolateral membranes of the gill epithelium cells (Towle et al., 1981; Towle & Kays, 1986). This  $\text{Na}^+/\text{K}^+$ -ATPase is synergistically stimulated by  $\text{NH}_4^+$  and  $\text{K}^+$ . In freshwater decapods, at high  $\text{NH}_4^+$  concentrations, the pump exposes a new binding site for  $\text{NH}_4^+$  that modulates the activity of the  $\text{Na}^+/\text{K}^+$ -ATPase independently of  $\text{K}^+$  ions (Romano & Zeng, 2007, 2010).

Histological damage in gills, and the mucus segregation observed in prawns exposed to pesticides, may hinder ammonia excretion. These effects are especially relevant in freshwater benthic crustaceans, mainly crabs and crayfishes. As mentioned above, the passive efflux of ammonium ( $\text{NH}_4^+$ ) is difficult because of the thickened gill epithelium, while ammonia excretion ( $\text{NH}_3$ ) is difficult because of the environmental concentration. Nitrogenous waste compounds are eliminated by active efflux, and histological damage provoked by pesticides hinders this excretion process. When decapods are not able to eliminate the ammonia produced by nitrogen compound metabolism, it accumulates in the haemolymph, with several effects on individuals. Ammonia modifies the release of cytokines and increases the activity of lysosomal hydrolases. Ammonia toxicity is mediated by the excessive activation of *N*-methyl-*D*-aspartate (NMDA)-type glutamate receptors in the brain. As a consequence, cerebral ATP is depleted, while intracellular  $\text{Ca}^{2+}$  increases, with subsequent increases in intracellular  $\text{K}^+$  and, finally, cell death (Weihrauch et al., 1999, 2004).

The intensity of the observed effects is related to pesticide concentration and animal resistance. Nevertheless, many of the described effects were achieved at concentrations that usually occur in the environment after aerial or terrestrial pesticide applications. The constant aggression provoked by biocides induces malfunctions in this vital organ, which eventually may cause the death of an individual.

#### 2.4.1 Histopathological effects on female gonads

Freshwater decapods modified their reproductive strategy when they conquered freshwater environments. Larval stages were abbreviated or suppressed, and females invest their energy in fewer but more expensive progeny, which hatch at a more advanced stage.

Gonads are characterised by fewer but bigger oocytes, with more energetic reserves for the extended embryonic stage. In subtropical regions, gonad development occurs during late winter, spring and summer, the same period when pesticide applications. The drift and runoff provoke the migration of biocides to aquatic environments, causing a continuous contact with females during gonad maturation.

Ovary growth in crustaceans has two different periods: endogenous vitellogenesis (vitellogenesis I) and exogenous vitellogenesis (vitellogenesis II). The first period is characterised by an autosynthesis of lipovitellin and slow oocyte growth. The second period is characterised by the input of exogenous vitellogenin (a vitellin precursor) from outside of the ovary, mainly from the hepatopancreas, and rapid oocytic growth. Along with all the compounds provided by the hepatopancreas, lipophilic pesticides migrate to the ovaries (Lubzens et al., 1995). The effects of these biocides include abnormalities in shape, as the loss of the typical spherical shape of ovarian follicles; abnormal oocyte area increase or decrease, depending on pesticide type; and oocyte atresia (Rodriguez et al., 1994, Lee et al., 1996). The abnormal development of the ovaries causes a reduction in the available oocytes for fecundation, with the consequent reduction in eggs and the future brood, decreasing the population over the short and medium term.

Once fecundation occurs, females carry their eggs in their pleon until juveniles or mysis hatch. If these females live in contaminated areas, the exposure to biocides causes different effects in eggs and embryos. The easiest observable effect is death, but embryo death may occur at relatively high pesticide concentrations. Eggs are surrounded by the chorion, which isolates them from the environment. In the case of freshwater decapods, the chorion is thicker than that present in marine decapods because it has to protect the embryo from the osmotic stress caused by the environment. This thicker chorion also isolates the embryos from biocides and other compounds (Lindley et al., 1999; Varó et al., 2006). This protective effect makes embryos more resistant to toxicants, in some cases more resistant than juveniles, with a median lethal concentration similar to adults in several cases (Key et al., 2003; Li et al., 2006). Furthermore, embryos are more sensitive to pesticides when they are close to hatching because of the thinning of the chorion, which allows more pesticide to enter into the egg. This effect is also observable in prawns exposed to different salinity levels, as embryos are more sensitive to osmotic stress when they are close to hatching (Ituarte et al., 2005).

