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Metals in Crawfish

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1. Introduction

1.1 Overview

Much of the information in the first nine sections of this chapter was derived and condensed from the Louisiana Crawfish Production Manual (McClain et al, 2007). The harvesting of crawfish (or crayfish) for human consumption has become an important industry in several areas throughout the world. In the United States, crawfish are common in Louisiana (located in the southern United States on the Gulf of Mexico) and throughout the southeastern states. Crawfish (McClain, 2005) are well suited for habitats that have seasonal flooding and drying, especially when the dry periods occur in the summer and fall. Periods of flooding allow the crawfish to feed, grow and mature. Dry periods help aeration of sediments, reduce the abundance of predators and allow for establishment of vegetation, which serves as cover and food resources when flooded. Crawfish are common in parts of northeastern Mexico, can be found extensively in southeastern Asia and are grown commercially in China. In the 1970s, crawfish were introduced into Portugal and Spain and thrived in the rivers and estuaries to the extent that commercial harvesting now contributes significantly to the food supply in these regions (Alcorlo et al, 2006) (Maranhao et al, 1999). In Louisiana there are numerous varieties of crawfish but the two dominant species are the red swamp crawfish (*Procambarus clarkii*) and the white river crawfish (*Procambarus zonangulus*). Both species dominate harvests in natural habitats and on crawfish farms, therefore, unless otherwise noted these two species are the species of crawfish to which the authors refer.

1.2 History

In Louisiana, crawfish has been a part of the human diet for centuries but the commercial sales of crawfish began only in the late 1800s. As the demand for crawfish outgrew the harvest from the wild, the crawfish aquaculture industry was born. Early crawfish aquaculture consisted only of re-flooding rice fields after the harvest to produce food for the farm family. Excess production from these fields was sold to nearby consumers. Over time the demand and supply of crawfish continued to grow and by the 1960s commercial processing of crawfish began. The peeling and packaging of crawfish tail meat allowed for the establishment of regional markets. Commercial processing required a steady supply of crawfish to meet demand. This increased demand was greater than could be supplied by harvests in natural habitats and lead to a great increase crawfish aquaculture in Louisiana. In the United States there are few commercial producers of crawfish outside of Louisiana.

Louisiana produces about 90 percent of the nation’s crawfish, 70 percent of which is consumed within the state (McClain, 2005).

2. Crawfish

2.1 Anatomy

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2.2 Biology

Both red swamp and white river crawfish are considered a temperate species of crustaceans that are able to tolerate moderately cold winters. There are some differences of note in these two species. White river crawfish spawn in the fall of the year and produce larger but less numerous eggs than do the red river crawfish which spawn year around. Red river crawfish have higher feeding rates at temperatures above 30° C. Additionally, the red swamp crawfish are more tolerant to the low dissolved oxygen content that sometimes occurs in crawfish ponds. This may be the reason that the red swamp crawfish dominate most crawfish production ponds. The two species are very similar in appearance but can be differentiated between by an experienced person. Red swamp crawfish is the preferred species because of this species greater reproductive potential and a more prolonged reproductive season. There is no evidence of natural hybrids between the two species.

2.3 Life cycle

Both species live about 2 years, have high juvenile survival rates and have reproductively active and inactive periods. After mating in open water, the female crawfish then stores the sperm in an internal receptacle. Egg development in the females takes place internally. Developing eggs in the ovary increase in size and change from a light color to black. More than 500 mature eggs are expelled and attached to the swimmerets under the tail of the crawfish. Fertilization of the eggs takes place externally when the female releases the stored sperm. During egg production the female builds a burrow and spawning takes place in the burrow. The incubation period for the eggs is approximately three weeks. The hatchlings remain attached to the female's swimmerets through the first two molting phases. After detaching from the mother, the young crawfish resemble adult crawfish and begin to feed. The female and young crawfish remain in the burrow for several weeks but then must leave because little food is available in the burrows. Pond flooding or heavy rainfall will encourage the crawfish to emerge sooner rather than later. Reproduction is somewhat synchronized in pond-raised crawfish because rice fields are flooded in the fall after the rice is harvested to coincide with the optimum time for reproduction. While red river crawfish spawn at anytime, autumn is the best time for white river crawfish to spawn. Peak production can best be attained when reproduction is properly timed in autumn.

Crawfish, like all crustaceans, must molt periodically to increase in size. Growth rate is dependent on variables such as: water temperature, oxygen levels, population density and food quality and quantity. Optimum harvest size can be reached in as little as 7 to 9 weeks, but usually takes 3 to 5 months after hatching. Crawfish molt approximately 11 times before reaching maturity. Though there are five major stages in the molt cycle, it is a continuous process. The inter-molt stage is the phase in which the exoskeleton is hard and fully formed. During this stage the crawfish feed to increase their energy and tissue reserves. In the pre-molt stage an underlying soft exoskeleton forms and begins re-absorbing calcium for the older exterior. During molting feeding stops and temporary shelter is sought. Molting takes place in a matter of minutes as the old exoskeleton splits between the carapace and the abdomen on the dorsal surface. The crawfish then withdraws by flipping its tail several times. After emerging from the old exoskeleton the crawfish is in the "soft" phase during which the new exoskeleton expands to a larger size than the previous exoskeleton. Calcification of the new exoskeleton takes place in two phases. During the first phase the

calcium stored by the crawfish in two hard gastroliths on each side of the stomach is transported to the new shell. The second phase of the hardening occurs as calcium is absorbed from the water. Once crawfish molt to a reproductively active stage, growth ceases. A medium size crawfish has a mass of about 25 grams and a head to tail length of 10 centimeters. Mature crawfish can be identified by dark coloration, enlarged claws and harden sexual structure. Adult males develop prominent hooks on the base of the third and fourth pair of walking legs. Females will mate several times after molting to the mature stage.

