CHAPTER 6

Crayfish: An Experimental Model for Examining Exposure to Environmental Contamination

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6.1 Introduction

6.1.1 Background

The introductory material presented here on the ecology, behavior and phylogeny of the crayfish is very cursory. This material is written to give the reader a very basic introduction into the potential of the crayfish as a toxicology model. There are excellent books that give a far more detailed overview of the many different aspects of the crayfish. The reader, if interested, should investigate these ref. 1–3.
6.1.2 Phylogeny and Distribution

Crayfish are Malacostracan Crustaceans and are placed within one of the largest taxon of crustaceans: the decapods. Decapod translates into ten feet because the crustaceans in this group have five pairs of walking legs. Currently, there are more than over 14,000 species within this order, which also includes shrimps, lobsters and crabs.\(^4\) Crustaceans within this order demonstrate bilateral symmetry, including a pair of compound eyes that are usually located on long stalks. In addition to eyes, these organisms possess numerous appendages dedicated to either mechanoreception and/or chemoreception, including pairs of antennae, antennules (lateral and medial), pereopods and three pairs of mouthparts (or maxillipeds).\(^5\)

Within the decapods, crayfish fall across three phylogenetic families, namely Astacidae, Cambaridae, and Parastacidae.\(^6\) Currently, there are over 600 recognized and named species of crayfish in the world.\(^7\) Crayfish within the families of Astacidae and Cambaridae are almost found exclusively in the northern hemisphere, whereas the Parastacidae are found across the southern hemisphere. Although absent from the African and Antarctic continents, crayfish are found across the globe in a large variety of different habitats. An excellent description of the distribution of different genera of crayfish can be found elsewhere.\(^8\) One of the most significant problems with regard to habitat and global distribution is the introduction of non-native species of crayfish. A number of species of crayfish have either been purposefully introduced for aquaculture reasons or accidentally introduced as a result of recreational fishing as crayfish are often used as bait for large predator fish.\(^9\)

6.1.3 Habitat and Range

Crayfish inhabit a large variety of freshwater habitats, including lakes, rivers, wetlands, caves and swamps. Crayfish in the genus *Astacus* are located across Europe and can be found in lakes, estuaries, and even brackish waters.\(^7\) The narrow-clawed crayfish (*A. leptodactylus*) can tolerate higher salinity waters as well as waters that can be eutrophic with lower oxygen content. In contrast, the noble crayfish (*A. astacus*) and the thick clawed crayfish (*A. pachypus*) tend to prefer colder climate fresh water rivers and streams that are rich in oxygen and free from pollutants.

Within the western part of the United States, the genus *Pacifastacus* is the dominant genus of crayfish. This genus originated west of the rocky mountains to the Pacific coast of the United States.\(^10\) More recently, this genus has been introduced to Japan and some areas of Europe.\(^7\) The signal crayfish (*P. leniusculus*) is one of the dominant invasive species of crayfish and one of the reasons is the large range of habitats where the crayfish can survive. The signal crayfish’s original habitats are typically small streams and rivers. The transport and introduction by humans has resulted in an explosion of different habitats in which this crayfish has been found. The
signal crayfish has been found in estuaries with salinities as high as 20 ppt and in lakes as deep as 100 m. This crayfish creates simple burrows in the banks of rivers and lakes, and can reach considerable densities because of its burrowing capacity.

In the northern to middle part of the United States, the genus *Procambarus* is a dominant genus. Among this group of crayfish is the commercially important red swamp crayfish (*P. clarkii*). As the name indicates, this species of crayfish can be found in swamps and other wetlands. This species of crayfish has a very low tolerance for salinity ranges (<10 ppt), but is tolerant of lower oxygen levels that are sometimes present within periodically flooded wetlands. As with the signal crayfish, *P. clarkii* has been introduced across the globe and can now be found within lakes and rivers as well as wetlands. Two other *Procambarus* species, both referred to as the white river crayfish (*P. acutus acutus* and *P. zonangulus*), prefer faster flowing rivers and streams and are not typically found in the same habitats as *P. clarkii.*

Another eastern North American genus is the *Orconectes*. Crayfish within this genus can be found from the Gulf of Mexico up to Central Canada. Crayfish within *Orconectes* are found in lakes and rivers and rarely inhabit estuaries or flooded wetland habitats. A number of species within this genus can construct elaborate burrows in lakes, river banks, and even some semi-terrestrial habitats. *Orconectes* species are tolerant of large changes in the temperature and oxygen content of water. The rusty crayfish (*O. rusticus*) occurs in lakes and rivers and can often be found associated with large rocks and debris within streams (Figure 6.1A,B). The virile crayfish (*O. virilis*), like the rusty crayfish, inhabits rivers and lakes but has a higher tolerance for muddy environments when compared to the rusty crayfish (Figure 6.1C,D). A third species (*O. limosus*) prefers more sediment-rich habitats often with slower flowing rivers that may have clay or silt substrates.

North America has a rich diversity of crayfish species and a third genus can be located east of the Rocky Mountains to the Atlantic seashore. The genus *Cambarus* consists of a wide diverse group of crayfish that can be found in habitats that range from cold, rocky, and fast-flowing rivers to stagnant drainage ditches. In addition, this genus contains species of crayfish that can build elaborate burrows in soft sediments. The devil crayfish (*C. diogenes*) are primarily burrowing crayfish that may have the widest distribution of habitats of any crayfish species. The devil crayfish can be found in marsh or swamp like habitats as well as the banks of rivers and lakes. The Appalachian brook crayfish (*C. bartonii bartonii*) is typically found in small rivers and streams, but has a range that extends from Georgia in the United States to the Hudson Bay in Canada.

The final genus covered in this chapter is located in Australia. This genus, *Cherax*, is another diverse group of crayfish that contain commercially important species in Australia. The marron crayfish (*C. tenuimanus*) is found in heavily forested regions that have permanently flowing streams and high rainfall. As with many other crayfish species, the marron crayfish has been
introduce to regions of Africa, Japan, and China for aquaculture reasons. The yabby crayfish (*C. destructor* and *C. albidus*) include crayfish that can get as large as the Maine lobster (*Homarus americanus*). These crayfish are found in habitats ranging from cold, fast-flowing alpine streams to slow, warm and almost stagnant swamps.\(^{18}\)

Although as a group crayfish inhabit a wide range of freshwater habitats, the central commonality, for the purposes of this chapter, is that all of these habitats are located near riparian zones that are often inhabited by human communities. Thus, these habitats have the potential to be altered or impacted by human activities that include the introduction of a wide variety of anthropogenic chemicals (e.g. pharmaceuticals, agricultural chemicals and metals).