In addition to lethality, constant exposure to pesticides may cause differences in incubation periods and several abnormalities in embryos. Among these abnormalities, biocides may cause hydropsy, abnormal eye spots and several atrophies in the eyes, the pleon and the dorsal spine (Rodriguez & Pisanó, 1993; Lee & Oshima, 1998). All these abnormalities provoke the death of the juvenile, either from internal malformations of organs or from the incapability to moult successfully. Additionally, abnormalities in pleopods and pereopods cause the inability to eat, find food or avoid predators.

Constant exposure to pesticides causes a reduction in functional oocytes, resulting in fewer eggs, a reduction in surviving embryos and a decrease in juveniles that will reach the adult stage, which in turn provokes effects on populations, the community and the ecosystem.

### 3. Reproduction effects

Freshwater environments impose a severe osmotic stress to the animals living there. Marine crustacean reproduction is characterised by a large brood, which hatches as larvae and

undergoes several stages up to the juvenile stage. Freshwater environments impose a severe osmotic stress on unprotected eggs and the free larvae stage. In the same way, developing embryos must be protected against this stress. When they conquered these environments, decapods developed different strategies to protect eggs and embryos. The primitive pelagic larval phases were suppressed; larval stages occur inside the egg, and the offspring hatch as mysis or juveniles. This internal development (i.e., inside the egg) imposes a greater protection to embryos against environmental pressures, especially in the susceptible larval stages. To support these internal stages, eggs increased in size and energy resources, mainly lipoproteins, because embryos grow inside the eggs and use their internal energy resources. Freshwater decapod females carry their eggs in the pleon, protecting them until the larvae or juveniles hatch. Because of their increased size, the number of eggs that a female can carry decreased, resulting in a concomitant decrease in the number of offspring (Ruppert & Barnes, 1994; Lee & Bell, 1999).

Several pesticides are highly lipophilic and are accumulated mainly in lipid reserves. During ovary development, oocytes accumulate lipids and lipoproteins, mainly lipovitellin, forming the vellum, which in turn will be used by the embryo as an energetic resource (the embryo “feeds” on the vellum). Attached to the lipovitellins, pesticides enter to the oocytes and accumulate on them. One explanation for the relatively greater resistance of females to organic pollutants is the distribution of these toxicants in the ovary, decreasing their concentrations in vital organs such as the hepatopancreas and delaying death (Sheridan, 1975; Menone et al., 2000; Wirth et al., 2001; Menone et al., 2004, 2006; Santos de Souza et al., 2008). The presence of pesticides in the oocytes implies that the embryo, beginning with fertilisation, is exposed to pesticides. Embryos grow and feed on the lipid reserves present on the vellum, with the consequent intake of pesticides. This may provoke not only the death of the embryos, with the release of dead eggs by the female, but also sublethal effects, such as abnormal size in eggs; deformation of embryos, such as tissue dropsy, atrophy, abnormal or depigmented eyes; and abnormalities in the pleon, telson, and spine, pereopods and pleopods (Rodriguez et al., 1994; Saravana Bhavan & Geraldine, 2001). Deformation may cause difficulties in hatching or in brood survival, as activities such as swimming and searching for prey or escaping from predators may be hampered, and even moulting may not be successfully completed (Fig. 6).

In the case of freshwater decapods, the amount of vitellins and the time that embryos spend inside the eggs are greater than found in their marine relatives. This provokes an extended exposure time to different concentrations of pesticides, which depends on the exposure of females during gonad development and pesticide concentration in the ovaries.

