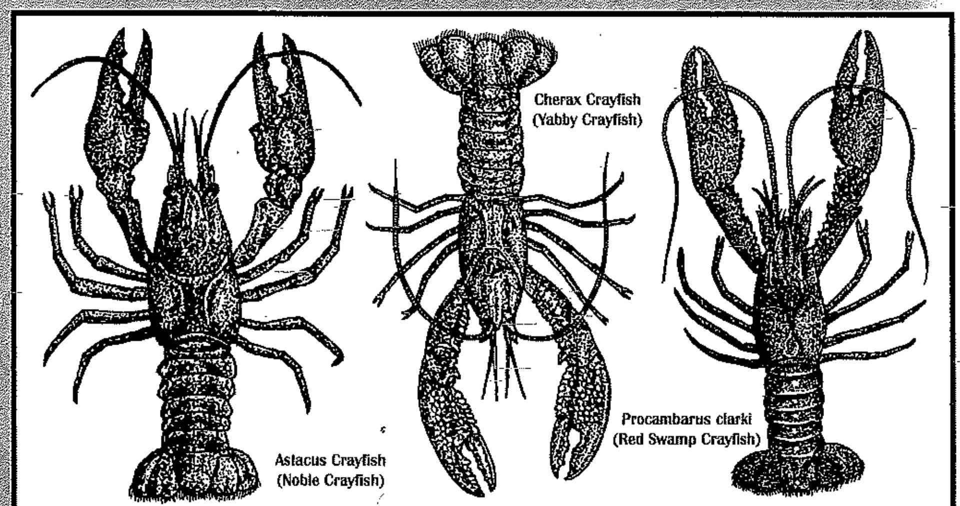
FRESHWATER CRAYFISH AQUACULTURE

in North America, Europe, and Australia

Families Astacidae, Cambaridae and Parastacidae



Jay V. Huner, PhD • Editor

Section III: Diseases of Louisiana Crayfish

INTRODUCTION

Diseases of crayfish encompass a range of problems with different levels of significance. Scientific literature addresses primarily metazoan parasites, only a couple of which may have an impact on commercial crayfish production. Crayfish culture as currently practiced in the United States is relatively free of significant disease problems. Only recently, with the development of high density systems for purging and for soft-shell production, have conditions similar to those experienced in more intensive aquaculture systems been encountered. This intensification has led to the emergence of some significant disease problems. Additionally, there are some agents that do not limit production, but may affect marketing due to their effects on the appearance of the product.

BACTERIA

Potential bacterial pathogens of crayfish include species of the genera *Aeromonas*, *Bacillus*, *Corynebacterium*, *Mycobacterium*, *Flavobacterium*, *Pseudomonas*, *Vibrio*, *Edwardsiella*, and *Acinetobacter*. Members of these genera are common inhabitants of the stagnant water and decomposing vegetation found in crayfish production ponds and have been isolated from the crayfish exoskeleton, hindgut, and hemolymph. They are also associated with opportunistic bacterial infections in other aquatic species and have been implicated in external and internal infections in cultured crustaceans.

The chitinous cuticle and lipid containing epicuticle provide crayfish with an effective barrier against the invasion of most microorganisms. However, chitinoclastic bacteria can cause a syndrome in crayfish and other crustaceans known as shell disease, which is characterized by black or dark brown lesions on the exo-skeleton (Figure 41). These lesions are due to melanization of a wound or damaged area of the exoskeleton associated with various bacteria, primarily of the genera *Aeromonas*, *Pseudomonas*, *Acinetobacter*, and *Vibrio*. The incidence and severity of this syndrome can increase under conditions where molting is infrequent (Figure 42), as in holding facilities or in nutritionally poor environments. Intensive systems also favor the development of shell disease, probably due to the infection of wounds caused by aggressive behavior, increased ease of pathogen transfer due to increased density, and poor water quality.

Systemic bacterial infections are generally considered to be rare in crayfish in commercial ponds. However, evidence indicates that significant levels of bacteria can occur in the hemolymph of pond raised crayfish (Scott and Thune 1986a) and that these increases are

Figure 41. Dark lesions on the lateral surface of a crayfish with bacterial shell disease. R. Thune.

Figure 42. Severe case of bacterial shell disease demonstrating a lesion that has penetrated the exoskeleton to the gill chamber. R. Thune.

dependent on high temperature and low dissolved oxygen levels. Stress associated with extremes of either of these factors seems to affect the ability of crayfish to control bacterial populations in the hemolymph. These bacteremias are atypical of those encountered in other cultured aquatic species in that clinical signs of disease are not apparent and that multiple bacterial genera are present in an individual, rather than one or two obligate or opportunistic pathogens.

A recent report (Thune et al. 1991) documents the first cases of bacterial septicemia in crayfish. *Vibrio mimicus* and *V. cholera* were the predominant organisms isolated in each of 15 cases examined. Of the 15 cases, 1 occurred in a holding tank, 4 in ponds, 5 in purging systems and 6 in soft-shell operations (Table 18). Epizootics occurred near the end of the pond harvest season in late May and early June, during periods of elevated water temperatures and/or depressed levels of dissolved oxygen. Diseased crayfish were lethargic and were easily identified by their failure to posture when

Table 18. Summary of conditions for vibriosis cases in crayfish in Louisiana for 1985-1990.