### 6.1.4 Life History Strategies

Although crayfish life history strategies are as varied as the different habitats described in the previous section, some generalized concepts can be developed.\(^{19}\) Female crayfish carry eggs on their swimmerets on the underside of the abdomen. Here the female oxygenates the eggs as well as provides support in the form of continual hygienic care. Eggs will typically incubate for two to four months depending on the species and environmental,
conditions such as temperature and oxygen. After hatching, the juveniles of some species of crayfish will immediately leave the mother to live a solitary existence. In a few burrowing species, the juveniles will remain with the mother for years. Sexual maturity is obtained after a few weeks up to a year depending on the species.¹

Life span also varies greatly across the different species of crayfish with *P. clarkii* found at the lower end of the spectrum (~1.5 years) and *Astacopsis gouldi* at the upper range (over five decades). For most of the species of crayfish, continual and allometric growth has been measured across the entire live span. The classical concepts of *r*-selected and *K*-selected reproductive strategies can be applied to crayfish, but produce mixed results. As mentioned above, crayfish can be quite short lived, mate once, have numerous offspring, fast egg development, and high juvenile mortality, which would place these species within an *r*-selected range of strategies.² Still, other species are quite long lived, mate multiple times throughout their life, have lower fecundity, slow egg development, and some parental care.² In addition, some species have a single adult morphology that is sexually active, while other species cycle through a reproductive and non-reproductive form throughout the adult lifetime. Those species that cycle often have morphological and physiological differences between the non-reproductive and reproductive forms. Beyond hormonal and sexually-related morphological changes, levels of aggression and chelae to carapace morphological differences are also seen.²

### 6.1.5 General Anatomy and Physiology

Being decapod crustaceans, crayfish have five pairs of pereopods (including the major chelae and walking legs), bilateral symmetry and an exoskeleton. The anterior pereopods are often modified into major and minor chelae. Many of the appendages (walking legs, antennae, and antennules) demonstrate a segmented morphology. In general, the body plan contains a cephalothorax (often called the carapace) and the abdomen. The end of the abdomen contains the telson. Many of the appendages and other body parts of the crayfish are covered with sensory hairs (chemo and/or mechano) located on body parts specialized into sensory appendages. The majority of these appendages (e.g. antennae, antennules, maxillipeds and chelae) are located anteriorly on the cephalothorax. In addition to these appendages, a pair of stalked eyes is found at the head of the cephalothorax.⁵

The circulatory system of crayfish is an open system with a heart and several arteries and sinus.⁵ While the heart serves as the major pumping organ, a minor role is played by the accessory pump, the cor frontale. The heart and the pericardial cavity are located dorsally with a major source of innervation travelling laterally to the gills on either side of the heart. The heart and gills are located in the cephalothorax region of the body. Also within this section is the digestive system, which is divided into three sections: the foregut (oesophagus and stomach), midgut (canal, caecum, and
hepatopancreas), and the hind gut. Of special interest for ecotoxicology is the hepatopancreas (or digestive gland), which functions to detoxify compounds within the crayfish body, as well as to produce digestive enzymes. Given the central function of this organ in the digestion of food and protection of the body from toxins, the physiological state of this organ can provide some insight into the effects of ecotoxins. Finally, crayfish have a well-developed excretory system located anteriorly of the heart with a bladder system. The bladder contains a nephropore located just ventrally of the eyes, which is used to release the bladder contents.

6.1.6 Crayfish Ecology

Crayfish are polytrophic and omnivorous. Essentially, these two distinctions mean that crayfish will consume a wide range of material, including detritus, live plant material, macroinvertebrates and small fish. In most environments, crayfish can play an important role in the structure of the food web because of their polytrophic status. Crayfish will also consume benthic macroinvertebrates and crayfish, in turn, are consumed by fish populations. In some aquatic habitats, crayfish are keystone species based on their central role in food web dynamics and, as such, can play a key role as bioindicators of habitat quality. Crayfish can function as shredding organisms in streams and lakes. “Shredders” are aquatic organisms that, through foraging, consume and break up terrestrial leaf material, which allows this material to be consumed by other aquatic macroinvertebrates. In addition, adult crayfish can have significant impacts on plant and algal communities in aquatic habitats. The nature of crayfish diets and the exact role that they play in aquatic food webs is dependent upon the size and age of the crayfish, the distribution of resources available, and the presence and distribution of predators. The presence of crayfish in habitats is often an indicator of increased diversity of macroinvertebrates, macrophytes, and even fish populations. Aquatic habitats have a wide range of ecosystem services, such as biofiltering of sediments and contaminants, and crayfish populations play a key role in influencing a rich diverse biological community that produces these services.

As a group, crayfish inhabit rivers, lakes, swamps, wetlands, and a wide diversity of substrates, including gravel, sand, mud, clays, and macrophytes. Within these habitats, the distribution of crayfish is often randomly spread across a heterogeneous landscape. Regardless of the location or habitat, the key point for this chapter is that all of these habitats have strong linkage to the terrestrial habitat through the riparian zone (which is the terrestrial habitat that serves as an interface between aquatic and terrestrial habitats). The importance of this point is that many different human activities (agricultural, industrial, wastewater treatment plants and industrial uses) often impact the riparian zone and subsequent habitat adjacent to the riparian zone. These areas are often where crayfish are located as they tend to inhabit the shallower areas in aquatic habitats.
6.1.7 Human Activities That Impact Crayfish

Although crayfish can be found in a wide diversity of habitats, any particular species often has a limited range of abiotic and biotic factors that determine suitable habitats. A number of different human activities have contributed to alteration to or degradation of crayfish habitats. Chief among these activities, and central to this chapter, is declining water quality through pollution. Outflow from industrial facilities may contain organic compounds, such as polycyclic aromatic hydrocarbons (PAHs) and polychlorinated biphenyls (PCBs), or even heavy metals, such as copper, zinc, lead or nickel. Runoff from large-scale agricultural fields can contain high concentrations of nitrogen or phosphorus as well as herbicides or insecticides. Wastewater treatment plants often contain untreated levels of pharmaceuticals that are contained within effluents or even find their way into streams through groundwater.

6.1.8 Using Crayfish as Bioindicators

Model systems for bioindicators should have a number of characteristics that can serve a broad and diverse field like ecotoxicology. These characteristics can be grouped based on their ecology, morphology and physiology. First, ecological attributes should include a well-developed taxonomy, a global distribution, high densities, long life span and low mobility. Second, morphological attributes include large body size for identification and physiology studies as well as low genetic variability. Finally, the physiology of any indicator species should be well-studied and include sensitivity to toxic substances and accumulation of toxins within specific organs. Crayfish have all of these attributes.

Crayfish can serve as an excellent model species to increase the knowledge base for toxicology. Overall, crayfish fulfill criteria described for bioindicators of environmental contamination. One of the most important aspects of crayfish that makes them an ideal bioindicator is the important role that they play as a keystone species within aquatic systems. Many different crayfish species have often been used as model organisms in toxicological research, which provides a solid research background for toxicological work. Attributes such as their size, excellent fecundity and relatively short life-span create opportunities for crayfish to be laboratory models for toxicity testing. There is a rich and diverse research literature on their basic physiological functions, which provides a baseline for sublethal testing. Given the large-sized and externally carried eggs, measurement and quantification for assays on fertility, reproduction success and mortality are readily done. The successful aquaculture of different crayfish species also opens doorways to examine how toxins affect the developmental processes.

Crayfish have often been considered bioindicator species of heavy metal pollution present in the aquatic environment as metals tend to accumulate
in their tissues. Traditionally, the toxicity of metals and agricultural chemicals has been investigated by examining the 24, 48, 72 or 96 hour (h) lethal concentration (LC) at which 50% of the population dies (e.g. 96 h LC$_{50}$). However, concentrations that cause high mortality rates may be well above environmentally relevant concentrations and thus may be of little biological significance. Crayfish display other quantifiable responses to sublethal concentrations of contaminants, which include, but are not limited to, changes in behavior, chemoreception, mate success, morphology, tissue structure and toxicant accumulation, anti-oxidant activation, enzyme function, and cardiovascular system regulation. This chapter focuses on a variety of responses by crayfish to sublethal concentrations of contaminants. In addition to lethality, other physiological changes have been recorded when crayfish were exposed to pharmaceuticals, agricultural chemicals and metals, either under laboratory conditions or when collected from contaminated field sites. These responses make crayfish an invaluable bioindicator of contamination. Further, given their importance in the aquatic food web, they may be used as a sentinel species when examining contamination from pharmaceuticals, agricultural chemicals and metals as the uptake and toxic responses seen in crayfish are similar to those in other aquatic species.