Because of the osmotic stress that freshwater environments present, freshwater decapods possess a thicker chorion for protecting embryos from external aggressions. This chorion also protects them from biocides, making eggs as resistant as adults in some freshwater prawns and crabs, leaving juveniles as the most vulnerable (Key et al., 2003; Li et al., 2006). When the embryo is close to hatching, the chorion narrows to allow embryos to hatch, also allowing external agents to come into contact with embryos, making them more vulnerable to external agents, as observed in the prawn, *Palaemonetes argentinus* (Ituarte et al., 2005).



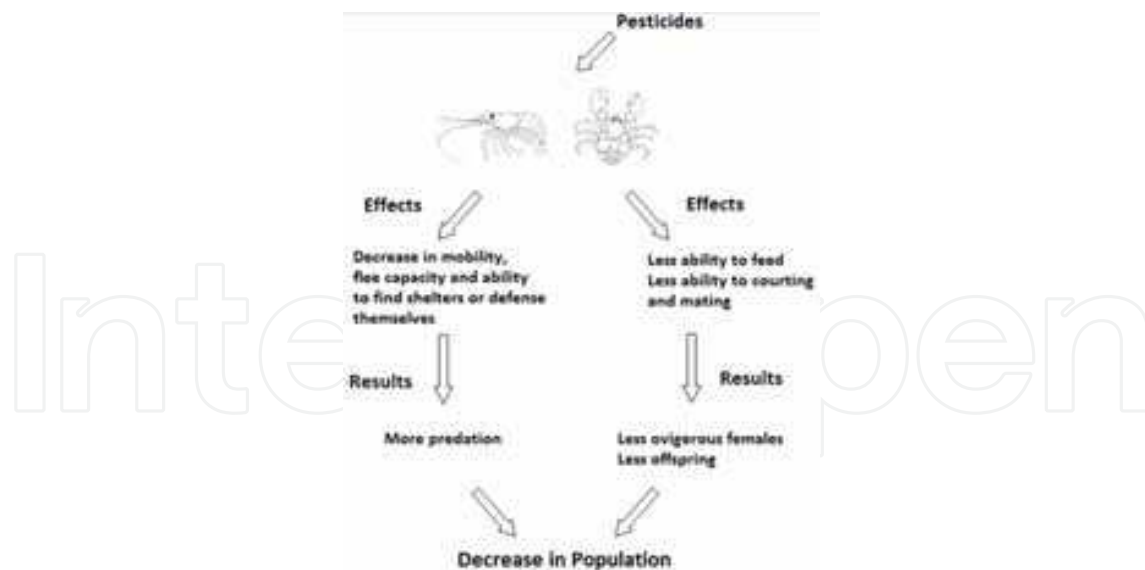


Fig.6. Pesticide effects in crustaceans' behavior and its results in population. Crustacean image modified from Collins et al. 2004.

The eggs of ovigerous females that live in contaminated areas may be resistant to pollutants, but toxicants may cause the death of juveniles after hatching, when they are not protected by a chorion. Moreover, moult events are a critical period for crustaceans, as their exoskeletons become softer and they are more vulnerable to external contaminants such as pesticides. In juveniles, the intermoult period is short, and the lethal effects of pesticides are increased during that critical period.

#### 4. Growth

Growth is an interesting aspect in decapods in that it includes both internal and external factors. The intermoult period and increase in size are affected by different factors, such as diet (mainly protein, and lipid level variation), interspecific interactions (searching for agonistic behaviour and hierarchical conditions), temperature, biocides (or xenobiotic elements). Moreover, the growth in many species shows isometry and/or allometry variations in the ontogeny, and thus growth pattern can be affected. The study methods are different according to a study's objectives. In some cases, the animals are evaluated in groups, e.g., with diets; in other cases, a study is conducted with isolated animals to observe the xenobiotic effects on growth through chronic assays.

The capacity of an organism for survival, growth, and reproduction involves competition for energy resources at the individual level (Schmidt-Nielsen, 1997). Toxicant-induced shifts in energy allocations to these life-history activities will have important consequences on population. For example, higher respiration rates of estuarine crustaceans sublethally exposed to a variety of pesticides reduced juvenile growth by lowering growth efficiency rates, suggesting that increased metabolic demands lowered the amount of assimilated energy available for production of new tissue (McKenney & Hamaker, 1984; McKenney & Matthews, 1990). The assessment of changes in growth and energy stores of toxicant-sensitive life stages have a direct link to ecological consequences of environmental stress and can be useful as biomarkers to diagnose early damage in aquatic populations (Newman & Unger, 2003).