Case	Organism	Temp. (C)	System
85-63	*V. cholerae*	26	Purging
85-69	*V. mimicus*	26	Purging
86-56	*V. mimicus/V. cholerae*	23	Pond
86-77	*V. mimicus*	23	Purging
86-110a	*V. mimicus*	30	Purging
86-111	*V. mimicus*	30	Purging
86-120	*V. mimicus*	30	Pond
87-123	*V. mimicus*	30	Soft-Shell System
87-136	*V. mimicus*	26	Pond
87-240	*V. mimicus/V. cholera*	26	Soft-Shell System
88-13	*V. mimicus*	26	Soft-Shell System
89-255	*V. mimicus*	28	Ponds
89-323	*V. mimicus*	28	Holding tank
90-297	*V. mimicus*	25	Soft-Shell System
90-327	*V. mimicus*	25	Soft-Shell System

From Thune et al. (1991)

threatened, yet they exhibited no gross clinical signs. Daily mortality rates range from 5 to 25%. Physiological, biochemical, and serological profiles for *V. cholera* and *V. mimicus* isolates indicated each crayfish isolate was virtually identical in biochemical profile with the exception of sucrose fermentation, which differentiates these two vibrio species (Davis et al. 1981). Serological evaluation indicated that each isolate expressed a single 0 antigen, serovar J, using the system and nomenclature of Adams and Siebeling (1984). Serovar J corresponds to Sakazaki serovar 6 (Sakazaki and Shimada 1977) and Smith serotype 14 (Smith 1979).

The predominant *Vibrio* species known to produce septicemias in

other crustaceans are *V. parahemolyticus*, *V. alginolyticus*, and *V. anguillarum*. Since crustaceans previously reported to be infected by *Vibrio* sp. are marine species, it is not surprising that halophilic *Vibrio* species have been the predominant species reported. According to Baumann et al. (1980) optimal NaCl concentrations for *V. anguillarum* are 60-100 mM, 160-180 mM for *V. parahemolyticus* and 200-260 mM for *V. alginolyticus*. *Vibrio cholera* requires an optimal range of only 5-15 mM NaCl, which may account for its association with the freshwater crayfish.

The typability of *V. mimicus* strains with *V. cholera* antisera is not unusual (Davis et al. 1981); however, the isolation of a single *V. cholera* non-01 serotype from affected crayfish from several parishes across south Louisiana over a period of several years is surprising because non-01 vibrios are serologically diverse (Smith and Goodner 1965; Sakazaki and Shimada 1977; Smith 1979; Adams and Siebeling 1984). That a single serotype was responsible for all reported cases indicates a common functional characteristic associated with the bacterial cell wall that favors the establishment of infections in crayfish. Additionally, the serovar in question may have public health significance. All 46 isolates of serovar 6 evaluated by Sakazaki and Shimada (1977) were of human origin, although the clinical status of the patients was not disclosed. Twenty-six of 107 isolates of serovar 14 evaluated by Smith (1979) were associated with gastrointestinal disease and 2 others with non-gastrointestinal illness. However, no documented case of human vibriosis has been correlated to crayfish consumption.

Although the overall impact of both the clinically inapparent bacteremias and vibriosis on the crayfish industry is unknown, high levels of bacteria associated with water temperatures greater than 27°C, low dissolved oxygen levels, transport from ponds to intensive systems, or a combination of these factors may effect crayfish production. It is possible that ponds drained late in the season, when the water temperatures are warm, will have reduced survival of crayfish in the burrows. This would lead to a loss of "brood" crayfish, potentially reduce recruitment of young the following year, and also reduce the crop of holdover crayfish the following fall. Additionally, increased bacterial levels may affect survival and quality of crayfish during transportation, storage, and handling after harvest, especially when they are placed in intensive systems.

FUNGI

Crayfish plaque fungus, caused by the fungi *Aphanomyces astaci*, is notorious for the elimination of native crayfish populations in many European waters and has been extensively studied in European crayfishes of the genera *Astacus* and *Austropotamobius*. The fungus attacks almost entirely in the soft cuticle in joints or between segments and can kill a susceptible crayfish in a week or two. North American crayfish appear to be resistant to severe pathological affects of *A. astaci*, although limited, chronic infections can be established (Unestam 1969b). Recently, the relationship between the North American crayfish and the crayfish plaque fungus has come into question due to the increased exportation of live crayfish to Europe. Both *Pacifastacus leniusculus* and *Orconectes limosus* are considered to be vectors of *A. astaci* in Europe (Alderman and Polglase 1988; Vey et al. 1983). Furthermore, *Procambarus clarkii* from Spain have been shown to be potential vectors in Swedish laboratory studies (Uribeondo and Söderhäll 1992).