### 6.2 Pharmaceuticals

As a class of compounds, pharmaceuticals are a relatively new group of exotoxins that has unfortunately grown over the past two decades. Pharmaceuticals can enter aquatic habitats through their use in farming, disposal of unused medicines in landfills, and wastewater treatment plants. The types of pharmaceuticals found in wastewater effluent are varied. Anti-inflammatory drugs, β-blockers, antibiotics, anxiolytics and endocrine compounds have all been detected in wastewater treatment plant effluent. Some of these compounds are removed by wastewater treatment plants; however, the fact that so many remain at detectable levels after treatment is concerning. Pharmaceutical compounds that remain in treated effluent may affect aquatic organisms, especially in immediate areas of wastewater effluent discharge. Although a growing area of research, many of the toxicological studies on pharmaceutically active compounds have been performed on pelagic organisms, such as cnidarians and daphnia. This work has shown that dosages as small as 1 ng L$^{-1}$ can have impacts on aquatic invertebrates. Benthic macroinvertebrates, like crayfish, will likely be exposed to different concentrations of compounds than pelagic organisms.

Few studies have examined the effects of pharmacological agents on crayfish, but those that do are alarming. For example, Kulkarni et al. showed that the presence of serotonin alters the reproductive cycle of crayfish. When female $P$. clarkii were treated with 15 μg g$^{-1}$ serotonin (5-HT), they demonstrated enhanced ovarian development owing to increased the
release of ovary-stimulating hormone. This resulted in larger oocytes with enhanced amounts of vitelline, while 5-HT blockers have been shown to inhibit development. Exposure to serotonin reuptake inhibitors, such as fluoxetine, the active ingredient in Prozac, was shown to enhance crayfish (*O. rusticus*) growth in both form I and form II males (500 μg L⁻¹ for 10 days). Additionally, Tierney *et al.* showed that exposure to 2 and 500 μg L⁻¹ fluoxetine limits locomotory behavior, indicating that crayfish growth and locomotion can be manipulated by short-term exposure to fluoxetine. In addition, Goetz *et al.* showed that long-term exposure to fluoxetine (100 ng L⁻¹) had significant effects on excitatory junction potential amplitudes of crayfish superficial extensor muscles, leading to long-term depression of potential amplitudes. BETAMAX VET is a cypermethrin-based pharmaceutical used in the aquaculture industry to treat salmon louse (*Lepeophtheirus salmonis*) infestations in salmon farms. This compound is lethal to crayfish and has been used as a control agent for the spread of the invasive crayfish *P. leniusculus*. Chloramine-T is a disinfectant used in medical and veterinary facilities that can enter aquatic habitats through wastewater treatment facilities. It was shown that 50 mg L⁻¹ was toxic to the narrow clawed crayfish (*A. leptodactylus*) and led to substantial loss of energy in these crayfish. Overall, Kuklina *et al.* found that crayfish tolerated short-term chloramine-T exposure, but had increased sensitivity to higher concentrations, making them an ideal model organism for examining environmental exposure to chloramine-T and other pharmaceuticals.

### 6.3 Agricultural Chemicals

In the United States, approximately 1 billion pounds of agricultural chemicals are used each year to control weeds, insects and other pests; however, the use of these chemicals may have adverse effects on non-target aquatic organisms. Agricultural chemicals can enter the aquatic ecosystem typically through non-point sources. Non-point sources (*e.g.* runoff, seepage and deposition from the atmosphere) are the dominant sources of pesticides found in streams. Typically aquatic organisms, like crayfish, are exposed to agricultural chemicals in the water, but they may also ingest them via their food source. A key parameter for assessing the potential of a lipophilic chemical in the environment is the use of the octanol–water partition coefficient (*K*ₐ₎). If a chemical has a high *K*ₐ₎, it will partition appreciably into biota and organic matter, and this coefficient has been used successfully when estimating bioconcentration factors. Substances with a high *K*ₐ₎ (>4) are persistent in the environment and have the potential to accumulate and have long-term effects. Most pesticides (herbicides, insecticides and fungicides) have a high *K*ₐ₎ and thus a strong hydrophobic character. Given this, these chemicals are mainly associated with particulate matter and sediments and may bioaccumulate in fatty tissues of organisms.
6.3.1 Herbicides

Herbicides are used mainly in agricultural areas, but are also used in urban areas to increase product yield and to control unwanted urban vegetation. Some of the most commonly used pesticides are s-triazine herbicides (e.g. atrazine, hexazinone, metribuzine, prometryne, simazine and terbutryne). Because of their chemical properties, triazines are highly toxic and are commonly found in groundwater and surface water following heavy rainfall.48,49 Triazines have been found to have toxic effects on various crayfish species and research supports the fact that crayfish may be used as an appropriate bioindicator when examining the toxicity of triazines. Velisek et al.50 used the signal crayfish (P. leniusculus) to evaluate the 96 h LC50 toxicity values for atrazine, hexazinone, metribuzine, prometryne, simazine, and terbutryne. They reported that the LC50s were 12.1 mg L\(^{-1}\) for atrazine, 13.9 mg L\(^{-1}\) for terbutryne, 14.4 mg L\(^{-1}\) for prometryne, 19.5 mg L\(^{-1}\) for hexazinone, 30.6 mg L\(^{-1}\) for metribuzine, and 77.9 mg L\(^{-1}\) for simazine. They also found that atrazine was the most toxic triazine examined.50 When crayfish were treated with atrazine, they had difficulty navigating around a tank and often remained in the corners.50 Belanger et al.51,52 also showed that exposing crayfish (O. rusticus and O. virilis) to 80 \(\mu\)g L\(^{-1}\) atrazine affected their chemosensory abilities. After an acute exposure, crayfish could not localize a food odor source and these chemosensory deficits were long-term. Mac Loughlin et al.53 showed that when juvenile red claw crayfish (Cherax quadricarinatus) were exposed to high concentrations of atrazine (2.5 mg L\(^{-1}\)) they exhibited decreased weight gain and decreased abdominal muscle protein. Further, they reported that the proportion of females increased progressively as the atrazine concentration increased, suggesting that atrazine is an endocrine disruptor in crayfish. Exposure to other triazines, such as prometryne (144, 1444 and 4320 \(\mu\)g L\(^{-1}\)), caused decreases to weight and growth rates, changes in the gills of marbled crayfish (Procambarus fallax), and a delay in development at the highest concentration.54 Exposure of crayfish to prometryne, terbutylazine, terbutylazine-desethyl and metribuzin has been shown to cause histological damage, as well as changes in oxidative stress and antioxidant biomarkers (see Section 6.5).54–57 Because triazines are among the most commonly used herbicides worldwide, it is important to study the effects of these substances on non-target organisms, like crayfish.