Crustaceans do not grow continuously but by periodically shedding the hard exoskeleton in a process called moult or ecdysis. Moulting is a very important physiological process because it not only allows for growth and development of these animals, which possess a rigid, confining exoskeleton but is also tied to metamorphosis during the early stages of the life cycle and reproduction during the adult stage (Passano, 1960). The process of ecdysis of decapod crustaceans is an antagonistic interaction by ecdysone and the MIH (moult inhibiting hormone), which originates from the Y-organ and X-organ/ sinus gland (XO/ SG) complex, respectively. The X-organ/ sinus gland complex is located within the eyestalks. A reduction in MIH in the haemolymph is believed to induce moulting and stimulate the Y-organs to synthesise and secrete ecdysone, which will be converted to the active moulting hormone 20-HE (20-Hydroxyecdysone). Moreover, a significantly lower level of 20-HE was recorded in the haemolymph during the interval of moulting (Chang, 1995).

Limb regeneration is also an aspect of moulting. In this case, the regenerate first develops as a limb bud folded within a layer of cuticle and becomes free to unfold when the individual undergoes ecdysis as part of the moulting process (Fingerman et al., 1998). However, low levels of pollutants (such as chlorinated compounds) had an inhibitory effect on moulting and limb regeneration in some decapods (Fingerman, 1985).

Growth rate is usually described in terms of independent moult periods. These consist of a description of the size increment for each individual moult (moult increment) and a description of the time increment between moults (intermoult period) (Hartnoll, 1982). In many decapod species, growth alterations by toxicant may be caused by variations in the moult increment, but principally by changes in the intermoult duration. A reduction in growth by the lengthening of the intermoult period was observed in juvenile prawns, *Palaemonetes argentinus*, during the first moult cycles exposed to cypermethrin (Collins & Cappello, 2006) and to chlorpyrifos and endosulfan insecticides (Montagna & Collins, 2007). In contrast, this same freshwater prawn showed a shortening in the intermoult period with a reduction in the moult increment at the highest concentration of glyphosate tested (0.070 ml l<sup>-1</sup>) (Montagna & Collins, 2005). These changes may involve perturbations to the X-organ and the sinus gland, which affect the production and storage of the inhibitory moult hormone or, more integrally, the neurohormonal system located in the eyestalks. Snyder & Mulder (2001) reported a delay in the onset of moulting of larvae of the lobster, *Homarus americanus*, exposed to heptachlor. This delay was correlated with both reduced levels of circulating ecdysteroids and increases of some P450-dependent detoxifying enzymes. Although it is known that 20-hydroxyecdysone itself can induce the expression of these enzymes, it is quite possible that this induction can also be produced by some toxicants.

## 5. Biocide effects on behaviour

Among the movements made by an animal, there are vital movements, such as breathing and cardiac movements; locomotive movements for prey finding and predator escaping; and behavioural movements, such as courtship and copulation. Every movement, even the simplest, depends on the harmony of every single movement to complete a desired action, i.e., for swimming, a prawn needs each pleopod to move in the right direction at the right time and with the right intensity to accomplish the final desired movement. Movements are transmitted through the nervous system and the synaptic gap by neurotransmitters, such as acetylcholine, while they are inhibited by enzymes, such as acetylcholinesterase, which stops the nerve impulse.