A related species, *Aphanomyces laevis*, has been described as primarily a wound parasite for red swamp crayfish, but can cause mortality in crowded, unhealthful conditions (Smith 1940). It differs from *A. astaci* in having narrower hyphae, smaller oogonia, and a thin smooth oogonial wall rather than a thickened rough one. *A. laevis* is generally considered to be more saprophytic than parasitic (Coker 1923) and could not reliably induce disease in inoculated crayfish (Smith 1940; Unestam 1969a).

Other fungi known to invade the cuticle and flesh of crayfish include the species of the genera *Fusarium* and *Ramularia*. *Fusarium* spp. can cause significant disease in marine shrimp and lobsters, although mortalities associated with this organism have not been described in crayfish. *Ramularia* causes "burn spot disease," which appears as round brown or black spots in the exoskeleton, 1 cm or more in diameter, and often with raised margins (Unestam 1973) (Figure 43). The development of both of these infections in

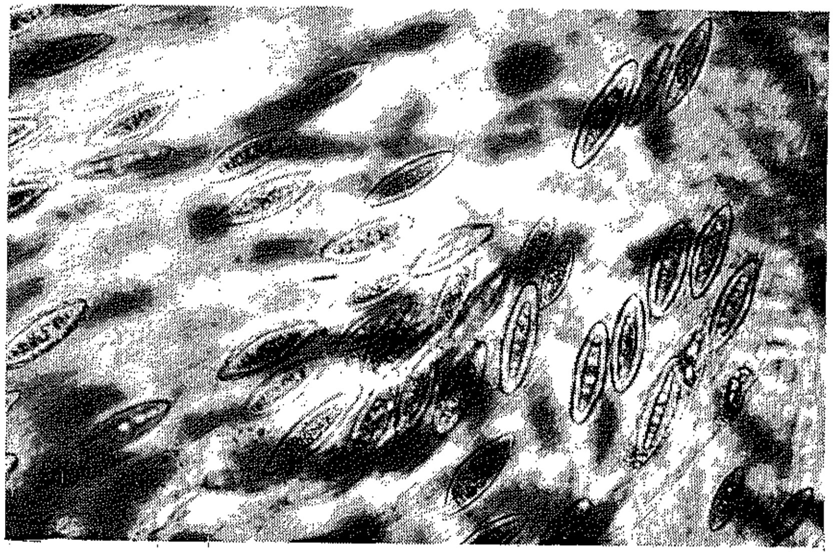
crayfish is favored by crowded conditions and breaks in the exoskeleton.

Psorospermium haeckeli is a unicellular organism of uncertain taxonomic status that is commonly seen in the tissues of Louisiana crayfish (Hentonnen et al. 1991). It has variously been classified as a protozoan (Grabda 1934; Schaperclaus 1954; Unestam 1975) and a nematode or trematode egg (Ljunberg and Monné 1968; Schaperclaus 1979), but current thought is that it is a fungal organism of some type, possibly a species of the dimorphic pathogenic fungi (Nylund et al. 1983; Alderman and Polglase 1988). The organism is detected microscopically as a distinctive ovoid object with a thickened cell wall and a highly vacuolated cytoplasm (Figure 44); however, thin cell wall forms are encountered regularly. There appears to be little or no host response to its presence in the tissue and no reports have been made of any mortality associated with the presence of this organism.

A fungal group known as Trichomycetes are common in crayfish

Figure 43. *Ramularia*, or "burn spot," on the dorsal aspect of a red swamp crayfish. R. Thune.

Figure 44. Characteristic form of *Psorospermium haeckeli* in crayfish tissue wet mounts. R. Thune.



intestines and occasionally on the cuticle. However there is some question as to whether these organisms should be considered parasites or commensals (Johnson 1977).

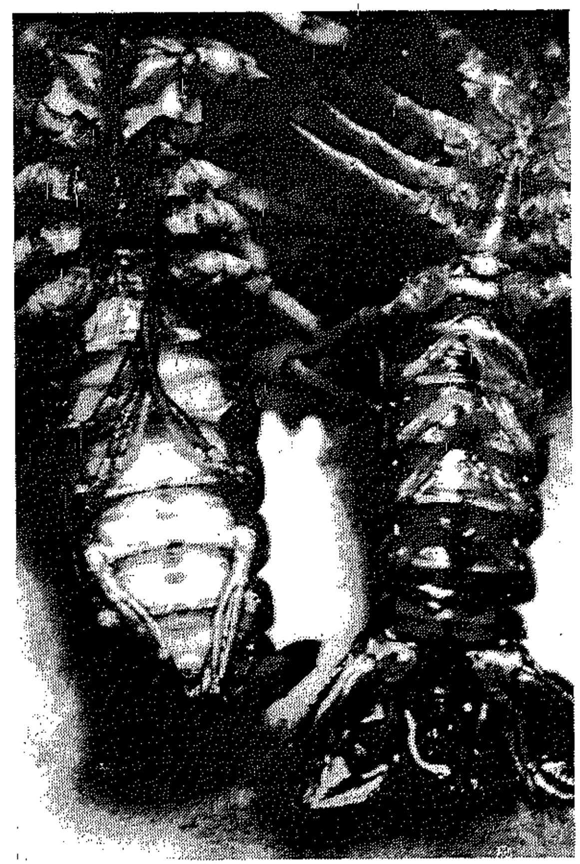
PROTOZOANS

Protozoa associate with crayfish as true parasites and as ectocommensals. Genera of the order Microsporida are the principal parasitic forms, while ectocommensals are genera of the subphylum Ciliophora.