Other herbicides, such as chloroacetamide herbicides (e.g. metolachlor), phenoxy herbicides (e.g. 2,4-dichlorophenoxyacetic acid (2,4-D), polychlorinated dioxins (e.g. 2,3,7,8-tetrachloro-dibenzo-p-dioxin (TCDD)), carbamates (e.g. Bolero) and glyphosate, have also been shown to cause changes in crayfish behavior and physiology after exposure to sublethal concentrations. Metolachlor is a herbicide that is heavily applied in agricultural areas in the United States.58 Exposure of crayfish (O. rusticus) to 80 \(\mu\)g L\(^{-1}\) interfered with the ability of crayfish to receive or respond to chemical signals from conspecifics and thus affected certain agonistic behaviors.59
Wolf and Moore\textsuperscript{60} also showed that when \textit{O. rusticus} were exposed to metolachlor (25, 50 and 70 \(\mu\)g L\(^{-1}\)), they did not respond to alarm cues or food odors. A reduction in chemosensory abilities can be detrimental for animals that rely heavily on chemosensory information for survival. 2,4-D is another widely used agricultural herbicide that has been shown to affect crayfish. Benli \textit{et al.}\textsuperscript{61} showed that the 96 h LC\(_{50}\) value was calculated to be 32.6 mg L\(^{-1}\) in crayfish (\textit{A. leptodactylus}) and also reported changes in behavior. Crayfish exposed to 30, 40 and 50 mg L\(^{-1}\) frequently stood in corners, had difficulty moving, began rocking, and walked in circles.\textsuperscript{61} Browne and Moore\textsuperscript{62} also noted changes in behavior of \textit{O. rusticus} in a Y-maze following exposure to 7.65, 14.07 and 32.69 mg L\(^{-1}\) 2,4-D. They showed that 2,4-D exposure inhibits the ability of crayfish to locate food by causing crayfish to walk at more rapid speeds, spend less time in the correct arm of the maze, and take significantly longer to locate food. Consequently, they consumed less food.\textsuperscript{62} The polychlorinated dioxin 2,3,7,8-tetrachloro-dibenzo-p-dioxin (TCDD) was toxic to crayfish (\textit{P. leniusculus}), with an LD\(_{50}\) of 30–100 \(\mu\)g kg\(^{-1}\); however, the toxicity of TCDD was delayed, with death occurring between 14 and 40 days after a single dose.\textsuperscript{63} Changes in cytochrome P450 induction were also noted.\textsuperscript{63} The 96 h LC\(_{50}\) for the carbamate Bolero (benthiocarb or thiobencarb) for crayfish \textit{Orconectes nais} and \textit{P. clarkii} was determined to be 2 mg L\(^{-1}\) and 6.5 mg L\(^{-1}\), respectively. Bioconcentration occurred in the hepatopancreas and muscle tissue following exposure and elimination of Bolero from crayfish was slow.\textsuperscript{64} Glyphosate acid, an active ingredient in Roundup\textsuperscript{8}, is another heavily used aquatic and terrestrial herbicide that has been shown to affect crayfish following exposure. Avigliano \textit{et al.}\textsuperscript{65} found that when juvenile crayfish (\textit{C. quadricarinatus}) were exposed for 60 days to 10 and 40 mg L\(^{-1}\) of glyphosate, that mortality was 33\% at the highest concentration and that lipid and protein levels, as well as growth, were all reduced in the 40 mg L\(^{-1}\) treatment. Frontera \textit{et al.}\textsuperscript{66} also showed that exposing \textit{C. quadricarinatus} for 50 days to glyphosate acid, either alone or in combination with polyoxyethyleneamine, caused lower somatic growth and decreased muscle protein levels. Collectively, crayfish are highly sensitive to various herbicides and display a variety of behavioral and physiological responses to sublethal concentrations, and thus serve as an invaluable tool when monitoring environmental contamination.

### 6.3.2 Insecticides

Chlorinated pesticides, such as dichlorodiphenyl-tri-chloroethane (DDT), are persistent in the environment (high \(K_{ow}\)) and have been used to control insects. Additionally, dichlorodiphenyldichloroethylene (DDE), a breakdown product of DDT, is also found in the environment owing to its persistence.\textsuperscript{47} Schilderman \textit{et al.}\textsuperscript{57} examined crayfish (\textit{O. limosus}) from the river Meuse (The Netherlands) and showed that crayfish from the heavily polluted site in the river had an accumulation of DDT and DDE in their hepatopancreas and an elevated level of DNA adducts. They suggest that crayfish are exposed to
persistent pollutants via the food chain or the sediment. Santerre et al.\textsuperscript{68} also collected and analyzed crayfish (\textit{P. clarkii}) tissue for the presence of organochlorines, organophosphates and pyrethroids. They found that 7\% of the crayfish sampled from Louisiana and Texas (United States of America) contained detectable residues of DDT (average concentration detected 0.047 mg L\textsuperscript{-1}). Crayfish (\textit{P. clarkii}) exposed to 1 mg L\textsuperscript{-1} \textsuperscript{14}C-ethion (an organophosphate insecticide) metabolized ethion into ethion mono-oxon, ethion dioxon, \textit{O,O-diethyl phosphorothioate}, \textit{O-ethyl phosphorothioate} and one unknown compound. These compounds accumulated in the gills and hepatopancreas.\textsuperscript{69} Additionally, exposure to ethion concentrations of 0.36 mg L\textsuperscript{-1} (1/4 96 h LC\textsubscript{50}) caused extensive ultrastructural alterations to both hepatopancreas and gill epithelial cells.\textsuperscript{69} Another organophosphate insecticide, fenitrothion, was shown to be toxic to the narrow-clawed crayfish (\textit{A. leptodactylus}) at a low concentration (15.75 μg L\textsuperscript{-1} 96 h LC\textsubscript{50}).\textsuperscript{70} Sarikaya \textit{et al.}\textsuperscript{70} showed that after a 24 h exposure, crayfish had decreased malondialdehyde levels at 5, 10 and 20 μg L\textsuperscript{-1} due to oxidative stress, as well as decreased mobility, walking in circles and loss of equilibrium. Overall, they found that fenitrothion was highly toxic to crayfish and that lipid peroxidation markers, like malondialdehyde, levels can be used as biochemical biomarkers for environmental monitoring. Diazinon is another organophosphorus insecticide that is widely used on agricultural crops. Burčić \textit{et al.}\textsuperscript{71} tested the toxicity of diazinon on three size classes of crayfish (\textit{O. limosus} and \textit{P. leniusculus}). They found that young-of-the-year crayfish were found to be the most sensitive to diazinon (96 h LC\textsubscript{50} = 0.15 mg L\textsuperscript{-1}), followed by juvenile crayfish (96 h LC\textsubscript{50} = 0.27 mg L\textsuperscript{-1}) and adults (96 h LC\textsubscript{50} = 0.51 mg L\textsuperscript{-1}). There was also a delayed effect of diazinon on adults was also detected (144 h LC\textsubscript{50} = 0.44 mg L\textsuperscript{-1}). This suggests that sublethal concentrations may cause functional damage in the long-term.

Pyrethroids are the most commonly used insecticides to control rice water weevils (\textit{Lissorhoptrus oryzophilus}) during rice farming; however, these pyrethroids (\textit{e.g.} lambda-cyhalothrin and etofenprox) are very toxic to juvenile crayfish (\textit{P. clarkii}), which are also farmed on the same land. Barbee and Stout\textsuperscript{72} found that the 96 h LC\textsubscript{50} for lambda-cyhalothrin was 0.16 μg L\textsuperscript{-1} and for etofenprox was 0.29 μg L\textsuperscript{-1}. Crayfish are often exposed to these chemicals during crop rotation when \textit{P. clarkii} are farmed on rice fields in Louisiana and pyrethroids are persistent in the soil after several applications to rice fields. Another pyrethroid, permethrin, caused 50–80\% mortality in crayfish (\textit{Procambarus} spp.) exposed to ponds to 1–3 μg L\textsuperscript{-1}.\textsuperscript{73} Barbee and Stout\textsuperscript{72} suggested that neonicotinoid insecticides should be used as an alternative to pyrethroids as they are two to three times less toxic (\textit{e.g.} 96 h LC\textsubscript{50} values: clothianidin, 59 μg L\textsuperscript{-1}; thiamethoxam, 967 μg L\textsuperscript{-1}; and dinotefuran, 2032 μg L\textsuperscript{-1}). Barbee \textit{et al.}\textsuperscript{74} also examined another potential replacement for pyrethroids for treating infestations of rice crops with rice water weevils, the insecticide chlorantraniliprole (anthranilic diamide insecticide). They showed that the 96 h LC\textsubscript{50} was 951 μg L\textsuperscript{-1} for \textit{P. clarkii}, three times less toxic than the pyrethroids currently used, and suggested that they are more
compatible with rice-crackfish crop rotations. Overall, many insecticides used in agriculture are toxic to crayfish, and may bioaccumulate and cause physiological changes. Crayfish can be used as indicators for biomonitoring studies when insecticides are used so that alternatives may be investigated if necessary.