Some pesticides are acetylcholinesterase inhibitors in crustaceans and other animals (Saravana Bhavan & Geraldine, 2001; Braga da Fonseca et al., 2008). The inhibition of this enzyme enhances the contraction of skeletal muscles and impairs movement. When exposed to biocides that provoke an acetylcholinesterase inhibition, decapods are affected in their vital, locomotive and behavioural actions, with several different implications for the individual and the community. A prawn, crab or crayfish that is not able to swim or run correctly will be more susceptible to predation. Freshwater prawns and crabs exposed to acetylcholinesterase inhibitor biocides had a stimulus that improves appendage movements. Nevertheless, these movements are not synchronised, and the total movement efficiency is lower than that of normal locomotion. Prawn jumps are uncontrolled, and they keep jumping in the same place, without escaping from the area; crabs walking becomes frenetic, and they jump and walk, but move more slowly than normal. The increasing of impaired movements, which provokes a greater demand on muscle activity, more rapidly tires the affected animals. After the initial excitation, animals become quiet because of this tiredness, with slower movements and even immobility, making them more susceptible to predation (Williner & Collins, 2003; Collins et al., 2004; Collins & Cappello, 2006; Montagna & Collins, 2008). In a natural environment, escape from natural predators will be more difficult if crustaceans are affected by this kind of biocide, enhancing predation and decreasing the population.

Moreover, impaired movements not only affect locomotion as a way of escaping from the risk area but also affect the capacity of crustaceans to quickly locate refuges. Some freshwater crabs are pleustonic; they live between the roots of aquatic plants. As these roots act as filters for suspended organic matter and planktonic organisms, crabs go to the periphery for feeding. When detecting predators, they quickly migrate to the inside of the roots or stay still as a way of camouflage. Prawns and crabs also use rocks or burrows as refuges, either made by themselves or by other animals, and they swim or run to these refuges or bury themselves when they detect predators. Some crabs, especially the bigger species, use their chelipeds to attack their predators as a way of intimidating them and allowing themselves to flee (Collins et al. 2006, Collins et al., 2007). All these actions require a complex sequence of movements. If these decapods are affected by biocides, uncoordinated movements or tiredness will hinder their ability to find refuges, leading to increased predation and decreasing the population (Fig. 7).

Coordination of movements is not only necessary for escaping predators but also for finding food resources. Freshwater crabs and prawns are omnivorous animals. Some groups are specialised to filter sand and clay, feeding on the microbiota inhabiting these sediments. Other groups eat algae, macrophytes and animals tissues. Animal food may come from carrion or from hunting live prey. Decapod prey includes insect larvae, cladocerans, copepods, benthic organisms such as annelids and molluscs, fishes, other crustaceans and even eggs, juveniles and adults of the same species (Collins et al., 2006, Collins et al., 2007).

The hunting of mobile prey, such as fishes and crustaceans, and the manipulation of molluscs, which enclose themselves in their shells, requires both coordination in movements and strength. These actions become more difficult if decapods are subjected to acetylcholinesterase inhibitors or narcotic pesticides, decreasing the feeding capacity. Combining this decreased feed capacity with the increase in the energetic expenditure provoked by the impaired movements, biocide exposure eventually causes a depletion in

energetic resources, with several detrimental results for survival, growth, gonad development and reproduction (Saravana Bhavan & Geraldine, 1997).

The coordination of movements is also important in behaviours, such as territorial defence, courtship, mating and copulation. Decapod crustaceans, like many others animal species, have a courtship routine that is more or less complex, depending on the species. Mate selection is related to size and previous learning, and some crab species have a kind of "aggressive" courtship during which the male subjugates to the female (Fig. 7).