The microsporida are intracellular parasites of invertebrates and lower vertebrates, with some species being quite pathogenic. Microsporideans infect crayfish when microscopic spores ingested by the host extrude a filament that deposits an infective stage through the gut wall. This infective stage presumably migrates to specific tissues to invade a host cell, where it divides repeatedly, producing

large numbers of spores. In crayfish the spores develop primarily in muscle and cause the tissue to take on a milky-white appearance (Figure 45), especially when substantial muscle tissue has been affected. In an advanced infection, animals are noticeably sluggish with an ineffectual tail-flick response. Histologically, normal muscle tissue is replaced by microsporidian cysts. Although little

Figure 45. Crawfish exhibiting the milky-white appearance of the abdominal muscle associated with a severe infestation of the microsporidean *Thelohania*. Normal crayfish on the right. R. Thune.



pathology or host response is evident, an infiltration of eosinophilic granular cells is apparent (Figure 46).

Microsporidiosis in crayfish, commonly known as porcelain disease in Europe, is primarily due to the genus *Thelohania* in the class microsporididea. Other genera of this class such as *Pleistophora*, *Nosema*, and *Inodosporaes* have been described from other crustaceans such as marine shrimp. *Thelohania* has microscopic uninucleate spores (Figure 47) produced by synchronous sporogony resulting in pansporoblasts containing 8 spores.

Ectocommensal protozoans of the phylum *Ciliophora* utilize the crayfish cuticle as a substrate for attachment and rely on bacterial populations in the water for nourishment (Fisher 1977; Couch 1983). Accumulations of suspended organic material often occur in commercial crayfish ponds in the spring, as indicated by increased turbidity. When this occurs, bacterial concentrations also increase, allowing ectocommensals to flourish (Figure 48). A study by Scott

Figure 46. Micrograph of a section of crayfish abdominal flexor muscle infested with the microsporidean *Thelohania.* Note the general lack of pathology and the infiltration of the tissue with large eosinophilic granulocytes (1). R. Thune.

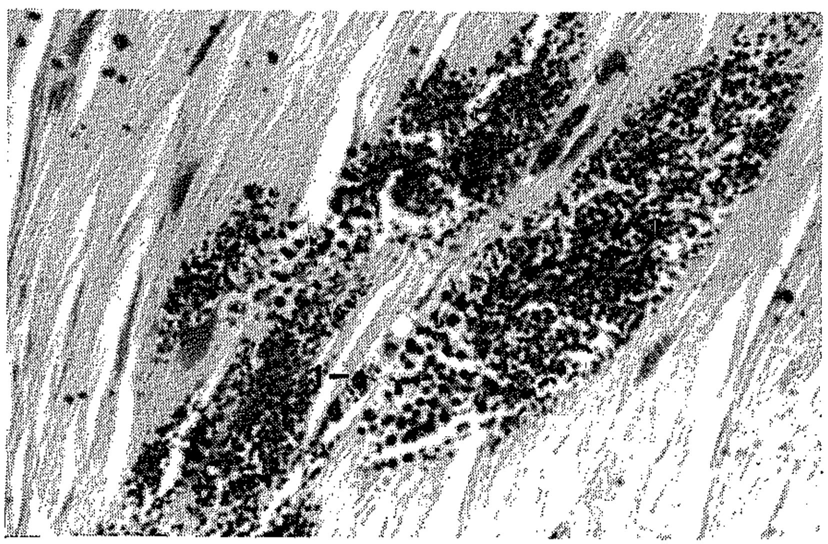
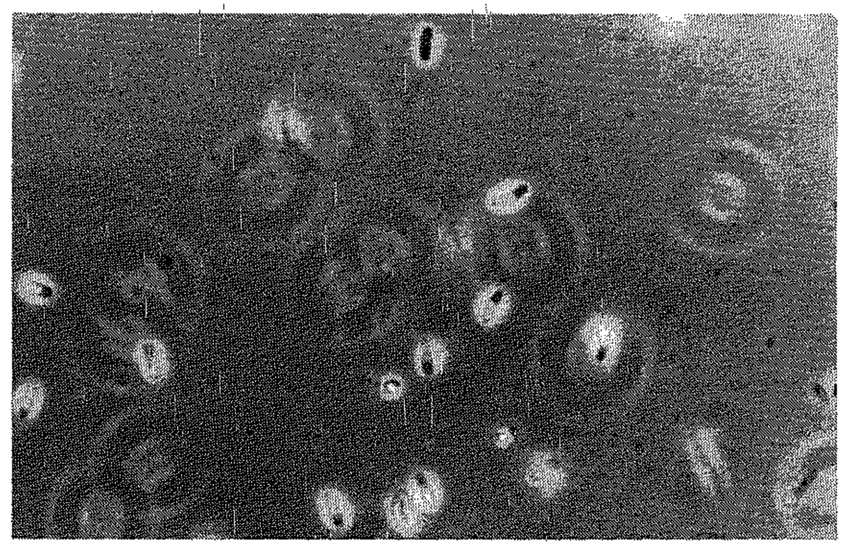