6.4 Metals

Heavy metals such as copper, cadmium, zinc, lead and mercury are among the most common anthropogenic chemical wastes that pollute aquatic systems. While trace levels of these metals are present in freshwater systems under normal conditions, discharges from agricultural, industrial and municipal sources can elevate heavy metal concentrations to levels that are lethal to crayfish. While crayfish are generally considered to be highly resistant to death caused by heavy metal contamination, sublethal concentrations have also been shown to have significant effects on crayfish through bioaccumulation in tissues and detrimental impacts on physiology and ecologically important behaviors. Generally, there are no significant differences between males and females in regards to affinity for heavy metal accumulation; however, differing accumulation trends in the tissues with the highest accumulation have been demonstrated between sexes in crayfish (A. leptodactylus) with males having more accumulation of metals in their tissues.

6.4.1 Lethality

To investigate acute copper toxicity in the rusty crayfish (O. rusticus), Hubschman used continuous flow exposure experiments for 24, 48, 72, and 96 h at concentrations up to 12 mg L$^{-1}$. Results from Hubschman’s work showed that a copper concentration of 3 mg L$^{-1}$ was sufficient enough to kill 50% of adult intermolt crayfish following a 96 h exposure. Additionally, Hubschman reported that an exposure of 1 mg L$^{-1}$ caused a 50% mortality rate in newly hatched crayfish within an exposure time that was 1/50th of that needed to cause the same rate in adults. Taylor et al. used comparative toxicological responses of C. robustus from both a metal-contaminated site and unpolluted site to copper concentrations ranging from 0.61 to 24.06 mg L$^{-1}$ in 24 h time intervals. This research revealed that crayfish that were reared in a polluted environment had higher tolerance to heavy metal exposures, reporting an LC$_{50}$ value for crayfish from polluted sites of 4.07 mg L$^{-1}$ and 3.48 mg L$^{-1}$ for those from unpolluted sites for a 24 h exposure.

Khan and Nugegoda established heavy metal LC$_{50}$ values for juvenile C. destructor following 96 h static-renewal exposures. The LC$_{50}$ were reported as follows: nickel = 327 mg L$^{-1}$; iron = 50 mg L$^{-1}$; copper = 494 μg L$^{-1}$; and cadmium = 379 μg L$^{-1}$. Aguirre-Sierra et al. established lethal concentrations of fluoride for the white-clawed crayfish Autropotamobius pallipes to
be 93.0, 55.3, and 36.5 mg L\(^{-1}\) for 48, 72, and 96 h exposures, respectively. For the majority of crayfish species, heavy metal concentrations that occur in the environment are not high enough to directly cause death, which has led to studies focusing on bioaccumulation and behavioral response following sublethal exposures.\(^{31}\)

### 6.4.2 Bioaccumulation

Like other crustaceans, crayfish are able to depurate and detoxify themselves of many heavy metals. The main organ that functions in metal detoxification is the hepatopancreas, which has the ability to concentrate metals from the hemolymph and digestive tract, storing them in intracellular vacuoles.\(^{81}\) Consequently, the highest levels of bioaccumulation are seen in this organ.\(^{82}\)

However, once concentrations within the environment (either in sediment or water column) are elevated, crayfish may no longer be able to detoxify metals, leading to death. Alcorlo \textit{et al.}\(^{83}\) found that bioaccumulation of heavy metals in the tissues of \textit{P. clarkii} was the result of the organism’s interaction with contaminated substrates. A period of exposure of 6 to 12 days to a heavy metal-contaminated environment was sufficient enough to lead to significant bioaccumulation of metal in crayfish tissues.\(^{83}\) For most heavy metals (copper, zinc, cadmium, lead, nickel, and mercury), bioaccumulation in various tissues, such as the hepatopancreas, abdominal muscles, green gland and digestive tract, exoskeleton, and/or gills, is time- and dose-dependent and may be indicative of metal concentrations in the surrounding environment.

Compared to other heavy metals, relatively high amounts of copper can be found in crayfish tissues given that copper is a crucial component of the respiratory metalloprotein (hemocyanin).\(^{84}\) When copper bioavailability exceeds a high threshold, species-, concentration-, and exposure-period-specific accumulation has been documented in the hepatopancreas as well as other tissues, such as abdominal muscle, exoskeleton, and gills [Finerty \textit{et al.}\(^{85}\) (\textit{P. clarkii} and \textit{P. astacusacutus}); Madden \textit{et al.}\(^{86}\) (\textit{P. clarkii}); Gherardi \textit{et al.}\(^{87}\) (\textit{P. clarkii} and \textit{Astacuspallipes}); Bruno \textit{et al.}\(^{82}\) (\textit{C. destructor}); Hothem \textit{et al.}\(^{88}\) (\textit{P. clarkii} and \textit{P. leniusculus})]. Maranhão \textit{et al.}\(^{89}\) reported that following a 96 h exposure to copper concentrations of 0.125–0.500 mg L\(^{-1}\), there was no significant accumulation of copper in tissues of \textit{P. clarkii}. The same study reported that following an 8 week copper exposure of 5 mg L\(^{-1}\), time-dependent copper accumulation was documented in the gills, exoskeleton, and abdominal muscles of \textit{P. clarkii}. The levels of copper in \textit{P. clarkii} gill, exoskeleton, and abdominal tissue were subsequently reduced when crayfish were placed in clean water following exposure.\(^{90}\) \textit{A. leptodactylus} demonstrated a similar pattern of accumulation and depuration following copper exposure.\(^{91}\)

Like copper, zinc is an essential metal for crayfish metabolism and thus the content of zinc in crayfish bodies is naturally high regardless of environmental conditions. However, this metal has been shown to accumulate
in the hepatopancreas and abdominal tissues of many species [Madden et al. 86 (P. clarkii); Gherardi et al. 87 (P. clarkii and A. pallipes); Bruno et al. 82 (C. destructor); Hothem et al. 88 (P. clarkii and P. leniusculus)]. Additionally, Bagatto and Alikhan 92 and Mackevičienė 93 found that zinc accumulated in the digestive tract tissues of C. bartonii and A. astacus, respectively. Zinc content in the hepatopancreas, gills, and abdominal muscle of C. tenuimanus was found to be highest in juveniles, owing largely in part to the higher permeability of the body surface, rendering them unable to regulate zinc as efficiently as older individuals.94 Similar results were documented in C. destructor juveniles.82

Conversely to copper and zinc, cadmium and lead are not involved in crayfish metabolism and consequently tend to increase rapidly along with increasing environmental concentrations. Accumulation of cadmium has been shown to be positively correlated with the level of environmental cadmium and proximity to the source of cadmium pollution.95,96 Crayfish are able to take up cadmium from both the surrounding environment and from food.97,98 Chambers 99 and Bruno et al. 82 showed that cadmium accumulated largely in the hepatopancreas, followed by the gills, exoskeleton, and abdominal muscles in C. tenuimanus and C. destructor. Additional studies have reported that cadmium largely accumulates in the hepatopancreas and abdominal muscle tissue [Finerty et al. 85 (P. clarkii and P. astacusacutus); Madden et al. 86 (P. clarkii); Gherardi et al. 87 (P. clarkii and A. pallipes); Bruno et al. 82 (C. destructor); Hothem et al. 88 (P. clarkii and P. leniusculus)]. C. destructor adults were found to have higher levels of cadmium accumulation in the exoskeleton and muscle tissues as compared to juveniles, seemingly related to molting frequency.82