Agonistic behaviour is common in decapods, especially in crabs, and it is characterised by a series of coordinated movements that lead disputes in which the animals involved are at risk of serious injuries, loss of pereopods and/ or chelae or death of during combat. The more common resources involved in the disputes include shelters, mates and/ or food. This behaviour may be affected by side effects of biocides; Williner & Collins (2003) and Collins & Cappello (2006), observed hyperactivity in freshwater crabs and prawns treated with cypermethrin. This hyperactivity capped oxygen consumption, resulting in an obligated hypoactivity during which there was a recovery state with reduced metabolism and lower oxygen consumption. This finding may show that decapods affected by biocides are exacerbating the agonistic behaviour in the beginning, with a subsequent negative effect on recuperation. Reproductive behaviour may also be affected. Palaemonid males court females by swimming, chasing after them until they successfully place a piece of spermatophore on the female's abdomen, and cypermethrin produces erratic movements in *Palaemonetes argentinus* (Collins & Cappello, 2006). This could affect both courtship and reproduction itself, especially in regard to "freezing" of the spermatophore, as this requires coordination and precision. It is also possible that a female would find "defective" males under the influence of toxic and remove their sperm packages to obtain offspring with higher fitness or more viable eggs. It has been found that the effect of stress on egg masses affects the viability of these eggs (Siegel & Wenner, 1984). Stress may also disrupt or alter the chemical communication of these animals, as studies show that in many crustaceans, this type of communication occurs, permitting these animals to determine states of dominance. It is also known that during courtship, the chemical perception needed to recognise the state of female receptivity may also be disrupted by the action of biocides. In addition, in relation to energy, oxygen consumption increase as a result of biocide action causing hyperactivity, reduces the energy available for reproduction, either reducing the number of eggs or the effectiveness of the fertility of eggs (Siegel & Wenner, 1984), or in relation to the behaviour during parental care (Fig. 7). Moreover, shrimp in estuaries, such as penaeid shrimp, when exposed to biocides, exhibit decreases in the percentages of proteins as energy resources (Galindo Reyes et al., 1996). This alteration in energy storage could affect animals not only directly but the energy available for reproduction. Huang & Chen (2004) show that endocrine abnormalities were related to levels of testosterone and vitellogenin in *Neocaridina denticulata* treated with toxic. These abnormalities could affect the reproductive behaviour and gonadal development of these shrimp. It is known that female crabs, particularly freshwater crabs, incubate the eggs in their abdomens until hatching, and in some cases keep their offspring alive for some time after hatching, requiring sufficient energy to do so (Senkman, unpublished data). The effects of biocides may provoke the death of eggs and juveniles and the development of abnormal juvenile behaviour caused by stress. During embryonic development, many crustacean females move their eggs with opening and closing movements of the abdomen in rhythm, and their pleopods are used to remove

bad eggs or foreign particles or microorganisms entering via the same motions for the abdomen.

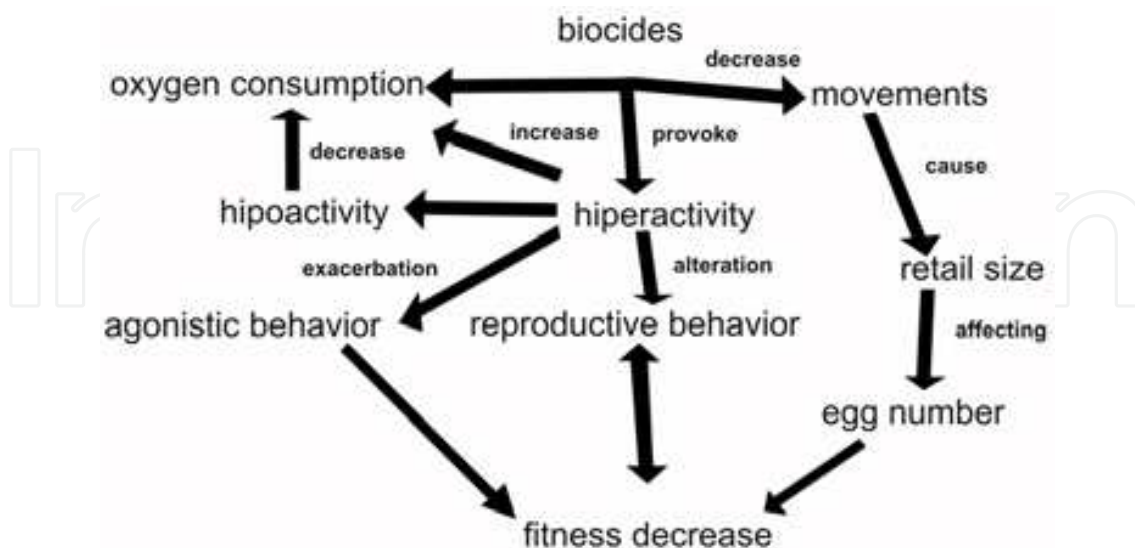


Fig. 7. Different effects that can occur when the animals are exposed to biocide in relationship to oxygen consumption, activities, movements, and reproduction. All these affect the fitness of the species.