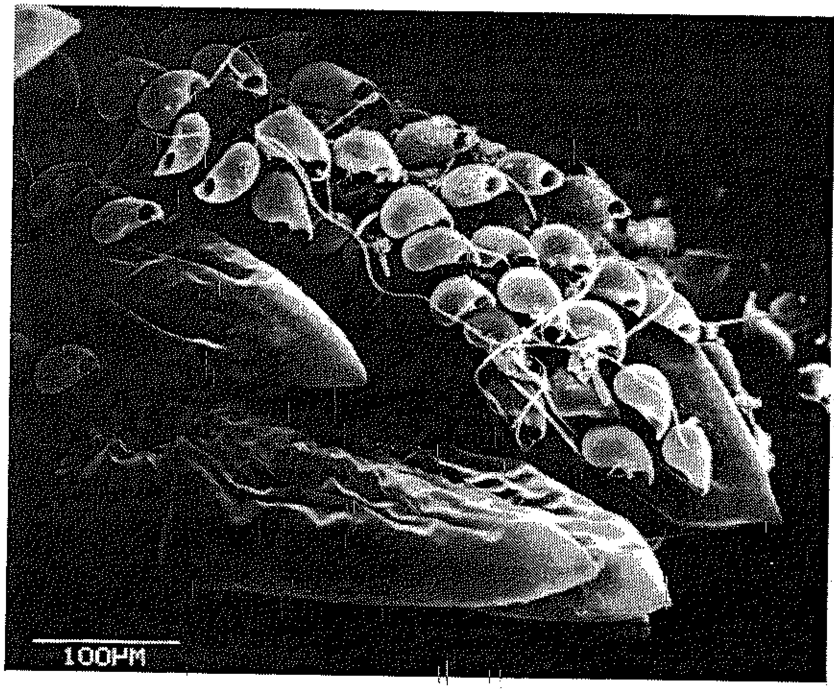
Figure 47. Phase contrast micrograph of *Thelohania* spores from the abdominal flexor muscle of an infested crayfish. R. Thune.



and Thune (1986b) indicated that 94% of crayfish sampled from commercial ponds were infested, with 65% of the infested crayfish carrying more than 100 ectocommensals per gill filament.

Ectocommensals most commonly associated with crayfish (Figure 49, A-D) are the peritrichs *Cothurnia*, *Epistylis*, *Lagenophrys*, and *Zoothamnium* and the suctorian *Acineta*, with the presence of any particular group depending on water quality. The peritrichs feed on bacteria and are favored by high bacterial levels in the water column (Sleigh 1973; Sawyer et al. 1979), while *Acineta* feeds primarily on free-swimming ciliated protozoans (Hall 1979; Sawyer et al. 1979). Turbidity is an excellent water quality indicator of potential peritrich infestations in commercial crayfish ponds (Scott and Thune 1986b), particularly for *Cothurnia* and *Epistylis*, the most commonly found genera. *Lagenophrys* is generally observed in low numbers, primarily in the spring. *Zoothamnium* is not common in the gill chamber of crayfish, but is often found on the exoskeleton.

Figure 48. Scanning electron micrograph of crayfish gill filaments carrying a heavy infestation of the ectocommensal protozoan *Cothurnia*. R. Thune.



Ectocommensal protozoans do not damage the gill surface (Vogelbein and Thune 1988), but their presence may result in increased susceptibility to low dissolved oxygen levels due to decreased gill surface area and impaired water flow through the gill chamber. Although several authors (Johnson 1977; Huner and Barr 1991) have postulated that ectocommensal protozoa can cause mortalities in commercial crayfish ponds, this has never been demonstrated with certainty. During periods when environmental conditions are favorable, crayfish can tolerate heavy branchial infestations without apparent harm and the infestations can spread to the exoskeleton, occasionally in high numbers without mortality (Figure 50). Whether or not these infestations increase the susceptibility of crayfish to the poor water quality conditions that periodically occur in com-

Figure 49. Ectocommensal protozoans commonly associated with Louisiana crayfish. A. *Epistylus*, B. *Acineta*, C. *Lagenophrys*, D, *Cothurnia*. R. Thune.