The accumulation of lead in crayfish tissue has been studied in both aquaculture and natural settings [Madden et al. 86 (P. clarkii); Gherardi et al. 87 (P. clarkii and A. pallipes); Bruno et al. 82 (C. destructor); Hothem et al. 88 (P. clarkii and P. leniusculus)]. The hepatopancreas has been documented as the main organ for lead accumulation, storing metal in vacuoles.82,93 Accumulation has also been observed in the digestive tract, abdominal muscle, exoskeleton, and antennal (green) gland.93,100 Following a 10 week exposure to 0.02 mg L$^{-1}$ of lead, accumulation in A. astacus was primarily in the hepatopancreas, carapace, and gills.101 Significant accumulation in the hepatopancreatic and gill tissues of P. clarkii was documented following 7 day exposure in a contaminated habitat.95 Lead concentrations in tissues of P. clarkii were significantly decreased after a 3 week recovery period from a 7 week exposure to 0.15 mg L$^{-1}$.

Methylmercury represents approximately 90% of the total mercury accumulated in crayfish tissues.88,102 In crayfish, mercury is largely accumulated in the abdominal muscle tissue [Finerty et al. 85 (P. clarkii and P. astacusacutus); Madden et al. 86 (P. clarkii); Simon et al. 103 (A. astacus); Loukola-Ruskeeniemi et al. 75 (A. astacus); Hothem et al. 88 (P. clarkii and P. leniusculus)]. Studies have demonstrated that mercury and methyl mercury also accumulated in the hepatopancreas, gills, and exoskeleton in Orconectes propinquus
that were fed mercury and methyl mercury dosed pellets.\textsuperscript{104} Crayfish have a marked tendency to accumulate methylmercury taken up from food and water, from which they also take up mercury.\textsuperscript{105}

Like other heavy metals, nickel has been shown to accumulate in crayfish tissues based on the concentration in the surrounding environment. In both \textit{A. astacus} and \textit{C. bartonii}, nickel accumulation was documented to be the highest in the exoskeleton, suggesting that this type of tissue might be involved in the excretion of this metal.\textsuperscript{92,93} Similarly, chromium was found to be at highest accumulation in the exoskeleton of \textit{A. astacus} as well as at high levels in the hepatopancreas and abdominal muscle of \textit{A. astacus} and \textit{P. leniusculus}.\textsuperscript{93,106} \textit{P. clarkii} primarily accumulated chromium in the gills and hemolymph following a 7 day exposure in a contaminated environment.\textsuperscript{95} Grosell \textit{et al.}\textsuperscript{107} reported that adult \textit{C. diogenes diogenes} accumulated substantial amounts of silver in gill, hemolymph, and hepatopancreatic tissues following a 96 h silver exposure at a concentration of 8.41 $\mu$g L$^{-1}$. Fluoride was shown to accumulate primarily in the exoskeleton for \textit{A. pallipes}.\textsuperscript{80}

Research has shown that the amount of bioaccumulation that occurs in crayfish tissues is positively correlated to the concentration of heavy metal pollution in the animal’s habitat.\textsuperscript{83,91} Goretti \textit{et al.}\textsuperscript{108} suggested the use of \textit{P. clarkii} as a potential bioindicator for heavy metal pollution in freshwater systems through establishing a toxic contamination index utilizing a ratio of bioaccumulation for cadmium, copper, lead, and zinc within the hepatopancreas and abdominal muscle. As the hepatopancreas has high detoxifying activity compared to the low activity of muscle, concentration values of heavy metals within those tissues can demonstrate the level of toxicity due to heavy metal pollution. This index was proposed to be used as an easy and useful tool that can assess the toxicity level of heavy metal-contaminated sites.\textsuperscript{108} Thus, crayfish can be used to identify and monitor areas within aquatic systems that are under pollution stress.\textsuperscript{83,88,109}

### 6.4.3 Physiological and Behavioral Impacts

In addition to accumulation in tissues, sublethal exposure to heavy metals can impact physiological processes of crayfish (for a complete review see Section 6.5). For example, cardiac arrhythmia followed by substantial levels of death was reported in \textit{A. astacus} following exposure to mercury chloride at concentrations of 0.1–0.8 mg L$^{-1}$.\textsuperscript{110,111} Inhibitory effects on the ovarian maturation in \textit{P. clarkii} exposed to mercury were reported by Reddy \textit{et al.}\textsuperscript{112} Adult \textit{C. diogenes diogenes} exhibited ionoregulatory disturbance and elevated production of metabolic ammonia following a 96 h exposure to 8.41 $\mu$g L$^{-1}$ of silver.\textsuperscript{107} Rowe \textit{et al.}\textsuperscript{113} found that the standard metabolic rate of the crayfish \textit{P. acutus} had approximately 30% higher standard metabolic rates following a chronic exposure to sediment contaminated with heavy metals that included arsenic, cadmium, chromium, copper, and selenium. This elevated metabolic rate supports that detoxifying and combating the
deleterious effects of heavy metal contaminants to energetic costly to crayfish and could subsequently lead to reduced fitness.

Sublethal heavy metal exposures can also have severe consequences on ecologically important behaviors. Burba investigated the effects of a 96 h exposure to a copper below the LC50 concentration on the exploratory and social behaviors of the noble crayfish (A. astacus). Crayfish exposed to copper showed decreased movement around a test arena and lack of thigm- and chemo-tactic behaviors. Additionally, agonistic behavior was more frequent between individuals exposed to copper than those that were unexposed. Escape responses (tail flips) in the white-clawed crayfish (A. pallipes) decreased significantly in animals exposed to 5.9–18.4 mg L−1 of fluoride. Sherba et al. demonstrated that the freshwater crayfish C. bartonii exhibited increased foraging latency and was not able to localize food sources following a 120 h exposure to 0.02 or 0.2 mg L−1 of copper. Similarly, significant differences were found in the overall orientation ability of the rusty crayfish (O. rusticus) to locate an odor source when previously exposed to copper (4.5, 45 and 450 µg L−1) for 120 h in combination with a source of copper pollution in the background of orientation trials. O. rusticus exposed to copper in any capacity showed altered orientation parameters as compared to unexposed crayfish, showing that copper impaired the crayfish’s ability to detect, process, and/or respond appropriately to chemosensory information. In another study, the antennular flicking rates of rusty crayfish exposed to 450 µg L−1 of copper for 120 h were significantly lower than unexposed animals, and were consequently less successful in locating a food odor source. Once placed in clean water, crayfish demonstrated significant increases in both antennular flicking rates and successful localization of odors, indicating that O. rusticus can recover olfactory behaviors following clean-up of a copper polluted environment.

In addition to foraging and orientation behaviors, the overall population densities of crayfish have been shown to be impacted by elevated levels of heavy metal discharge. Allert et al. reported that mean population densities of the crayfish Orconectes hylas were negatively correlated with sediment pore-water metal concentrations (lead, zinc, cadmium, nickel, and cobalt), with lower densities found at sites close to and downstream from lead–zinc mines. Similar results have been reported for O. neglectus neglectus, O. luteus and O. virilis. During a 28 day in situ experiment, the survival and biomass of O. hylas were significantly lower in populations of crayfish located at sites in close proximity to historical lead–zinc mining activities. Similar results following a 56 day in situ experiment were reported for O. luteus.