2.4 Crawfish borrows

Crawfish dig unbranched vertical burrows usually less than one meter deep. The burrows are used for purposes other than reproduction. The burrows serve as refuge from predators and provide a moist environment necessary to survive dry periods. Crawfish burrows are built over several days time by an individual crawfish and the burrow diameter is dependent on the size of the crawfish. At the bottom of the burrow is chamber slightly larger than the crawfish. The water level in a burrow is dependent on the moisture conditions in the surrounding soil. The entrance to a completed burrow is sealed with a mud plug. Crawfish burrows often have a soil stack or chimney above ground which is formed by the excavated dirt. Once sealed in the burrow the crawfish is confined to the burrow until the plug is softened by heavy rainfall or pond flooding.

2.5 Population dynamics

In most forms of aquaculture, known numbers and sizes of juveniles are used to stock the ponds. Stocking with juveniles is not used in Louisiana crawfish aquaculture. Population within a pond depends upon reproduction from either stocked or already present mature crawfish. Density of the population depends upon brood stock survival, successful reproduction and survival of the offspring. Density can be adversely affected by factors over which the farmer has no control such as: by low oxygen levels, predators or pesticide exposure from nearby farming operations. Research has revealed that density greater than 15 crawfish per square yard results in slow growth rates and smaller sized crawfish at maturity. These factors have made control over population levels one of the most elusive aspects of crawfish aquaculture.

2.6 Nutrition

Crawfish are omnivores, detritivores (consumers of decomposing organic matter) and most recently as obligate carnivores because it has been found that they “require” some animal matter in their diet to optimize growth. A crawfish diet includes living and decomposing plant matter, seeds, algae, microorganisms, epiphytic organisms, and many invertebrates such as worms, snails and insects. They will also eat small fish and other smaller crawfish. Living and decomposing plants are often the most abundant food source in a crawfish pond, yet contribute very little directly to the nourishment of crawfish. These plants do supply most of the nutrients in the ecosystem of a crawfish pond. The decomposing plants and its associated microorganisms (commonly referred to as detritus) are consumed because it has high food value than living plants. The amount of detritus that can be utilized by crawfish as the mainstay of their nutrition is limited. This microbe rich detritus is the main food

source for insects, worms, snails and some small vertebrates that then consumed by the crawfish. These organisms provide high quality nutrition for crawfish.

Supplemental feeds are not often utilized in Louisiana crawfish aquaculture ponds. The primary means of providing nutrition to crawfish in aquaculture is by establishing and managing a forage crop in the crawfish ponds. Once the ponds are flooded in the fall the forage crop and detritus produced by it must provide a constant and continuous supply of nutrients for the food web on which the crawfish relies for its nutrition.

3. Crawfish aquaculture ponds

3.1 Location and layout

Ponds are located in open flat areas in soils with a sufficient amount of clay in the soil. Soils that can be shaped into a ball have enough clay to be suitable. The clay content is important to hold water during flooding and to maintain the integrity of the crawfish burrows. Water resources for periodic flooding of the ponds are also necessary. The pond must be at an elevation above the water levels of surrounding canals and ditches. On farms where crawfishing is rotated with rice crops, several factors need to be considered. Ponds must have adequate all-weather access because aquaculture of crawfish requires almost daily harvesting and pond management during the January through June harvesting period. Because crawfish farming is labor intensive, only 10 to 50 percent of a farmer's rice acreage will be selected for crawfish aquaculture. Farmers usually do not rotate the best rice producing fields with crawfish farming.

There is no standard size for a crawfish pond. A size range of 10 to 40 acres is prevalent in Louisiana. Most crawfish producers manage fewer than 150 total acres. For the purpose of trapping, long fields with few levee crossings are the most efficient. Consideration must be given to other nearby farming operations where the aerial application of pesticides may contaminate downwind crawfish ponds.

3.2 Design and construction

Whether constructing a pond for permanent crawfish production or using an existing rice field for rotation with crawfish, many considerations need to be taken into account. The perimeter levees should be 3 meters wide at the base to prevent leakage from crawfish burrowing. Levees that are 1 meter tall are adequate to maintain the minimum of 20 to 25 centimeters of water necessary for crawfish production. The fall of the land should not exceed 15 centimeters between levees. Fields with steeper grades result in water depth variations that hamper forage growth and reduce harvesting efficiency. Drains for the field should be sized based on field size, projected rainfall and irrigation pumping capacity. One 25 centimeter diameter drain is sufficient per 10 acres of pond. Interior levees within the pond should be 2 meters at the base and be 15 centimeters above the water level in the pond. These interior levees should be spaced 50 to 100 meters apart to facilitate water circulation. Wide or deep interior ditches should be avoided if possible. These ditches provide the least resistant flow of water within the pond which can lead to poor circulation in the rest of the pond. Poor circulation of water can lead to stagnation of the water and cause lower production of crawfish. Deep areas within the field will also make periodic draining the fields difficult.

Crawfish pond with a row of traps/ Courtesy of Joel Richert

4. Production systems

4.1 Monocropping system

Monoculture or “single crop” aquaculture of crawfish is used on small farms or in areas where agriculturally marginal land is available. Ponds devoted to numerous production cycles are typically used for this strategy. The size of these operations range from 300 acres with little management or input to small 15 acres sites that are highly managed. The big advantage to monocropping is that farmers manage the acreage for maximum crawfish production without concerns of other crops, such as pesticide contamination and seasonal limitations. The yields from this kind of system range from 100 kg/ac in large low input ponds to more than 600 kg/ac in small intensively managed ponds. “Permanent” ponds yields will generally increase over a period of three to four consecutive years of production.

Rice is the standard forage crop used in a monocropping system. It is planted in the summer months after the crawfish harvesting season. Emphasis is on the growth of stem and leaf production for use as forage. The rice planted is not harvested for grain at maturity, rather it is allowed to die and begin decomposition. One of the advantages of permanent ponds is earlier, intense harvesting of crawfish. The early harvesting of crawfish is economically important because early harvest is almost always rewarded by high market prices. The disadvantages to monocropping include: 1) the need to construct dedicated ponds, costs must be amortized over only one crop, and crawfish overcrowding can occur after a few years which leads to stunted, low-priced crawfish that are difficult to market. Production schedules for monocropping can vary, but generally follow the schedule on Table 4.1.