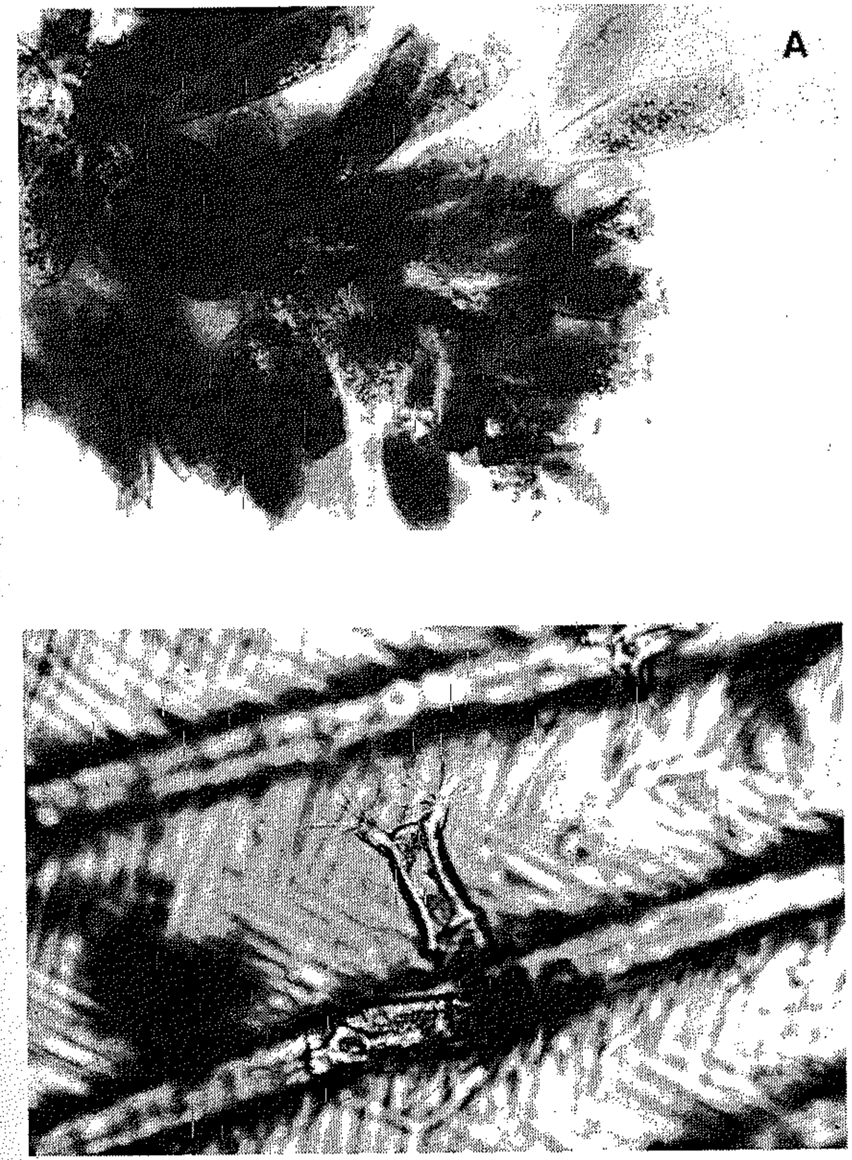
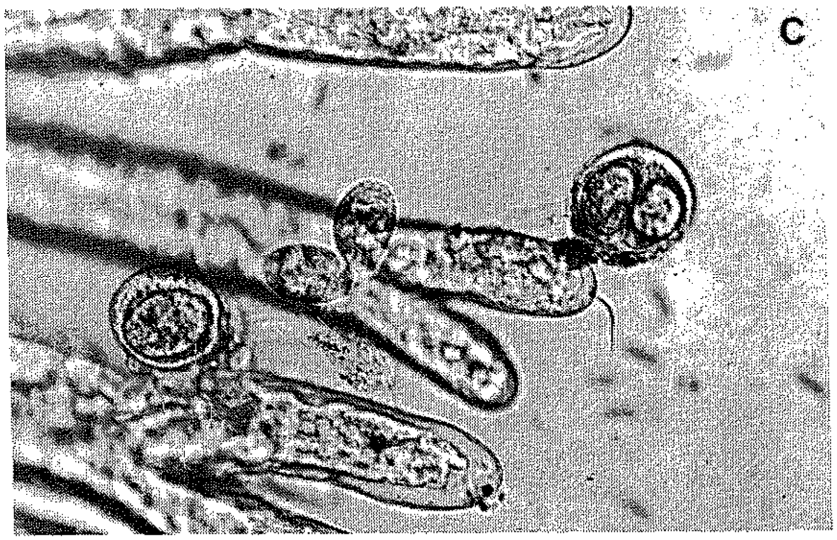


Figure 49 (continued)