6.5 Physiological Responses to Contaminants

When exposed to sublethal concentrations of contaminants in aquatic ecosystems, crayfish do not always display changes in behavior, reproductive
effort, external morphology and/or death. They may have physiological changes that may be examined using molecular, histological and analytical methods. Typically, when organisms are exposed to contaminants, they experience reductions in physiological processes; however, in some cases they are increased. Evaluating the 96 h LC_{50} or population size changes are techniques that are heavily used in toxicology research to test the lethality of a toxicant; however, concentrations that cause high mortality rates may be well above environmentally relevant concentrations and thus may be of little biological significance. Sublethal concentrations of contaminants are more likely to be encountered in aquatic environments and lead to changes in the physiology of crayfish, rather than death. Moreover, crayfish can be used as a sentinel species as they display changes in tissue structure, cytochrome P450 levels and antioxidant activity, acetylcholinesterase activity, oxidative stressors, DNA structure, and cardiovascular, respiratory and metabolic function, as well as other physiological changes (e.g. enzymatic function) in response to changes in abiotic and biotic factors.

Crayfish absorb xenobiotics into their body via the gills and subsequently accumulate them in the hepatopancreas. This may lead to structural changes in the hepatopancreas (Figure 6.2). Desouky et al. demonstrated that when crayfish (*P. clarkii*) were treated with the insecticide ethion (1 mg L^{-1}), it accumulated in both the gills and hepatopancreatic tissue. When the crayfish were then placed in clean water for 7 days, the concentrations of insecticide residues were decreased in both the hepatopancreas and gills. When *P. clarkii* where exposed to 1/4 the 96 h LC_{50} (0.36 mg L^{-1}), it caused ultrastructural changes to both the gill and hepatopancreatic epithelial cells, which included vacuolation, degradation and distinct cell lysis in the hepatopancreas. Infiltration of hemocytes, cytoplasmic vacuolation and changes in the plasma membrane structure were visualized in gill tissue. Exposure to environmentally relevant concentrations of the herbicide metribuzin for 30 days also caused histological changes in crayfish (*P. leniusculus*), which included disintegration of the tubular epithelium in the hepatopancreas. Chupani et al. also demonstrated that exposure to peracetic acid (2 mg L^{-1}), a powerful disinfectant used to eliminate zoospores (*Aphanomyces astaci*) that cause crayfish plague, produces slight damage to gill, hepatopancreatic and antennal gland tissue in crayfish (*P. leniusculus*). These changes were more pronounced when the environmentally relevant concentration (10 mg L^{-1}) was used. Gill tissues were infiltrated with hemocytes and had disorganized epithelial cells and malformed lamellar tips. Tissue changes were reduced to normal levels after a 7 day recovery period. Exposure to xenobiotics does not always cause histopathological changes in the hepatopancreas. Stará et al. found no changes in the hepatopancreas of *P. clarkii* after exposure to 0.51–1444 μg L^{-1} of the herbicide prometryne; however, changes in antioxidant activity were observed for 11 and 25 day exposures. Overall, results suggest that histological changes in crayfish tissues vary depending on the type of xenobiotic they are exposed to. Changes in the histological organization of both the gills
and hepatopancreas of crayfish may be used as a bioindicator of contamination, but should be examined in combination with other measures of toxicity (e.g. cytochrome P450 activation and antioxidant activity).

Cytochrome P450 oxidase are proteins that use a variety of small and large molecules as substrates in enzymatic reactions. Each isoform has a broad spectrum of catalytic activities and substrates. These proteins have been shown to play an important role in the transformation of xenobiotics and endogenous chemicals in crayfish and other crustaceans, and are abundant in hepatopancreas microsomes. The cytochrome P450 system has been characterized in the hepatopancreas and green gland of P. clarkii. Ashley et al. demonstrated that crayfish (P. leniusculus) exposed to an extremely low dose (3 μg kg⁻¹ body weight) of 2,3,7,8-tetrachloro-dibenzo-p-dioxin (TCDD) exhibited significant induction of cytochrome P450, measured spectrally, while TCDD-related histological changes were not observed. Fenitrothion is a powerful organophosphorus insecticide used to control rice
stem bores. When crayfish (P. clarkii) were exposed to 200 μg L\(^{-1}\) for 1–2 weeks, induction of cytochrome P450 activity was recorded, as well as the inhibition of acetylcholinesterase activity.\(^{126,127}\) An increase in EROD activity (the catalytic form of cytochrome P4501A) was also increased after exposure of P. clarkii to fenitrothion.\(^{127}\) This suggests that cytochrome P450 induction in crayfish can be used as a biomarker for exposure to environmental pollutants.

Antioxidant enzyme system activation (e.g. superoxide dismutase and glutathione reductase) can also be investigated to determine if these systems are activated during exposure to xenobiotics. Metribuzin is used in both agriculture and recreational areas, and runs off into local streams and rivers.\(^{128}\) When crayfish (P. leniusculus) were exposed to 0.52 μg L\(^{-1}\) (environmentally relevant concentration) and 3.06 mg L\(^{-1}\) metribuzin for 10 and 30 days, changes in oxidative stress (thiobarbituric acid reactive substances) and antioxidant enzymes [total superoxide dismutase (SOD), catalase (CAT) and glutathione reductase (GR)] were observed in muscle, gill and hepatopancreatic tissues.\(^{56}\) Crayfish (P. clarkii), exposed to prometryne (0.51 μg L\(^{-1}\), 0.144 mg L\(^{-1}\), and 1.144 mg L\(^{-1}\) for 11 and 25 days) also displayed changes in SOD, CAT and GR antioxidant enzyme activity in muscle and hepatopancreatic tissues.\(^{57}\) P. leniusculus exposed to peracetic acid (2 mg L\(^{-1}\) and 10 mg L\(^{-1}\)) demonstrated decreases in SOD activity in both gill and hepatopancreatic tissues after a 3 day exposure to 10 mg L\(^{-1}\) when compared to controls and crayfish treated with 2 mg L\(^{-1}\), while CAT activity remained unaffected in all treatment groups.\(^{122}\) GR activity was significantly reduced in the gill tissue of crayfish treated with 10 mg L\(^{-1}\) peracetic acid and increased in crayfish exposed to 2 mg L\(^{-1}\) after a 7 day exposure. All enzyme levels returned to normal after a 7 day recovery period.\(^{122}\)

Malondialdehyde (MDA) activity can also be used as a marker of oxidative stress. Crayfish (A. leptodactylus) demonstrated decreased MDA levels when they were exposed to 5, 10, and 20 μg L\(^{-1}\) of fenitrothion.\(^{70}\) Increased levels of oxidative stress can cause decreases in survival and reproductive rates and thus can be considered a marker for overall general health.\(^{129}\) Taken together, these results suggest that antioxidant enzymes in crayfish can be evaluated and may serve as potential biomarkers for monitoring exposure to xenobiotics. Changes in antioxidant levels can be assessed in a sentinel species, like crayfish, and used to avoid toxic responses to aquatic animals before changes to overall population fitness occur.

For biomonitoring purposes, the presence of DNA adducts or DNA adduct formation can be used as a measure of the toxicity of substances in the aquatic environment. These adducts are formed when a segment of DNA bonds to a chemical, changing the structure of the DNA.\(^{67}\) Crayfish have the ability to form DNA adducts when exposed to xenobiotics. For example, Schilderman et al.\(^{67}\) examined crayfish (O. limosus) collected from the River Meuse (The Netherlands) to determine DNA adduct levels and correlated them with heavy metal residues, chlorinated pesticides and the seven indicator congeners of PCBs from four different locations. Overall, they found
that DNA adduct levels can be used as a dosimeter for the internal dose of aromatic compounds, such as polycyclic aromatic hydrocarbons and PCBs, and that the highest hepatopancreatic DNA adducts were found to be significantly higher at the most polluted site.\textsuperscript{67} Additionally, a Comet assay can be used to assess DNA damage or double strand breaks in crayfish exposed to changes in abiotic and biotic conditions. Malev \textit{et al.}\textsuperscript{130} showed that when \textit{A. leptodactylus} were exposed environmental stressors (\textit{i.e.} increased environmental temperatures), significantly more DNA damage was present in cells obtained from the hemolymph. These studies demonstrate that changes to crayfish DNA integrity can be used as a bioindicator of environmental health.