4.2 Crop rotation systems

In rotation systems, crawfish is alternated with a plant crop in order to grow two crops in one year. Rotational strategies have the advantage of efficient use of labor, farm equipment

Summary of Major Crawfish Production Strategies With Common Practices by Month.			
Months	Crawfish Monoculture	Crop Rotational Systems	
		Rice-Crawfish-Rice	Rice-Crawfish-Fallow or (Rice-Crawfish-Soybean)
Jul - Aug	Forage crop planted or natural vegetation allowed to grow	Rice crop harvested in August and stubble managed for regrowth	Rice crop harvested and stubble managed for regrowth
Sep - Oct	Pond flooded and water quality monitored and managed	Pond flooded in October and water quality monitored and managed	Pond flooded in October and water quality monitored and managed
Nov - Dec	Harvest when catch can be economically justified	Harvest when catch can be economically justified	Water quality monitored and managed
Jan - Feb	Crawfish harvested 2-4 days per week according to catch and markets	Crawfish harvested 2-4 days per week according to catch and markets	Crawfish harvested 2-4 days per week according to catch and markets
Mar - Apr	Crawfish harvested 3-5 days per week according to catch and markets	Crawfish harvested 3-5 days per week until late April, then pond drained and readied for planting	Crawfish harvested 3-5 days per week according to catch and markets
May - Jun	Crawfish harvested until catch is no longer justified; then pond drained	Rice planted in May and rice crop managed for grain production	Pond drained and soybeans planted or harvest proceeds as long as catch is feasible; pond then drained and left fallow
July - ...	Repeat cycle	Repeat cycle	Harvest soybeans in October, plant rice in March/April, stock crawfish in May, repeat cycle

Louisiana Crawfish Production Manual

Table 4.1

and land. Additionally, some fixed costs and the costs of a plant crop can be amortized over two crops rather than just one. Rotational systems can vary based on the type of land, crops already in production on a farm or past experience of the farmer.

4.3 Rice-crawfish-rice rotation

Rotation of rice and crawfish takes advantage of the seasonality of each crop which allows for the production and harvest of each crop in one year. Rice is grown and harvested in the summer months while crawfish grown during the fall, winter and spring in the same field in a twelve month period. In rice rotation systems the initial stocking takes place 4 to 7 weeks after the rice is planted. After the rice is harvested in July or August, the residual rice stubble is fertilized with a nitrogen based fertilizer in order to establish a re-growth crop of rice for forage. After a fall flooding of the pond, management practices are similar to the monocropping system. (See Table 4.1) The major disadvantage to this rotational system is that neither crop can be managed to maximum efficiency. In Louisiana rice yields are maximized when it is planted in early spring. Draining the ponds and planting rice in early spring shortens the crawfish harvest season. Delaying the planting of rice will adversely affect rice yields. Care must be taken in the use of pesticides and herbicides that are helpful to the rice crop but may be harmful to the crawfish crop. Yields for each crop can vary greatly depending upon the management emphasis. Generally the crop that is given preferred treatment has higher yields at the expense of lower yields for the less preferred crop.

4.4 Crawfish-rice-fallow (or rice-crawfish-other plant crop)

The big difference in these rotational strategies is that rice is typically not cultivated in the same field in consecutive years in order to control rice diseases and weeds. Like rice-crawfish-rice rotation crawfish culture follows rice cultivation; therefore crawfish

production does not take place in the same ponds from one year to the next. In the first mentioned rotation method, the field will be left fallow for a year following the crawfish harvest. Under the second method of rotation, if soybeans or another crop is planted after the crawfish harvest, then three crops per field can be harvested in a two year time frame. These two approaches require sufficient acreage to allow staggered crops in different fields within the farm. This rotational method is used on much of the acreage used in crawfish aquaculture in Louisiana because it has advantages over rotation within the same field. Each of the crops in this type of rotation can be better managed because each crop has the necessary time to maximize growth and harvest. Specifically for crawfish production, the crawfish ponds do not have to be drained in late spring to rice. This yields a lengthening of the crawfish harvest season into early summer because soybeans or other crops can be planted later in the year. The disadvantages of this rotation system include: the need to re-stock crawfish each year, lower population densities and the late season harvest frequently is frequently plagued by low market prices for the crawfish.

5. Stocking

5.1 General guidelines

Crawfish farming relies on reproduction of the resident adults to sufficiently populate the ponds for each harvest. Established ponds seldom have a need to be re-stocked because the crawfish that remain after the harvest serve as brood stock for the next year. Stocking is usually only necessary in: 1) new ponds, 2) after a fallow year or crop rotation other than rice, 3) after severe drought or 4) after extensive levee renovation.

5.2 Stocking considerations and procedures

Red swamp crawfish is the preferred species for stocking because of their longer reproductive season. The size of the mature broodstock is of little concern, because even though large crawfish produce a higher number of offspring, there would be fewer of them per purchased kilogram of crawfish. The time for stocking depends upon the reason for stocking. April to July is the best time to stock a new pond in a monocropping system or restock a pond that has skipped a season. When re-stocking a rice field, stocking should be done about 45 days after planting when the rice plants are large enough to withstand the crawfish without damage and when the need for harmful pesticide applications have passed. Careful handling of the broodstock from trapping to release is important. Keeping the crawfish clean, moist, and at temperatures around 20° C. This is best accomplished by avoiding direct sunlight, wind, and by covering the sacks of crawfish with wet tarpaulins or burlap sacks. When stocking, crawfish should be dumped directly in the water. Because crawfish are mobile, there is no need to equally spread them over the entire pond. The crawfish should however be stocked in each section of a large pond. The temperature of the pond being stocked should not differ greatly from the temperature of the crawfish themselves at the time of stocking. Stocking rates of 25 to 30 kg/acre is generally recommended but many farmers stock up to 40 kg/acre if post-stocking survival is unpredictable. The female population should exceed 50 percent because a male crawfish can mate with more than one female. Survival rate of the broodstock is extremely important to the stocking process. Water temperature, dissolved oxygen levels and water levels in the stocked pond must be maintained.