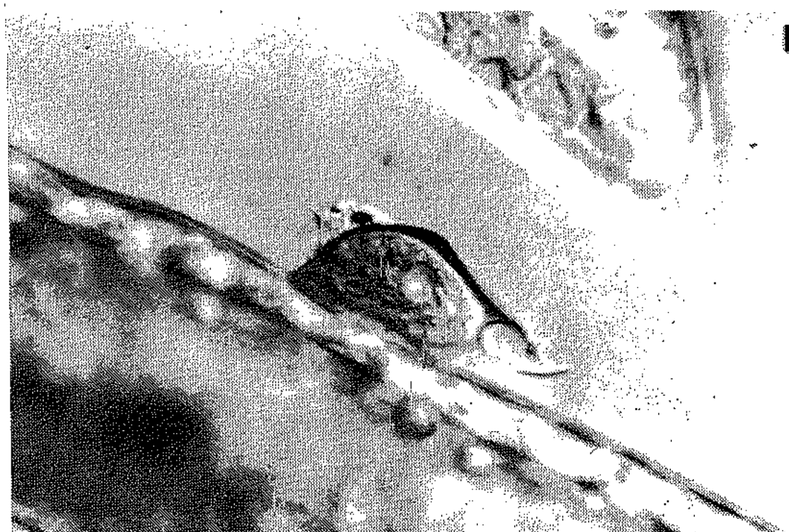
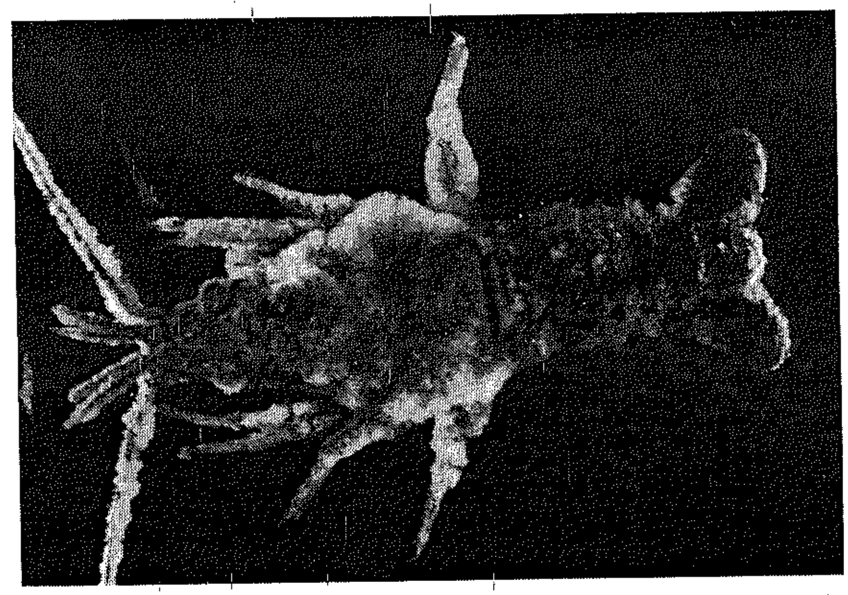


Figure 50. Extremely heavy infestation of *Epistylus* on red swamp crayfish from a pond with depleted forage during early winter. Numerous animals collected from this pond had loads ranging from a few patches to that pictured. R. Thune.



mercial ponds requires further study. However, critically low dissolved oxygen levels are most prevalent in the fall when ectocommensal levels are low due to lower food availability for the protozoans, and to the fact that young, rapidly growing crayfish molt frequently, which reduces their protozoan load. Thus, if ectocommensal protozoans limit commercial crayfish production in ponds, the effect would occur during occasional low dissolved oxygen levels in the spring, when suspended nutrient levels increase and mature crayfish that molt infrequently are predominant. Some mortalities of crayfish in soft-shell production systems have been associated with heavy infestations of protozoans on the gills. These crayfish exhibited a "wooly" appearance and died in molt (Huner unpublished).

One other group of ciliates found on crayfish belongs to the subclass apostomatia and is characterized by having a complex life cycle. One stage, the phoront, encysts on the crayfish cuticle. When the host molts, it exencysts and becomes a trophont which feeds on exuvial fluids or tissues of the dead or molted host. After a series of mitotic divisions, free-swimming tomites that can attach to new hosts are produced (Lee et al. 1985). Common genera in North American fresh and brackish water are *Hyalophysa*, *Gymnodinioides*, and *Terebrospira*, with *Hyalophysa* being more common in freshwater (Johnson 1977).

PARASITIC WORMS

Digenetic Trematodes

Only two groups of parasitic worms are described in crayfish. The first of these, members of the phylum Platyhelminthes, subclass Digenea, are characterized by having a complex life cycle involving two or more hosts. Most worms that parasitize crayfish are present as juvenile forms known as metacercaria, which develop into adult worms when ingested by the appropriate final host. Table 19 lists the most common metacercaria of Louisiana crayfish, their location in the tissues, and other hosts involved in the life cycle.

One particular digenea, *Paragonimus kellicotti*, deserves special mention because the adult develops in the lung of crustacean-eating mammals, including cats and dogs. Clinical signs, including dullness and intermittent cough, are mild. Human infections, although rare, have been reported. *Procambarus clarkii* also serves as an intermediate host for *P. westermani,* a potential human pathogen, in Japan (Hamajima et al. 1976). Thorough cooking eliminates the possibility of human infestation with either parasite. The metacercaria are found encysted in the heart of naturally infected crayfish.

Two digenetic trematodes, *Allocorrigia filliformes* and *Alloglossoides cardicola*, that utilize the crayfish as the final host and reside in the antennal gland have been described. Turner (1984) studied *A. filiformes* in *P. clarkii* and determined that the worm resides with its anterior end in the nephridial tubule of the antennal gland and its posterior end often extending into the excretory bladder. No host response was evident to the worm itself, but melanized nodules

Table 19. Common Metacercaria for Louisiana Crayfish.