Exposure to xenobiotics has also been shown to cause cardiovascular, respiratory and metabolic changes in crayfish. Exposure of crayfish (\textit{A. astacus}) to 0.1 mg L\textsuperscript{-1} mercury (HgCl\textsubscript{2}) produced cardiac arrhythmias, which were believed to be related to metabolic disturbances. These arrhythmias ultimately lead to death.\textsuperscript{110,111} Copper exposure in \textit{P. clarkii} lead to a reduction of both cardiac and ventilatory activity that was concentration-and time-dependent.\textsuperscript{131} Lead (Pb) decreased the respiration rate in crayfish (\textit{P. clarkii}). Oxygen uptake of the whole animal generally decreased with increasing Pb concentration, though not significantly. The histology of gill filaments of crayfish treated with 200 mg L\textsuperscript{-1} Pb indicated a general disorganization.\textsuperscript{132} Ahern and Morris\textsuperscript{133} found that exposure of the crayfish \textit{C. destructor} to 100 or 0.5 mg L\textsuperscript{-1} Pb reduced oxygen consumption, along with reduced heart rate, although ventilation rate was unchanged. There was also a reduction in oxygen transfer factor across the gills after 21 days and a reduction in cellular metabolism.\textsuperscript{134} Standard metabolic rates (SMR) in crayfish (\textit{P. acutus}) were also altered following exposure to contaminants. Rowe \textit{et al.}\textsuperscript{113} found that crayfish that had chronic exposure in a site contaminated with trace elements had higher SMRs when compared to crayfish collected from a reference site (25.1 vs. 19.2 J g\textsuperscript{-1} day\textsuperscript{-1}). Growth of animals from contaminated sites was also lower than that for animals from control sites. The authors suggest that SMR comparisons can be used as a biomarker for contamination as many other species demonstrate elevations in SMRs when exposed to pollutants.\textsuperscript{113} Generally, exposure to xenobiotics at environmentally relevant concentrations can slow growth and/or lead to death in crayfish when changes in the metabolic or cardiovascular system are involved.

In general, other physiological disturbances have been noted when crayfish are exposed to various contaminants from metals to pesticides. For example, digestive enzymes are inhibited by metal contaminants. Cadmium (Cd) exposure reduced amylase activity in gastric juice of the crayfish \textit{P. clarkii}.\textsuperscript{135} Changes in ion (Na\textsuperscript{+}) regulation, elevated metabolic ammonia production and substantial silver accumulation in the gills, hemolymph and hepatopancreas were also noted in crayfish (\textit{C. diogenes diogenes}) when they were exposed to 8.41 ± 0.17 μg L\textsuperscript{-1} of silver for 96 h.\textsuperscript{107} A combination of metals and pesticides has also been shown to cause changes in esterase
activity in the crayfish *P. clarkii*. Vioque-Fernández *et al.* found that examining acetylcholinesterase and carboxylesterase activity can be useful as a biomarker of pesticide exposure, including organophosphate and carbamate insecticides. Further, muscle pyruvate kinase activity was significantly lower in crayfish (*C. quadricarinatus*) exposed to 10 mg L\(^{-1}\) of the herbicide glyphosate \([N-(phosphonomethyl)glycine]\) in freshwater. Further, no changes in lactate dehydrogenase were observed, indicating reduced metabolic intensity of cells. Avigliano *et al.* demonstrated that alanine and aspartate aminotransferase activities (ALAT and ASAT, respectively) can also be used as indicators of tissue damage following exposure to glyphosate. Crayfish (*C. quadricarinatus*) that were exposed for 60 days to 40 mg L\(^{-1}\) of pure glyphosate, an active ingredient in the herbicide formulation Roundup\textsuperscript{R}, were found to have higher hemolymphatic ASAT : ALAT ratios than control crayfish. This suggests that there may be damage to several tissue types as crayfish may mobilize lipids and proteins under stressful situations. Overall, various physiological changes in crayfish can be used as biomarkers to determine if sublethal exposure to metals and/or pesticides is affecting the physiology of aquatic animals.

Physiological responses to environmental stressors are typically deleterious in nature. We see that exposure to environmentally relevant concentrations of xenobiotics can cause measurable physiological changes in the crayfish before death occurs. These endpoints can be easily examined using a variety of molecular, analytical and histological techniques. Any one or several of these physiological changes should be examined after exposing crayfish to potential environmental contaminants in order to evaluate their sublethal toxicity before they are released into the environment.

### 6.6 Conclusions

As more and more sources of freshwater are deemed unsuitable for consumption and/or agricultural use without some means of treatment, growing strain is placed on existing resources. Global focus has been aimed at finding efficient and effective methods of assessing, restoring, and/or maintaining healthy aquatic systems. This chapter has summarized crayfish ecology and how exposure to elevated levels of environmental contamination from anthropogenic sources can impact crayfish survival, physiology and behavior. The research included in this chapter establishes a solid case for the use of crayfish as a bioindicator for assessing and monitoring contaminated aquatic systems, as well as for using this invertebrate as a model organism in toxicological studies of aquatic pollutants. Foremost, crayfish are found in nearly every freshwater system worldwide, making toxicological studies using this organism broadly applicable. Abundant, widespread crayfish populations with narrow home ranges and low migration mean that crayfish tissue specimens are indicative of the locations from which animals were obtained. Crayfish are easily captured, can be easily maintained and cultured in laboratory settings, they produce large numbers of offspring.
from a single individual, and most species have body sizes that provide sufficient tissue for analyses. These animals rely on chemical information from the surrounding environment for all of their behaviors, and possess olfactory receptors that are in direct contact with the fluid environment. Consequently, crayfish are highly sensitive to minute chemical fluctuations and modifications to their behaviors can serve as a more sensitive, less invasive means of environmental monitoring and assessment.

Research has also demonstrated that the uptake and toxic responses of many environmental pollutants are similar in crayfish and other aquatic species, such as teleost fish, signifying that crayfish are a useful model for monitoring environmental conditions and determining remediation strategies. However, the LC50 values reported by Khan and Nugegoda were higher than those documented for other aquatic species, providing evidence that juvenile *C. destructor* is less sensitive to heavy metals in the environment. This suggests that LC50 calculations should be coupled with physiological and behavioral assessments when determining toxicological endpoints and safe concentrations in the environment. Owing to the propensity for bioaccumulation in tissues, crayfish have the potential to transfer pollutants and toxins to organisms in higher trophic levels given their role in both aquatic and terrestrial food webs. This propensity also provides means for establishing toxicity contamination indices as measures of environmental contamination. Further, crayfish are listed as the second most imperiled aquatic group in the United States, suggesting that research should focus on their conservation. As this chapter has illustrated, crayfish are most often non-target organisms of the anthropogenic chemicals, providing urgency to studying the effects of xenobiotics on these animals. By continuing and increasing the use of crayfish as model organisms and bioindicators, knowledge can be gained for conservation and preservation of these crucially important organisms and their habitats. Lastly, crayfish may also serve as a sentinel organism for other aquatic organisms, allowing environmental managers to determine environmentally appropriate concentrations of xenobiotics.

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