6. Forage management

6.1 Forages

Establishment of a forage-based production system is important in order to produce the complex ecological community necessary to provide a high quality food source for the crawfish. The food sources rely on a continuous influx of plant matter that is in turn consumed by bacteria and other microorganisms. The detritus produced by these decomposers is the fuel for the food web on which the crawfish rely. The forage crop must be able to produce adequate portions of plant material on a consistent basis throughout the growth and harvest seasons. Overproduction of plant material at one time is wasted because it cannot be stockpiled for later use. Decomposition of large amounts of material in a short span of time can also lead to oxygen depletion in the water. Too little plant material can lead to insufficient detritus to support the food web. Selected agronomic crops are the most effective forage resources for crawfish. These crops are most effective because of their ability to flourish in the flooded pond environment and their predictable plant material yield.

6.2 Types of forage

Rice is by far the most often used forage plant in Louisiana. Rice is semi-aquatic and grows well in flooded crawfish ponds. Many varieties of rice have been used as forage. In 2004, Louisiana State University released the first rice specifically developed for use in crawfish monocropping. "Ecrevisse" rice exhibits much greater forage biomass production, greater growth under the extended flood conditions of a crawfish pond and has an improved ability for post-winter re-growth than the commonly used domestic rice varieties. A sorgam-sudangrass hybrid that is commonly used by cattlemen for grassing and hay, is a well suited alternative forage crop for crawfish monocropping systems.

7. Water quality and management

7.1 Overview

Water quality is influenced by both environmental and biological factors. Some environmental factors such as rainfall and temperature are beyond control. Factors such as what type of vegetation planted, when planting occurs and how the vegetation is managed are within the controls of the producer and can affect water quality. Additionally, maintaining optimum water levels and the timing of flooding ponds can have a positive effect on water quality.

7.2 Quantity and supply

Surface and subsurface water are both used for flooding in crawfish aquaculture. Surface water that is pollution-free and free of predatory fish is cheaper than subsurface water but is usually not reliable as to quality or quantity. Wells provide predator-free water on demand but require a large investment and reoccurring pumping costs. Pumps are usually powered by diesel engines. Subsurface water has no oxygen and must be aerated before entering the pond. Subsurface water must also be monitored for high iron content and hydrogen sulfide. A pumping capacity of 300 to 400 liters per minute per surface acre is ideal. This pumping rate is sufficient to exchange all the water in a pond in 4 to 5 days. Flushing a pond

completely is important in the early fall when water is flooded on to the vegetation. During the spring, warm weather causes rapid plant decay which causes a high demand for dissolved oxygen in the water. Low oxygen levels in the water cause high mortality rates and stress which reduces growth. The ability of the farmer to flush the ponds of oxygen deficient water in a timely manner is important. Few crawfish farmers have the capacity to supply water in the ideal quantities, therefore following an intense management plan can make up for lack of pumping capacity. Filling ponds to one-half normal depth will enable a pump with a smaller pumping capacity to flush the pumps quickly enough when necessary. However, if less than optimal water depth is utilized, more intensive monitoring of water quality is necessary because smaller volumes of water can change characteristics quicker than larger volumes of water.

7.3 Quality of water

Water quality variables include: 1) total hardness, 2) total alkalinity, 3) pH, salinity and other dissolved materials, and 4) dissolved oxygen. Dissolved oxygen is by far the most important because low oxygen is responsible for more crawfish mortality than any other factor. Temperature of the water is a factor because warm water cannot hold as much oxygen as cooler water. In water that increases from 21° to 26° C the rate of oxygen use due to decomposition doubles. The source water after aeration should have a pH in the 6.5 to 8.5 range and total hardness and alkalinity should range from 50ppm to 250 ppm in calcium carbonate. Most water and soil in Louisiana that is used for crawfish production meets these parameters. Crawfish are fairly tolerant to salt water, however areas along the gulf coast that are subject to salt water intrusion should not be used as crawfish farms. Young hatchlings will die at 15 ppt salinity and juveniles die at 30 ppt salinity after a week of exposure.

8. Harvesting

8.1 Overview

The harvesting of crawfish utilizes baited traps that are periodically emptied. Trapping may begin as early as November and continues through the harvest season which usually ends by late June. In Louisiana, two-thirds of the crop is harvested from March through early June. In 2008, the most recent year in which accurate numbers are available, approximately 58 million kg of crawfish were harvested from aquaculture operations in Louisiana. The value of 2008 crawfish farm harvest was 115 million US dollars (Isaacs and Lavergne, 2010). Trapping is responsible for over half of the production expenses. The cost of bait and labor are the major harvesting costs. Crawfish yield within a pond can vary greatly from day-to-day and is governed by many factors. Water temperature, crawfish density, mass molting and weather are some of the variables that cause fluctuations in harvest.

8.2 Traps and baits

The “pyramid trap” is currently the industry standard in Louisiana. (Figure 8.1) Traps are constructed of 3/4-inch or 7/8-inch mesh wire formed into a three-sided pyramid. A 15 cm plastic collar on the opening at the top acts as handle. Traps are 60 cm wide and from 1.1 m to 1.4 m tall with one-way openings in the three bottom corners of the trap. Crawfish can

easy enter the baited trap but cannot exit through the openings. The traps are secured in place on the bottom of the pond by rod that runs through the trap into the mud. The bait used in the trap is often the single highest expense in crawfish production. Bait cost depends upon the type, amount used, number of traps and trapping frequency. Various types of small fish, butchered larger fish or formulated baits are the most common baits used. Early in the season the fish baits are most commonly used because the strong scent of the fish is better at attracting crawfish in the cooler waters of that time of year. As the water warms, less expensive formulated baits are used. The formulated baits are densely packed cylinders (approximately 3 cm in diameter and 7 cm long) of cereal grains, grain by-products, favoring and a binder.