	Location	First Intermediate Host	Final Host
Crepidostomum cornutum	Hepatopancreas, heart, cephalothorax	Sphaeriid clam, *Musculium*	Fish
Gorgodera amplicava	Stomach wall		Amphibians
Microphallus opacus	Hepatopancreas	*Amnicola*	Various vertebrates
Maritrema obstipum	Gill filaments and Hepatopancreas	*Amnicola*	
Macroderoides typicus	Cephalothoracic musculative	*Heliosoma*	*Amia calva*
Paragonimus kellicotti	Heart and surrounding membranes		Cats, mink, skunks, raccoons, fox, and other crawfish-eating mammals
Ochetosoma	Abdominal muscle	Physid snails	Watersnakes

containing trematode ova were observed within the interstices of the nephridial tubule. Turner (1985) describes *A. caridicola* as residing in the lumen of the nephridial tubules, where minute tegumental spines abraded the tubule epithelium. However, no host response to either worm was observed.

Spiny-headed worms

The second group of parasitic worms found in crayfish are the spiny-headed worms, represented by a single species, *Southwellinia dimorpha* (phylum Acanthocephala). *Southwellinia dimorpha* uses the crayfish as an intermediate host, encysting in the anterior portion of the abdomen, usually in association with the intestine. Adult worms of *S. dimorpha* are found in the small intestine of the white ibis, *Eudocimus albus* (Schmidt 1973). An embryonated egg containing the infective acanthor stage is released in the feces. Development proceeds in the intermediate crayfish host when the egg is eaten and the acanthor is released to penetrate the gut wall and encyst in the tissues surrounding the intestine. The worm then becomes parasitic, absorbing nutrients and developing into a rice-grain-sized white to pink cyst (Figure 51) containing the infective stage, called a cystocanth. The intermediate host must then be eaten by the final host where the adult develops and the life cycle is completed.

Southwellinia dimorpha seems to be more prevalent in ponds that are not completely dewatered and dried during the summer. If heavy infestations occur, market acceptability can be adversely affected due to the unsightly appearance when the tail is removed. In

Figure 51. Rice-grain-sized cystocanth cyst of *Southwellinia dimorpha* (1) along the intestine of a red swamp crayfish just posterior to the cephalothorax/abdomen junction. R. Thune.

addition, some cysts tend to remain attached to the tail meat when peeled, necessitating additional processing to remove them prior to packaging in commercial peeling operations.

MISCELLANEOUS

Members of the order Branchiobdellida in the phylum Annelida have an anatomy similar to oligochaete worms and leeches. Holt (1973, 1975) separates them from oligochaetes by the lack of setae, the fixed number of segments, the presence of suckers and the unpaired gonopores. He differentiates them from leeches by the anterior position of the testes and by the different number of segments. They are generally ectocommensal on the crayfish carapace, but some species are parasitic in the gill chamber of the host (Holt 1973, 1975). There are several reports of branchiobdellids found away from the host (Young 1966; Bishop 1968; Holt 1973), but little is known of their life cycle. It is presumed that they are dispersed primarily by body contact and that infestations are increased and maintained on a given host by the hatching of eggs commonly found on the carapace (Huner and Barr 1991).

A variety of other invertebrates are found inhabiting the surface of the crayfish carapace. Certain hemipteran insects known as water boatmen lay their eggs on the shell of crayfish and can become numerous when populations of these insects are high and crayfish molting frequency is low. A variety of ostracod crustaceans, rotifers, temnocephalid flatworms, leeches, and nematodes are also found as free-living inhabitants of the crayfish cuticle, but, as with the other ectocommensal organisms described previously, they cause little damage and are purged during molting.

Uropod swelling is commonly observed in crayfish but has no apparent adverse effects. This syndrome seems to be more apparent in the spring when *Procambarus* spp. are growing rapidly (Huner and Barr 1991).

Older mature *Procambarus* spp. may undergo severe muscular atrophy followed by death, presumably associated with old age. Lindqvist and Mikkola (1979) first described this syndrome histologically in a *P. clarkii* population in Kenya.

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Cultivation of Freshwater Crayfishes in Europe

Hans Ackefors

Department of Zoology
Stockholm University
Stockholm, Sweden

Ossi V. Lindqvist

Department of Applied Zoology
University of Kuopio
Kuopio, Finland

Section I: Introduction

The recorded history of the use of crayfish as well as of their culture and management in Europe extends back hundreds of years, at least to the Middle Ages. In central Europe, Emperor Maximilian I of the Habsburg Empire and the Archbishops of Salzburg gave written orders on crayfish catching. Monasteries of the time in the fifteenth century had their own ways of cultivating, holding, and cooking crayfish (Spitzy 1973), apparently to be consumed especially as a lenten fare. Earlier still, in the old Roman Empire, crayfish appear in a recipe from the third century A.D. published by Caelius in *De re culinaria* (Lagerqvist and Nathorst-Böös 1980).

In Scandinavia, written records on the interest of the Swedish royal house in crayfish come from the sixteenth century (Abrahamsson 1969). The Wasa Kings introduced the noble crayfish *Astacus astacus* from Germany in the early sixteenth century and kept them in ponds. Most likely natural crayfish populations existed