Biocides are known to affect moulting events in some decapod crustaceans, affecting their growth and keeping many adult individuals in sizes below the average body size of conspecifics. It is estimated that the size of individuals is an important factor in mate choice, as there is a direct relationship in many crustaceans between adult size and the number of eggs a female is capable of carrying, so the effect of biocides may include the number of eggs, or indirectly the reduction of average adult size.

## 6. Relationships between the external medium and an animal's body

In assessing organisational levels, it is necessary to analyse those relationships beyond the physical dimension of the animal's body and that provoke the defined interactions. Among these relationships are the connections between the various components of a community, i.e., trophic webs. According to the environment, the trophic web may be more simple or complex, e.g., with more connections and interactions between components or with greater or lesser possibility of prey choice by top members.

In these communities, whether they are subjected to fumigation or the biocides that enter the physical environment with runoff caused by rain, there will be species that are more sensitive than others, and these pollutants can make these species disappear or decrease their numbers extensively. This alteration will also be reflected in species that use this directly affected species as food, leading to increased competition among predators for fewer prey species. In this way, a decrease in diversity and a simplification of the system occurs.

In addition, all community members are in contact with the biocide, which may accumulate in organisms. When predators eat contaminated prey the toxic conditions of the biocides from the lower elements of the food chain are transferred to the other trophic levels of the chain, magnifying their effects.



Thus, the direction of flow of energy and matter through the food web can be affected, changing both direction and intensity, affecting the ability of each species and population to persist.

The movement of populations occurs and can be induced by abiotic, biotic and human factors. In the last case, this movement can be induced after rainfall, when biocides accompany rainwater. The xenobiotics in sediment, suspended in colloids or dissolved in rainwater are trapped. These biocides can cause changes in abiotic conditions (pH, conductivity, nutrients) and water and sediment qualities. The different factors make it difficult to identify a cause and/or cause-effect relationship, but these factors increase stress and impair various species' activities under various conditions. According to the timing of a rainfall event, the population may or may not be in its most vulnerable condition, based on its endogenous cycles (e.g., moult, reproduction).

## 7. Conclusions

In the modern world with the farmland activities, the aquatic communities are affected by different pesticides used in these agro-ecosystems. Herbicides, insecticides, fungicides are the most common elements used, and in some cases with several millions of liter what are used in the systems nearby to aquatic environments. This occurs due to the input of toxic compounds to water bodies by several ways, such as drift, and runoff provoking a risk to the fauna, and thus, creates the need of a constantly updates. The biological communities in rivers with floodplain and their tributaries are abundant, and with very high diversity. An interesting group is the decapods crustaceans by their abundance. These included prawns, crabs, pseudo-crabs and crayfish of South America. Populations of decapods could be reduced by lethal effects on the individual, and chronic alterations modify their fitness. The pesticide exposures cause damage in several tissues as hepatopancreas, gills, muscles and gonads, affecting the aquatic fauna. Among physiological functions, growth and reproduction could decrease by alterations in growth, gonad tissues, genetical material, and eggs development. Changes in metabolism, cell composition of the hepatopancreas, neuro-hormones have effect on behavior, growth rate, reproduction efficiency and survival due to the exposition of pesticide. Even more, these variations could affect the trophic web, and alter the transfers of material and energy into the aquatic systems. The proposed focus gives a snapshot from the macroscopic view of the ecosystem - community together to the molecular view. The different levels of organization with their temporal and spatial scale are necessary to achieve a better idea of the problem facing modern society with pesticides.

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## **Pesticides in the Modern World - Risks and Benefits**

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This book is a compilation of 29 chapters focused on: pesticides and food production, environmental effects of pesticides, and pesticides mobility, transport and fate. The first book section addresses the benefits of the pest control for crop protection and food supply increasing, and the associated risks of food contamination. The second book section is dedicated to the effects of pesticides on the non-target organisms and the environment such as: effects involving pollinators, effects on nutrient cycling in ecosystems, effects on soil erosion, structure and fertility, effects on water quality, and pesticides resistance development. The third book section furnishes numerous data contributing to the better understanding of the pesticides mobility, transport and fate. The addressed in this book issues should attract the public concern to support rational decisions to pesticides use.

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