Louisiana Crawfish Production Manual / Courtesy of Joel Richert

8.3 Trapping machinery and strategies

The most common method of collecting the harvest from the traps is by use of a specially designed paddle boat driven by a gasoline engine. The aluminum boats have flat bottoms and are typically 4.5 to 6 meters long and 1.5 to 2 meters wide. The 12 to 24 horsepower air-cooled engine drives a hydraulic pump to propel a metals cleated wheel attached to the boat. The boat is equipped with a sacking table on which the contents of each trap are dumped. Two or more sacks (capacity approximately 18 kg. each) temporarily attached to the table hold the crawfish as they are harvested. Many tables are designed to cull smaller crawfish and to remove bait and other debris from the catch. After dumping a trap, the operator re-baits the trap and returns it to the pond. The boat is in continuous motion and does not stop unless a problem occurs. A single operator can service about 150 to 200 traps per hour. Traps are placed in rows with 15 to 20 meters between rows and individual traps. The traps are emptied and baited usually three to four days per week. If prices and demand are high, traps may be run more often.

Courtesy of Joel Richert

9. Marketing

9.1 Live crawfish

Crawfish are sold live by farmers to wholesalers and live crawfish comprise most of the retail sales to consumers. The most common method of preparing crawfish for consumption, whether in households or in restaurants, is by boiling in well seasoned water. In times when supply exceeds demand, producers of large crawfish receive a better price for their crop. In times of over-supply, large crawfish are sold for immediate consumption and smaller crawfish are processed for later consumption.

9.2 Processed crawfish

A portion of the annual crop is processed and sold as fresh or frozen abdominal (tail) meat. Processing involves cooking, peeling and deveining the tail meat. The amount of tail meat obtained from a crawfish is approximately 15 percent of the live weight of the whole crawfish. The prepared crawfish meat is then sold in 1 pound (.45 kg) plastic bags. The processed crawfish meat is used to make jambalaya (a seasoned rice dish), etouffe (a stew-like preparation served over rice), or any other number of “cajun” dishes for which Louisiana is famous.

10. Environmental

10.1 Crawfish use as a bio-indicator

This section, as well as, sections 10.2 and 10.3 was taken from a literature review written by the authors of this chapter entitled *Determination of Inorganics and Organics in Crawfish*

(Richert and Sneddon, 2008). Numerous environmental studies have been done using crawfish as a bio-indicator to monitor pollution. Crawfish have been useful as vectors to monitor contaminants in water and soil because they are prolific, relatively sedentary, easily recognizable and have a reasonably long life span. Additionally, they are in constant physical contact with the water and surrounding soil. Their position in the food web is high enough that some biomagnification can occur from eating contaminated organisms from lower positions in the food web. Three recent studies have shown that crawfish can be useful in monitoring the levels of a variety of metals. Schmitt looked at the potential ecological and human risks associated with metals in fish and crawfish from activities in the Tri-States Mining District (TSMD) in Northeast Oklahoma (Schmitt et al, 2006). Crawfish and six species of frequently consumed fish were collected in 2001-2002 from the Spring River and Neosho River which drain the TSMD. Whole crawfish were analyzed in composite samples. Metals concentrations were found to be higher in the samples from the sites most heavily affected by the mining and were lower in the reference samples tested. The levels of Pb, Cd, and Zn exceeded current acceptable levels. Human consumption of crawfish from the area was restricted based on the results of this and previous studies. Monitoring contamination caused by a release of pollutants in 1998 in the Aznalcollar-Los Frailes, Spain mining region was the subject of a study (Sanchez Lopez et al, 2004). Immediately following the spill there was much destruction to the aquatic ecosystem of the Guadiamar River. Inductively coupled plasma-mass spectroscopy (ICP-MS) was used to measure levels of Cu, Zn, Pb and Cd. American red crawfish (*Procambarus clarkii*) were collected from various sites within the affected area. The crawfish collected in the areas near the toxic release showed much higher levels of the tested metals than did the crawfish collected from areas not directly affected by the spill. Additionally, a translocation experiment using red swamp crawfish (*Procambarus clarkii*) at different sites along the Guadiamar River was carried out in order to determine the ability of this species as a bio-indicator of heavy metal (Cd, Cu, Zn, Pb, and As) contamination (Alcorlo et al, 2006). Caged uncontaminated crawfish were placed at three different sites which had different levels of contamination. The crawfish were then harvested after six days and again after twelve days. Analysis by ICP-MS showed that in as little as six days the crawfish were already accumulating the metals. The study also showed that the metals not involved in crawfish metabolism tended to increase with higher exposure and longer exposures.

10.2 Inorganics or metals

Inorganics or metals are most frequently determined in crawfish using spectrochemical techniques such as flame and graphite furnace atomic absorption spectrometry (FAAS, GFAAS), inductively coupled plasma-optical emission spectrometry (ICP-OES), and most recently inductively coupled plasma mass spectrometry (ICP-MS). It is beyond the scope of this chapter to describe these widely used and accepted techniques and the reader is referred to several texts that describe principles, instrumentation, and use of these techniques. However, in most cases these spectrochemical techniques perform best when the sample is in solution form, preferably in an aqueous or slightly acidic form. Typically microwave digestion is using nitric acid is used to put the analyte in solution. The toxic metals such as cadmium, mercury, and lead have been the most widely determined in crawfish.

10.3 Reviewed studies

Following a gold mine disaster in Romania, heavy metal content (Pb, Cd, Cr, Ni, Hg, As) was determined in silver carp, crawfish, sediment, and water (Francuski et al, 2000). Samples were collected from the Tisa River on February 28, 2000, upstream from the dam near Novi Becej in Rumania. Results showed heavy metals in all samples were significantly increased especially the Cd concentration in crawfish, which was three times higher than the maximal allowed concentration.

A study was performed to investigate direct and direct plus trophic contamination routes of crawfish (*Astacus astacus*) by inorganic Hg(II) or methylmercury (MeHg) (Simon and Boudou, 2001). Direct exposure was based on low contamination conditions, 300 and 30 ng/L in the dissolved phase, respectively, during 30 days at 20°C. Trophic exposure was based on daily consumption of the Asiatic clam (*Corbicula fluminea*), previously contaminated during 40 days with similar exposure conditions. The Hg concentrations in the bivalves were very similar: 1,451±287 ng/g for Hg(II) and 1,346±143 ng/g for MeHg. In the crustaceans, Hg bioaccumulation was determined at the whole-organism level and in eight organs (gills, stomach, intestine, hepatopancreas, tail muscle, green gland, carapace, and hemolymph), after 15 and 30 days of exposure. Analysis of the results showed marked differences between Hg(II) and MeHg accumulation in favor of MeHg: for the direct route, the ratio between metal concentrations was close to 8; for the trophic route, no significant increase in Hg accumulation

was observed for Hg(II) even when the ratio between Hg concentration in the direct plus trophic contamination route and Hg concentration in the direct contamination route was 1.6 for MeHg, with an estimated trophic transfer rate close to 20%. Mercury organotropism was also specifically connected to the exposure conditions, especially at the biological barrier level according to the route of exposure: gills and carapace for the direct route and digestive tract including hepatopancreas for the trophic route.

Another study evaluated potential human and ecological risks associated with metals in fish and crawfish from mining in the Tri-States Mining District (TSMD) in northeast Oklahoma (Schmitt et al, 2006). Crawfish (*Orconectes spp.*) and fish of six frequently consumed species (common carp (*Cyprinus carpio*); channel catfish (*Ictalurus punctatus*); flathead catfish (*Pylodictis olivaris*); largemouth bass (*Micropterus salmoides*); spotted bass (*M. punctulatus*); and white crappie (*Pomoxis annularis*) were collected in 2001–2002 from the Oklahoma waters of the Spring River (SR) and Neosho River (NR), which drain the TSMD. Samples from a mining-contaminated site in eastern Missouri and from reference sites were also analyzed. Individual fish were prepared for human consumption in the manner used locally by Native Americans (headed, eviscerated, and scaled) and analyzed for Pb, Cd, and Zn. Whole crawfish were analyzed as composite samples. Metals concentrations were typically higher in samples from sites most heavily affected by mining and lowest in reference samples. Within the TSMD, most metal concentrations were higher at sites on the SR than on the NR and were typically highest in common carp and crawfish. Higher concentrations and greater risk were associated with fish and crawfish from heavily contaminated SR tributaries than the SR or NR mainstreams. Based on the results of this and previous studies, the human consumption of carp and crawfish could be restricted based on current criteria for Pb, Cd, and Zn. Overall, the wildlife assessment is consistent with previously reported biological effects attributed to metals from the TSMD. The results demonstrated the

potential for adverse effects in fish, wildlife, and humans and indicate that further investigation of human health and ecological risks to include additional exposure pathways and endpoints is warranted.

A translocation of red swamp crawfish to different sites in the River Guadiamar in southwest Spain was performed to assess the ability of the species as a bio-indicator of heavy metal and metalloid pollution (Alcorlo et al, 2006). Crawfish were caged and exposed to a polluted environment for 6–12 days at three sites with different pollutant concentrations. Tissue (exoskeleton + gills, hepatopancreas, abdominal muscle) were dissected and analyzed by inductively coupled plasma-mass spectrometry (ICP-MS) to assess Cd, Cu, Zn, Pb, and As concentrations. Both exposure times resulted in significant bio-accumulation of some metals in crawfish tissue versus their environmental concentration. According to overall metal concentration, crawfish tissue ranked as follows: hepatopancreas/viscera >exoskeleton/ gills >abdominal muscle. Essential metals for crawfish metabolism (Cu and Zn) always occur in high concentrations independent of their environmental concentration due to the ability of crawfish to manipulate concentrations for their metabolic profit. Metals not involved in crawfish metabolism (Cd, Pb, As) tended to increase with increasing environmental concentrations and with longer exposure times. Thus, crawfish could be used as bio-indicator of these pollutants because their dose and time-dependent accumulation may be reflective of concentrations of nonessential metals in polluted wetlands. Future guidelines for plans to monitor pollution in Mediterranean rivers and wetlands should account for implementing crawfish incubation for 6 days and their subsequent metal content analyses as a routine.

In Spain a study completed using crawfish (*Procambarus clarkii*, males and females) that were exposed simultaneously to Cd and Zn during 21 days (Martin-Diaz et al, 2006). Exposure concentrations were those determined at the Guadiamar River after the Aznalcollar mining spill (SW, Spain): 10 and 30 mg/L of Cd and 1000 and 3000 mg/L of Zn. Three biomarkers (MT: metallothioneins like proteins, VTG: vitellogenin/vitellin like proteins and histopathology) together with heavy metal bio-accumulation were determined in soft tissues of male and female crawfish. At the concentrations tested, increasing Cd exposure resulted in increasing Cd bio-accumulation and increasing sublethal effects (induction of MT, VTG, and histopathology damage in tissues). Nevertheless, although increasing Zn exposure showed increasing VTG induction and histopathological damages, a positive relationship was not determined with MT induction. The only differences found between sexes were at the highest Cd exposure concentration related to bio-accumulation in hepatopancreas tissues. Biomarkers responses to heavy metal contamination in this crawfish, even VTG induction not before tested in heavy metal contamination assessment in crustaceans, resulted in potential tools for the monitoring of heavy metal environmental contamination.

Biomagnification of some essential metals (Fe, Zn, Cu) and toxic metals (Pb, Ni, Cd, Cr, Co, Mn) was determined in sediment, three types of fish (*Oreochromis niloticus*, *Synodonthis*, *Clarias gariepinus*), and crawfish from the Ondo State coastal region, Nigeria (Asaolu and Olaofe, 2005). Metal biomagnifications in fish and crawfish was many times greater than in water; in sediment it was several thousand fold greater than in organisms and water. Among metals in water, Fe was the most abundant, with average concentrations of 146.7 and 74.3 mg/L in wet and dry seasons, respectively; Co was least abundant at concentrations of 2.4 and 1.6 mg/L. The Fe concentration was found to have an average

concentration of 50.9 mg/kg in *C. gariepinus* and was the most abundant metal in fish; Cu, with an average concentration of 0.3 mg/kg in *O. niloticus*, was the least abundant metal. Biomagnification of most metals in both seasons varied widely from site to site. This was confirmed by a coefficient of variation from 31 to 144% and 29 to 130% in wet and dry seasons, respectively. Results showed that fish, crawfish, and sediment can be used to monitor metal pollution in Nigerian coastal seawater.

Water quality assessment in the Aznalcollar area of Spain was attempted using multivariate methods based on heavy metal concentrations in red swamp crawfish (*Procambarus clarkii*) (Sanchez Lopez et al, 2004). Trace levels of four heavy metals, Cu, Zn, Cd, and Pb, were detected in crawfish from 11 different stations. Principal component analysis (PCA) highlighted a gradient of contamination between the sampling stations. Cluster analysis (CA) distinguished three groups of stations. Discriminant analysis also differentiated three groups. The group centroids of the first discriminant function were used to devise an index that varies according to the source of the crawfish. These standardized values are proposed for use as a water quality index. The ability of this index to successfully predict environmental quality was proved with random samples.

Contamination of the American red crawfish from the Guadiamar riverside is due to the disastrous toxic spill that occurred on April 25, 1998, in the mining area of Aznalcollar-Los Frailes, Spain (Sanchez Lopez et al 2003). A high concentration of heavy metals in the waters from the mine pool and their spill to the River Guadiamar was the cause of the destruction of a great number of animal and vegetable organisms. An inductively coupled plasma-mass spectrometry (ICP-MS) method for the total determination of heavy metals (Cu, Zn, Pb, and Cd) in whole bodies of American red crawfish (*Procambarus clarkii*) was used. Metals were extracted from the matrix in a closed-vessel microwave digestion system with nitric acid and hydrogen peroxide. A study of the uncertainty of the method for the determination of metals was carried out; at a concentration of 5 mg/L, the uncertainty was below 34%.

10.4 Research from McNeese State University in Lake Charles, Louisiana

Due to a national emergency in the early 1940's, Southwest Louisiana and Lake Charles was chosen as a place for petrochemical refining and associated petrochemical industry. Currently there are about fifty different petrochemical companies in this area. Due to the lack of or enforced environmental laws and accidental spillage, the area was polluted, particularly with selected metals. While this has significantly improved since the 1970's, there is still a legacy of contamination. Lake Charles is situated around 50 miles from the Gulf and can only be reached via a canal. This has to be continuously dredged which disturbs dormant or at least less accessible metal pollutant. Further complicating this area was a direct hit from Hurricane Rita in late September 2005 and to a lesser extent Hurricanes Gustav and Ike in September 2008.

Initial work in this laboratory at McNeese State University in Lake Charles, Louisiana was started back in 1997 when Dr. Joseph Sneddon was asked by a colleague, Dr. Mary G. Heagler from the Environmental Sciences Department to assist with the determination of lead using flame atomic absorption spectrometry with crawfish digestion via classical acid reflux in the meat of locally caught crawfish (Briggs-Reed and Heagler, 1998). The authors' data supported the hypothesis that the lead accumulated in the meat or digestive tract as an

indicator that lead existed in the sediments of the soil in which the crawfish lived and ate. The concentration found ranged from 0.25 to 0.40 micrograms of lead per gram of crawfish. They recommended purging of the crawfish prior to human consumption to reduce the possibility of lead consumption.

Approximately ten years later this laboratory embarked on several major projects in the determination and interpretation of metals in crawfish. Initial work involved an undergraduate chemistry major project to determine copper, iron and zinc in the crawfish from Southwest Louisiana (Hagen and Sneddon, 2009). The results showed no significant differences in these metal concentrations between male and female crawfish, and no significant differences between crawfish from a pristine area and near a major highway. The concentrations of iron in the meat compared to the whole crawfish was about four times higher and was assumed to be due to the shell of the crawfish containing high levels of iron.

In early 2007 and through 2010, two major field trials were initiated (Richert and Sneddon, 2008b and Moss et al., 2010). Six metals (cadmium, copper, nickel, lead, iron and zinc) were determined by inductively coupled plasma-optical emission spectrometry (ICP-OES) in both tail (meat) and whole body of the crawfish and also in the soil in a four-month season (February through May) 2007 in Southwest Louisiana (Richert and Sneddon, 2008b). Cadmium or lead were not found in the meat or the whole body. Nickel was found in some samples of the meat but mostly in the April and May samples. Copper, iron and zinc were found in both tail and whole body of the crawfish. Limited soil sampling showed no cadmium, nickel, or copper but levels or concentrations of iron, lead and zinc were found in the soils. Moss et al. (Moss et al., 2010) provided a mini-review of crawfish aquaculture and determined the following concentrations (microgram of metal per gram of dried sample of metal), 95 % confidence interval and range from February through May 2009 as follows: cadmium, 0.49 ± 0.14 , 0.34-0.79, copper, 34.9 ± 5.3 , 23.8-44.2, nickel, 1.83 ± 0.54 , 1.08-3.39, lead, 18.0 ± 4.0 , 9.9-23, zinc, 47.4 ± 4.63 , 41.3-55.8 were relatively constant with a slight increase in iron, 620.4 ± 205.8 , 328.8-1072.8 over the four months. The temperature of the crawfish ponds were monitored weekly but had no noticeable effect on the metal concentrations. Also sampled were the soil the crawfish lived in with copper and zinc concentrations decreasing with increasing water temperature and noticeable effect with the other four metals. A comparison to the study by Richert and Sneddon (Richert and Sneddon, 2008b) showed no significant differences in the six metal concentrations from one season to the next.

Two projects were undertaken in the laboratory for controlled studies for the uptake of copper, lead and zinc in crawfish (Neelam et al., 2010) and selenium-lead interactions (White et al., 2012). Ten gallon aquarium tanks were spiked with up to 100 part per million (ppm) of the three metals and the crawfish introduced into the tanks. Not surprisingly all three metals were absorbed by the crawfish. Copper showed the highest absorption by the crawfish. Lead showed a constant increase in absorption with increased spiked of lead. It was noted that there was no obvious correlation between the metal absorbed and the amount of metal in the aquarium. It was noted that higher amount of lead were absorbed compared to copper and zinc. White et al. (White et al., 2012) determined whether the relationship between selenium and lead is one of an antagonistic or synergistic nature. Experiments were conducted on the freshwater crustacean crawfish (*Procambarus clarkii*). Crawfish were exposed to a known concentration of lead, dissected then analyzed to

determine the accumulation of lead as follows: gills>exoskeleton>organs>edible meat. Duplicates of the lead exposed crawfish were exposed to a concentration of approximately 10ppm (mg/L) selenium for a week to determine any adverse physiological effects. Within 48 hrs of exposure of selenium, the control crawfish were experiencing lethargy and signs of paralysis. The same symptoms began to occur to the previously lead exposed crawfish within 72 hours of selenium exposure. Analysis of the selenium exposed crawfish revealed time dependent and tissue specific adsorption of selenium identical to the concentrations of the lead exposed crawfish: gills>exoskeleton>organs>edible meat.

After seven days of living in a complete state of paralysis, duplicates were placed in pure water to determine the ability of the crawfish to purge the selenium and regain mobility. Within 24 hours of purging, 88 percent of the paralyzed crawfish had regained full motor skills. Analysis of the purged crawfish showed a significant decrease in the concentration of selenium in the chitin rich exoskeleton and gills. However, the lead concentration in the gills and exoskeleton of the purged versus non-purged crawfish did not show any significant decrease indicating the covalent bond between the nitrogen and lead is much stronger than the chelating ionic bond between the selenium and lead.

Currently in progress is the use of crawfish shells for the uptake and removal of metal ions in aqueous solution (Vootla et al, 2011, and Beeram et al, 2011). Using ICP-OES, the samples were analyzed for various concentration of lead at three different volumes, 40-mL, 500-mL, and 3000-mL. Crawfish shells, with the meat removed, were dried and pulverized to a 150-mesh size. The results showed that with an increase in volume of water, the capacity to remove lead by the crawfish exoskeleton by the same amount of shell powder decreased. However, lead absorption by the same amount of shell powder in all phases was good in terms of efficiency. The phase III study (3000-mL) showed that 0.5-g of crawfish absorbed the maximum amount of lead. Moreover, both raw and boiled crawfish shells have the same or similar capacity to uptake lead from water (Vootla et al., 2011). Crawfish shell is a good source of chitin constituting about 23.5 percent on a dry basis. Using this value, the number of moles of chitin were calculated theoretically and compared to the number of moles of Pb that it can uptake. For 0.5 g of crawfish shell powder taken, the amount of Pb uptake per gram of ground crawfish powder is calculated as shown in equation (1) and the values shown in equation (2) and equation (3).

$$\text{weight of crawfish} \times \frac{0.235 \text{ g of chitin}}{1 \text{ g crawfish}} \times \frac{1 \text{ mole chitin}}{\text{molar mass chitin monomer}} \times \frac{\text{molar mass of Pb}}{1 \text{ mole Pb}} \times \frac{1 \text{ atom of Pb}}{1 \text{ monomer of chitin}} \quad (1)$$

$$0.5 \text{ g} \times \frac{0.235 \text{ g}}{1 \text{ g}} \times \frac{1 \text{ mole}}{203 \text{ g}} \times \frac{207 \text{ g Pb}}{1 \text{ mole}} \times \frac{1 \text{ atom Pb}}{1 \text{ monomer of chitin}} = 0.1198 \text{ g Pb} \quad (2)$$

$$\frac{0.1198 \text{ g Pb}}{0.5 \text{ g of crawfish shell}} = 0.240 \text{ g of Pb per 1g of crawfish shell} = 240,000 \text{ ppm Pb} \quad (3)$$

These calculations were obtained by assuming this model as 100 % chitin efficient crawfish shell. Based on 100% absorbance, one monomer uptaking one atom of Pb, the approximate value obtained was 240,000 ppm. The experimental value (amount of Pb that has up taken) obtained by analyzing the powder in this phase is approximately 200,000 ppm which is 83%.

Lack of 100% efficiency may be because some of the chitin molecules are inside the chunk of powder and not available for binding to the metals.

Work by Beeram et al., (Beeram et al., 2011) is still very much in progress as of early August 2011. This work concentrates on the use of the whole crawfish and shells (as opposed to ground crawfish shell by Vootla et al., (Vootla et al., 2011)) for the removal of various selected metals. Results are very preliminary but suggest surface area (ground versus whole) do play a part in the uptake rate.

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Aquaculture and the Environment - A Shared Destiny

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Aquaculture is the art, science and business of cultivating aquatic animals and plants in fresh or marine waters. It is the extension of fishing, resulted from the fact that harvests of wild sources of fish and other aquatic species cannot keep up with the increased demand of a growing human population. Expansion of aquaculture can result with less care for the environment. The first pre-requisite to sustainable aquaculture is clean water, but bad management of aquatic species production can alter or even destroy existing wild habitat, increase local pollution levels or negatively impact local species. Aquatic managers are aware of this and together with scientists are looking for modern and more effective solutions to many issues regarding fish farming. This book presents recent research results on the interaction between aquaculture and environment, and includes several case studies all over the world with the aim of improving and performing sustainable aquaculture.

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